



PROCEEDINGS OF THE  
NATIONAL WORKSHOP ON ANIMAL BIOTECHNOLOGY

*Held at*

MADRAS VETERINARY COLLEGE, MADRAS.  
On July 26 - 28, 1990.

*Sponsored by*

Department of Biotechnology,  
Ministry of Science and Technology,  
Govt. of India, New Delhi.

AND

Tamil Nadu Veterinary and Animal Sciences University  
Madras.

*Organised by*

Department of Animal Biotechnology,  
Madras Veterinary College, Madras 600 007.

AND

Fisheries College, Tuticorin.

EDITORS : Dr. V.D. Padmanaban and Dr. M. Devaraj

**Proceedings of the National workshop  
on Animal Biotechnology**

Released by

**Thiru. D. JAYAKUMAR**, B Sc , B.L.,  
Hon'ble Minister For Animal Husbandry,  
Fisheries and Forest.  
Govt. of Tamilnadu.

**on 20-7-1991**

Published by

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Dept. of Animal Biotechnology,  
Madras Veterinary College,  
Madras-7.

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July 26 - 28, 1990**

**EDITORS**

**Dr. V.D. Padmanaban and Dr. M. Devaraj**



2. Evaluation of leaves of some wild plants as supplementary Source in the diet of Mrigal (*Cirrhinus mrigala*) (Hamilton 1822) fingerlings  
– *R. Pappathi and Samuel Paulraj* 76
3. Status of Shrimp Feeds: Formulation, Manufacture and Marketing  
*N. Sukumaran, Md. Kaleemur Rahman and M. Devaraj* 82
4. Karyological Studies on Toda Buffaloes in Comparison with Murrah and their Crosses  
– *V. Thiagarajan, A.R. Krishnan and V. Ulaganathan* 89
5. Polypeptides of Rinderpest Virus  
– *G. Dhinakar Raj and P.P. Bhat* 96
6. Strategies to Maximise Freezability and to Minimise Cryo – Injuries in Rooster Sperms  
*A.J.S. Austin and N. Natarajan* 101
7. Physical and Biochemical Aspects of Murrah Buffalo Bull Semen and their Relationship with Freezability  
– *A. Mahalinga Nainar, B.M. Easwaran, M. John Edwin and V. Ulaganathan* 106
8. Comparison of Superovulatory Response of Cross Bred Cows to FSH and PMSG Treatment  
– *D. Kathiresan and T.G.Devanathan* 111
9. Effect of Two Different Routes of PGF<sub>2</sub> Alpha Administration in Crossbred Cows  
– *D. Kathiresan, A. Palaniswamy, Cecilia Christopher, S. Balasubramanian and S.R. Pattabiraman* 114
10. An Attempt to Separate X and Y Bearing Bovine Sperms  
– *P. Kanakaraj, B.M. Easwaran and M. John Edwin* 117
11. Reproductive Performance of Tellicherry Goats  
– *S. Thilagar, M. Mohan, R. Manickam, R. Balagopal and K.G. Sadasivam Pillai* 120
12. Prospectives of Embryo Transfer in Goats  
– *S. Thilagar, D. Kathiresan, M.S. Dewan Muthu Mohammed and V.D. Padmanaban* 123
13. Relationship of Serum Alkaline Phosphatase with Production Traits in Broiler Rabbits  
– *K. Venkoba Rao, P. Thangaraju, V. Ulaganathan, Mustaq Ahmed and B.M. Easwaran* 126

14. Protein Pattern Variations in the *Cyprinus carpio* Muscle on Exposure to Endosulfan  
- K. Riji John, N. Jayabalan and G.M. Rosalind 130
15. Studies on the Autoimmune Responses in Tilapia  
- Md. Kaleemur Rahman, K. Riji John and G.M. Rosalind 138
16. Antibiotics as Growth Promoters in Common carp *Cyprinus carpio* Fry  
- S. Vijayan, R. Suresh and V. Sundararaj 143
17. The Effect of 17 Alpha Methyl Testosterone on Growth in Black Molly, *Poecilia sphenops*  
- S. Felix, N. Sukumaran, V. Sundararaj and I. Rachel 148
18. Application of Molecular Techniques in the Diagnosis and Control of Rabies  
- R. Jayakumar and S. Nedunchellian 152
19. Studies on Immunisation Against *Schistosoma nasale*  
- R. Anandan and E. Ebenezer Raja 156
20. DNA Hybridization in Veterinary Disease Diagnosis  
- P. Ramadass 160
21. Evaluation of Coagglutination Test for Rapid Detection of Salmonella Enterotoxin  
- H. Rahman, V.D. Sharma and V.B. Singh 163
22. Studies on the biological control of horse - flies by hymenopteran insect, *Telenomus* sp  
- S.A. Joseph and G. Karunamoorthy 169
23. Micro Elisa for Detecting Newcastle Disease Virus Antibodies in Chicks  
- Parimal Roy and V.D. Padmanaban 176
24. Detection of Infectious Bursal Disease Virus by Reversed Passive Haemagglutination Method  
- K. Nachimuthu, G. Dhinakar Raj, A. Thangavelu and R.A. Venkatesan 180
25. Production and Evaluation of Anti - Idiotypes For Rinderpest  
- A. Thangavelu and B. Sharma 183
26. Sub - Unit Vaccine for Poultry against Newcastle Disease Virus  
- K. Vijayarani, K. Kumanan, A. Albert and V.D. Padmanaban 189
27. Adaptation of Sheep Pox Virus to BHK<sub>21</sub> Cell Line  
- J. John Kirubaharan and V.D. Padmanaban 194

28. Embryo Vaccination for Marek's Disease  
 - *V. Ramaswamy, N. Dorairajan and V.D. Padmanaban* 201
29. Strategy of Split Dose Vaccination for Foot and Mouth Disease in Lambs  
 - *N. Dorairajan, V. Ramaswamy and V.D. Padmanaban* 204
30. A Report on the Outbreak of Ranikhet Disease in Japanese Quail Layers  
 - *S. Elankumaran, K.S. Palaniswami, V.N. Appaji Rao, John Francis Gnanbaranam and G.A. Rahamathulla Khan* 208
31. Characterization of A Newcastle Disease Virus from Japanese Quails in Lay  
 - *S. Elankumaran, K.S. Palaniswami, John Francis Gnanbaranam, V.N. Appaji Rao and G.A. Rahamathulla Khan* 210
32. Screening of Enzootic Bovine Leucosis in Imported Exotic Bulls  
 - *V.N. Appaji Rao, S. Elankumaran, K.S. Palaniswami, G.A. Rahamthulla Khan and John Francis Gnanabaranam* 214
33. Enzyme Linked Immunosorbent Assay for the Detection Antibodies To Leptospirosis  
 - *K.S. Venkataraman, J. Ramkrishna and N. Raghavan* 216
34. Usefulness of Enzyme Linked Immunosorbent Assay in the Diagnosis of Brucellosis  
*V.K. Srinivasan, J. Ramkrishna and N. Raghavan* 220
35. Rapid Diagnosis of Canine Coronaviral Enteritis  
 - *P.I. Ganesan, L. Gunaseelan and M. Thanappa Pillai* 223
36. Detection of Blue Tongue Virus Antigen in the Sera of Sheep by Agar Gel Immuno Diffusion Test  
 - *R. Govindarajan, Johnson Rajeswar, Vajravelu Jayakumar and J. Doraiswamy* 226
37. Purification of Duck Hepatitis B Virus Surface Antigen by Poly Acrylamide Gel Electrophoresis  
*G. Sridhar, T. Valliammal, S.P. Thyagarajan and J. Ramkrihna* 230
38. Evaluation of A Tissue Culture Strain of Newcastle Disease Virus for Vaccine Production  
 - *K. Kumanan, A. Padmaraj and V.D. Padmanaban* 233
39. Studies on the Screening of Fishes Grown in Sewage - Fed Water for *Enterobacteriaceae* (*Shigella* spp.) Group of Pathogens  
 - *P. Rajasekaran* 237

	Page No.
40. Parasite Induced Changes on the Haematology of Fresh-water Catfish, <i>Mystus guilto</i> (Hamilton – Buchanan) – S. Felix and P. Natarajan	242
41. Bottlenecks In Increasing the Production in Poultry Industry in Tamil Nadu – R. Balasubramanian	247
42. Pre and Post Weaning Performance of Broiler Rabbits – P. Thangaraju, K. Venkoba Rao, N. Kandaswamy, B.M. Easwaran and V. Ulaganathan	254
43. Association of Milk Yield, Major Milk Constituents and Mineral Contents in Crossbred Cows and Buffaloes Milk – P.N. Padmanabhan and V. Ulaganathan	258
44. Possibilities of Fish Rearing in Sewage water – Godavari Kamalanathan	263
45. Sewagefed Fishculture with Reference to Different BOD Levels – S. Balasubramanian, M.R. Rajan and Samuel Paul Raj	266
46. Treatment of Waste Water from Seafood Processing Plant for Fish Production – A. Srinivasan, V. Ramadhas and R. Santhanam	275
47. Utilization of Sewage Nutrients for Mass Culture of Algae for Fish Feed – B. Manimaran, R. Santhanam and V. Ramadhas	279
48. New Vista for Economic Utility of Coastal fallow Land by Prawn Culture with Saline Ground Water – J. Bojan and K.R. Ramesh Babu	283
49. Effect of The Plant Enzymes (papain) on the Acceleration of Fermentation Process in Fish Sauce Preparation – K. Rathnakumar, M.H. Bhandary and T.M.R. Shetty	287
50. Effect of Enzyme on the Quality of Fish Sauce – P. Velayutham and G. Jegatheesan	294
51. Biotechnology As a Tool in Aquaculture Its Relevance in Indian Context – M.J. Prince Jeyaseelan, J.D. Jameson and V. Sundararaj	299
<b>6. List of Participants</b>	<b>308</b>
<b>7. Author Index</b>	<b>310</b>



## TAMIL NADU VETERINARY AND ANIMAL SCIENCES UNIVERSITY

### FOREWORD

The National Workshop on Animal Biotechnology, jointly sponsored by the Department of Biotechnology, Government of India, New Delhi and Tamil Nadu Veterinary and Animal Sciences University, held at Madras during July 26-28, 1990 was organised by the Department of Animal Biotechnology, Madras Veterinary College, Madras and Fisheries College, Tuticorin. The workshop was well attended by experts in Veterinary and Animal Sciences and Fisheries Sciences from the length and breadth of the country. The three days celebrations went on with business like precision. The number of papers were so many and the discussion thereon was so interesting that it necessitated simultaneous sessions on the last day. The plenary session witnessed fruitful debate leading to meaningful recommendations. The organisers won the appreciation of one and all.

I am happy that the organisers have been able to bring out the proceedings of the workshop in the form of a book within a short period. If the recommendations are implemented faithfully, the purpose of this Workshop shall have been fulfilled. I wish to place on record my appreciation of the magnificent work carried out by Dr.V.D.Padmanaban, Professor and Head, Department of Animal Biotechnology, Madras Veterinary College, Madras-7 and the active support from Dr.M.Devaraj, Dean, Fisheries College, Tuticorin. I wish to acknowledge the relentless work carried out by the staff of Madras Veterinary College, and Fisheries College, Tuticorin in the successful conduct of this workshop.

This proceedings would not have been published but for the support of the Department of Biotechnology, Government of India. I wish to thank Dr. S. Ramachandran, Secretary, Department of Biotechnology for his unstinted support and personal interest shown.

Madras  
15-3-91

*Richard Masillamony*

**P. Richard Masillamony,**  
Vice-Chancellor.

## PREFACE

When we undertook the ambitious task of conducting the National Workshop on Animal Biotechnology, we received instantaneous support from the Department of Biotechnology, New Delhi and the Tamil Nadu Veterinary and Animal Sciences University by way of generous grant from the former and active support from the latter. As we went through the process, we received support from all quarters we approached. Our colleagues at Madras Veterinary College and Fisheries College gave us commendable support but for which the workshop would not have been what it was. The encouragement from the participants and various officials made us to venture publishing the proceedings in the form of a book. M/s. Akshara, our printers did this job with professional excellence.

We wish to place on record our sincere thanks to the Secretary, Department of Biotechnology, New Delhi and the Vice-Chancellor, Tamil Nadu Veterinary and Animal Sciences University, Madras for their support, encouragement and guidance.

We are thankful to the Secretary to the Government of Tamil Nadu, Department of Animal Husbandry and Fisheries, Advisor, Department of Biotechnology, New Delhi, the Deputy Director General (AS), ICAR and the Deputy Director General (FS), ICAR for the encouragement.

We are grateful to the officers of the Department of Biotechnology and our University, Director of Animal Husbandry, Madras. Managing Director, TAPCO and Director of Fisheries, Madras for their cooperation and support.

We are thankful to the numerous sponsors such as Indian Overseas Bank, Indian Bank, State Bank of India, M/s. George Maijo, M/s. Vishal Scientific Co., M/s. Spinco., M/s. RIA, M/s. Electronics and Electricals, and a host of others for their financial support.

We will be failing in our duty if we do not thank our Colleagues who spared no effort in making this workshop a great success.

The Finance Officer of the University was very meticulous in keeping the accounts and our special thanks are due to him.

*V.D.Padmanaban.      M.Devaraj.*

**EDITORS.**

# NATIONAL WORKSHOP ON ANIMAL BIOTECHNOLOGY

JULY 26-28, 1990

JOINTLY SPONSORED BY

DEPARTMENT OF BIOTECHNOLOGY, NEW DELHI  
AND  
TAMIL NADU VETERINARY AND  
ANIMAL SCIENCES UNIVERSITY, MADRAS

## PROCEEDINGS

### INAUGURATION

The Workshop organised by the Department of Animal Biotechnology, Madras Veterinary College and Fisheries College, Tuticorin was held at Madras Veterinary College. The registration of delegates started from 10.00 A.M. on 26.7.1990.

The workshop was inaugurated by Hon'ble Union Minister for Science and Technology at the Anna Auditorium. Hon'ble Minister for Animal Husbandry and fisheries, Government of Tamil Nadu presided over the function which started at 2.30 P.M. on 26.7.1990 with prayer.

Hon'ble Union Minister for Science and Technology declared open the Animal Biotechnology Laboratory in the immediate presence of the Hon'ble Minister for Animal Husbandry and Fisheries, Government of Tamil Nadu and other dignitaries. The Union Minister released the Souvenir and the first copy was presented to the Hon'ble Minister for Animal Husbandry and Fisheries, Government of Tamil Nadu.

Hon'ble Union Minister of state for Science and Technology in his inaugural address congratulated Tamil Nadu Veterinary and Animal Sciences University which is unique in being the first University of its kind though deemed University status has been given to IVRI, Izatnagar, NDRI, Karnal and Central Institute of Fisheries, Bombay. Hon'ble Minister said that Human dependence on variety of animals for clothing, companionship, ornaments and sports has been known. Selection of animals has been going on for a long time. It became a science from the period of Mendel. Men's approach towards his food has been changing from food gathering to cultivation, intensive cultivation and so on. He wanted diversification of food basket.

Animal Biotechnology, Hon'ble Minister said is a new tool to the new University for a true transformation. Hon'ble Minister outlined the development through AI wherein the annual genetic improvement is not more than 2% and the next stage, embryo transfer where female is also fully used. Hon'ble Union Minister emphasized that just as there has

been a shift from vegetative growth to grain, i.e. different partition of final biomass, in the Animal Husbandry sector better feed with growth hormone, should be formulated. Growth hormone which could prevent the Nitrogen going as waste as urea in excreta and utilise the same in terms of milk and flesh. Long action and selective action should be achieved.

The next area Hon'ble Union Minister emphasized is the Ruminant Nutrition where the indigestible biomass could be converted by using enzymes such as cellulase and hemicellulase obtained through recombinant DNA technology.

Hon'ble Union Minister said that it could be appropriate that the entire gamut of Biotechnology as it relates to animals and fishes are dealt in one University as every operation involves many aspects. The Union Minister cited the example of shifting the inequality between the number of sperms and ova by in vitro fertilisation, sexing, splitting zygotes etc. which is applicable to any other living system. He wished the workshop every success and assured support from central government for meaningful projects and proposals.

Hon'ble Minister for Animal Husbandry and Fisheries in his Presidential address welcomed all the delegates and dignitaries on behalf of the state. He cited that the Tamil Nadu Veterinary and Animal Sciences University was started by Hon'ble Chief Minister for development of society and for improvement of society by exploiting the research achievements of the scientific work. Hon'ble Minister said that Biotechnology, one of the recent branches of

biology, has already proved itself and he was happy that the infant university within a year of its inception through its research and extension work has impressed every one so much that the Department of Biotechnology has come forward to jointly sponsor the workshop. The Hon'ble Minister was happy the Tamil Nadu Veterinary and Animal Sciences University has created Animal Biotechnology laboratory within a short span of time.

The Hon'ble Minister requested Hon'ble Union Minister to kindly look into the recognition of the University by U.G.C. and I.C.A.R. and also requested for big financial support from central Government, I.C.A.R, Department of Biotechnology and other agencies for the new University.

Hon'ble Minister emphasized that the research in Animal Biotechnology should be beneficial to poor farmers downtrodden and other weaker sections.

He assured the Central Government that necessary support would be given to the proposed National Marine Biological Resource Centre and said that for shrimp industry, already 5000 HA of land has been earmarked and part of the lands has been given. Hon'ble Minister wanted the delegates to discuss about research as well as extension.

Dr. S.Ramachandran, Secretary to Govt. of India, Department of Biotechnology, New Delhi in his Keynote address congratulated the Government of Tamil Nadu on the formation of Tamil Nadu Veterinary and Animal Sciences University by pooling training, teaching and research facilities available in premier

institutions of the state involved in the Animal Husbandry and Fisheries sections. He stressed on the need for continuous updating of facilities, infrastructure and manpower to produce Science and Technology of high quality and to serve the country more effectively. He complemented Madras Veterinary College as one of the outstanding examples for several decades for producing outstanding graduates. He said that Animal Husbandry involves inter disciplinary approach. He conceded that Animal Husbandry has received less attention and suffered from lack of impetus *vis a vis* with agriculture and declared that it is time for changing this situation and provide greater impetus to Animal Husbandry. He felt that the day is not far when the Indian Council for Veterinary and Animal Husbandry research will be formed.

Dr.S.Ramachandran said that there is enormous scope in Animal Husbandry activities. He said Agriculture is dependent on animals for draught, transport, milk etc. for producing more than double by 2000 AD. He wanted increase in production without increase in number. He informed that several task forces have studied and came out with the importance of embryo transfer and in increasing production of milk from 500 kg/animal/lactation to 1500 kg/animal / lactation and for doubling the existing yield of 50 million tonnes of Dairy products with reduction in number. He said that the country has to depend on Animal power and would need 60-80 MW of Animal power approximating to 120 million cattle to produce 250 million tonne of food by 2000 AD.

He complemented Tamil Nadu Veterinary and Animal Sciences University in getting the first calf by embryo transfer. He stated that Department of Biotechnology has already gone ahead in this field with one Main centre at Gujrat, four regional centres and 6 state centres. He said that Department of Biotechnology would start a centre in every state and provide infrastructural facilities. He complemented Tamil Nadu Veterinary and Animal Sciences University for the ET programme in sheep and goats and said Department of Biotechnology also has launched embryo transfer in camels.

Dr.S.Ramachandran said that India has got 7000 KM of coast line with 70000 KM of rivers and inland waterlines with water for at least 4 to 6 months in a year. He said that we are not exploiting the wealth which foreign countries are exploiting very close to us. He stressed the need to develop indigenous feed. He wanted a big fillip given to this sector and said Department of Biotechnology will provide all help wherever necessary to deserving projects.

Dr.Ramachandran stressed on the urgent need for development of hightech feed for intensive and semi intensive prawn culture. He cited how small countries like, Sri Lanka, Taiwan etc. could get a yield of 10 to 20 tonnes/ha whereas our yield is at 1. tonne/ha. He wanted the scientist to evolve indigenous feed with FCR of 1.8 to 2.0 using agro residue and waste materials and convert them as value added fodder and feed.

He outlined plans of the Centre to set up National Marine Biological Research Centre or centres and

thanked the authorities of Tamil Nadu for providing all the help in this regard.

He concluded his address with the remarks that funds would not be a problem if only meaningful projects are put forth and time bound results directly applicable to farmers are achieved with suitable methods for technology transfer.

Dr.S.Ramachandran wanted effective technology generation, transfer, absorption and utilisation as such a system from start to finish will alone be successful. He wanted that scientific programme, should be of relevance, beyond the realm of the research centres, for adoption in the field and drawn in cooperation with the industry and farmers.

Earlier Dr.P.Richard Masillamony, Vice-Chancellor, Tamil Nadu Veterinary and Animal Sciences University, welcomed the chief guest, dignitaries, delegates, faculty members, retired staff, press, Doordharshan, AIR, staff and students. The Vice-Chancellor indicated that the delegates who have come from all parts of the country would discuss on the base document prepared by an expert committee under the aegis of the Department of Biotechnology, New Delhi on increasing the production and productivity from marine and freshwater aquaculture and poultry and bring out recommendations which can be followed throughout the country.

The following offered felicitations:

Thiru Rahumankhan, M.L.A. and Vice-Chairman, Small Savings department, Government of Tamil Nadu emphasized that the scientists available at Tamil Nadu Veterinary

and Animal Sciences University are second to none but they lack infrastructural facilities. He was happy about the efforts taken by Tamil Nadu Veterinary and Animal Sciences University in trying to increase production and productivity of animals and again stressed the need for providing facilities to the scientists.

Dr.(Mrs) Manju Sharma, Advisor to Government of India, emphatically stated that the Department of Biotechnology, Government of India, New Delhi would support programmes in modern biology especially those leading to increase in production and productivity of animals, including fishes leading to better availability of food.

Thiru D.Sundaresan, Secretary to Government, Government of Tamil Nadu stressed on the need for qualitative and quantitative improvement and hoped that the Department of Biotechnology would come in a big way to support the young Tamil Nadu Veterinary and Animal Sciences University. He thanked the ICAR for the support and for recommending the recognition of the university and hoped that through the good offices of the Hon'ble Union Minister, U.G.C. will accord formal recognition soon.

Dr. R.M. Acharya, Deputy Director General (Animal Sciences), ICAR, complemented the Government of Tamil Nadu on the formation of Tamil Nadu Veterinary and Animal Sciences University and stressed the need for bestowing more attention to Animal Sciences including Fisheries sector both at the National level as well as at state level.

Dr.P.V.Dehradrai, Deputy Director General (Fisheries Sciences), ICAR, complemented the Government of Tamil Nadu for taking the rightful step in forming a separate university for Veterinary Animal Sciences including Fisheries. He wanted the available potential of germplasm or genepool to be completely exploited.

Dr.T.K.Govindarajan, Director of Animal Husbandry, Madras, requested the Department of Biotechnology and Tamil Nadu Veterinary and Animal Sciences University to give greater emphasis for embryo transfer to get more superior calves from elite mothers and also to develop high tech vaccines and diagnostic kits.

Thiru S.Machendranathan, IAS, Director of Fisheries emphasized the need for bringing more area under aquaculture and also the need for mechanisation. He stressed on the need for produce diversification and product development with intensive fish culture using the talent available.

Thiru R.Rathinaswamy, IAS, Managing Director, TAPCO wanted steps to be taken to transfer the available technology to the farmer fully. He wanted the Department of Biotechnology, Government of India and the Universities all over the country to plan effective methods for such a transfer. He wanted the Department of Biotechnology and Tamil Nadu Veterinary and Animal Sciences University to take up frontier areas of research in poultry especially feed, health cover, upgradation of germplasm and transgenic animals.

Dr.V.Gnanaprakasam, Registrar i/c, Tamil Nadu Veterinary and Animal Sciences University proposed a vote of thanks. The inaugural function ended with National anthem.

The delegates and dignitaries went round the new laboratory facilities created and the exhibition organised portraying the activities of the Department of Animal Biotechnology, Madras Veterinary College, Madras and Biotechnology Unit, Fisheries College, Tuticorin.

## REPORT ON TECHNICAL SESSIONS

The Workshop consisted of five technical sessions besides a Plenary session. More than 120 delegates all over the country attended the workshop both from Veterinary and Animal Sciences as well as Fisheries faculties.

The first session on Feed and Nutrition started at 8.30 A.M. on 27.7.90. Dr.P.V.Dehradrai, Deputy Director General (Fisheries Sciences) was requested to chair the session.

Dr.R.Natanam, Professor and Head, Department of Animal Nutrition, Veterinary College and Research Institute, Namakkal delivered for key note address on "Biotechnological innovations in the field of Animal and Poultry Nutrition".

Dr.P.K.Mukopadyaya, Scientist, CIFA, Dhali, Orissa, delivered his key note address on "Fresh water finfish and shellfish nutrition and feed development in India".

Dr.R.Paulraj, Scientist, Central Marine fisheries Research Station delivered his key note address on "Marine finfish and shellfish nutrition and feed development in India".

Six papers were presented during this session by scientists.

The house discussed in detail all the papers as well as key note addresses and suggested their recommendations for future course of action in this sector.

The second session on Genetics and growth promotion started at 11.30 A.M. on 27.7.90. Dr.T.K.Govindarajan, Director of Animal Husbandry, Madras was requested to chair this session.

Dr.V.Ulaganathan, Director, Centre for Animal Production Studies, Tamil Nadu Veterinary and Animal Sciences University, Madras delivered his key note address on "Genetics and growth promotion for enhancing livestock and poultry production".

Dr.Kaleemur Rahman, Fisheries College, Tuticorin delivered his key note address on "Genetics and growth promotion in mariculture".

Fifteen papers were presented in the field of Veterinary and Animal Sciences and nine papers were presented in the field of Fisheries during his session which prolonged even after lunch and the session witnessed fruitful discussions on many aspects of this branch of biotechnology. The session came to a close by 3.30 P.M. after drawing recommendations for adoption.

The third session on Health care and disease control started at 4.00 P.M. and was initially chaired by Dr.S.C.Adlakha, Far Eastern Regional Research Office, U.S.Embassy, New Delhi and later by Dr.M.C.Prasad, Assistant Director General (AH), ICAR, New Delhi.

Dr.Shaila Subba Rao, Indian Institute of Science delivered her key note address on "Nucleic acid hybridisation technique and its use in diagnosis of *viral diseases*". Her address was followed by key note addresses on Health care and Disease control in fresh water aquaculture by Dr.S.C.Mukherjee, Scientist, CIFA, Dhali, Orissa and on "Diseases of Marine Finfishes and shellfishes" by Dr.P.Vedavyasa Rao, Scientist,

CMFR Research Station, Mangalore.

More than 25 papers were listed for presentation during this session. Only 10 papers could be presented till 7.00 P.M. when the session was adjourned for the next day.

On 28.7.90 two sessions were conducted simultaneously. At the Conference Hall, the session on "Health care and disease control" was continued with Dr.Shaila on the chair. This session came to a close with the presentation of scientific papers followed by discussion and framing up of recommendations for future action.

At Swarajya Hall, the IV session on Management was chaired by Dr.K.Alagarwami, Director, CIBA, Madras, Dr.P.Kothandaraman, retired Dean, Madras Veterinary College, delivered his key note address on "Management of poultry". Dr.M.Devaraj, Dean, Fisheries College, Tuticorin delivered his key note address on "Management of Marine Fisheries and Mariculture". Twelve papers were presented by scientists from different parts of the country during this session. The session ended with recommendations for further action.

The fifth session on Post-harvest technology was chaired by Dr.P.Kothandaraman, retired Dean, Madras Veterinary College, Madras. Dr.J.C.Nair, Indian Overseas Bank, Madras delivered his address on "Bank's role in increasing the production and productivity of animals including fishes". Two key note addresses were delivered during this session. Dr.K.Nageswara Rao, Scientist, Central Poultry Training and Research Centre, Hessarghatta, Bangalore delivered his key note ad-

dress on "Post-harvest technology in relation to poultry".

Dr.P.V.Prabhu, Head of Division, CIFT, Cochin delivered his key note address on "Post-harvest technology in Inland fisheries. This session ended after presentation of scientific papers and drawing up of recommendation."

The Plenary session started at 2.30 P.M. on 28.7.90. In the absence of Thiru D.Sundaresan, Secretary to Government, Animal Husbandry and Fisheries Department, Government of Tamil Nadu, Dr.P.Richard Masilamony, Vice-Chancellor, Tamil Nadu Veterinary and Animal Sciences University, chaired this session. Thiru S.Machendranathan, IAS, Director of Fisheries, Government of Tamil Nadu and Thiru R.Rathinaswamy, Managing Director, Tamil Nadu Poultry Development Corporation, Madras acted as Co-Chairman.

Dr.V.D.Padmanaban, Chairman and Contact Person, National Workshop on Animal Biotechnology, welcomed the gathering, briefed about the workshop and thanked all concerned for their unstinted cooperation and advice.

The Chairman/Rappoteurs of respective sessions presented their recommendations in the respective session. Each and every recommendation was discussed in length and adopted unanimously with modifications if any.

The Chairman, National Workshop on Animal Biotechnology was requested to communicate the recommendation and take appropriate follow up action.

Dr.S.C.Chopra, Director, Central Buffalo Research Centre, proposed a

toast to the organisers about the nice arrangements and also how they were engaged for almost 12 hours a day. On behalf of the delegates he thanked the organisers.

Dr.N.Natarajan, Dean i/c, Madras Veterinary College, proposed vote of thanks and the plenary session as well as the workshop, came to a close.

# **TAMIL NADU VETERINARY AND ANIMAL SCIENCE UNIVERSITY**

## **RECOMMENDATIONS OF THE NATIONAL WORKSHOP ON ANIMAL BIOTECHNOLOGY**

**HELD BETWEEN JULY 26-28, 1990**

**AT**

**MADRAS VETERINARY COLLEGE**

**SPONSORED JOINTLY BY**

**DEPARTMENT OF BIOTECHNOLOGY, NEW DELHI  
AND  
TAMIL NADU VETERINARY AND ANIMAL SCIENCES  
UNIVERSITY, MADRAS.**

### **NUTRITION**

1. THERE IS URGENT NEED TO FORMULATE HIGHTECH FEEDS USING PROBIOTICS, HORMONES, ANTI-HORMONES AND OTHER GROWTH PROMOTORS. IN DOING SO, CARE MUST BE TAKEN TO AVOID AGENTS WHICH WOULD BE INJURIOUS WHEN THE PRODUCE IS CONSUMED BECAUSE OF THEIR RESIDUAL LEVELS.
2. RESEARCH ON DEVELOPMENT OF INDIGENOUS CONVENTIONAL AND NON-CONVENTIONAL FEEDS SHOULD BE STRENGTHENED IN A BIG WAY. NATIONAL AND INTERNATIONAL COLLABORATION SHOULD BE SOUGHT FOR THIS PURPOSE.
3. QUALITY CONTROL OF FEED SHOULD BE ASSURED. UNIVERSITIES SHOULD BE THE REFERENCE CENTRES FOR CHECKING THE QUALITY OF FEED TILL SEPARATE QUALITY CONTROL AGENCY IS FORMULATED AT NATIONAL LEVEL AND REGIONAL LEVEL.
4. STUDIES ON MANIPULATION OF RUMEN ECOSYSTEM AND FISH GUT ECOSYSTEM TO UTILISE HIGH FIBROUS FEEDS AND CROP RESIDUES SHOULD BE UNDERTAKEN.

## GENETICS AND GROWTH PROMOTION

1. GERMPLASM IDENTIFICATION, DESCRIPTION AND CONSERVATION SHOULD BE UNDERTAKEN USING BASIC MOLECULAR BIOLOGICAL TECHNOLOGIES TO EVOLVE AND PROVIDE HIGH YIELDING AND BETTER VARIETIES OF ANIMALS, POULTRY AND FISHERIES.
2. KARYOLOGICAL AND PLOIDY STUDIES, CHROMOSOMAL MANIPULATIONS AND SEX REVERSAL SYSTEM FOR IMPROVING PRODUCTION AND PRODUCTIVITY SHOULD BE TAKEN UP IN ANIMALS, POULTRY, FRESHWATER AND BRACKISHWATER FAUNA.
3. GENETIC ENGINEERING SHOULD BE TAKEN UP SO AS TO ACHIEVE GENE TRANSFER WITH ULTIMATE GOAL OF DEVELOPING TRANSGENIC ANIMALS WITH DESIRED TRAITS LEADING TO INCREASED PRODUCTION AND PRODUCTIVITY.
4. STEPS MUST BE TAKEN TO DEVELOP ALTERNATE METHODS FOR EYE-STALK ABLATION IN INDUCING MATURING AND SPAWNING OF PRAWNS.
5. STEPS MUST BE TAKEN TO DEVELOP APPROPRIATE CULTURE TECHNOLOGIES FOR SINGLE CELL PROTEINS OF MARINE AND FRESHWATER ORIGIN.
6. EMBRYO TRANSFER TECHNOLOGY HAS TO BE HARNESED FOR INCREASING PRODUCTION BY EXPANDING ELITE MOTHER COLONIES UTILISING THE LESS PRODUCTIVE NATIVE CATTLE AS SURROGATE MOTHERS. CRYOPRESERVATION AND MICROMANIPULATION OF EMBRYOS MUST BE STANDARDISED AND EXPLOITED.
7. STUDIES ON MASS CRYOPRESERVATION OF MAJOR CARP DIPLOID ZYGOTES FOR OFF-SEASON STOCKING OF FRESHWATER BODIES MAY BE INITIATED. THE TECHNIQUE MAY BE EXTENDED TO TRIPLOID ZYGOTES ALSO.

## HEALTH CARE AND DISEASE CONTROL

1. STUDY ON DISEASES OF FRESHWATER, BRACKISH WATER AND MARINE FISHES SHOULD BE AUGMENTED. TO START WITH FISHERIES SCIENTISTS COULD BE TRAINED AT PG LEVEL IN VETERINARY COLLEGES AND A SEPARATE PG PROGRAMME COULD BE EVOLVED FOR THIS PURPOSE.
2. QUICK, SENSITIVE, SPECIFIC DIAGNOSTIC AIDS SHOULD BE EVOLVED FOR USE AT FIELD LEVEL FOR DISEASE DIAGNOSIS. BIOTECHNOLOGICAL INNOVATIONS SHOULD BE EXPLOITED FOR THIS PURPOSE.
3. SURVEILLANCE FOR EMERGING DISEASES SHOULD BE UNDERTAKEN. THE EXISTING QUARANTINE LABORATORIES SHOULD BE STRENGTHENED AS A NATIONAL LEVEL AGENCY BOTH WITH INFRASTRUCTURAL FACILITIES WITH BIOTECHNOLOGICAL GADGETS

AND ALSO WITH TRAINED MANPOWER.

4. DISEASE SURVEILLANCE AND DISEASE FORECASTING SHOULD BE STEPPED UP AT ALL LEVELS FOR THE BENEFIT OF LIVESTOCK, POULTRY AND FISHERIES FARMERS.
5. AN AUTONOMOUS NATIONAL ANIMAL AND FISHERIES HEALTH AGENCY HAS TO BE FORMED TO COORDINATE MINISTRIES OF UNION AND STATE GOVERNMENTS, AGENCIES LIKE ICAR, DBT AND OTHER CENTRAL INSTITUTES SO THAT A NATIONAL APPROACH COULD BE LAUNCHED IN PROVIDING HEALTH CARE AND DISEASE CONTROL TO LIVESTOCK, POULTRY AND FISHERIES.
6. HIGH TECH VACCINES SHOULD BE EVOLVED WITH THE FOLLOWING CRITERIA:
  - (1) SAFE
  - (2) POTENT
  - (3) EASILY ADMINISTERED
  - (4) PREFERABLY MULTICOMPONENT
  - (5) INDUCING LONG DURATION OF IMMUNITY
  - (6) CAUSING LEAST STRESS WITHOUT AFFECTING PRODUCTIVITY
  - (7) ECONOMICAL; AND
  - (8) NOT REQUIRING COLD CHAIN.

### MANAGEMENT

1. MANAGEMENT PROGRAMMES ARE HIGHLY IMPORTANT FOR ENHANCING AND SUSTAINING PRODUCTION FROM ANIMALS, POULTRY AND FISHERIES AND APPROPRIATE MANAGEMENT TECHNIQUES MAY BE EVOLVED FOR OPTIMUM UTILISATION OF RESOURCES IN THEIR DIVERSITY.
2. UNSCRUPULOUS MEDICATION AND VACCINATION IN THE POULTRY SECTOR HAS RESULTED IN A CONFUSING HEALTH SITUATION AFFECTING PRODUCTION AND SCIENTIFIC HEALTH MANAGEMENT PROGRAMMES INVOLVING NEWER DIAGNOSTICS AND VACCINES WITH BIOTECHNOLOGICAL INPUTS SHOULD BE STRENGTHENED.
3. HORMONAL APPROACH USING STEROIDS, CLONED GROWTH HORMONE GENES AND TRANSGENIC GENES IN SEX MANIPULATION AND GROWTH PROMOTION IS RECEIVING INCREASING ATTENTION FOR IMPROVING PRODUCTION. SIMULTANEOUS RESEARCH ON THE LONG-TERM EFFECTS OF SUCH HORMONES ON THE HEALTH OF THE ANIMALS, POULTRY AND FISHES SUBJECTED TO THE TREATMENTS AND ON THE HEALTH OF HUMANBEINGS WHO ARE THE ULTIMATE CONSUMERS OF SUCH PRODUCTION SHOULD BE STUDIED.

## POST-HARVEST TECHNOLOGY

1. WAYS AND MEANS TO ENHANCE THE SHELF LIFE OF THE PRODUCE AND ENHANCE ITS MARKETABILITY HAVE GOT TO BE FOUND OUT BOTH AT LABORATORY LEVEL AS WELL AS AT COMMERCIAL LEVEL, SO AS TO AVOID LOSS THROUGH MIDDLEMAN AND DUE TO DECOMPOSITION.
2. VALUE ADDED PRODUCTS SHOULD BE GIVEN TOP PRIORITY SO THAT THE AVAILABLE PRODUCE COULD BE MARKETED AFTER SIMPLE PROCESSES AT A REASONABLY HIGHER PRICE THEREBY INCREASING THE PROFITABILITY TO THE POOR FARMERS.
3. AGENCIES SUCH AS UNIVERSITIES/RESEARCH INSTITUTIONS/DEPARTMENTS ALL OVER THE COUNTRY SHOULD STUDY INDEPTH THE VARIOUS APPROACHES FOR ENHANCING THE MARKETABILITY OF THE PRODUCTS.
4. WHILE THERE IS A GREAT NEED TO AUGMENT AND MANAGE THE SUPPLIES OF RAW MATERIALS FROM AGRICULTURAL LIVESTOCK AND FISHERIES SECTORS FOR PRODUCTION OF NUTRITIONALLY BALANCED FEED FOR POULTRY AND FISH/PRAWN FARMING, THE QUALITY OF PRESENTLY AVAILABLE RAW MATERIALS SHOULD BE IMPROVED THROUGH BIOTECHNOLOGICAL INPUTS AND JUDICIOUSLY USED TO CATER TO THE NEEDS OF THE PROTEIN PRODUCTION SECTORS.
5. AN INTER-DISCIPLINARY APPROACH FOR CONVERTING "WASTE INTO WEALTH" WITH THE MEDIATION OF BIOTECHNOLOGY IN AREAS SUCH AS SEWAGE TREATMENT AND POLLUTION CONTROL IS HIGHLY NECESSARY FOR UTILISING SUCH TREATED MATERIALS AND WATERBODIES FOR PRODUCTION OF HEALTHY FOOD.
6. AN INTEGRATED STUDY ON AMELIORATIVE MEASURES INCLUDING HOUSING, NUTRITIONAL AND CLIMATIC ASPECTS IN RESPONSE TO SEASONAL CHANGES IN ENVIRONMENTAL FACTORS SHOULD BE TAKEN UP.
7. LONG TERM PLANNING FOR DEVELOPMENT OF FISHERIES, LIVESTOCK AND POULTRY SECTORS SHOULD CONSIDER THE GLOBAL CLIMATOLOGICAL CHANGES THAT ARE TAKING PLACE PARTICULARLY THE FORECASTS ON GLOBAL WARMING AND SEALEVEL RISE AND APPROPRIATE ANTICIPATORY RESEARCH SHOULD BE INITIATED.
8. RECOGNISING THE FACT THAT WOMEN PLAY A KEY ROLE IN MANAGEMENT OF PRODUCTION AND MARKETING IN THE RURAL SECTOR, THEIR INVOLVEMENT IN APPLICATION OF BIOTECHNOLOGICAL TOOLS IN PRODUCTION OF LIVESTOCK, POULTRY AND FISH SHOULD RECEIVE DUE ATTENTION.

## GENERAL

1. ALTERNATE FEED SOURCES SHOULD BE IDENTIFIED AND PROFITABLY UTILISED. RECYCLING OF WASTE MATERIAL SHOULD BE THOUGHT OF. BICONVERSION OF CRUDE FEED INTO EASILY DIGESTABLE FEED SHOULD BE UTILISED TO PROMOTE INTEGRATED FARMING IN ORDER TO ENHANCE PRODUCTION IN LIVESTOCK, POULTRY AND FISHERIES.
2. PRODUCTION ASPECTS OF QUAILS, DUCKERY AND GUINEA FOWL SHOULD BE STUDIED AND PROMOTED SINCE A NEAR SATURATION POINT HAS BEEN REACHED IN POULTRY INDUSTRY AND ALTERNATE TASTE IS DEMANDED BY CONSUMERS.
3. EVERY EFFORT SHOULD BE MADE TO EXPLOIT THE LIMITLESS POTENTIAL TO PRODUCE QUALITY FOOD FROM ANIMALS INCLUDING POULTRY, FRESHWATER FAUNA AND MARINE FAUNA. TO START WITH, THE EXISTING TECHNOLOGIES ARE TO BE TRANSFERRED TO THE FIELD LEVEL FOR LARGE SCALE APPLICATION TO INCREASE PRODUCTION.
4. COLLABORATION WITH PRIVATE ENTREPRENEURS SHOULD BE SPEEDED BOTH AT FINANCIAL LEVEL AS WELL AS AT TECHNOLOGICAL LEVEL.
5. BOTH BASIC AND APPLIED RESEARCH SHOULD BE UNDERTAKEN IN A MEANINGFUL RATIO SO AS TO ACHIEVE MAXIMUM DEVELOPMENT AND PRODUCTION.
6. AS A LONG TERM PROGRAMME RESEARCH IN BIOTECHNOLOGY SHOULD BE STEPPED UP. FOR THIS PURPOSE, BIOTECHNOLOGY DEPARTMENTS/UNITS HAVE TO BE STARTED IN VETERINARY COLLEGE/FISHERIES COLLEGES AND VARIOUS RESEARCH INSTITUTES ALL OVER THE COUNTRY. MULTIDISCIPLINARY APPROACH SHOULD BE FOLLOWED IN FORMULATION SUCH DEPARTMENTS/UNITS. FACULTY BARRIERS SHOULD ALSO BE REMOVED TO FACILITATE COMPLEMENTARY RESEARCH.
7. THROUGHOUT THE COUNTRY BIOTECHNOLOGY SHOULD BE INTRODUCED INTO THE P.G. CURRICULUM. FOR THIS PURPOSE, AN UNIFORM SYLLABUS AND CURRICULA SHOULD BE DEVELOPED AND INTRODUCED.

**KEY NOTE ADDRESS****Bio - Technological Innovations in the Field of animal and poultry Nutrition**

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India being an agro - based country, holds about more than 70% of its people in the villages. Among the rural population, particularly the small farmers and landless labourers depend on small scale Animal agriculture for their subsistence. Hence, it is but natural to devote more attention in planning for further developmental avenues concerning animal production.

Our country though claims to have about 25% of the world's cattle population, we are highly deficient in various livestock products. The analysis of this situation reveals that one of the main reasons for low productivity of our stock is malnutrition, under nutrition or both, besides the poor genetic potential of the animal. On perusal of the demand and supply position of feeds and fodders, a deficiency of 36% of green fodder, 40% of dry fodder and 44% of concentrate is observed. Moreover, there is a constant increase in the number of animals with very high production potential, consequent to the implementation of newer techniques in animal breeding. This is sure to cause a renewed demand further for the feeds and fodder. Urbanization and other allied activities have dwindled the area available for fodder production, year after year. Be-

sides, the currently available fodder and feeds are quality wise equally low in nutrients density and digestibility. To provide quality feed at optimum level to our livestock, in the existing situation, the most promising approach would be to improve the quality and utilisation of the available fodder, feeds, crop-residues and other industrial by - products more efficiently. This is the area where bio - technology in the field of animal nutrition, has very important implications to increase animal productivity and feed conversion efficiency.

The biotechnology includes a number of powerful techniques such as recombinant DNA manipulation, monoclonal antibody production, tissue culture, protoplast infusion, protein engineering, bio - additives and use of immobilised enzyme.

**I. Quality of Forages:**

Forage occupies a very pivotal role in economic production of milk and meat. However, most of forages and feeds have one or other antinutritional factors such as protease inhibitors, phytohaemoagglutinin, oxalates, cyanogens, tannin etc. These factors affect nutrient utilization and in some cases induce chronic intoxication thereby reducing

growth and feed efficiency. It is reasonable to suppose that the techniques of genetic engineering supported by traditional plant breeding practice may lead to the development of new varieties or cultivars free or very low of these factors.

## II. Nutrition of Animals:

**Ruminants:** In view of the key role that the rumen microbial population plays in the digestion of feeds by cattle and sheep, it is not surprising that considerable interest is currently focused on the potential of some of the newer techniques - particularly that of recombinant DNA for modifying the rumen microbial ecosystem with the objectives of enhancing efficiency of production from the host animals.

(i) *Enhance cellulolytic activity:* Cellulose is a major component of natural ruminant feed and an increase in its degradation will not only provide volatile fatty acids and amino acids but also enhance voluntary feed intake.

Among rumen bacteria *Bacteroides succinogens* and *Ruminococcus flavefaciens* are able to degrade hemicellulose; *R.albus* another cellulolytic bacteria which produces propionate as a major end product is advantageous for meat animals.

This activity assumes that the rate-limiting step in cellulose degradation is cellulolytic activity and not the physical nature of the cellulose. With advent of newer techniques, it is now possible genetically to 'design' or 'engineer' bacterial strain having specific characteristics.

(ii) *Ability to cleave ligno-hemicellulose complex:* The major factor which governs the digestibility of

forage cell walls is the degree of lignification. Only a limited range of micro-organism can degrade lignin such as white rot fungi, brown rot fungi and the bacteria notably actinomycetes. All lignin-digesters are aerobic, because enzymic degradation utilizes O<sub>2</sub> to cleave the phenolic ring; however, in an environment such as the rumen, it will be necessary to isolate a gene encoding an enzyme which cleaves, a non-oxidation mechanism. Perhaps, one may have a doubt of its possibility. However, recent study showed that on wet land ecosystems such as marshes or swamps, lignin and lignified plant tissue are biodegraded slowly in the absence of O<sub>2</sub>.

(iii) *Reduction in methanogenesis:* The production of methane accounts for between 5 - 8% of the gross energy; the partial or complete inhibition could be advantageous in terms of increased feed efficiency, in ruminant livestock. This could be achieved by the selective inhibitors of methanogenic bacteria and by the establishment of a bacterial species which converts hydrogen and carbon to acetate and butyrate. Inhibition could be achieved through the use of a specific bacteriocin; Another means to overcome hydrogen accumulation is by introducing the *Eubacterium limosum* which converts hydrogen and carbon to acetate and butyrate.

(iv) *Decrease in proteolytic and / or deaminase activities:* A decrease in proteolytic and / or deaminative activity of microbial species should increase the supply of amino acids for the host animal. This has to be followed depending up on quality of feed protein.

(v) *Increase in biuretase activity:* The organisms in which biuretase activity has been stimulated, are to be isolated and genes cloned subsequently into other rumen bacteria, so as to ensure slow but continual supply of  $\text{NH}_3$  - N to the rumen micro - organisms for their microbial proteins synthesis.

(vi) *Increased microbial production of specific amino acids:* Shortage of specific amino acids to the host animal can limit some of its production for examples methionine for wool growth and for milk production. This defect can be overcome by insertion of the codon for methionine into DNA of bacterial species normally present in the rumen.

(vii) *N-fixation:* The suggestion of N fixation is certainly an extreme speculation. Recently, a novel cellulolytic N - fixing bacteria has been isolated from wood worms and this helps not only to degrade the cellulose but also maintains N equilibrium.

Even if this objectives achieved, there are many technical difficulties to be overcome. There is a possibility that the genetically modified organism may interfere the delicate biochemical interdependence; in addition the modified organism may be unable to compete for survival with the unmodified organism with in the rumen ecosystem.

**BIO - ADDITIVES:** i) *Yeast culture:* Studies conducted in North America and Europe demonstrated that the effectiveness of yeast culture on increasing milk yield by improving the flow of nutrients to the intestine and their subsequent absorptions. Yeast cultures were also found to improve feed intake and

stimulate cellulolytic activity due to the influence of isoacids, amino acids, peptides or by re - modulation.

(ii) *Probiotics:* In the preparation of silage, a number of microbial inoculants are being added to improve the quality. The predominant bacterial species is *Lactobacillus*. This helps in the acidification as well as in the increase of some organic matter and N digestibility and thereby enhances the voluntary feed intake.

*Non-ruminants: Protein quality :* Most of the cereal grains protein suffer from low concentration of lysine and methionine, followed by tryptophane and threonine. Such imbalance in amino acids results in poor utilization of protein. The newer techniques associated with biotechnology namely genetic manipulation could be applied for the improvement of the nutritional value of the grain protein using certain strategies, namely

a) Decreasing the amount of the low lysine storage protein (Prolamins).

b) Manipulating the amino acid composition of one or more of the prolamine so as to increase the lysine content.

c) Increasing the amount of other lysine - rich seed protein (glutelin).

d) Selecting for mutants with higher lysine by reducing inhibition of key biosynthetic enzyme example aspartate kinase.

Progress could be achieved by genetic knowledge to develop suitable cultivars rich in these amino acids.

*Enzymes:* Enzymes are used to improve the digestibility and utilisation of nutrients. The various en-

ymes that have been experimented with are -

- Beta glucanase
- Bacterial diastases
- Pentosanases
- Fungal cellulase
- Amylases
- Proteases

Attempts should be made to harvest these industrial enzymes and its usefulness assessed by extensive investigation. Since a compounded feed is a mixture of different ingredients a multi - enzyme system is preferred than a single enzyme preparation. The use of enzyme pretreated feeds would provide the following opportunity for the feed manufacturer.

- a) the use of cheaper raw materials without reduction in performance
- b) less inclusion of processed raw materials.
- c) improved performance of rations.

**Probiotics:** The gut microflora play an important role in protecting animals/birds against pathogenic bacteria thus the microflora which inhabit the gut and give protection by bacterial competition/bacterial interference/competitive exclusion is of significance for better animal production practices. Yeast cultures (*Saccharomyces cerevisiae*) and probiotics (*Lactobacillus* sp.) have been used and found to exclude salmonella and coliform related problems.

Biotechnology can be used to grow and make available new and powerful biocide to eliminate salmonella and coliform from feed, plants and processing units.

### III. Newer Feed Resources:

The notable development is the formation of single cell protein, suitable for use either by man or for incorporation in feeds for farm livestock example, the bacterium *Methylophilus methylotrophus* on methanol is currently used as a protein source replacing soyabean partially in calf milk replacers and in diets for piglets, laying hen, broilers and turkey.

### IV. Biodegradation of Poultry Waste:

Poultry and animal waste can be converted into a high protein feed - stuff by biological digestion and degradation, oxidation or by the action of micro - organisms and algae.

These processes might help to solve the accumulating problem of disposal of poultry and animal waste, which in some cases are not suitable as soil fertilizers and cause pollution problems. The following methods may be adopted to convert the wastes as feedstuffs for poultry:

- a) Housefly pupae meal - - caged layer manure degraded by housefly larvae.
- b) Earthworm meal - - another biodegradation of caged layer manure.
- c) Liquor and residue from a ditch used for oxidizing swine liquid manure.
- d) Aerobic fermentation of poultry manure.
- e) Meals produced from algae grown in ponds of sedimented animals waste and sewage.

**V. Future Prospects:**

The outlook for biotechnology applications to improve the quantity and quality of the world's food supply is good. The technology is so powerful that it is allowing manipulation of the genome in ways hard to imagine only a decade ago. Now it is possible to remove single genes from nature and put them into systems in which they make a positive impact.

In the near future may be by the end of this century, the mechanism of expression of genes in plants and animals will be well understood so that crop varieties that are nutritionally superior and resistant to diseases, herbicides, insecticides and pesticides may be available. Similarly milk, meat and wool production may be improved by transplanting of growth hormones genes.

**KEY NOTE ADDRESS****Freshwater Finfish and Shellfish Nutrition and Diet Development Studies in India**

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The fact that nutrition plays a pivotal role in aquaculture just as in any other field of animal husbandry hardly needs any emphasis. In most of the cases major part of the operational cost of aquaculture is due to feed and has the largest expense. Nutritional research aimed at development of diets for various important species of finfish and shellfish to meet their physiological needs is rather new in this country although some studies were conducted in the past on the feed and nutrition of carps in particular (Alikunhi, 1952; Das & Krishnamurthy, 1961; Mitra & Das, 1965; Lakshmanan *et al.*, 1967; Singh & Bhanot, 1970; Chakrabarty *et al.* 1971; Mahajan & Yadav, 1974).

An attempt is made herein to summarise the information generated in India in recent years on various aspects of nutrition and diet development of important freshwater species of fish and prawns. A few reports of studies from abroad have also been included. The purpose of this review is to collate published information as a means of establishing a baseline to prioritise future research.

**Carps**

The country had a century old system of carp culture mostly based on empirical and traditionally in-

herited methods. The subsequent development of carp polyculture and breakthrough in induced breeding of all the carp species led to studies on nutritional requirements, feed formulation, feed digestibility and conversion and so on.

**Nutritional Requirement Studies**

(i) *Protein, amino acid and carbohydrate*: Sen *et al.* (1978) conducted series of experiments with purified test diet, to ascertain optimum level of protein and carbohydrate requirement in the carps *Labeo rohita* and *Cyprinus carpio*. They observed that optimum growth of carp spawn, fry and fingerlings could be reached with test diets having 45% protein (Casein) and 26% carbohydrate (dextrin), Das & Tripathi (1979) found that protein requirement of grass carp was 36% and that it did not vary between 20° and 29°C when a diet comprising leaf protein concentrate was used. Renukardhya and Varghese (1986) estimated the protein requirement of *Catla catla* and *Labeo rohita* to be around 30%. Protein requirement of fry of *Catla catla* was worked out by Singh & Bhanot to be 47%. They also found protein : energy ratio to be 124.8 and protein efficiency ratio 0.42. Singh (1989) found the protein requirement of silver carp fry to range from 37 - 42% at 23 - 28.7°C

but noted that growth rate was doubled at 28°C when fed on a diet having 42% protein. Pandian (1989) found the protein requirement of carps and tilapias in the range of 25 - 30%. Mohanty *et al.* (1990) reported that fry and fingerlings of *Labeo rohita* and *Cirrhina mrigala* have similar protein requirement and that it varies from 40 - 45%. Recently Mohanty and others (Personal communication, 1990) indicated that though the gross protein requirement of Indian major carps vary, their amino acid requirements are similar.

(ii) *Vitamin and mineral requirements:* Gupta *et al.* (1979) found that vitamin E when fed @ 5 mg/kg body weight/day to catla, rohu and cyprinus, induced complete spawning. Mahajan & Agarwal (1980) studied the effects of ascorbic acid on growth and survival of mrigal hatchlings and reported that 600 mg/kg feed was the optimum dose. Mahajan & John (1979) indicated that Vitamin B<sub>12</sub> and folic acid were more important than thiamin and inositol for mrigal during the first year of its growth. Cobalt, boron, manganese and molybdenum at 0.01, 0.05, 1.0 mg/day/fish respectively were found to enhance the growth and survival of Indian major carp fry and fingerlings (Sen & Chatterjee, 1979a, b). Effects of different levels of pyridoxine on blood parameters were studied in *Cyprinus carpio* by Sakthivel & Sampath (1989). 4 deoxypyridoxine was used to accelerate deficiency effects. For an exposure of 120 days, test fish fed with pyridoxine level below 5 mg/kg showed significant reduction in RBC, Hb content, hematocrit, MCV and MCH leading to anaemia. The fish could restore the normal

levels of blood cells during the recovery treatment of 30 days. The minimum level of pyridoxine required for healthy condition of *Cyprinus carpio* should be 5 mg/kg feed. Sinha (1989) studied ascorbic acid requirement of the hatchlings of *Catla catla* and found that ascorbic acid @ 600 mg/kg diet gave the highest rate of survival (82%) and growth.

(iii) *Artificial/supplementary feeds and their utilisation:* Venkatesh & Shetty (1978 a, b) found that aquatic weeds - *Hydrilla* and *Ceratophyllum* were better digested (60 - 65%) than the terrestrial grass, hybrid napier (58%). The digestion coefficients of crude protein in these plants varied from 58.96 - 87.97% while that of crude fat varied from 63.85 - 87.12%, crude fibre digestion was recorded to vary from 38.97 - 44.80% in case of aquatic weeds and 32.87% in the case of hybrid napier. Investigations (CIFRI, 1979) on protein utilisation by grass carp fed on aquatic weeds and land vegetations indicated that protein utilisation was the best with *Lemna minor* (52%) followed by *Wolffia arrhiza* (50.6%), *Hydrilla* (48.6%) and cabbage leaves (45%). Sen *et al.* (1980) studied the relationship between the rate of feeding, growth and conversion in Indian major carps. Experiments conducted with a mixture of rice polish and ground - nut oil cake mixed in equal proportion by weight at 1,2,3,4,5 & 6 percent of the initial body weight of the carp fingerlings showed that 5% level of feeding recorded maximum growth and best feed conversion in *L.rohita* and *C.mrigala* (Kumar, 1980). Singh *et al.* (1980) made observations on the digestion and absorption in *L.rohita*

fed on diets comprising fish meal, groundnut oil cake, wheat bran and yeast 25:65:5:5 with protein content of 45% compared to that of conventional feed containing groundnut oil cake and wheat bran (1:1). Significantly better conversion and higher rate of growth could be observed with this formulated feed. Hajra *et al.* (1987) conducted comparative digestibility trials with grass carp *C. idella* fed on two different feeds of plant origin *Cyanodon dactylon* Pers. and *Spirodela polyrhiza* Linn containing 23.2% and 7.9% fibre content. The digestibility coefficients of dry matter, crude protein and gross energy declined significantly when the fish was fed on *Cyanodon dactylon*. Digestibility coefficients of dietary fat also declined with increased level of fibre. Fibre contents of the feeds, however, did not have any significant effect on its digestibility. Roy and Patra (1987) utilised a flow through system to measure digestibilities of diet by way of collection of faecal matters. The leaching of nutrients from faecal samples collected through the system was negligible and there was close agreement between the digestibility values of dry matter, protein and lipid obtained with faecal samples collected by intestinal dissections and the results obtained with faeces collected through such system. Studies carried out by Mohanty *et al.* (1988) on protein utilisation and metabolism of Indian carps indicated poor protein retention efficiency in *Catla catla* but the highest in *C. mrigala*. As for Protein : Energy ratio, a value of .95 obtained with 38% protein and 4 Kcal/g of energy resulted in maximum growth of fry of *Labeo rohita* (CIFA Annual Report, 1989). Nan-

deesha *et al.* (1989) found a rhythmicity in the protein digestibility of common carp and suggested that the fish may be fed for a day on low levels of protein of plant origin followed by three days of high protein of animal origin to effect saving on feed costs. Singh (1989) conducted studies on the digestibility of crude protein and energy from conventional and non - conventional fish feedstuffs and pelleted diets in mrigal and grass carp. The crude protein digestibility from conventional plant feedstuffs and pelleted feeds ranged between 73.9 - 92.4% in mrigal. A non conventional feedstuff sal seed cake partially substituted in carp diet gave high crude protein digestibility coefficient (93.9%) in mrigal but 82.0% in grass carp. The protein digestibility coefficients for feedstuffs of plant origin ranged between 71.9 - 94.5% in grass carp. The energy digestibility from feedstuffs and pelleted feeds of plant origin ranged between 2729.4 - 3063.9 Kcal/kg in mrigal with exception of sesame oil cake which gave comparatively low energy digestibility (1901 Kcal/kg). The energy digestibility for sesame oil cake and sal seed oil cake was low (2008.2 and 2230.4 Kcal/kg). Das and Tripathi (1990) studied in detail the various digestive enzymes of grass carp from different diet treatments. The studies indicated that pattern of distribution and activity of the digestive enzymes depend on the type of feed ingested by the species. It also indicated that cellulose activity is of inherent systemic origin and that exclusively derived from intestinal microflora.

*Supplementary feeding and diet evaluation:* The commonly used supplementary feed for carp consists of

mixture (1:1) of rice bran and one of the locally available oil cakes. Das (1958) found that when carp spawn was reared in manured nurseries providing various artificial feeds with different combinations maximum growth was obtained with feed having a combination of hydrolysed protein and Carbohydrate (50:30). According to Mitra & Das (1965) til oilcake, rice powder and black gram account for higher survival and growth of carp spawn than rice bran. Lakshmanan *et al.* (1967) evaluated various feed mixtures and found that an artificial feed comprising a mixture of dried finely powdered and sieved notonectids, small prawn and cow pea in 5:3:2 ratio gave better results in enhancing growth and survival of the spawn than rice bran and mustard oil cake mixture. Singh (1970) and Singh & Bhanot (1970) suggested powdered algae (*Oedogonium*, *Zygnema*, *Spirogonium* etc.) mixed with other ingredients viz., potato starch, fish meal, yeast as feed for carp spawn. Chakrabarty *et al.* (1973) conducted trials on the usefulness of various feed mixtures vis - a - vis zooplankton in spawn rearing and found better performance of carp spawn when fed on zooplankton, silkworm pupae (Tasar silk worm), soyabean and wheat bran. Chakrabarty and Kar (1975) observed that protein concentrate containing 55% protein to be quite good for mrigal spawn and common carp fry. Varghese *et al.* (1976) conducted feeding experiments using pelleted feeds (containing fish meal, rice bran, groundnut oil cake, rice flour and mineral mix.) for common carp and recorded 50% more production than compared to those fed with a mixture (1:1) of rice bran and

groundnut oil cake. Jeyachandran and Paul Raj (1976 & 1977) conducted series of trials with common carp and found that silkworm pupae and prawn wastes were very well utilised. Tripathi *et al.* (1979) obtained high survival (more than 80%) of spawn using a diet comprising a mixture of fish meal, groundnut oil cake and rice bran (1:1:1) containing 26.7% protein, 32.5% carbohydrate and an energy content of 300 Kcal/100 g. Kumar (1980) studied intake of formulated feeds by *Labeo rohita* and found feeding @ 5% level resulted in good conversion and growth with minimum wastage. Jayram & Shetty (1981) formulated two pelleted fish feeds and studied their water stability/leaching of nutrients. Paul Raj and Kutty (1983) used the seed kennels of wild trees such as *Gliricidia*, *Albizia* and *Enterolobium* as a substitute of ground nut cake. Mohanty and Swamy (1986) obtained good growth in *Labeo rohita* using diet comprising blood meal, rice bran and groundnut oil cake (1:3:6). Devaraj and Keshavappa (1986a,b) used subabul and lucerne as ingredients in carp feed and found that they could be incorporated upto 25% and 80% respectively with beneficial results. Bhatt *et al.* (1986) formulated two diets based on soyabean meal and squilla meal. These diets were evaluated in relation to fish meal based diet by the growth performance of rohu, common carp and silver carp. Although net return was the highest with fish meal based diet the nearly similar relative and absolute conversion rates of the three diets indicated the two new sources of protein thus evaluated could be used for partial replacement of fish meal rather than

its complete replacement. Singh *et al.* (1986) conducted experiments on rohu/mrigal fry and fingerlings and showed that fortification of conventional fish feeds of rice/wheat bran and groundnut oil cake with multivitamins and minerals and trace elements increased the efficiency of feeds significantly both at high (23 - 30°C) and low (15 - 20°C) temperatures. Venugopál and Keshavanath (1987) reported higher conversion rates in common carp with feed containing silage as a substitute for fish meal. Swamy *et al.* (1988)

all four different diets tested (having 30 - 45% protein) or mrigal fingerlings growth performance was the best at 40% dietary protein level with fish meal and ground nut oil cake. Nandeeshá *et al.* (1988) formulated three feeds using dried worm meal obtained from *Eudrilus eugeniae* as a replacement for fish meal and evaluated in a culture trial with common carp *Cyprinus carpio* for a period of 84 days. In one of the diets where fish meal was partially replaced by earthworm meal and 5% sardine oil was added induced best growth of fish compared to diets fully replaced by earthworm meal. Patra and Roy (1988) undertook studies to evaluate the influence of the submerged aquatic weed *Hydrilla verticillata* on growth and flesh protein and lipid contents of *Labeo rohita* and suggested that the weed may advantageously substitute costly ingredients of the conventional feeds. Nandeeshá *et al.* (1989) formulated pelleted feeds with sericulture wastes and evaluated these in carp culture (rohu, catla and silver carp). A comparison was also made with that of fish meal based standard diet. Relative conversion ratio was better with

pelleted feeds containing deoiled silkworm pupae. The economics of production indicated possibility of substituting fish meal with deoiled silkworm pupae and silkworm faecal matter. Das and Singh (1989) reported their observations on the possibilities of using biogas effluent as carp feed substitute in the form of dough or pellets. Das & Karmakar (1989) studied the diurnal rhythm of intestinal amylase activity in *Labeo rohita* and determined preference for food intake time and feeding intensity with a view to evolve a feeding strategy.

#### Air - Breathing Catfish and Other Air - Breathers

These exist a unique group of teleosts which are different from others by virtue of their adaptation such as development of accessory respiratory organs and occurrence of diving reflexes. They are known as air - breathing fishes. In India there are more than 20 such species of which Singhi (*Heteropneustes fossilis*), magur *Clarias batrachus*, common murrel (*Channa punctatus*), striped murrel (*Channa striatus*), chital (*Notopterus chitala*), large murrel (*Channa marulius*) and koi (*Anabas testudineus*) are important food fishes.

Unlike carps on which considerable information pertaining to nutrition is now available, studies on nutritional requirement and supplementary feeding for airbreathing catfishes are limited. Till now selection of feed ingredients in formulated supplementary feeds for such species is to a great extent arbitrary since the knowledge of nutrition is still tentative. Although different types of compounded diets are being tried as

supplementary feed, information relating to nutritional requirements, food intake, digestibility of feeds and associated physiological changes in relation to growth are scanty.

(i) *General nutrition and feed formulation:* Tripathi & Sharaf (1974) for the first time conducted experiments on development of feeds for *Clarias batrachus*. Srinivasachar *et al.* (1976) studied food intake and conversion efficiency of *Heteropneustes fossilis* in relation to light and dark periods and found that light had hardly any influence on these parameters. Sen *et al.* (1976) studied fatty acid composition of muscle, liver and visceral lipids of murrels and found that level of 20:4006 occurred in relatively high amounts in majority of lipids analysed. Vivekanandan *et al.* (1976) studies the influence of biotic and abiotic factors on feeding and growth of channid fishes. Singh (1977) developed a method for the study of bioenergetics of the airbreathing fishes based on studies on metabolism, assimilation and growth. Thakur (1978) using the index of preponderance as proposed by Natarajan & Jhingran (1961) studied the food of post-larval, juvenile and adult stages of *C. batrachus*. Pal *et al.* (1977) conducted trials with six different compounded feeds for *Anabas* spawn using wheat flour, rice bran, soyabean powder, prawn meal, zooplankton and cooked hen egg. Reddy & Shakuntala (1979) studied the effects of different feeding rates on food intake, growth and conversion efficiency of *Heteropneustes fossilis* using the oligochaete worm *Tubifex tubifex* as food. They observed that an amount of worm substance equivalent to 12.73% live body

weight/fish/day which a fingerling of *Heteropneustes* could consume thereby increasing specific dynamic action from 14 mg/g/day to 70 mg/g/day. Sundararaj *et al.* (1982) while studying circadian meal timing in air-breathing catfishes demonstrated that maximal food intake occurred when fish were fed during dusk. Niamat & Jafri (1984) studied the growth response and survival of *Heteropneustes fossilis* fed compounded pelleted diets and recorded a 100% survival with pelleted feed. Venkatesh *et al.* (1986) studied growth and metabolism of the catfish *Clarias batrachus* fed with different compounded diets based on fish meal, meat meal, ground nut oil cake and silkworm pupae having identical crude protein level. Specific growth rate, PER, intestinal proteolytic enzyme activity tissue protein synthesis, levels of serum protein and Ca:P ratio indicated that satisfactory growth occurred in fish when fed with fish meal based diet followed by silkworm pupae. Mukhopadhyay & Hajra (1986) studied the intestinal protein breakdown and liver protein synthesis in *Clarias batrachus* fed isonitrogenous diets having variable energy level. Mukhopadhyay *et al.* (1986) in an experiment on growth response of *Clarias batrachus* found that at constant dietary protein level, weight gain, feed efficiency and protein utilisation increased with increase in dietary energy level upto 3831.7 Kcal/kg (P/E = 87.59). Dietary carbohydrate and to some extent dietary lipid showed a protein sparing action indicating that part of protein could be replaced by lipid calorie for reducing the cost of catfish diet. Pakuska *et al.* (1986) investigated food consumption in

*C. batrachus* and found that food requirement for maintenance in the species is 24 mg/g fish/ day and fish weighing 3 g can consume a maximum of 142 mg/g fish/day. Dehadrai & Mukhopadhyay (1987) studied the effects of non - protein nitrogen supplementation in the feed of *Clarias batrachus*. Urea substitution (3% weight of feed) replacing approximately one fifth of dietary protein (8.6 g protein = 1.38 g nitrogen) and balancing the total with cellulose showed a near normal growth performance of the species compared to control. Patra & Ray (1988) found that animal protein source such as dried goat blood meal or fish meal when incorporated in the diet of *Anabas testudineus* gives better growth performance. The parameters like protease activity and protein synthesis also corroborated the finding on growth. Ray & Patra (1989) conducted a 60 day laboratory feeding trial to examine the effects of different dietary protein sources on the weight gain, feed conversion, PER, protein synthesis and protease activity of *Anabas*. The diet containing carcass waste, rice bran and ground nut cake proved to be the best for the species. Krishnan & Reddy (1989) studied the effects of food quality together with quantity of food on feeding energetics of the species was not dependent on the type of the food offered but the optimum feeding levels were influenced by the nature by the nature of the diet.

**Vitamin nutrition:** Barua & Das (1975) elucidated the mechanism of dehydroretinol (Vitamin A<sub>2</sub>) biosynthesis in *H. fossilis*. They found that anhydrolutein is present naturally in the liver oil of fish and can be found

from lutein. Barma & Goswami (1977) reported that B - carotene is converted into retinoic acid by *H. fossilis* and in some cases into retinol. Further lutein has been shown to be converted into dehydroretinol in the apparently normal and healthy fish. They also showed (Goswami & Barma, 1981a) that the conversion of lutein into 3 - dehydroretinol takes place in the intestine within 4 - 6 hours after feeding lutein. Goswami & Barma (1981b) while studying distribution of retinol and dehydroretinol in freshwater fish (both carp & catfish) found a specific taxonomic group can be separated out as retinol predominant or dehydroretinol predominant group. Calcium uptake in experimentally induced scorbutic snakehead *Channa punctatus* fed vitamin C deficient diet for 210 days using <sup>45</sup>Ca as tracer was reported by Mahajan and Agarwal (1980). The results showed decreased absorption and utilisation of calcium by gill, skin, muscle and bone of scorbutic fish from surrounding water. Agarwal and Mahajan (1983) reported that in *C. punctatus* fed with 4 - deoxypyridoxine, leucopenia appeared after 180 days and the other pyridoxine deficient group developed leucopenia only after 240 days. Studies (Swarup & Srivastav, 1982; Swarup *et al.*, 1984; Srivastav *et al.*, 1985) indicated that fish appear to have exactly the same vitamin D metabolites as found in higher vertebrates; furthermore both vitamin D<sub>3</sub> and 1,25 - dihydroxycholecalciferol have been found to stimulate intestinal calcium uptake in catfish *Clarias batrachus*.

### Freshwater Prawn

The culture of freshwater prawn - particularly the *Macrobrachium* group is of recent origin and has recently become the favourite species for aquaculture in India. Unfortunately nutritional studies on such species like *M. rosenbergii* and *M. malcomsonii* are very limited in this country although some useful information on nutrition and diet development are available from abroad.

Ling (1969) a pioneer in rearing *Macrobrachium rosenbergii* did not use compounded diet for juvenile. He used fresh animal tissues and fed the prawns. Sic & Beaty (1974) found that *Macrobrachium* fed a soyabean diet bound with 5% collagen did not gain weight whereas those fed diet having a high amylase binder grew well. Soyabean meal bound with corn starch or agar produced relatively low weight gains. Balzas *et al.*, (1973) found that an all vegetable protein source diet gave results superior to that of fish. All diets had 35% protein. Balzas *et al.*, (1974) prepared a series of diets for *M. rosenbergii* with protein levels of 15, 25 and 35%. These diets were based on soyabean meal, tuna meal, shrimp meal and combination of soy - tuna and soy - tuna - shrimp. Averaging the results achieved by each protein source, at all protein levels led to the conclusion that only the soy - tuna diet gave significantly greater gains than either soy, tuna or shrimp individually. Watanabe (1975) studied requirements for amino acids for *M. rosenbergii* and found that methionine, phenylalanine, theronine, tryptophan, tyrosine and valine were essential. The apparent non -

essentiality for lysine in this case was probably due to analytical difficulties. Balzas & Ross (1975) reported a food conversion rate of 1.46 over a 244 day trial with soya - tuna - shrimp diet. Sandifer & Joseph (1976) conducted a long term feeding trial with *M. rosenbergii* fed a diet with 3% shrimp head oil. The prawn showed 15 times more carotenoid than when this was cultured under control diet treatment. In both the cases the levels of W3 fatty acids in the tissue mirrored those in the diet. The diet containing shrimp head oil produced larger prawn and after 12 weeks the total biomass produced was twice as high as control.

Balzas & Ross (1976) in a later trial when better tank management was feasible, re - examined the effects of soy - tuna and soy - tuna - shrimp diets at 15, 25 & 35% protein levels. Juveniles of *M. rosenbergii* were used. The other diets were included at 25% protein level which used soy - tilapia, copra (coconut meal) - tilapia and activated sludge - tilapia - duckweed meals as protein sources. In these trial even with soy - tuna - shrimp diet, significantly greater weight gains were obtained as dietary protein level rose. Growth rates of control shrimp receiving no compounded diets indicated that all shrimp were feeding heavily on algae present in the tanks. The authors concluded that protein levels in excess of 35% may produce maximum growth in *M. rosenbergii* but that lower levels may be possible in diets having a favourable aminoacid balance. Costa - Pierce & Laws (1985) found that a volatile chemoactive substance - trimethylammonium hydrochloride (TMAH) was potential-

ly attractive and that cost effective application of TMAH to commercial crustacean diets may increase ingestion rates.

From a perusal of literature, it is evident that nutritional studies so far conducted emphasised mostly on dietary trials. Basic nutritional studies with specific metabolic parameters at blood and tissue level which contribute to the expression of nutrient needs of the species are necessary as biochemical evidence. The fish/prawn will respond to gross changes in the nature of the diet by adaptive changes at cellular level particularly by alteration in the activities of certain key enzymes. Such knowledge may aid in predicting growth models for culture ecosystem.

The following deserve serious consideration:

- In the field of fish nutrition the aspect for which there is little information is the environmental effects on the feed utilisation. Not much information is available on the influence of circadian feeding times on feed consumption, overall efficiency and growth.
- The pathobiological aspects associated with nutritional diseases such as avitaminosis, hypervitaminosis and the effects of deficiencies and excesses of other nutrients remained virtually unstudied.
- There is a need to study requirements of mature/brood fish and prawns as well as post-larvae fry. This has particular relevance to catfish and prawns. The requirements of protein:calorie ratio, quantitative amino acids and fatty acids, Ca/P ratio are unknown.
- Fishes being ammonotelic (Ammonia being the primary product of nitrogen excretion) can readily use protein as energy source. This use of protein may result in high concentration of ammonia especially in the intensive culture system if the ratio of protein energy: non-protein energy is disproportionately high and can also result in increased feed cost. Instead of emphasising on protein only, lipids and carbohydrates need be included in diets at levels that will maximise use of protein for growth and tissue build-up without affecting the organoleptic qualities. The amount of non-protein sources that should be appropriately incorporated in fish diet is not fully understood as yet.
- Despite higher participation of protein in the energy metabolism of fish in comparison to that of homeotherms, the protein needs for maintenance seem to be relatively low in fish. The concept of protein need for maintenance in fish energy-protein interrelationship requires to be delineated.
- The information on the use of feeding stimulants/feeding enhancers are lacking. While their presence in trace amounts can effectively increase consumption/rate of feeding and palatability - studies to maximise food consumption have not so far been taken up on a large scale.

**KEY NOTE ADDRESS****Feed and Nutrition of Marine Finfish and Prawns**

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The greatest challenge for global development in the twenty - first century would be that of providing adequate levels of nutritious food to the growing human population. To meet this challenge, the efficiency of existing food generating systems should be engineered to achieve maximum efficiency through biotechnological methods and new avenues of food production should be explored. The aquatic environment comprising the freshwater, brackish-water and marine ecosystems offers tremendous scope for augmenting protein - rich food production. Man has been tapping the natural food resources of these ecosystems, since the dawn of times, mainly by hunting. On a smaller scale aquaculture has been in practice for several centuries, particularly in the Asian countries. However, the momentum that aquaculture has gained worldwide during the recent times is probably unparalleled in other branches of food production. The production of fish and shellfish through culture increased from a level of 6 million tonnes in 1975 (Pillay, 1976) to 10.6 million tonnes in 1988 (Rhodes, 1988). An average growth rate of 7.5% per year would mean a world aquaculture production of about 22 million tonnes by the year 2000 A.D.,

which would be about 20% of the total world fisheries production (Rabanal, 1987).

**Production Trends from fish and Prawn Culture Systems in India**

Recent research and development efforts in India have amply proved that by adopting scientific culture procedures, adequate feeding and efficient management practices, substantial increase in production could be obtained from finfish and prawn culture systems. Production rates exceeding 10 tonnes/ha/yr have been achieved in static earthen carp culture ponds at the Central Institute of Freshwater Aquaculture, Dhauli, Orissa, and 50 tonnes/ha/yr in the golden tilapia culture farm in Chingleput near Madras. Efforts are being made to realise a production rate of 25 tonnes/ha/yr in carp culture ponds and 250 tonnes/ha/yr in the golden tilapia culture farm through intensive methods of culture with high stocking densities, optimum fertilization, artificial feeding and water quality management. In the prawn culture systems in the coastal areas, production rate as high as 7 tonnes/ha/crop has been achieved, although several farms in Nellore District of Andhra Pradesh are consistently realising a production rate of 1 - 1.5 tonnes/ha/crop of

4-5 months. *Penaeus monodon* and *P. indicus* are the two prawn species currently cultured in coastal ponds. Culture of marine and brackishwater finfish is still in an infant stage in India due to several constraints in the artificial propagation of finfish species. The potential species for culture are the mullets (*Mugil cephalus*, *Liza macrolepis*, *Liza parsia*), the pearl-spot (*Etroplus suratensis*), the sea bass (*Lates calcarifer*) and the milkfish (*Chanos chanos*).

#### Importance of Feed in Culture Systems

Production of fish and prawns in extensive culture systems mainly depend upon natural food. Natural food production could be augmented by judicious application of organic as well as inorganic fertilizers in optimum dosages. According to New (1987) production levels achievable in prawn ponds are approximately 100 - 300 kg/ha/yr without fertilization and 600 - 1000 kg/ha/yr with fertilization. The overall productivity depends on the natural biogenic potential prevailing environmental conditions as well as on the kinds and levels of fertilizers used. In general production rates exceeding 1 tonne/ha/yr are rarely achieved without artificial feeding in prawn ponds; whereas in fish culture systems substantially higher levels of production could be achieved through judicious application of fertilizers.

Artificial feeds can be broadly grouped into (i) supplementary feeds and (ii) complete feeds. Supplementary feeds, by and large, are empirical formulations, which do not provide the essential nutrients in adequate levels and balanced proportions as in the case of complete

feeds. Supplementary feeds can be either made of a single ingredient or a mixture of ingredients; but, usually low-cost agricultural and animal wastes, and by-products are used. Thus the production achievable through supplementary feeding depends on the quality and quantity of the supplementary feed and on the natural food produced in the ponds.

In semi-intensive and intensive culture systems provision of nutritionally balanced complete feeds is essential to achieve maximum production. In these systems, feed forms the major operational input and often accounts for 40 to 60 percent of the running expenditure. Therefore, it is of foremost importance that the feeds are scientifically formulated to provide all the essential nutrients and energy in adequate levels and balanced proportions, optimally processed and judiciously supplied to achieve maximum efficiency at optimal cost.

#### Feed Development Strategies

The production of nutritionally adequate feeds for fish and prawns requires research, quality control and biological evaluation. A well balanced diet not only results in higher production but also provides the nutrients necessary to hasten recovery from diseases or aid the animal in overcoming the effects of environmental stress. Design and fabrication of complete artificial diets for finfish and prawns need information on a variety of biological, physiological, biochemical and nutritional aspects, besides the intensity of culture operations. Information is required about the levels of dietary essential nutrients that promote maximum growth at optimum intake,

the preferred form of diet, feeding behaviour; the role of attractants, feeding stimulants and deterrents; digestive capabilities of the species, and, also about the feed ingredients and additives that should go into the feed for proper ingestion and utilization by the animals.

#### Balance of Nutrients

A balanced feed formula must include an energy source plus sufficient indispensable amino acids, essential fatty acids, specific vitamins and minerals to sustain life and promote growth. In the case of prawns a source of sterol (cholesterol) and phospholipids (phosphatidylcholine and phosphatidylethanolamine) are found to be essential.

#### Protein and essential amino acids:

All the finfish and prawn species so far studied seems to require the same ten essential amino acids: arginine, histidine, lysine, leucine, isoleucine, methionine, phenylalanine, threonine, tryptophan and valine. In the case of prawns, tyrosine may be essential if the phenylalanine level is inadequate. While the quantitative essential amino acid requirements have been established for several species of freshwater finfish, quantitative requirements have not been clearly established for prawns, since prawns are found to be poor utilisers of chemically pure amino acids. At present, the amino acids profile of prawns and that of their best natural dietary protein source are used as a criterion for balancing amino acids levels in prawn diets.

In complete grow-out feeds, protein level in the range 35-40% would be adequate for most species

of prawns. *Penaeus japonicus* being a carnivorous species, needs relatively high dietary protein levels (50 to 55%). In general, marine prawns require relatively high levels of animal protein of marine origin. Diets with fish meal, clam meal, mussel meal, crab meal, prawn meal, prawn-head meal, squid meal and squilla meal as predominant protein sources have been efficiently utilized by prawns. A mixture of several marine protein sources seems to be better than individual ones for *P. indicus*. A number of other animal protein sources such as slaughter house waste, silkworm pupae, meat meal, meat and bone meal are also being incorporated in some diets. Among plant protein sources, soyabean meal provided the best response and is invariably used in prawn feed formulations (Gopal, 1986). Groundnut oil cake, sesame cake, coconut oil cake etc. have also been used in prawn feeds. Compounded diets made from several known ingredients are potentially more efficient due to the 'complementing effect' of amino acids than a single ingredient, which may be too high or too low in one or more of the essential amino acids. Supplementation of methionine and lysine, the first two limiting amino acids, is reported to improve the feed efficiency.

Among the finfish, protein requirements have been established for fry and fingerlings of the milkfish, *Chanos chanos* and the mullets, *Liza macrolepis* and *L. parsia*. In all the species the optimum protein for maximum growth was within the range 35-40% (Lim *et al.*, 1979; Kandasami *et al.*, 1987; Kiron, 1989). A variety of plant and animal protein sources have been tested in the diets of fin-

fish. In *L. macrolepis*, plant protein sources such as coconut oil cake, groundnut oil cake, sesame cake, field bean, bengal-gram, and horse-gram were found to be inferior to casein (Easterson *et al.*, 1989). In *Liza parsia*, groundnut oil cake among plant sources and fish meal among animal sources produced better performance; yet the best response was observed in the fish fed compounded diets with a mixture of plant and animal protein sources (Kiron, 1989).

#### Lipids and fatty acids:

Lipids as a group are important nutrients in the diet of marine fish and prawns: as sources of energy, essential fatty acids, sterol, phospholipids and as carriers of fat-soluble vitamins. Lipids are also important factors in the palatability of feeds. In complete feeds for prawns about 8 to 10% lipid containing a blend of essential fatty acids and phospholipids should be ensured. Although many species of prawns can tolerate lipid levels exceeding 10%, in large scale feed manufacture excess of lipids may lead to pelletability problems. In all the species of marine prawns so far studied highly unsaturated fatty acids such as eicosapentaenoic (20:5w3) and docosahexaenoic (22:6w3) fatty acids are found to be essential. In general a mixture of plant and animal (marine) lipids should be ensured to provide a blend of w6 and w3 fatty acids. The absence of *de novo* synthesis of 18:2w6, 18:3w3, 20:5w3 and 22:6w3 has been established for *P. japonicus*, *P. monodon* and *P. merguensis* (Kanazawa *et al.*, 1979) and for a number of other crustaceans. Besides, 18:2w6 and 18:3w3 are poor-

ly converted into HUFA of the same series (Kanazawa *et al.*, 1979; Colvin 1979; Chandge 1987). In *Penaeus indicus*, inclusion of 1% linoleic as well as 1% linolenic acids resulted in growth improvement over the control diet deficient in these fatty acids. While more than 1% of these fatty acids had no beneficial effect, levels of 3% and above were found to be detrimental to the growth. Linoleic acid was also found to be inferior to that of linolenic acid in efficacy. Of the diets tested, a diet containing lipids rich in 20:5w3 and 22:6w3 along with 18:2w6 and 18:3w3 alone produced the best response. Exclusion of polyunsaturated fatty acids from the diets of juveniles severely affected the efficiency of utilization of the ingested protein, and FCR. Besides, diets containing linoleic and linolenic acid when fed to larvae of *P. indicus* induced complete mortality (Chandge, 1987) as in the case of *P. japonicus* (Kanazawa *et al.*, 1985).

Phospholipids are the major lipids in crustaceans. Gopakumar and Nair (1975) reported that phospholipids constitute 62% of the total lipids in *P. indicus*. In common with other life forms, the major phospholipids in crustaceans are phosphatidylcholine and phosphatidylethanolamine which are important from the nutritional point of view (Sargent, 1976). Inclusion of 1% lecithin from short-necked clam in the purified diets promoted growth in *Penaeus japonicus* and inclusion of soy lecithin into purified juvenile lobster diets eliminated mortality associated with a 'moult-death syndrome' (Conklin *et al.*, 1980). In *P. indicus* exclusion of lecithin from purified diet induced high mortality

in larvae during the metamorphosis and all the larvae died before reaching the post-larval stage. A dietary level of 2% lecithin is adequate in the diets of larvae, post-larvae and juveniles of *P. indicus* (Chandge, 1987).

Cholesterol is an essential nutrient in the diet of crustaceans since cholesterol is not biosynthesized *de novo* (Zandee 1964; Kanazawa *et al.*, 1971). In crustaceans, cholesterol is the precursor for physiologically important hormones such as steroid hormones, brain and moulting hormones and vitamin D. Sterols are also found to be important components in the cellular and sub-cellular membranes particularly in the hypodermis in arthropods (New, 1976). About 0.25 to 0.5% cholesterol has been found to be sufficient in the diet of most species of prawns including *P. indicus* (Chandge, 1987).

Gross lipid requirements of fry of three species of marine finfish found in India have been determined. All the three species, *C. chanos*, *L. parsia* and *L. macrolepis* have been found to require about 6% of lipid in the diet for maximum performance (Kiron 1989; Paulraj and Thirunavukkarasu, 1988). Among lipid sources, cod liver oil and sardine oil were the best individual sources for *L. parsia* fry, followed by soyabean oil. A diet containing a mixture of sardine oil, groundnut oil and soyabean oil produced the best response in the fry (Kiron 1989), when reared in low-saline water of 15 to 25%. Since the salinity of the medium has been reported to alter the fatty acid composition in euryhaline finfish, more detailed investigations are required on the lipid

and fatty acid requirements in relation to salinities.

#### Carbohydrates:

Carbohydrates are the cheapest source of energy available for incorporation in the diets of animals. Among the carbohydrates, penaeid prawns are found to utilize polysaccharides like starch, dextrin and glycogen, and maltose more effectively than monosaccharides like glucose or galactose (Rahman *et al.*, 1979). In *P. indicus* starch and maltose were found to be good sources of carbohydrates (Ali, 1989). Gelatinised starch as high as 40% could be incorporated in *P. indicus* diets. However, starch digestibility was observed to be maximum at a dietary level of 20% in the diet (Hemambika, 1990). A variety of carbohydrases have been recorded in the mid-gut gland of *P. indicus*, but alpha amylase is the dominant one. Postlarval prawns were observed to have greater amylase activity, and the enzyme activity declines with the growth of the prawn to the adult stage (Hemambika, 1990).

Studies with milkfish and mullets indicate that gelatinised starch could be incorporated at levels in the range 35 to 45% without any adverse effect on performance. The most commonly available carbohydrate sources are tapioca, maize, wheat, rice, rice bran, wheat bran and millets.

#### Vitamins:

Vitamins are distributed in feedstuffs in small quantities and form a distinct entity from other major and minor food components. Despite the recognition of their importance in the diets of prawns, due to inherent problems posed by the

aquatic medium, the quantitative requirements have not been clearly established for various prawn species. Leaching of vitamins, especially the water soluble ones is a major constraint. Another factor is the contribution from gut microbial flora in certain species, which masks the actual requirement. It has also been observed that vitamins and their precursors, since already present in the raw materials, blanket applications of vitamins premixes in multi-ingredient diets may result in some excesses (New, 1976). Conversely, as the vitamin requirements remain unknown, formulated diets may still be deficient in certain vitamins. All the water-soluble and fat-soluble vitamins are considered essential in the diets of prawns and finfish, and are incorporated in feeds. Considerable amount of most of the water-soluble vitamins are lost during processing and storage of feeds, and by leaching after introduction into water. Therefore, excess of these vitamins are added in diets. In *Penaeus indicus*, optimum response was achieved with 0.8g ascorbic acid, 0.5g choline chloride, 0.02g thiamine hydrochloride, 0.075g calcium pantothenate, 0.03g pyridoxine hydrochloride and 0.025g nicotinic acid per 100g diet. In *P. japonicus* 2.0g ascorbic acid per 100g diet was necessary for maximum growth and survival. In recent years, several attempts are being made to improve the water stability of these vitamins in feeds. Microencapsulation appears to be a promising method for control of leaching of vitamins and the microencapsulated vitamins can be added in feeds. A variety of ascorbic acid derivatives with vitamin C activity are also being developed. One

of the most significant observations, in *P. indicus* is the occurrence of post-moult deaths under vitamin deficiency (Gopal, 1986). Besides, two prominent deficiency symptoms noticed in *P. indicus* are 'black lesions' induced by ascorbic acid deficiency and 'partial moulting' under pantothenic acid deficiency (Gopal, 1986).

In *Liza parvia*, exclusion of riboflavin, niacin, pantothenic acid, ascorbic acid, choline, inositol, thiamine and pyridoxine from the diets induced symptoms like anorexia, erratic movements, photophobia, fin degeneration, body lesions, haemorrhagic damage besides poor growth and food conversion. The specific deficiency symptom noticed were corneal opacity for riboflavin, gill damage in pantothenic acid, and scoliosis and lordosis in ascorbic acid deficiency (Kiron, 1989).

#### Minerals:

Among the major minerals calcium (Ca), phosphorus (P), magnesium (Mg), sulphur (S), potassium (K), sodium (Na), chlorine (Cl) and fifteen trace elements: iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), nickel (Ni), cobalt (Co), molybdenum (Mo), iodine (I), selenium (Se), chromium (Cr), fluorine (F), tin (Sn), silicon (Si), vanadium (Va) and arsenic (As) reported essential for terrestrial animals are also assumed to be essential for fish and prawns (New, 1987). Specific dietary essentiality and requirements for various minerals have not been fully established for marine prawns and finfish, although some information is available.

Prawns and finfish can absorb a variety of minerals through the in-

gestion of water and through exchange from their aquatic environment across body tissues such as gill membranes, besides that obtained through assimilation of food. In *P. japonicus* approximately 0.83 mg of calcium is absorbed per day per g of body weight from the environment containing 0.44 mg/ml of Ca. Ca may not be a limiting factor as the natural waters are quite rich in calcium, and relatively high levels of calcium are also present in the feedstuffs, especially in feedstuffs of animal origin. In contrast, natural waters contain very little phosphorus and therefore diet should contain adequate levels of phosphorus. Some types of phosphorus are known to be unavailable to fish. Generally animal sources of phosphorus are best absorbed. The phosphorus from plant sources is poorly available as it is mostly bound to phytin. The bioavailability of inorganic sources of phosphorus varies depending upon the source. New (1987) gave the following provisional availability factors for fish and prawns: plant and plant products 30%; animal products 70%; microbial products 90%; monobasic calcium, sodium and potassium phosphate 95%; dibasic calcium phosphate 70%; and tribasic calcium phosphate 65%. In *P. japonicus* best growth was obtained with a diet supplemented with 1.4% of P and 1.24% Ca and a Ca and P ratio of 1.2:1 has been suggested for optimum growth. In *P. indicus* Ca 0.53% and P 1.05% promoted optimum growth (Ali, 1989). The dietary levels for other minerals and trace elements are yet to be studied. In *P. indicus* Cu 13.6 mg and Zn 23.6 mg. per 100g diet promoted optimum growth (Ali, 1989). The role of Co, Zn and

trivalent chromium needs proper elucidation as these trace elements are considered to promote growth significantly in trout and carp at relatively low levels.

### Additives

#### Binders:

Artificial feeds for fish and prawns should contain adequate levels of a binder or a mixture of binders to render the required water stability. Gelatinised starch would be quite adequate in most of the finfish diets; whereas, in prawn feeds inclusion of an additional binder may become necessary, if the feed is exposed in water for periods exceeding 6 hrs. and that the feed ingredients are not finely ground. Substances like gum acacia, guar gum, alginates, carboxymethyl-cellulose, sea weed meals can be used at 1-2% level to provide the desired feed stability in water. However, by evolving suitable feeding strategies (feeding frequency, time of feeding, size of meal etc.) it should be possible to do away with the need for additional binder.

#### Antioxidants:

Marine prawn and finfish require relatively high levels of polyunsaturated fatty acids in their diets which are highly susceptible for oxidation. Antioxidants are included in vitamin premixes or added to lipids during manufacture. Commercially available antioxidants are mostly used in levels indicated as follows: butylated hydroxytoluene (BHT) 0.2%, butylated hydroxy anisole (BHA) 0.2% and ethoxyquin 0.015% or even less. Correct requirements are yet to be worked out. Natural antioxidants include ascorbic acid, lecithin and vitamin E, which

can be included at much higher levels taking into account their efficacy and economics.

#### Preservatives:

Added to feeds to improve shelf-life and to control the rate of deterioration, particularly due to fungal attack. Sodium, potassium and calcium salts of propionic acid, benzoic or sorbic acid are most widely used; the recommended levels in diet are: Ca, K or Na sorbate, Na and Ca propionate no limit, sodium benzoate-0.1%. The specific levels of incorporation into fish and prawn diets need elucidation.

#### Chemo-attractants-and-feeding-stimulants:

Synthetic chemicals or natural ingredients containing chemicals which induce feeding behaviour in prawns and fish. Free amino acids and nucleotides are the most important ones. Some species of finfish are known to require specific feeding stimulants. In general, a mixture of L-amino acids, glycine-betaine, inosine or inosine-5' phosphate are considered as 'Universal feeding stimulants' for fish (Mackie and Mitchell, 1985). Inosine and inosine phosphate are found in relatively high levels in finfish and invertebrate tissues. During spoilage of fish, inosine will get decomposed to the inactive hypoxanthine. Squid, shrimp, clam and polychaete extracts are known to be excellent natural stimulants for prawns and carnivorous fastidious finfish such as sea bass and red sea bream. Artemia powder is an attractant for European sea bass, mussel meat for *Lates-calcarifer*. Among individual amino acids, glycine and taurine were found

to be attractants for juvenile *P. indicus*.

#### Pigments:

Carotenoids are the most important among substances which render colour (biochromes). Fish and prawns can not synthesize the pigments *de novo*, but can alter the molecules by oxidation. Most of the crustaceans and finfish are carotenoid innovators. B-carotene and its oxidative derivatives-crypto, xanthin, zeaxanthin, canthaxanthin and ataxanthin are the preferred carotenoids which are found commonly in a number of invertebrates, particularly crustaceans and polychaetes.

#### Antimicrobial agents:

In aquaculture systems, the cultured animals are predisposed to pathogenic infections. In order to achieve maximum efficiency in production and feed efficiency these infections should be prevented or controlled. The recent trend in high-tech or industrial aquafeeds has been to include certain broad spectrum medicines such as oxolinic acid, oxytetracycline, terramycin etc. to prevent outbreak of diseases.

#### Anabolic agents:

In order to improve growth above the physiological maximum, the species must be either genetically manipulated or given a substance which will act pharmacologically to improve the metabolic and or digestive efficiency, and to promote protein deposition and hence growth (Matty, 1988). The most successfully used hormone for growth promotion in fish culture is 17 - methyl testosterone, which is effective in doses as low as 2 mg/kg diet. However, the

use of steroids in animal feeds is banned in several countries. The other potential growth promoting hormones are thyroxine, triiodothyronine, insulin and growth hormone for finfish. Recently recombinant bovine somatotrophine has been successfully tested in some species like channel catfish, but such studies are lacking for marine fish and prawns. Glucosamine, the chitin precursor has been found to promote growth in prawns. Antibiotics such as virginiamycin, terramycin have been found to promote growth in certain fresh water species, when incorporated in the diets.

#### Feed Manufacture

The steps involved in feed manufacture include procurement and storage of selected ingredients and additives, evaluation of their quality, disintegration of large particles, microgrinding, sieving, weighing of individual ingredients, premixing of vitamins and additives, mixing of all the ingredients, steam cooking, extruding through desired die apertures, cutting to desired size, drying and storage. Important considerations in manufacture of aquatic diets include good water stability, fine grinding and homogeneous mixing of ingredients and additives and optimum processing to achieve good stability without altering the nutritional integrity of the feeds.

Moist and semi-moist feeds are preferred by majority of finfish species. Floating-pellets also could be used in grow-out phase for certain species. For feeding prawns in grow-out systems dry pellets are the most suitable. Pellets can be easily manufactured on large scale, stored

transported and dispensed into culture systems. Pellets of sizes ranging from 1.0 mm to 4 mm diameter are produced. Dry pellets can be crumbled or powdered/sieved to obtain the desired particles and can be fed to post-larvae and juveniles of prawns.

#### Feeding Strategies

In order to achieve maximum efficiency of a given feed from a culture system, the feeding strategies employed are also very important. The ration offered should be regulated with reference to the biomass of the fish or prawn being cultured. It is generally known that young fish and prawn require more food to meet the demands for fast growth, as compared to sub-adults and adults. Similarly, changes in temperature, salinity and dissolved oxygen in the water, and other stress inducing factors significantly alter the food requirements. Factors inducing high metabolic rates such as excessive activity, forced swimming, aggression and nervous excitability must be avoided if high efficiencies from a feed are to be achieved. The number of meals offered each day and their size should be also regulated. For instance penaeid prawns may require a greater proportion of their diet at late evening and night hours than during the day. The feed dispensing methods are also very important. Methods should be evolved so that each of the cultured organisms obtains a share of the feed offered for achieving uniform growth. At present, the most common practice in prawn culture systems is to offer feed in trays placed on the peripheral areas of the pond bottom, and in some cases broadcasting is also done.

More appropriate feeding strategies should be evolved to meet specific needs.

### Larval Feeds

In the case of finfish during the prelarval stage and in penaeid prawns during the naupliar stages yolk provides the essential nutrients for meeting the energy demand. But after yolk absorption, the larvae need an exogenous source of food to satisfy their nutritional needs. At present production of larvae in hatcheries and their subsequent rearing in nurseries depend mainly upon the provision of selected species of live-food organisms. Live food, invariably, provides the essential nutrients in adequate levels and balanced proportions to the larvae. Besides, the presence of attractants, feeding stimulants and digestive enzymes in the live food and the soft texture are considered advantageous to the larvae which invariably have poorly developed digestive organs and digestive enzymes. But separate facilities, raw materials, energy input and man-power are required for production of live food, and thus the prolonged use of live food can be costly and variations in quality can adversely affect survival and growth of larvae and fry. So, the development of weaning diets received priority in several countries and now some species are exclusively reared on artificial feeds.

Unlike natural foods, artificial diets are not subject to seasonal variations in supply or nutritional composition and can be thoroughly quality controlled during fabrication (Meyers, 1979). However, there are certain inherent problems in the development of complete artificial

feeds as live food replacements for the first feeding stages of larvae of marine fish and prawns. Information on specific nutritional requirements, diet preference, degree of buoyancy required in the diet, rehydration characteristics and size and attractability of food particles are not available.

The various types of artificial diets that have been considered for weaning larvae are wet microparticulate diets (tissue suspension, egg-custard diet), dry microparticulates (spray-dried, vacuum dried, freeze-dried), microbound diets, microcoated diets and microencapsulated diets (Paulraj, 1989).

Microencapsulated diets have been successfully used in the larval rearing of several marine prawns and, finfish and one company (Fripak, UK) is producing the feed on commercial scale. A major advantage of microcapsules is that there is minimal loss of nutrients within the aqueous environment and the capsules can be produced in a range of sizes, thereby, suitable sizes can be offered to the larvae as they grow. Depending upon the capsule wall material Kanazawa (1986) proposed several categories of microencapsulated diets. However, nylon-protein coated capsules were found to give poor results with turbot, sole, cod, and red sea bream. Feeding frequencies, ration offered and particle size are important considerations in the utilization of microdiets by larvae. The development and use of any successful larval diet ultimately depends upon the economics, feed-price and fry-price relations.

### Research Needs

Systematic nutritional studies in cultivable marine finfish and prawns in India were initiated only during the eighties with the establishment of a nutrition laboratory at the Central Marine Fisheries Research Institute, Cochin. A good deal of nutritional data have been obtained for the prawn *Penaeus indicus* and mullets, *Liza macrolepis* and *L. par-sia*. However, further research is needed on the following lines to develop efficient diets and effective feeding strategies for marine fish and prawn culture.

- Quantitative amino acids, fatty acids, vitamin and mineral requirements.
- Availability of amino acids in prepared feeds
- Inter-relationship between nutrients, and optimum protein and energy ratios.
- Digestive physiology of cultivated species.
- Nutritional needs of larvae and broodstock.
- Nutritional energetics of fish and prawns; ecological energetics in culture system.
- Nutrition in relation to environmental factors (salinity,

temperature and dissolved oxygen).

- Identification of attractants, feeding stimulants, anabolic agents including genetically engineered hormones and bioactive substances of plant, animal or microbial origin, and digestive enzymes.
- Identification of novel nutrient and ingredient sources.
- Effect of anti-nutrients, preservatives and antioxidants.
- Development of optimum feed processing techniques
- Development of optimum feeding strategies-feeding level, feeding frequency and feed dispensing methods.
- Development of genetically engineered strains of live-food organisms marine yeast, algae, zooplankton etc. with specific nutritional attributes.

### Acknowledgement

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## KEY NOTE ADDRESS

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# Genetics and Growth Promotion

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The advent of Biotechnology has many potential impacts on animal breeding. The purpose of this paper is not to attempt any comprehensive review of possibilities, rather to enquire into the new consequences of a few technologies and to study the ramifications of such developments.

### Genetic Engineering

Genetic Engineering uses recombinant DNA techniques to transfer genes. There are several methods for effecting gene transfer. The most important among them are:

1. Use of retrovirus vectors;
2. Use of embryonic cells;
3. Direct microinjection of DNA into fertilized ova at an early stage of development.

Of these methods, the first is most promising. Despite some limitations on the length of DNA which can be incorporated into a retrovirus, the method has the great potential advantage of enabling the production of transgenic animals with single additional copies of the gene to be transferred.

It is not the DNA transfer techniques as such that is difficult. What proves to be more difficult is the discovery of suitable candidate genes for

transfer and their regulation to perform in the right tissues at appropriate times. This is because that the number of major genes affecting performance is rather limited. Further, discovering the DNA sequences corresponding to the observed genetic differences may represent a major problem. When a gene product is known or can reasonably be looked for in a particular tissue, the prospects are quiet good, but when these are unknown, the task is a daunting one. Gene for growth hormone has been a candidate for transfer. The dramatic growth hormone gene had been transferred (Palmiter et al, 1982) led to the dreams of obtaining such changes in farm animals. Similar transfers were made in pigs, the outcome was not in increasing growth but many development problems of undesired kinds (Hammer et al, 1985). The regulation of gene expression has to be studied in detail to prevent such pitfalls (Wagner, 1986). Continuous research have been taken in international laboratories to develop transgenic fowls. Positive results are forthcoming in this report.

### Application of Genetic Engineering

Ways in which the new methodology can be applied are as follows:

1. Production of new products.

2. Enhanced efficiency in production of existing products.

3. Modification of present products to enhance or reduce certain components.

Production of new product means production of new and novel products using farm animals. The new product can be a new kind of food or fibre. For certain pharmaceuticals products the use of mammals rather than micro organisms would have their advantages both in terms of ease of production and relative purity of the final product.

Improved efficiency has probably got the greatest general attraction since the efficiency in production of milk, meat, fibre, etc from animals will benefit both the farmer and the consumer. Baumann *et al.* (1985) have reported startling increases in milk yield through daily administration of exogenous growth hormone. The prospect of being able to build such advantage into the animal would seem to be promising and a likely field for intense activity.

The third general area of application of gene transfer would be in the modification of present products., for example protein in milk, fat in the carcass etc. To enhance milk proteins, the transfer of additional casein genes or the introduction of more effective promoters seem obvious choices for initial trial. Relatively little work has been done for reducing components. It is hoped that the use of antisense RNA to negate particular coding messages may be useful in this respect.

### Chromosome Analysis

With the coming of genetic engineering, older techniques such as

analysis of chromosomes by counting and banding techniques may seem to be outmoded. However, the knowledge of chromosomes, linkage groups of naturally occurring and transplanted genes and other cytogenetic analysis may be a useful adjunct to genetic engineering. The cytogenetic analysis of wild sheep and goat revealed that they shared a common ancestor with chromosome number as  $2n = 54$  chromosomes and the goat  $2n = 60$  chromosomes. The divergence of species may be far from complete and hence hybrids between domestic sheep and their ancestors (quoted by Gray, 1972) have been reported. Such opportunities should have been exploited or indeed explored fully.

Wider crosses between goat and sheep is also a possibility, even though the majority of workers have found the cross to be non viable. This was found to be due to immunological problems experienced by the fetus (Hancock, McGovern and stamp, 1968), rather than due to chromosome imbalance, since there is a report of one hybrid female surviving and producing lambs (Bunch, Foote and Spiller, 1976). Even if it is not possible to overcome immunological problems, new methods of embryonic micro manipulation can make it a possibility. The suggested method would be to introduce hybrid cells into a developing blastocyst of the sheep or the goat thus producing chimeras in some of which the hybrid might be expected to produce germ-line cells. Any genes introduced in this way could be exploited by selection in the normal manner.

### Sex Determination

A method for predetermining the sex of offspring has long been the ambition of many research scientists. The ability to separate X-bearing and Y-bearing sperms into separate fractions for determining the sex of offspring would indeed be a major achievement. Failing this, methods for sexing the developing embryo may also be advantageous, particularly if coupled with the use of embryo transfer methods. Ideally the methods employed should be non-destructive so that conception rates are maintained and embryo survival not adversely affected by the treatment.

Progress in sex determination is reported regularly, almost every year. The reports have in the course of time been shown to have one common feature—they do not work. The reasons for the failure are often not far to seek. For example, the difference in size between X-bearing

and Y-bearing sperm is such that electrophoretic separation is unlikely to be effective. Similarly attempts to detect the H-Y antigen on sperm are handicapped because of the secretion of this antigen from the somatic cells of the male parent. Future progress in the area is, however, not without hope. For example, Keeler, Mackenzie and Dresser (1983) have reported the separation of sperm into two fractions according to their fluorescence after staining with a vital dye. Preliminary evidence is presented of X and Y separation but unfortunately with present equipment, the method is too slow to be of any practical consequence. For the future, more radical methods of genetic engineering may be necessary to reach the desired endpoint. For example, McClaren and Burgoyne (1983) have shown that by the combination of sex reversal gene and chromosome translocation, it is possible to produce mice with progeny of predominantly one sex.

**KEY NOTE ADDRESS****New Concepts in Animal Disease  
Diagnosis**

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**E**arly diagnosis and identification of the causal agent form the most important criteria for adopting appropriate measures for the treatment, prevention and control of an animal disease. The conventional methods used for this purpose depend upon the study of clinical signs, pathological lesions and epidemiological manifestations; confirmatory laboratory investigations involve macroscopic or microscopic examination of tissues and body fluids, cultural methods using selective growth media, immunological methods of identification of organisms or their antigens, measurement of specific antibodies in the blood and visualisation of the organisms or a product of its interaction with the host system. Many of these techniques are lacking rapidity and accuracy. They also make use of large quantities of biological reagents thereby making them expensive in terms of production, storage, transportation and application. They lack the needed sensitivity to detect clinical disease early, subclinical or latent form of the disease or to differentiate clinically similar diseases caused by more than one causative agent.

Modern methods have brought in many improvements over the conventional methods thereby considerably obviating the above mentioned shortcomings. Identification of microorganisms or their antigens is now possible *in vivo* or *in vitro* using the more sensitive methods of radio-immuno-assays (RIA). Similarly, the identification of such antigens is also possible through the method of tagging fluorescent dyes to corresponding antibodies and examining for specific fluorescence under ultra violet light (Fluorescent Antibody Technique - FAT). The RIA and FAT are also largely used to identify the specific epitopes of the infectious agent which determine the specific immune response of the host animal.

More specific qualitative and quantitative assays for immune response for definitive diagnosis have also been made possible through the use of enzyme linked immunosorbent assay (ELISA) systems. The ELISA systems and their amplifications identify the antigen-antibody complexes through enzyme markers and their optical colour intensities.

The development of molecular bio-chemistry has provided rapid and sensitive methods for the precise diagnosis of many animal diseases by

detecting the specific protein subunits or epitopes of the infectious micro-organism as elicited by the gene sequences in the genetic material (RNA or DNA). Several glycoproteins, nucleoproteins and lipo-polysaccharides for specific bacteria, fungi or viruses have been identified by these methods. Techniques have also been developed for separation of peptides associated with microbial cell membranes or viral coat proteins based on their molecular weight by using polyacrylamide gel electrophoresis (PAGE) labelled with protein binding dyes or radio active precursors.

The application of genetic engineering techniques in recent years has added much precision to the diagnosis of diseases. Through the application of hybridoma technique and production of monoclonal antibodies (MAbs), it is now possible to identify the specific antigenic components of surface proteins in organisms and to delineate the specific epitope induced cellular changes which help in early detection of several disease, such as those due to protozoa - plasmodium, trypanosome, leishmania, theileria and toxoplasma; helminths - ascaris, schistosomes, fasciola, taenia, trichinella and echinococcus; bacteria-neisseria, haemophilus, brucella and mycobacteria; and viruses - FMD, RP, herpes, rabies, canine gastroenteritis, blue tongue, sheep pox, feline panleucopenia, swine pseudorabies, Marek's disease and Ranikhet disease.

The monoclonal antibody profiles form very useful tools for constructing antigenic maps for purposes of type/strain differentiation in several animal pathogens. They also provide

information to understand the complexities of immune network by delineating and differentiating T-cell populations and characterizing the major histo-compatibility complexes of antigens. The MAbs are able to recognise the antigenic and pathogenic sites for characterization of strains of micro-organisms. They are also useful for providing quality assurance of recombinant vaccines. Since the MAbs are easily produced in tissue culture, it is possible to make cocktails of MAbs of defined reactivity. Further, they are easily manipulatable for use in antigenic targetting in electron microscopic or cytological studies.

There are, however, certain disadvantages of MAbs. They are too very specific, hence they may miss certain immunologically responsive antigenic sites which the polyclonals will pick up because of their broad reactivity. Further, the MAbs used in one assay may not be useful in another. They are also single chemical substances which are easily affected by freezing and thawing, proteolytic enzymes, protein precipitation, etc. Additionally, the biological effects on binding demonstrated in tissue culture or *in vitro* may not represent the *in vivo* situation.

With the availability of specific MAbs and, ELISA field diagnostic kits have been developed. It has been observed that ELISA plates can be precoated with specific rabbit antisera and stored for long periods at 4°C or at -20°C and used for detection of FMD or SVD viruses. In simple tests, microtitre plates have been replaced by nitro-cellulose paper sheets and such 'dot-ELISA' kits have been found to be capable

of assaying simultaneously adenovirus and rotavirus in faeces. A competitive ELISA test has also been developed for blue tongue antibodies. Most of these ELISA tests require a source of virus antigen either prepared in cell culture systems or as expressed antigen using the gene alone. An example of application of this method has been demonstrated in expressing the nucleoprotein of rabies virus from a DNA clone in the baculovirus which was subsequently grown in insect cell culture.

Methods based on analysis of nucleic acid genomes of disease producing organisms have been recently discovered. These methods depend upon the ability of certain enzymes, known as, restriction enzymes to cleave DNA or RNA at specific sites. The fragments so formed are separated by PAGE. By using two dimensional electrophoresis of RE fragments of DNA or RNA segments 'finger prints' are obtained which are specific for viruses and other microorganisms and are useful for purposes of classification and diagnosis. Much use has been made of this technique in *Mycobacterium paratuberculosis*, blue tongue, pseudorabies, enterotoxigenic *E.coli* and viral gastroenteritis.

By cutting and electro-eluting the genome segments, the genomic DNA or RNA is transcribed into complementary strands of DNA (cDNA) and inserted into a plasmid so that large amounts of DNA copies of DNA or RNA are available. These clones are adopted for use in specific hybridization assays for diagnostic purposes. For instance, a cDNA derived from FMDV used as probe could detect viruses of O, A and C

types of FMD in cells which would not yield virus in tissue culture thus detecting even subclinical or latent infections. Similarly, nucleic acid clones derived from the closely related viruses, such as RP and Peste des Petits Ruminants (PPR) viruses could be differentiated. Conditions have now been standardised for DNA/DNA, DNA/RNA and RNA/RNA genetic hybridizations on solid matrices. The 'Southern blot' hybridization technique is used to characterise genome segments of DNA, whereas, the 'Northern blot' hybridization is used as diagnostic test for viral RNA or mRNA.

On principles similar to Southern and Northern blotting, a rapid and simple method called 'dot blot' has been developed. In this method, the DNA or RNA is extracted from the infected tissues, concentrated and denatured. An aliquot of the denatured extract is dotted onto nitro-cellulose strips and the labelled probe is placed on the extract and hybridized. Dot blot assay can also be applied to tissue extracts. Using this method, the diagnosis of blue tongue is done in less than 3 days as compared to conventional methods which take about 3 weeks to even 3 months.

*In situ* hybridization is another method which has the advantage of detecting the genome of the infectious agent with the cells and tissues remaining intact. Pseudorabies virus in pigs and IBR can be identified by this method. Another direct method, called 'Sandwich hybridization', has been developed in which the DNA probe is immobilized on to membrane filters or nitro-cellulose papers and hybridized with the target nucleic acid and then reacted with a second

single stranded probe which does not cross react with the nucleic acid preparation bound to the membrane filter or paper sheet.

In 'colony hybridization', bacterial colonies are transferred after cultivation directly from the agar plates to a filter, cells lysed, DNA denatured and the hybridization performed.

The sensitivity of a hybridization assay is determined by the specific activity of the labelled probe used. When <sup>32</sup>P-labelled probes are used, the highest sensitivity is obtained while the enzymatic detection procedures are many times less sensitive.

In situations where the nucleotide sequences of the probe are known, it is possible to use synthetic oligonucleotides for specific diagnosis. The sequence can be copied many times by repeated short reactions in a procedure called, 'Polymerase Chain Reaction (PCR)'. This method has been used for the detection of FMD virus RNA in four hours as compared to 24 hours needed for nucleic acid hybridization. It can also detect the target nucleic acid in quantities much smaller than what

is normally required for hybridization methods.

Several promising areas of animal disease diagnosis have been brought out by the application of knowledge of nucleotide sequencing. It is now possible to apply RNA sequence analysis as a routine procedure in accurate subtyping of FMDV. By comparing the nucleotide and amino acid sequences of the FMDV capsid protein VPI, possible regions involved in serotype and subtype specificities have been identified. In epidemiological studies, nucleotide sequencing has been used for the identification of vaccine virus related outbreaks and determining the relationship among geographically distinct disease outbreaks.

In the application of the various modern approaches detailed above, it is essential that the economic feasibility, capability and preparedness to absorb the technology in terms of manpower, equipment, chemicals and laboratory facilities should receive due consideration. It would be necessary to develop centralised production and supply of standardised reagents and other requirements.

## KEY NOTE ADDRESS

## Health Care and Disease Control in Freshwater Aquaculture

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Like any farmed animal, fish is equally susceptible to a broad spectrum of diseases. In the modern world, aquaculture is achieving new heights of productivity and the fishes are now raised in confined areas with high population densities. Fish, whether farmed or wild, suffer from diseases. The hazards of disease transmission is, however, far more in the former than in the later. Disease problems are becoming the most seriously limiting factor in aquaculture. Management of fish health thus assumes special status and has become an integral part of aquaculture management.

Success of aquaculture farming, largely depends on the health status of the cultured fish. In order to achieve optimum production it is essential to keep the fish as healthy as possible throughout the culture period. Therefore, regular and proper monitoring of fish health is an essential and integral part of scientific aquaculture practices.

Unlike any other animal diseases, the nature of fish disease outbreaks and their treatment and control measures assumes special status due to some peculiar and specific problems which requires proper understanding before plan-

ning any strategy for disease management.

Fish live in an aquatic environment where some of the most essential parameters such as water level, temperature, pH Oxygen, Carbon-dioxide etc. fluctuate widely and may become lethal at times.

Metabolites get accumulated in the system and come in inimical contact with the vital organs of the fish such as gills, skin, buccal cavity etc.

Due to increased use of organic manures, fertilizers, feed etc., some of the gases such as hydrogen sulphide, ammonia, carbondioxide etc. are formed which are lethal to fish at 0.01, 0.03 and 30ppm. respectively. Some eco - biological components of the pond ecosystem play a major role in the production of such gases.

Sometimes as a result of temperature fluctuations, the fish are put to a lot of stress beyond their normal tolerance limit.

The stressful environment for fish becomes congenial environment for the disease agents where they increasingly flourish.

Outbreak of disease is the function of three interacting factors i.e. environmental stress, virulent

pathogen and susceptible fish species. However, environmental factors or stress is the most potential factor among the three.

Fish live inside water and out of sight, as a result, detection of any disease at its early stage becomes difficult. Outbreak of disease is considered to be the result of some basic environmental problem. Until therapy and corrective measures are taken up simultaneously, fish disease outbreaks are difficult to control.

In commercial aquaculture, where the ponds are relatively big in size, pond treatment becomes an expensive proposition, dip treatment impractical and individual treatment through injection impossible. Antibiotic therapy through feed also does not ensure the desired level of the drug in the fish mainly due to leaching effect of the drug in the water and decreasing appetite during sickness.

Most of the clinical symptoms such as emaciation, ulcerations etc. are seen commonly in many diseases which eventually make the diagnosis difficult on the basis of clinical symptoms alone.

Most of the pathogenic bacteria are facultative and remain in water with the fish and cause disease only when the balance between the host and the pathogen become destabilised due to environmental factors.

Even without the active role of the parasites or other pathogenic organisms the fish do suffer from environmental diseases.

Such a diverse nature of fish disease problem makes it essential to have the knowledge of the ecobiology

of the environment, the anatomy and physiology of the concerned species of fish and the nature of the associated parasites and pathogens. In India, traditional aquaculture has in recent years have turned into a scientifically based economic and commercial activity involving heavy inputs and diseases of all kinds are now known to occur on an increasingly larger scale. The occurrence of ulcerative disease in the north - eastern region, West Bengal, Bihar, Orissa and Uttar Pradesh also indicates that certain diseases can spread easily and rapidly across the national geographic boundaries.

#### **Approaches for prophylactic measure.**

We already know that various ecological parameters and components of the pond environment influence health status of the fish apart from its own genotypic and physiological characteristics. Right from the stage of fertilized eggs onwards upto their full growth and marketing, various prophylactic measures are to be adopted at different phases of aquaculture for ensuring adequate protection against ill health and disease. The basic approaches for prophylactic measures are selection of quality stocking material for different phases, management of the aquatic environment, prophylactic medication and feeding.

##### *i. Selection of quality stocking material:*

There should be planned selection of stocks to improve quality, production and disease resistance. Application of genetic principles in

stock development can result in an improvement of fish health.

*ii. Management of the aquatic environment:*

Proper and efficient management of the aquatic environment is very important from health point of view. Abrupt and wider fluctuations in some of the environmental parameters cause stress and predisposes the fish to some diseases. Therefore, maintenance of a high quality environment is of utmost importance in the prevention of many diseases. Before introduction of the stocking material, the pond environment should be thoroughly cleared and made free of any pathogen or parasites. This objective is achieved by the application of chemicals like bleaching powder  $\text{Ca}(\text{OCl})\text{Cl}$ , calcium oxide, calcium hydroxide, malathion, potassium dichromate, potassium permanganate, copper sulphate etc. These chemicals kill most of the harmful pathogens and parasites. Intermediate hosts and other carriers of disease organisms should be destroyed before stocking the ponds. After filling the pond, it should be kept without fish for 10 - 15 days which help in completely killing the obligate parasites and pathogens, as they can not survive longer without the host. Depending on the situation, periodic application of chemicals help as prophylaxis against many possible disease outbreaks.

Proper disinfection of fishing equipments and culture establishments is essential for preventing disease outbreaks. In some of the hatcheries U-V light is used for treating the water against the pathogens.

*iii. Prophylactic measures:*

Initially the pond should either be dried or disinfected. Bleaching powder (chlorinated lime) should be applied @ 50 ppm in the pond. It readily kills all the wild fish species, molluscs, tadpoles, frogs, crabs etc. and also disinfect the pond soil. In nursery and rearing ponds it is desirable to use malathion @ 0.25 ppm, 4 - 5 days prior to stocking. It eliminates the larger copepod population which prey upon young fish larvae and also serve as vectors or carriers of many infectious disease agents. Some of the common crustacean parasites are also killed by its application.

*Chemoprophylaxis:*

Chemoprophylaxis is being increasingly used against many viral, bacterial, fungal as well as parasitic diseases. It may be employed through feed, through injections or through bath or dip. Use of fish therapeutic treatment is a very effective measure particularly when a particular disease organisms which may be present on the host fish. Among several methods of application of prophylactic treatments dip, bath, oral or perenteral routes of drug administration are most commonly practiced. Occasional application of potassium permanganate @ 2 - 3 ppm is recommended for increasing dissolved oxygen concentration and hauling prophylaxis. Dip treatment in 500 - 1000 ppm solution of potassium permanganate for a minute before releasing the fish back to the pond or exposure for few minutes to the fry or fingerlings before stocking is also a practical prophylactic measure. Instead of Pot. permanganate 2 - 3% salt solution can also be used.

**Therapy:**

Therapy can be topical or external application, systemic treatment via the feed and parental treatment through injection. The therapeutics are broadly classified as

- antibacterial agents
- antiprotozoal agents
- metazoan parasiticides.

Sulfonamides are a group of potential antibacterial drugs which are given orally. In fishes they are rapidly absorbed through the intestinal tract and are very effective against gram positive bacteria like streptococcus and staphylococcus. Sulfamerazine and sulfamethazine are effective against columnaris disease and other gram - positive bacterial diseases, when administered with the feed @ 100 - 200 mg/kg body weight for 2 - 3 weeks. When used as a 10 ppm bath once a week for 2 - 3 weeks it is very effective against columnaris disease, ichthyophthiriasis and external fungal disease.

Drugs like nitrofurazone, furazolidine, furacene etc. are potential drugs against many bacterial and protozoan diseases. Nitrofurazone is very effective when applied @ 50 - 75 mg/kg body weight upto 20 days. They are effective against gill disease, bacterial septicaemia, columnaris disease and protozoan disease like costiasis & 'Ich' when applied @ 1-10 ppm as bath for 5-10 minutes. It can also be used in the feed @ 2 - 4 mg/kg of fish per day for 3 - 5 days. As chemoprophylaxis, it can be used @ 0.4 - 0.8 mg/kg of fish per day for a longer period.

Among the antibiotics, oxytetracyclin is perhaps the most widely used and effective drug in fish dis-

ease therapy. It is very effective against wide range of gram positive and gram negative bacteria. Bacterial gill disease, bacterial haemorrhagic septicaemia, columnaris disease, Ich and saprolegniasis is nicely controlled by this drug when administered through feed @ 50 - 75 mg/kg body weight for 10 - 15 days or intraperitoneal injection @ 20 - 30 mg/kg fish.

Streptomycin is another potential antibiotic which can be used for treating many bacterial diseases including columnaris diseases and bacterial septicaemia. It should be used @ 20 - 25 mg/kg as intraperitoneal injection. When applied in combination with penicillin it is very effective against large number of bacteria including *Flexibacter columnaris*.

Quinine hydrochloride is very effective against protozoan parasites of the skin eg. *Ichthyophthiriasis* and *Trichodina* Sp. of infection and also against monogenea. Doses vary from 10 - 20 ppm and exposure should be longer. The treatment should be repeated for 3 to 10 days depending on the severity. Quinine sulphate can also be used effectively. These drugs are fatal when injected parenterally.

Metal containing compounds like salts and oxides of arsenic, mercury, copper, potassium and sodium etc. are used as medicine in aquaculture. Copper sulphate is widely used as a fungicide, algicide or a bacteriacide. It works nicely against *Branchiomyces* and *Saprolegnia*. It is also very effective against some external protozoan infections caused by *Costia*, *Ichthyophthirius*, *Trichodina* etc. and also against monogenetic trematodes, leeches and molluscs as

well. It should be used @ 1 ppm if calcium carbonate level in the fresh-water is less than 50 ppm while the dose can be double if the level of  $\text{CaCO}_3$  lie between 50 - 200 ppm. A mixture of copper sulphate with acetic acid (500 ppm each) can be very effectively used as dip for 1 - 2 minutes against fungal and protozoan diseases.

Potassium permanganate is used very effectively both as prophylactic and curative treatment for external parasites, bacterial, fungal and protozoan infections. It is externally effective when applied against acute infection of argulus showing external lesions (500 - 1000 ppm dip). When applied @ 500 ppm for four minutes as short bath it successfully controls outbreak of columnaris disease. It is used @ 2 - 3 ppm in the treatment of pond.

Sodium chloride is equally practical, cheap and easily available which can be used to cure wide range of parasitic, fungal and bacterial infections. In gill hyperapasia of common carp where several antibiotics have failed to bring any improvement, short bath in 3% sodium chloride solution has completely cured the disease when applied twice at an interval of a week.

Malachite green is yet another medicine against large spectra of pathogenic organisms. It is effectively used against ectoparasites, bacteria, fungus (*Saprolegnia*, *Achlya*), protozoa (*Costia*, *Ichthyophthirius*, *Trichodina*) and also against trematodes. Effective doses are 0.5 ppm for 1 hour against fungus, 1 : 15000 solution for 10.30 secs. against wide range of parasites and 1 - 3 ppm for 1 hour as flesh treatment.

Against 'Ich' this should be used @ 0.05 to 0.1 ppm in combination with 50 ppm of formaldehyde.

#### Metazoan parasiticides and peasticides:

Usually vermifuge or vermicide is applied to eliminate the worms in fishes. Dosage vary from 8-9 g/100kg fish/day for 3 days. Organophosphorus insecticides are widely used in aquaculture to combat ectoparasites such as crustaceans, monogenetic trematode and leeches. Malathion belongs to this group of compounds and acts very effectively against *Argulus*, *Ergasilus* and trematode parasites when applied @ 0.25 ppm in the pond. These applications are required in weekly intervals to prevent recurrence. When the infection is severe causing haemorrhagic lesions over the body dip treatment with  $\text{KMnO}_4$  (500 - 1000 ppm) for about a minute should also be done. Neguvon or chlorofos is another drug of choice of this group which is widely used against metazoan parasites. This is applied @ 0.25 ppm.

#### Immunoprophylaxis:

Immunization is an effective way of preventing communicable diseases. Immunization programme is gradually emerging as one of the most important measures for preventing fish diseases. Vaccine against some of the bacterial diseases of carps are now commercially available in most of the developed countries. Vaccine against *A. hydrophila*, *F. columnaris*, *E. trada*, *A. salmonicida*, *Yersinia ruckeri*, *V. anguillarum* and several viral pathogens, such as IPN, SVC, VHS, IHN etc are being tried on large scale. Recently immunization against

Ich has been reported to be successful. Attempts are underway to develop possible immunization of fish against myxozoa.

#### *Monitoring Health Status*

In intensive rearing system, mass mortalities of fish commonly occur due to pollution or predation, the environmental parameters are to be monitored and suitably manipulated as far as practicable to make it congenial for the cultured fish.

Apart from adopting proper prophylactic measures it is essential to check frequently the health status of the reared fish which help in timely detection of anything going wrong. This timely diagnosis is very important which enables to decide the specific follow up action and management measures that are needed to save the stock.

Periodical netting of a part of fish population at least once in a month is a good practice that allows to check the growth rate and other defects.

#### *Procedure for fish health monitoring*

The fish should be first observed to detect any changed behaviour : eg,

- (i) erratic swimming behaviour
- (ii) resting near the margins
- (iii) loss of balance
- (iv) Excessive or lack of mucous secretion.
- (v) change in the normal pigmentation
- (vi) erosions of scales, fins and lesions on the body etc.
- (vii) loss of appetite etc.

A representative sample on a random selection basis should be examined in detail in the laboratory using modern scientific methods for disease diagnosis.

#### *PRINCIPLES OF THERAPY IN FISH DISEASES*

Though the principles of disease control in fish are almost similar as those applied to other higher animals yet the variability of the aquatic environment and the delicate nature of the fish poses special problems is determining the type & effectiveness of the treatment as the Pisciculturist has to act within many limitations. Medications hold disease organisms in check, retard their growth or even kill the pathogen.

#### *Planning of treatment:*

Planning of effective treatment depends on several factors such as:

- a) Correct diagnosis of the disease
- b) Knowledge about the pathogen
- c) Prognosis of the disease
- d) Economics of the treatment operation
- e) Tolerance range of the fish to drug or chemical
- f) Water quality of the pond
- g) Type of fish i.e. species, size age, physiological condition.
- h) Properties of the drug or chemical
- i) Other courses of action.

Treatment may be applied in very many ways and the particular type of treatment to be applied is to be decided as per the specific situations encountered. There are three

ways of applying the treatment such as:

- a) Adding chemicals to the water
- b) Adding chemicals to the feed and
- c) Administering chemicals directly to individual fish.

*Types of treatment*

Dip, flush, short bath, indefinite baths feeding and injections.

*Conclusion*

When we go for intensive aquaculture programme there is a

tendency to load the pond sometimes beyond its carrying capacity with various inputs like - stocking material, feed manures and fertilizers, drugs and chemicals. As a result of this overloading the pond environment frequently becomes adverse. Hence frequent monitoring of fish health is very important as it is not easy to keep the environmental parameters within the physiologically acceptable limits of the cultured fish, the range of which are generally very narrow.

**KEY NOTE ADDRESS****Diseases of Marine Finfishes and Shellfishes of India**

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The biotic and the abiotic diseases of marine fishes, crustaceans and molluscs exploited in the capture and culture fisheries impair their normal biological activities, reduce their quality as food and often cause considerable loss of production and in certain cases, mass mortalities. Due to the difficulties of initial diagnosis of diseases in the wild population and the differentiation of the affected population from the healthy ones, the stock loss is generally included under the natural mortality and due to the same reasons much less is known about the diseases affecting the exploited population in the wild. In the farming of these animals in an environment unlike their natural habitat, in relatively high stocking densities, in non-optimal water quality situations and feeding the stocked population with nutritionally deficient diets, disease incidences are frequent. Over the past three decades considerable progress has been made in the study of the diseases of tended population. The information available on the diseases and parasites among the commercially exploited marine fishes, crustaceans and molluscs of India is briefly reviewed here.

**Marine Fishes**

Although information on the diseases of freshwater fishes of India, particularly those cultivated, is available to some extent, studies on the diseases of marine fishes of the country are not many. Pathogenic bacteria, fungi, protozoans and parasitic trematodes, cestodes, nematodes, leeches and crustaceans have been reported from the marine fishes of the country. Apart from these, dietary deficiencies, environmental stress as well as pollution and toxic algal blooms in the water bring forth diseases.

While no viral diseases have so far been reported from the marine fishes of India, the important bacterial diseases recorded are finrot, gillrot, tailrot, haemorrhagic septicaemia, withering of scales and ulcerative lesions. Among these, the finrot disease appears to be the most common bacterial disease and is recorded in *Chanos chanos*, mullet fry, *Etroplus suratensis*, *Tachysurus* spp., *Pomadasis* sp., *Chorinemus* sp. and *Sphyraena* sp., Species of *Pseudomonas*, *Vibrio* and *Aeromonas* have been isolated from the infected fins. However, no detailed studies on these diseases are available.

The reports of fungal diseases among the marine fishes of India are

only a few. The epizootic *Saprolegnia ferax*, producing dark marks on the eggs of *Hilsa ilisha* and resulting finally on the failure of their hatching has been described. Similarly, dermatomycosis caused by *Penicillium* sp. has been observed in *Anguilla bicolor bicolor* producing anorexia, weakness and visible infection.

Although protozoans are the most common and widespread associates as commensals, symbionts, parasites and pathogens in several marine hosts, the reports on the diseases caused by them in the Indian marine fishes relate to the sporozoan infection in tunas, myxosporidiosis in the cat fish, *Tachysurus thalassinus*, giant perch, *Lates calcarifer* and in two species of *Otolithus* and microsporidian infection in *Nemipterus mesoprion* and *Saurida tumbil*. The sporozoans, *Hexacapsula* sp. and *H. nepothunni* cause 'Jelly meet' disease of the muscle in *Thunnus (Parathunnus) obesus sibi* and *T. (Neothunnus) albacares*.

The metazoan parasites of the marine fishes of the country comprise of monogenetic and digenetic trematodes, cestodes, nematodes, acanthocephalans, leeches, and crustaceans. Although a variety of monogenean and digenean trematodes infect the fishes, the latter are known to be serious pathogens of fish. Generally, monogenetic trematodes are found attached to the gills or the operculum of the host and damage the tender respiratory folds causing light to moderate hypertrophic changes of the gill epithelium. On the other hand, the digenetic trematodes are found in the lumen of the intestine and pyloric stomach or encysted on the fin of the

host, or as in the case of *Tetrochetus coryphaenae* found in *Diodon hystrix*, in the rectal wall. Digenetic trematodes are also found in the air-bladder of catfishes.

Both the adult and larval cestodes infect fishes. While the adults are generally found in the intestine, larval forms are encountered in almost all the visceral organs. The direct tissue damage relates to connective tissue reaction, proliferation of fibrous connective tissue and accumulation of inflammatory cells at and around the site of attachment. Certain haematological and biochemical changes in the host are also observed as in the case of tetraphyllidean plerocercoid *Scolex pleuronectis* infecting the gall bladder and pyloric caeca of *Trichiurus savala*, and *Penetrocephalus ganapathi* on *Saurida tumbil*. The haematological changes cause anaemic conditions in the host and the biochemical changes result in the depletion of carbohydrates, fat and protein.

The nematode parasitism is dealt with by a few workers. While the majority of nematodes lie freely in the intestinal lumen of the host, *Philometra rajani* is recorded from the ovaries of *Polynemus polydactylus*, *Sciaena coiter*, *Otolithus argenteus* and *Pennahia aneus* and suggested to affect the eggs. It is also found to be more specific to female fish. The nematodes occurring free in the intestinal lumen though not cause serious tissue damage, are found to cause measurable haematological and biochemical changes.

The records of endoparasitic acanthocephalan on marine fishes are only a few and belong to the

genera *Naoechinorhynchus*, *Acanthosentis*, *Arhythmacanthus*, *Heterosentis*, *Filisoma*, *Raorhynchus*, *Rhadinorhynchus* and *Serrasentis*.

Among the metazoan parasites, copepods constitute the most common and wide spread group and have received the attention as early as 1898. Following this, a series of investigations carried out by several workers have greatly contributed to our knowledge on the variety of copepod parasites and their hosts. Generally, the copepod parasites are found on the body-surface or attached to the gills or opercular chamber. When the parasites attach to the gill tissue/filaments, they cause destruction and hypertrophy of the delicate gill tissue at the point of attachment and results, often in the fusion of gill filaments or their break off, thus reducing the total respiratory area, and consequently in the general fatigue and disturbance to the host. These, further make them susceptible to other infections. Certain copepods such as *Lernaenies hemiramphi* parasitic on *Hemiramphus xanthopterus* and *H. far*, penetrate the vital organs of the host having copious blood supply and impair their function and bring forth anaemic condition and depletion of energy reserves. Similarly, *L. ramosus* infecting *Nemipterus japonicus* produces ulcer-like opening at the attachment point, destroying the scales. The skin surface becomes hyperaemic and swollen and the muscle bundles degenerate at the penetration-area.

The information on isopod parasites of marine fishes is limited to a few reports in *Nerocila sundiaca* on the food fishes of west coast: *N. pigmentata* parasitic on *Opisthop-*

*terus tardoore*; *nerocila* sp. on *Ilisha melastoma* and *I. indica* from the Bombay and Andhra Pradesh coast respectively; *N. pigmentata* on *Nematalosa nasus*; *N. pulicatensis* on *Mystus guilo* and *N. trivittata* on *Arius nenga*; *Cymathoa-cremita*, *Codonophilus hemiramphi*, *Irona far* and *Livoneca* sp. on *Parastromateus niger*, *Hemiramphus xanthopterus*, *H. far* and *Nemipterus japonicus* respectively. These parasites are found in the buccal cavity or branchial chamber or attached to the body. Although they do not cause serious damage to the host, when present in the buccal cavity they interfere with feeding and in the branchial chamber with the respiration of the host. Sometimes, they also cause erosion or lesions the gill tissue.

Leeches of the family *Piscicolidae* are known to infest a variety of marine fishes and other animals such as turtles. Information is available on 23 species belonging to 12 genera, their geographic and host distribution, and salient features of their biology, ecology and behaviour.

In addition to the above important diseases and parasites affecting the health, certain planktonic organisms such as *Noctiluca*, *Hornellia*, *Trichodesmium* and *Gonyaulax* when bloom in great abundance bring about discolouration to the sea water, commonly known as red or green water tides, and cause large scale fish mortality. While the exact causative factor of this phenomenon is not yet clear, it is reported that the toxic effects of this bloom or the oxygen deficient water occurring during the dying phase of the bloom either directly or indirectly results in the fish mortality. However, some believe that the shoaling fishes avoid

these red or green water areas and escape the adverse effects.

In the recent years, the pollution of coastal waters by domestic sewage, agro-industrial wastes and oil spills or release, is increasingly threatening the fish health. Studies carried out at certain areas in the coastal waters around Bombay, Goa, Cochin, Tuticorin and Madras, where appreciable industrial effluents are discharged into the inshore waters have indicated that pollution of these waters by such discharges is one of the major causes for the fish mortalities observed in certain years.

## CRUSTACEANS

### Prawns and Shrimps

Among crustaceans, the majority of diseases reported relate to prawns and shrimps, obviously due to their commercial importance, economic value and demand. Various bacterial diseases such as myxobacteriosis, haemorrhagic septicaemia, vibriosis and enteric bacterial infection have been reported in penaeid prawns of the country. Among the bacterial diseases, vibriosis caused by *Vibrio anguillarum* is the most frequent disease found in *Penaeus indicus* cultivated in the brackish water fields. Recently brown spot disease caused by *Vibrio* and *Aeromonas* sp. is also reported in *P. indicus*. The bacterium *Escherichia coli* is found to infect the larvae of *P. indicus*, *P. monodon*, *M. affinis* and *M. dobsoni* cultured in earthen ponds in the brackishwater areas while *Pseudomonas fluorescens* causing haemorrhagic septicaemia is encountered mainly in *P. indicus* and *M. monoceros*. Decay of body surface caused by *Staphylococcus aureus* and

*E. coli* in *P. indicus* has also been observed.

Among the diseases caused by fungi, large scale mortality in larvae and juveniles of *P. monodon* raised in the hatchery has been reported due to heavy infection by fungus *Lagenidium* sp. Similarly, the fungi *Saprolegnia parasitica* and *Leptolegnia marina* have been recorded from the juveniles of *P. monodon* caught from the backwaters of Cochin.

The protozoan parasites, *Zoothamnium rigiduro* and *Stenter coeruleus* have been reported in *M. monoceros*. Besides these, *Epistylis* sp. together with *Zoothamnium* sp. have been encountered in *P. monodon* causing hypoxia. Occasionally, these parasites have been found to affect the juvenile prawns in the culture ponds where dissolved oxygen level in pond water decreases to 1.0 ppm due to non-flushing of pond water with tidal water.

The 'cotton' or 'milk' shrimp disease caused by microsporidian parasites in the natural populations of *P. indicus*, *P. semisulcatus*, *M. monoceros* and *M. brevicornis* caught off Madras, Mandapam, Tuticorin and Cochin has been reported on several occasions. The taxonomy, pathogenicity and histopathology of microsporidian parasites affecting penaeid prawns have been studied in detail.

Large number of metacercarian cysts infecting *M. monoceros* inhabiting the Cochin Backwater have been reported. Encysted digenean trematode metacercariae have been found attached to the antenna and other anterior appendages of post-larvae and juveniles of *P. indicus* and *P. semisulcatus*. However, no

morphological changes or any damage were noticed in these appendages. Instances of isopod bopyrid parasites infecting the branchial chamber or attaching to the appendages have been reported in *P.indicus*, *P.semisulcatus*, *P.merguiensis*, *P.japonicus*, *Parapenaeopsis stylifera*, *M.monoceros*, *M.dobsoni*, *M.brevicornis*, *M.lysianassa* and *Palaemon tenuipes* from natural population. Although no gross effects of infestation on the normal body growth of the prawn are observed, examination of the gill lamellae and reproductive organs of the infested prawns suggested indirect effects on the normal respiratory and reproduction activities of the hosts.

The 'soft' prawn syndrome in *P.indicus* affects the quality of the prawn and contributes to considerable loss of production. The biological, ecological, bacteriological, histological and certain biochemical aspects of the syndrome have been studied. This syndrome is generally encountered in cultured prawns, during adverse ecological conditions such as low salinities, and combinations of higher temperature and salinities. Similarly, environmental stresses brought out by sudden changes in water temperature, salinity or dissolved oxygen level brings forth necrosis of abdominal segments in *P.indicus*.

A tumour on the carapace of *P.indicus* from the grow-out ponds at Narakkal has been reported. While the organs such as ovary and heart, and the abdominal muscles of the tumour carrying prawn are found to be normal, the hepatopancreas showed certain abnormal features.

### Lobsters and Crabs:

Information on the diseases and parasites of lobsters of India is scanty and relates to the epizoic infestation and a chiniostomatid copepod of the genus, *Chomiomyzon* associated with *Panulirus*. Among crabs rhizocephalian parasites *Heterosaccus ruginosus* and *Sacculina* sp. on the portunid crab, *Portunus sanguinolentus* have been reported. The externa epizoites infesting *Scylla* spp. and *Portunus pelagicus* from Pulicat lake include 2 species of sea anemones, serpulid tubes, 5 species of cirripeds and 3 varieties of *Ocotasmis cor* and 3 species of bivalves. A chiniostomatid copepod parasite is also recorded on *Portunus* sp.

### MOLLUSCS

Information on the diseases of commercial molluscs of India is rather limited. Although diseases caused by viruses such as Baculovirus, reo-like virus (RLV), herpes like virus (HLV) and Picarnolike virus (CBV); bacterial like *Vibrio*, *Pseudomonas*, *Mycobacterium*, *Myxobacterium*, *Chondrococcus*, *Aeromonas*; protozoans such as haemoflageellates, ciliates, myxosporideans, microsporideans and Coccidia; helminths and parasitic copepods are known to bring forth severe diseases and mortalities in oysters and bivalves farmed at several parts of the world; from India a fungal disease caused by *Myotomus ostrearum* is reported in *Crassostrea gryphoides*. This fungus atrophies the muscle of the oyster. Trematode helminth parasite, *Bucephalopsis haimaenus* infects the gonad of *Crassostrea madrasensis* and impairs the reproductive capacity of the oyster.

Similarly, *Tylocephalus* sp, a cestode parasite found in *Pinctada fucata* is also known to affect the reproduction of the oyster. Besides, the cestode, *Tetrarhynchus unionifactor*, trematodes, *Muttua margaritifera*, *Musalia herdmani* and *Aspidogastu margaritifera*, and nematodes *Ascaris melagrinae* and *Cheiracanthus uncinaten* have been found to be the common helminth parasites of oysters of India.

The shell boring polychaetes and sponges cause considerable damage to the valuable molluscan shells. A number of fouling organisms such as barnacles, ascidians, and bryozoans affect the quality of the shells. The peacrabs, *Pinnotheres* spp found in the body cavity of oysters and bivalves are known to damage the gills, mantle, gonad and digestive gland of the hosts. Bucephalid cercaria are found to parasitise *Meretrix casta*. Similarly, the parasitic ciliates have been reported in the molluscan population of the mangrove swamps in Hooghly — Matlah estuarine complex.

#### GENERAL REMARKS

Most of the accounts of the diseases of marine fishes and shellfishes of India report upon the descriptions of the pathogen; only a few works are extended to the pathobiological considerations. In recent years, great emphasis has been laid on the understanding of the relationship between the host and the pathogen and pathogenesis to elucidate the mode and mechanism of infection, nature of virulence and epizootiology. In nature, it is seen that both the hosts and the pathogens live together, and under certain conditions, the potential pathogens prevail and ensue the

disease cycle. Thus the studies on the external and internal responses and interactions between the pathogen and the host have received great attention in recent years. In transmission experiments carried out in certain diseases, it has been shown that the pathogen when transmitted through oral route is not infective, but the same pathogen when introduced intramuscularly becomes infective. Just as the studies on pathogenesis of the diseases are imperative, histopathological investigations serve as a powerful tool, facilitating proper diagnosis of the disease and for understanding the effect of the pathogen on various systems and their functional organisation. Similarly, the biochemical studies of the host greatly help to understand the significant changes in the biochemical profile of the host that otherwise do not show any external symptoms of the disease or their effect. As information on these aspects of the diseases affecting the marine fishes and shellfishes of India is not available at present, considerable input is required on all these aspects as well as on the specialised subjects such as mycology and bacteriology.

With the enhanced developmental efforts for the production of fishes and shellfishes through high density and intensively managed culture systems, it is natural to expect increasing problems of diseases among the tended population. This emphasises the immediate need to recognize the biological and environmental stresses which lead to disease manifestation, to early diagnosis, its prevention and/or control.

Disinfection of water containers in which the larvae and post-larvae

are held, and other implements, is necessary to overcome the disease problem in controlled conditions. The term "Mariculture medicine" coined by Klontz to denote the medical aspects of mariculture includes 1) recognition that disease does exist, 2) definition of the disease, 3) Correction of the disease and 4) prevention of recurrence of disease. Six basic approaches such as adequate husbandry practices, use of genetically resistant strains, adequate balanced diet, use of vaccines, use of antimicrobial compounds and prevention of movement of infected stock have been recognised to control diseases. The antigenic relationship of certain virus isolates are being studied to develop polyvalent antisera and effective vaccines against the virus. Similarly, the extracts from certain tropical marine invertebrates have shown to render the test animals such as blue crabs, cray fish, prawns and fishes more resistant to bacterial infection. Immunology and Chemotherapy are emerging as important means of disease control. Thus valuable information on different aspects of disease control are now available for fishes and shellfishes of India are very much limited and this field requires immediate attention. One of the constraints encountered in this respect is the lack of trained fishery

pathologists in the country to work on the subject.

With the expansion of the culture fisheries in the different coastal regions of the country, the seed collected from the wild and those produced in the hatchery are transported freely to distant places for further culture and production. These seed material are not at present subjected to screening for diseases or for the potential diseases. As this activity is envisaged to be stepped up in the near future, and as such free movement of live material carrying the potential pathogens could spread the diseases in the new places of introduction, it would be prudent to consider now itself some effective measures of health and sanitary control over the transportation of live fish and seed. In other words, some legal basis for disease control, particularly in aquaculture, is found to be essential in our country not only for stock improvement, but also to ensure sustained production. All these necessitate comprehensive studies on different aspects of diseases and information concerning the identification, nature and occurrence of pathological organisms, reliable and standard diagnostic methods and criteria for disease free status of aquaculture establishments.

**KEY NOTE ADDRESS****Management in Relation to Animals  
with Particular Reference to Poultry**

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**E**nvironment is of great importance to the livestock and poultry farmers in obtaining the fullest genetic expression from either domestic animals and/or birds.

An animal's or bird's environment is the total of all external conditions that affects its development, response and growth. Literally it includes the equipment and type or slope of floor as factors of environment and they may be important factors. It is often convenient, however, to separate the factors into physical, social and thermal factors. Physical factors are such things as space, light, sound, pressure and equipment. The social factors are the number of birds or animals per cage or pen, behaviour and peck order. Thermal factors are air temperature, relative humidity, air movement and radiation (Esmay, 1978).

There are few places in the world where the natural climate is continuously optimum for domestic animals, throughout the year. Man is interested in livestock and poultry production in regions that are increasingly farther removed for optimum growth and production.

Our country has wide variety of climates and terrains such as hot arid zones of Rajasthan, the tropical

forests of eastern regions of the country, moderate climates of the coastal areas, high altitude arid zones, of western regions of Himalayas and high altitude humid zones of eastern Himalayas, heavy rainfall areas of Assam, primitive environment of rural areas and pollution infested atmosphere of urban areas.

The climate of Madras is described a tropical maritime monsoon type "the city is located in the east coast of India." The region is benefited by both southwest monsoon and northeast monsoon rains, the latter predominating. The year can be divided into four seasons - cold (December, January, February), summer (March, April, May), southwest monsoon (June, July, August), and northeast monsoon (September, October, November). May is the hottest month and January is the coldest month. The maximum temperature of the day occurs between 1 p.m. and 2 p.m. but in hot months it may occur around midday, just before the sea breeze sets in; the minimum temperature of the day occurs just before sunrise.

Mean relative humidity is high throughout the year and varies between about 65 percent in May - July and about 8 percent in October

- December. June is the month with the least relative humidity. Relative humidity increase sharply by about 20 - 30, with the onset of seabreeze and continues high as long as sea breeze lasts.

Mean annual rainfall is 121 cm, and mean number of rainy days is 58. About two thirds of the annual rainfall occurs during northeast monsoon. Rainiest months are October and November. The rainfall for each month or whole year is by no means constant from year to year.

The Poultry Research Station was established in the early forties at Madras to serve the interests of poultry farmers in the Madras Presidency. Since then the development activities related to poultry keeping were pursued. In the late forties Madras Veterinary College had its poultry unit established. Between fifties and sixties, the poultry unit grew into a poultry field laboratory serving the interests of the research workers of the various departments of the college. Beyond sixties, several studies were initiated to improve fertility, hatchability, growth and egg production as related to breeding, feeding and health aspects. Environmental aspects and management factors were given due consideration. Post-graduate students of various departments had the desired benefit of these developments.

With the inception of the Poultry Research Station, the back yard or free range system of poultry keeping was transformed into semi - intensive type of rearing with wiremesh run and a night shelter. Feeding was mostly with grains and greens. Birds were still subjected to considerable climatic stress due to seasonal

variations in climate of summer and monsoon months. Again most of housing conditions were similar to the temperate type of management conditions.

In the early sixties, the college poultry field laboratory was accommodated in available sheds which were originally meant for large animals with high walled structures. The inadequate ventilation meant adverse host - parasite relationship which favoured parasitism at the cost of host. Coryza and coccidiosis hampered brooder management. Added to this, monsoon weather conditions worsened the situation due to water logging. Consequent to the low lying status of the poultry field laboratory site, between sixties and seventies, several improvements were made especially in terms of houses. A well ventilated hatchery was set up. Runs with night shelter accommodation were substituted with two third covered pen with one - third run (i.e) per run shed type enclosure allowed for a little more intensive. Initially sand litter was used. Later they were substituted with gable/monitor type roof fully covered houses with better materials such as saw dust/wood shavings/paddy husk/groundnut shell etc. as litter. The side walls which were just 9 inches pen run type were increased to 1 1/2 to 2 feet. The floor which were either earthened or sand litter were substituted with cement rendered better flows.

All these meant improvement in the birds' environment to facilitate better hatchability, growth and production among brooder, grower and layer status. Simultaneously improvements were effected in feed for-

mulation to suit the age and the stage of development and production. Health aspects were also looked into to provide sanitary surroundings with suitable hygienic measures.

In the late sixties the Poultry Research station was transferred under the control of Director of Veterinary Education and Research and became an annex to the Madras Veterinary College. The Poultry field laboratory at Madras Veterinary College was dismantled and re-erected and this became a part of the Poultry Research Station. Beyond seventies several improved buildings were put up at the Poultry Research Station based on the experience gained at Madras Veterinary College for hatching, brooding, growing and laying operations. Considerable improvements were made possible with the introduction of newer strains of layer and broiler stocks. Health cover and ameliorative measures to overcome the losses due to climatic stress associated with seasonal variations. In the late seventies additional high roofed, well ventilated double monitor roofed houses with extraction levels were constructed. Management in terms of sanitation, vaccination and disease control measures were also improved. Planned approaches were made to have suitable replacement schedule to fall in line with housing accommodation.

Changed agricultural practices such as grass land conversion, irrigation, free movements of stock, geographical relocations within industry and expanding urban developments meant more and more intensiveness in stock rearing. Problems of parasitism prevailed despite several accomplishments

either due to drug resistance or tissue residues with the use of chemicals.

As outlined earlier, semi-intensivism of early forties was substituted by intensivism in early sixties with better nutritional management. By seventies with introduction of several exotic strains, the management procedures in terms of medication, vaccination, deep litter management etc. and better health cover was arranged. Further improvement in deep litter houses and introduction of cages facilitated economical poultry keeping. Developments in the later years led to ushering in of Poultry industry.

The tropical jungle fowls taken out to distant parts of the world centuries back (especially, to temperate surroundings) got transformed into superior performing layers/broiler stocks and got reintroduced to the tropics. Even in the early forties, the performance of these birds remained suppressed due to the inco-ordinate conditions as well as management procedures provided and prevailed then.

It took well over a century for the scientists in temperate countries to make the fancy breeder's spade work to bear fruits in terms of producing hybrid pure and hybrid stock for dissemination the world over. In the last fifty years, the experiences of the scientists in temperate zone and our own experiences in the tropical zone has been responsible for the steady and sustained growth of the poultry industry as we see to day. Poultry industry occupies a place in animal enterprises in our country.

A little peep into the past at this juncture will help us to look back on the improvements we have made in breeding, freeing and health aspects in terms of management. This would be beneficial to chalk out future course of action to make this prosperous industry more viable and enterprising in the future as well. Environmental studies at Madras Veterinary College with the primitive environment in the midsixties revealed that the seasonal variations due to climatic stress is one of the major concerns. Studies were conducted on the hatchability, fertility, growth and egg production.

White Rock which were heat susceptible suffered more than White Leghorns which were heat tolerant. In both breeds, cold season hatch gave the best fertility and hatchability result. Summer depression was more severe in White Rock. The southwest monsoon hatch also had the depression. The cold season and north east monsoon season hatches had comparable performance in White Leghorns, while White Rocks had considerable decline even in northeast monsoon hatch also.

Seasonal study on growth rate of White Rocks as well as monthly study on growth rate did not reveal significant difference. Compensatory growth phenomenon was responsible for such a result since the period of growth was 12 weeks which was quite long duration - wise.

Egg production in White Leghorn and White Rock as related to season of hatch revealed the highest percentage in White Leghorns for northeast monsoon hatch season followed by southwest monsoon, summer and cold season hatches while in White

Rocks, it was southwest monsoon hatch followed by summer, cold and northeast monsoon hatches. Heat tolerance of White Leghorns and the actual interpolation of summer period within the laying schedule are factors to be reckoned in this regard.

White Leghorn layers in cages produced significantly better in a fan environment as compared with natural environment.

Eggs stored for 7 days hatched during the months of July to January were set for hatch as day 1 through day 7 separately. In general fertile hatchability declined beyond the fourth day of storage. The sixth and seventh day storage showed drastic decline. This decline was most from June to October. November accounted for partial recovery. December and January showed the least decline even on the sixth and seventh day. Management practice was altered by having two hatches at 4 day intervals instead of one at the end of seven days. Later with cool cellar storage of 10 days, further improvements were made possible by hatchery sanitation practices. Seven different saniticing procedures of egg, litter, nest and incubator alone and combination thereof were tried. Litter or incubator sanitation alone gave the best result. Incubator sanitation was followed subsequently to get good hatchability. In an attempt to better further, continuous and conventional incubator fumigation was tried and container fumigation was found to be more advantageous in getting higher hatchability.

The beneficial effect of incubator sanitation was found in the sig-

nificantly improved growth of White Leghorn upto 8 weeks of age as well.

Litter sanitation was better achieved with raking of litter at regular intervals. 7 day raking interval resulted in low total coliform and mould counts. 14 day and 28 day intervals recorded higher microbial counts, with no raking the levels were very high for total and coliform counts while it was noticed that mould counts were lower than that of 28 days level but anyway higher than that of 7 or 14 day level.

In the mid sixties, broiler performance was compared upto 16 weeks in natural, cooler and fan environments. Fan environment was superior than natural or cooler environments. Relative humidity in the fan environment was the least while it was highest in the cooler environment (evaporative cooler). Performance of cooler environment was no better than the natural environment in spite of little lower temperature.

Floor space comparison of 0.8 1.0 and 1.2 square feet with broilers had no significant variation in the final 10th week body weight except for the fact that the crowded group had an economical advantage in having better feed efficiency. Perhaps the trial in not reflecting the summer weather effect.

A wire floor Vs paddy husk deep litter floor comparison with broilers revealed the wire floor superiority partly. However, the comparison between southwest and northeast monsoon seasons were inconsistent.

A cage Vs deep litter floor comparison revealed that it is advantageous to grow broiler in cage

both in summer and cold seasons for better finishing weights.

Protein levels of 20, 22 and 24 percent with three energy levels of 2600, 2800 and 3000 kilocalories in broiler diet was compared and 20% with any energy level recorded poor weights. 22% with any combination gave almost similar performance. 24% with 2600 Kcal diet fed group performed the best, while any higher levels had performance lower than 22% diet formulation. Caloric needs, if not taken care of, lower body weights are imminent even with a superior commercial stock.

Protein levels of 16 to 20% were tried in New Hampshire broilers, 24% level gave the highest body weight at 10 and 12 weeks; 26% level gave a lesser weight than 24% level but a weight higher than 22% level. 16% and 18% levels had poor performance. At 20 and 22% levels there was substantially higher performance than 16 and 18% levels, further bodyweight of 12 weeks 16% was obtained at 10 weeks with 22% levels and final bodyweight of 12 weeks with 18% was obtained with 24% level at 10 weeks.

Amelcorature study with house modifications and management procedures in broilers was carried out. Caged birds performed better than control on floor both in summer and cold seasons. The first summer was more severe than second summer. The first summer was dry and second summer was interspersed with showers. Burring roof insulation, other management/housing modification better designed and conducted over a stretch of time and repeated over and again will make the summer management much more

productive in the years ahead, Ventilation and light as well as radiation effects need sufficient thought for designing better microclimatic conditions within the poultry houses.

The broilers available in the mid sixties took nearly sixteen weeks to get 3lb body weight. By seventies it took only 14 weeks to get the same body weight. With different strains five years later, it took only 10 weeks to attain the same weight. Presently with the commercial strain, it is attainable much earlier than 8 weeks. From the nature of the environment in one and the same place, a little less conducive at one stage and a little more conducive at yet another, stage, made the strains of different inherited potential perform differently depending on the quality of the environment and the associated management practices. The same has been the case with the layers of different origin as well.

Experimentations thus far has been of a piece-meal character though elucidative aviculture and an integrated approach still remains a far cry. Environment is more often taken for granted as near ideal. Feed formulations are still oriented towards crude protein level. Energy levels to meet the variations in the climatic condition are yet to be workedout in any great detail. Aminoacid content and the correlating of the protein quantity and quality with the micro elements variations needs quite a deal of doing and redoing. Vaccination procedures and medication schedules need a strong recommendation for adoption in common in any one area to avoid too many vaccination schedules and wasteful over medication.

Integrated studies with housing condition, climatic conditions nutritional conditions, sanitary conditions taken together only, will be able to establish superior performance especially when dealing with breeding stock. Commercial poultry keepers have the advantages of our earlier studies as well as studies reported in the literature locally and abroad. The concentration of poultry in the pockets are on the increase. The incidence of emerging disease as well as occurrence of confusing health problems arising every now and then calls for a retrospective consideration and a determined and concentrated effort by the scientists of different disciplines to solve the future problems which are bound to be of a difficult nature and rather difficult to ignore for long.

Tropical environment was once thought to be hostile for animals. Poultry production do not remain to be so. It has been proved to be otherwise. Further enlightenment is possible only with an integrated and dedicated approach.

Any amount of experimentation in research laboratories/station would be of little avail, unless and otherwise these results permeate into the farmer's fold. Only with such transformations, the poultry industry would stand to benefit. It would be appropriate to take stock of what is happening to the poultry products especially.

Performance of different strains in the three poultry pockets namely Erode, Namakkal and Rajapalayam were compared. In general the strains had a hen house average of around 140 eggs. Some strains had a comparable hen day average but

there was a substantial difference on hen housed bases. Caged birds had a significantly higher production than deep litter housed birds. Birds fed compounded feed had a higher production than birds fed home mixed feed. Between localities Erode excelled in performance followed by Namakkal and Rajapalayam.

It is evident that strains, housing system and feeding system and locations as such had impact on the production pattern. Management varies between these locations in terms of houses and general management. A continuous assessment of these areas at periodical intervals in terms of bird concentration, changing practices, medication schedules, adequate housing conditions, management to overcome seasonal climatic variations as temperature, humidity wind velocity and rainfall condition, feed formulation and several other factors may be necessary to make the management work with superior commercial strains. Strong and weak points in each location will help to solve the problems and also to promote better production. Vaccination and medication schedules coupled with management factors will help to resolve confusing health situations. Seasonal variations has to be taken care to resolve confusing health situations. Seasonal variations has to be taken care of with housing improvements with suitable management programmes. Environment and management need to be blended to make poultry production more viable and quite profitable with least stress and maximum comfort to the birds.

A well replicated experiment may give very clear results at a particular time in a particular laboratory with a particular strain of bird but

another experiment may get entirely different result elsewhere. In fact this should not discourage us, because a critical study of the results from different works may be just as valuable as the detailed analysis of birds within a single experiment. It is often the discrepancy which emerges between well conducted experiments which lead to new creations which would never have been obtained from a single experiment.

The change from free range to deep litter, wire floor, cages etc. had its own advantages and disadvantages depending on the perfection and imperfection all inbuilt with such variations. Disease, parasitism, physical damage and welfare as reflected by behaviors and production as such are bound to be different with differing situations.

The data on All India coordinated project on poultry meat with same strains of chicken recalls the fluctuating results between the countries over the years. The economic characters as broiler weight, morality, feed efficiency, egg production etc. are found to vary considerably with the same strain involved in two stations. This suggests that the management being a variable factor makes a straight comparison based on the data but is rather misleading.

The welfare of domestic poultry especially those kept under intensive conditions is a subject on which many, often divergent conflicting views are held. This divergence may be the result of either of insufficient knowledge of the facts of a particular poultry husbandry system or a differing interpretation of these facts.

Recommendation of Article 3 of the council of European convention

on the production of animals kept for farming purposes states, that animals shall be housed, provided with food, water and care in manner which having regard to their species and to their degree of development, adaptation and domestications is appropriate to their physiological and the ethological needs in accordance with established experience and scientific knowledge.

From the normal and aesthetic points of view, opinions vary widely among different sections of community as to acceptable system of animal husbandry. Conflict exists between the emotionally desirable and economically practicable and conciliation is between humane treatment of animals on the one hand and human needs on the other. The explosive increase in the human population creates a shortage of food for which intensive methods of husbandry afford a temporary solution; on the other hand it would be wrong to inflict avoidable hardship on animals merely for the purpose of increasing dividends by reducing costs.

When the intensive methods are marginally within the function and physiological capacity of the species and optimal standards of husbandry

are not applied, breakdowns occur and the result is suffering and diseases. In such circumstances the animals can be protected only by raising the standard of management or reducing the degree of intensiveness. Both are desirable.

Management lends itself to no glib generalisation or rigid stratifications, for it has many purposes. It is subject of constant change and all the more so with livestock and poultry enterprises which is not what they were then or ever before. As it is rightly said it is outlandish to deal with today's problems with yesterday's solutions. Problems do arise as we solve more and more of them. Management should be in time with the changing livestock situations. Contribution of management is unique and has no substitute. Let us strive to manage better for a better tomorrow.

To conclude it would be quite appropriate to quote the unique statement of Pericles the ruler of Greece in 5th century B.C. who exemplified the singular nature of management conclusion. "I have around me a garland, the compliments of their men, it is the tie that binds them together that is my own."

**KEY NOTE ADDRESS****Certain Development and Management Issues in Marine Capture and Culture Fisheries in India**

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**T**his address outlines some of the key development and management issues concerning the marine fisheries sector in the country. The major issues in respect of marine capture fisheries include disproportionate allocation of investment capital between the crucial sectors of infrastructure, bycatch utilisation, product diversification, domestic marketing, development of new generation of fishing fleets and introduction of modern devices and techniques to reinforce, upgrade and promote infrastructure development. Coastal aquaculture issues are those concerning the slow progress in commercial growth of mariculture, lack of policy incentives to semi-intensive and intensive shrimp farming and lack of government initiative in brackishwater finfish aquaculture. Institutional mechanisms that could bring about steady growth in all sectors of marine fisheries are also dealt with.

**I. Marine Capture Fisheries****1. Investment mismatch**

Growing at the annual rate of about 3%, marine fish production in

India reached about 2.27 million tonnes in 1989 from 0.50 million tonnes in 1950. Investment in the fisheries sector increased significantly from Rs.2.78 crores in the first plan (1951-90) to Rs.569.54 crores in the seventh plan (1985-90) with a substantial portion of it going into the marine fisheries sector. The growth in gross annual yields, however, has not matched the growth in gross investment owing mainly to the mismatch between investment in different infrastructure sectors. For example, the heavy investment in fishing harbour was not matched well by investment in the equally vital harvesting and postharvest infrastructure, particularly in offshore fishing fleets and in nationwide cold chain for domestic marketing of frozen fish. Deepsea fishing and domestic marketing received low priority as investment in these sectors centre round finfish production and trade essentially for the domestic market. As the inshore grounds to a depth of 50m are near optimally exploited except along the Orissa-West Bengal coast, the annual yield which is stagnating around the present level for the last several

years would not improve unless fishing is extended beyond the 50m isobath for the finfish stocks. Tapping the natural production of wildfish stocks within our continental shelf is much more important than raising them in farms.

## 2. Bycatch utilisation

Finfish bycatch from the so called deepsea shrimp trawlers (Mexican trawlers), of which there are well over 180 vessels at present, operating along the Orissa-West Bengal coast, all well within the 50m isobath, is almost totally discarded at sea because of limited fishhold which is used exclusively for shrimps, lobsters, squids and cuttlefishes for export. This singular export obsession with the government and the industry is the sole reason for the near total neglect or poor development of large vessel based finfish sector in the country.

In the first instance, there should be a legal ban on bycatch discard at sea and mandatory requirement on Mexican trawlers operation along the Orissa-West Bengal coast to land bycatch at Roychowk port or any other port nearest to the fishing grounds through carrier vessels. Batteries of sailboat carrier vessels towed by mechanised boats could be deployed regularly for the transportation of bycatch from the fishing grounds, as in the case of purseseine operations along the Kerala, Karnataka and Goa coasts.

At an average of about 10 tonnes of bycatch per boat per day, the Mexican trawlers alone could land about 2000 tonnes of finfish daily, and at an average of 250 fishing days a year, the annual bycatch of this fleet

is in the vicinity of half a million tonnes – a real wealth in terms of raw materials for high grade fish meal for the feed (poultry, cattle and shrimp feeds) industry or in terms of various value added products like fish pickles for the domestic market where the demand is growing rapidly thanks to trade promotion efforts by the Cochin Integrated Fisheries Project and the Fisheries College, Tuticorin. The advantage with fish pickle is that it does not require any major machinery, it could be produced in large quantities by the small industrial sector by means of simple machineries, it has much longer shelf life of 6 months to one year, there is no need for cold chain network and could be sold through grocery shops and other retailers throughout the country.

## 3. Second generation trawlers

As we step up the efforts to utilise the bycatch, fleets of second generation trawlers of 37', 42', 47' and 52' overall length (OAL) should be developed for every minor, medium and major fishing harbour for exploiting the finfish stocks in the intermediate depths between the 50m and 150m isobaths. This development which has already started in the mid 1980s in the States of Gujarat, Maharashtra, Karnataka and Andhra Pradesh and recently in Tamil Nadu, should extend to all the remaining states to bring the intermediate depths into optimum exploitation. Development of this fleet should be seen as an immediate priority as with bycatch utilization, value added products and domestic marketing network. It is only through this fleet the nation could look forward to any meaningful

increase in marine production to bridge the persistent gap between actual and potential yields, to promote fishermen welfare and to increase domestic supplies and consumption.

The catch from this fleet will have to be used primarily for the pickle industry for domestic market and surimi for the Japanese market to ensure sustained offtake and economic prices. Therefore the private sector may be encouraged to put up pickle and surimi plants in the vicinity of all fishing harbours where second generation trawlers shall operate.

Arguments that the second generation trawlers (engaged essentially in bottom trawling, high opening bottom trawling, midwater trawling or pelagic trawling depending on resource characteristics) are not economically viable, do not hold as seen from the substantial profit margin in surimi and pickle trade.

#### 4. Third generation trawlers

As larger deepsea vessels of 57' to 82' OAL develop for the 150m to 200m deep grounds, they should be equipped with appropriate machineries and automation for storage, preservation and industrial production of pickles and surimi on-board for various markets (domestic or export) and tastes (spicy, sweetened or sour).

#### 5. Finfish export

To the extent possible, finfish export must be promoted by diversifying production, product and market. Considering the established markets, the following products and markets may serve the basis for expanding production and diversification of products and markets: (1) the spiny

threadfin bream (*Nemipterus japonicus*) and groupers (*Epinephelus* spp) are considered delicacies by the Chinese in Hong Kong, Singapore and Malaysia; (2) fish fillets, a simple value added product from almost any finfish species of suitable size a few intermuscular bones are in ready demand in most West European markets; the marine catfish, for example, is very ideal for fillets; (3) fillets of priya (*Priacanthus macracanthus*) is a potential delicacy in the US market; (4) fresh chilled red snappers, breams and groupers find ready market in Japan where only limited supplies reach from New Zealand; (5) smoke cured shark meat is a delicacy in most Far Eastern countries; (6) frozen shark meat is a demanded product in both Spain and France; (7) dry fish has excellent prospects in Hong Kong and Singapore, while (8) fish sausage has a flourishing market in the Philippines.

However, sophisticated infrastructure like transshipment facilities, fully refrigerated ships for bulk frozen fish cargos and airshipment of fresh chilled fish cargos should develop to support and sustain export of finfish to various market destinations. Since these infrastructures take time to develop and depend on the country's industrial growth, the immediate focus and thrust in trade in respect of offshore finfish catch should be in terms of value added products like pickles and surimi.

#### 6. Tuna fishing

As far as tuna is concerned, the immediate priority is steady increase in the production of oceanic skipjack from the Laccadives from the present

3000 tonnes to the potential 50,000 tonnes per year by removing the existing bottlenecks regarding the issue of licences to private operators from the mainland, by introducing large number of smaller mechanised pole and liners among the local fishermen and by installing large number of tuna aggregating devices. In the case of *Thunnus* group of tunas, the effort should be on the exploitation of highly productive oceanic southwest coast (Cape Comorin to Goa) and mid east coast (Madras to Visagapatnam) for bigeye and yellowfin by a small fleet of about six longliners on a sustained basis.

## 7. New infrastructure and devices

(i) *Construction of second and third generation trawlers:* Design and construction capabilities of our domestic shipyards in the public and corporate sectors for deepsea fishing vessels have improved considerably in recent years, but there is greater scope for improvement. There are 5 FRP boatyards with a capacity of 50 boats per year and 23 deepsea shipyards with a capacity of 59 vessels per year. With 33% subsidy support in vogue since 1984 for domestically constructed deepsea vessels, over ten yards are now engaged in active construction and vessels complete in all respects are being delivered to owners.

(ii) *Simple alternatives to fishing harbours:* Coasts where harbour facilities do not exist within reasonable distances could be provided with marina type berths which serve as floating quays and landing stages for vessels of upto 67' OAL for bunkering and unloading the catches. Creation of such simple alternatives will accelerate develop-

ment of new generation fishing fleets for finfish production along coasts separated by long distance ports. Such berths, made of armoured bunds and wavebreaks of floating pontoons of timber or concrete, have the advantage of the quay being at a constant level with the deck of the boat, thereby eliminating the unloading problems associated with the rise and fall of tides.

(iii) *Simple alternatives to slipways:* Since slipways for vessel maintenance and repairs are limited only to about 25 localities along the Indian coast, shiplifts or marine hoists could be used as alternatives in localities not served by slipways. Shiplift is a relatively modern development for handling small boats out of water. The civil engineering works for shiplift are simple and inexpensive and it takes less space than a slipway. The marine hoist used in lifting boats outside water, is of comparatively low cost, greater flexibility and particular benefit in localities where tidal range prohibits grids. There are several western manufactures who produce standard models of upto 100 tonnes lift capacity.

(iv) *Package type onboard preservation units:* Since fish taint fast under tropical conditions, package type ice units (for flake ice, plate ice or cube ice) and associated storage bins could be installed with considerable advantage on offshore vessels which remain at sea for a week or two. This could ensure the stipulated quality of raw material for the various products and markets. The domestic engineering industries should be encouraged to take up manufacture of package type ice units and storage bins and all off-

shore fishing vessels encouraged to carry them onboard.

(v) *Fuel saving devices:* Fuel economy on mechanised boats could be effected by fitting specially designed nozzles (9° and 12.5°) and propellers (kalpan type) to wooden trawlers. A nozzle is a ducted propeller arrangement which increases propeller thrust at low vessel speeds for the same engine power. To maintain existing propeller thrust while trawling, lower engine power (i.e., with less fuel consumption) is required. On smaller inshore trawlers, kort nozzle could be put to use either to drag a bigger net for the same fuel consumption, depending on the proportion of trawling to steaming time. This nozzle effects 25% saving on fuel, thus resulting in a gain of Rs.70.00 per trawler per day or Rs.300 million for a fleet of 15,000 trawlers per year.

(vi) *Fish aggregating devices:* Since trawling is fuel intensive, passive fishing gears like traps, lines and gillnets aided by fish aggregating devices (FADs) are found to be very productive. FADs could in fact be tried all along our coast, but areas of high potential include the Laccadives and Andamans archipelagoes, the Gulf of Mannar, the Wadge Bank, the Kalva (perch) ground along the southwest coast at 75 to 115m depths and the Kori Great Bank off the Gujarat coast.

FADs made of concrete rings of about 3' diameter and 1' height or of concrete or stone blocks chained together and stationed on the seafloor off Madras recently have been found to increase catches by traditional boats operating gears around them. Considerable commer-

cial success has recently been reported for Malaysian inshore grounds installed with tyre reefs. These artificial reefs are regularly exploited by the artisanal fishermen using huge metal traps which are periodically lifted to the surface to empty the catches.

Surface to column FADs seem to have great potential in our exclusive economic zone where most demersal stocks are known to undertake diurnal vertical migration for the purpose of oxygen replenishment in their blood pigments and for feeding in the surface during nights, but descending down to the hypoxic depths below 100 m for feeding on the deep scattering layer during daytime. Surface to column FADs within 100 m depth from the surface may serve to arrest such vertical migration and concentrate schools around them, which may be harvested by means of huge metal traps.

(vii) *Fishing diversification:* While trawling for shrimps in inshore grounds would continue to be bottom trawling, offshore trawling has to be essentially high opening bottom trawling (HOBT) or midwater trawling as the mainstay of fisheries in offshore grounds is of finfish stocks and the productivity in HOBT double that of conventional bottom trawling. Therefore, the second and third generation vessels should necessarily adopt these devices in their fishing operations.

Along the Kerala coast, small mechanised trawlers of the 28' to 32' class considered capable of trawling grounds to a depth of 50 m only owing to winch and wrap limitations, have been able to extend their operations upto the 100 m isobath by in-

creasing the size of their winches to carry much longer wraps for deepsea trawling. The extended operations beyond the 50 m isobath are paying rich dividends with significant catches of squids and cuttlefishes. Such diversification is timely and important for the other states as well.

Squid jigging from aboard indigenous sail boats or motorised boats has already emerged as a major fishing activity in localities like Kilakarai where a fleet of over 180 local boats is engaged in this fishery. Squid potential exists almost all along our coast, particularly West Bengal, Orissa, Palk Bay, Gulf of Mannar, Kerala and Maharashtra where jigging should be popularised urgently and production stepped up for the booming export market.

## II. Coastal Aquaculture

### 1. Mariculture

Technically sound and commercially viable hatchery and production technologies have been developed in India in the last decade for various bivalve species, for all of which extremely good markets exist both in India and abroad. However, commercial adoption was virtually absent owing to the failure of the governments in creating the necessary awareness among the prospective users of the technologies, and this in turn is attributable to the woefully inadequate technology transfer programmes.

(i) *Pearl Culture:* The joint venture Tamil Nadu Pearls Limited where the Southern Petrochemical Industries Corporation Limited (SPIC) and the Tamil Nadu Fisheries Development Corporation (TNFDC) were equity partners collapsed not

because of technology, but reportedly because of mismanagement. A measure of the soundness of the technology can be gained from over 80% success in the incidence of pearl formation among the nucleated pearl oysters in the Mandapam farm. Satisfied with the quality of pearls from Tamil Nadu Pearls Limited sold in the Bombay market, the Seths of Bombay have established pearl culture farms in the Gulf of Kutch where millions of spats are airlifted from the Tuticorin hatchery of the Central Marine Fisheries Research Institute (CMFRI).

(ii) *Meat bivalves:* Green mussel, brown mussel, edible oyster and clams are fast growing filter feeders of enormous biomass production capacity per unit time and space (100 to 450 tones per ha per year). Bivalve farming involves absolutely no cost on account of feed and the only major investment is on the creation of appropriate substrata for the animals. In spite of the knowhow for technologically and commercially sound hatchery and growth systems developed by the CMFRI in the last decade, existing and potential markets both in India and overseas for processed or live bivalves for human consumption, growing demand for bivalve meat for the shrimp feed industry and shells for the lime and cement industries, existence of ideal sites amidst the Laccadive and Andaman archipelagoes, the innumerable sheltered estuaries, bays and coves along the entire coastline and the opportunity for subsea farming on submerged rafts, commercial farms are yet to develop in the private or joint sector. That the huge underground intake tunnel of the atomic power

plant at Kalpakkam near Madras gets frequently choked with massive loads of mussels and that the CMFRI has demonstrated commercial farming of mussels at Muthukadu near Madras are evidence of the prospects for commercial mussel farming even along the east coast where mussels do not apparently exist. The potential demand for mussels and other bivalves could be gauged from the fact that the shrimp feed industry alone would require over 75,000 tonnes of bivalve meat for the envisaged 250,000 tonnes of feed per year.

(iii) *Rock lobster*: Lobster farming could be tagged on to coastal mussel and oyster farms as the various types of substrata used in farming are excellent collectors of puerulus (where adult lobsters abound) and these post-larvae to early juvenile lobsters could be tapped for raising market size lobsters on locally landed low value fish bycatch from trawlers. The CMFRI technique of eyestalk ablation coupled with *ad libitum* feeding is very productive and worthy of commercial adoption.

(iv) *Seacucumber*: Seacucumber harvest from the wild and the individual size of wild caught seacucumber are on the decline owing to overexploitation. Modest searching of intertidal and immediately subtidal seas with hatchery raised young seacucumber being undertaken by the CMSRI, Tuticorin, should be stepped up with the active involvement of the governments of Tamil Nadu, Gujarat and Andamans where commercial species of seacucumber occur. Present technical snags in raising hatchery produced

post-larvae to market size in artificial farms need to be overcome.

(v) *Seaweeds*: Coir rope frame cultivation of seaweeds developed by the CMFRI is technically and commercially unsound, and hence, open-sea farming practices evolved by the Japanese for certain species and the estuarine plot cultivation system developed by Peru for certain other species may be adopted, and commercial farms put up to bridge the existing supply gaps and to meet the growing demands.

## 2. Brackishwater shrimp aquaculture

The area of about 50,000 ha of brackishwater cultivated for shrimps at present is essentially under extensive farming where the two critical inputs of seed and feed do not receive much or any attention at all. Sound indigenous hatchery technologies capable of generating sufficient seed for semi-intensive to intensive farming exist in the country and many commercial hatcheries like the TASPAC in Andrapradesh and OSPARC in Orissa could be developed to produce seed for all the one million ha of potential farms. There is, however, much little progress in the development of high grade feed because of prohibitive costs which today's extensive farmers are not in a position to afford, particularly in the absence of any water quality management which is critically important for optimum feed intake and conversion. Subsidised feed could at best be a short term panacea, but in the long run small extensive farmers should be able to upgrade their farms into intensive systems.

Since all the commercial species of *Penaeus* spend their post-larval to early juvenile life in the estuarine ecosystems where salinities generally range from about 5 ppt to 30 ppt and their adult life in the sea where the mean salinity is 35 ppt, ideal farms should provide such progressive increase in salinity from about 5 ppt to 10 ppt for the post-larvae (pl 20) to early juveniles through 10 ppt to 25 ppt for juveniles to early adults to 25 ppt to 35 ppt for the growing adults. But the actual situation in most farms is far from ideal, and as long as this situation persists, semi-intensive to intensive farming is hardly possible. Creation of farms providing optimum salinity conditions is capital intensive and beyond the means of ordinary farmers. Bank loans, now available only for the maiden crop which in most instances results in losses, should be extended on a continuing basis so that the profits could be ploughed back into farm improvements.

Small farmers as well as big farmers and companies capable of substantial capital investment in high grade intensive farms should receive the necessary policy and administrative support in terms of liberal land allocation, import of feedmills and related machineries and feed grade mineral and vitamin mixes and attractants, concessional tariff for power, soft loans, removal of all existing social obstacles and removal of sand bars forming seasonally across river mouths to ensure year round supply of seawater to farms. The present policy irritants in the way of bigger companies in the fray should be removed. In so far as their 1000 ha potential intensive farm at Panaspatha in Orissa, the

TISCO is faced with all kinds of social problems from the local communities and hurdles from the government. Arguments that policy incentives to big companies like TISCO may be at the cost of small farmers are ill conceived, particularly in the context of these companies offering employment at two per family in the area in their farms.

### 3. Brackishwater finfish aquaculture

Among the finfishes, the seabass, pearlspot, grey mullets and milkfish are potential cultivable species for which seed production technologies have not developed in the country owing to lack of any government initiative, except stray instances of success in the case of mullets. The Central Institute of Brackishwater Aquaculture and the Fisheries Colleges in maritimes states should be charged with the responsibility technology development over a specified time frame as the first step towards organised commercial farming.

### III. Institutional Mechanism

It is rather unfortunate that we do not yet have a viable institutional system for fostering local grassroots level marine fisheries development in the capture sector or in the coastal aquaculture sector. The only development institutions are the traditional hierarchies of offices of the Departments of Fisheries and the Fisheries Development Corporations interspersed with establishments of various central research and training organisations with much little co-ordination or linkages between them. Such an institutional system or structure offering wide range of services including research, development and training lacks accountability. It should, therefore,

be supplemented or complemented with independent district development institutions one each for marine capture fisheries, mariculture and brackishwater aquaculture like the Fish Farmer's Development Agencies (FFDAS) which have been successful in bringing about faster growth in the freshwater aquaculture sector all over the country in the last one decade of their existence. Institutions of the FFDA type recommended here for the different sectors of marine fisheries and their envisaged functions are outlined below.

(i) *Marine Capture Fishermen Development Agency (MCFDA)* : The inshore grounds to a depth of 50m are all almost optimally exploited all along the Indian coast except the northeast coast. There is little scope for the small fishermen to increase the catches from the inshore belts, and therefore, the present fishing limits should expand by systematic development of new generations of trawlers of 42' OAL upwards. The proposed MCFDAs that should be fashioned after the FFDAs will undertake the responsibility of systematic development of marine capture fisheries in their respective districts by focussing particular attention on the issues spelt out in section I above.

(ii) *Marine Farmers' Development Agency (MFDA)* : This proposed agency, which should also be fashioned after the FFDAs, shall be charged with the responsibility of systematic mariculture development

of bivalves, lobster, seacucumber and seaweeds on the line indicated in the text above. Its main functions would be identification of suitable mariculture stations for the different candidate species, arranging inputs and capital for the construction of suitable farm substrate, training of farmers, marketing and avoiding conflicts with the capture fishermen.

(iii) *Brackishwater Fish Farmers' Development Agency (BFFDA)* : It is heartening that the government of Tamil Nadu has just announced the establishment of BFFDAs in three of its coastal districts with central assistance. The state has already allocated 2000 acres of brackishwater area out of a total of 15,000 ha to small and medium farmers and a few export houses for shrimp farming while the second instalment of allocation will take place soon. Land allocation and establishment of BFDDAs are taking place actively in most maritime states. The mandate of the BFFDAs is to initiate active efforts towards systematic shrimp farming by providing training, survey and design details, water quality management and soft loans to the farmers in their respective districts.

While the BFFDAs will focus mainly on the needs of the small and medium farmers, large scale shrimp industrial estates, both in the public and private sectors, are also being contemplated for intensive shrimp farming.

## KEY NOTE ADDRESS

# Post Harvest Technology in Relation to Poultry.

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**P**oultry has developed in our country and has been raised to the level of agro - based industry. All facets of poultry husbandry activities involving breeding, feeding, health coverage and management are improved on scientific norms except post - harvest technology.

Although India is producing 22880 million eggs and 400 thousand tonnes of poultry meat per year, technology has not yet developed to retain its quality until it reaches to the consumer.

Post - harvest technology in relation to poultry comprises of all aspects involved to retain quality of egg and meat until it reaches the consumer. They are enumerated with a brief discussion here.

### I. Egg Processing

- (A) Primary Processing
- (B) Further Processing

### II. Poultry Processing

- (A) Primary Processing
- (B) Further Processing

#### (A) Primary processing of egg:

Primary processing refers to the operation carried out for shell egg.

#### Egg collection, Cleaning, Grading and Cooling

\* Collect the eggs 2 or 3 times a day.

Dirty eggs are only subjected for cleaning.

\* Cleaning: (1) Wet cleaning;  
(2) Dry cleaning.

*Cleaning solution:* Contain a detergent and a sanitiser.

Chlorine - 50 ppm

*Dry cleaning:* Sand paper or steel wool.

*Grading:* Grouping of eggs according to weight and quality. Bureau of Indian Standards (B.I.S.) has laid down standards for grading eggs.

\* *Oil coating of eggs:* Light parafin oil (viscosity 50 - 60) containing 0.25% penta chlorophenol.

\* *Cooling:* Egg temperature immediately after laying is 41°C. Cool the eggs to 16°C at farm to retain its quality. Relative Humidity 70 - 80%.

#### *Cooling methods:*

1. Egg cooling room
2. Home made egg cooler (Wooden rack with shelves).

3. Earthen pot.
4. Underground pit.
5. Cold storages.

– 8 to – 10°C & RH 85% – 6 to 8 weeks. – 13°C & RH 75 – 80% – 1 month.

#### — EGG PACKAGING PLANTS —

\* Main areas:

1. For ungraded eggs.
2. Processing area for egg grading and packaging.
3. Grading egg store.
4. Laboratory
5. Packing material store
6. Other accommodation needed (offices, rest rooms, further processing area).

#### Storage and Transportation

\* *Short period* – Refer cooling methods explained above.

\* *Transport* – Eggs are transported from South to North India.

\* Good transport vehicles are needed, because of long distance travels of eggs for about 3000 kms.

\* Closed and refrigerated trucks are necessary.

#### B. Further processing of eggs:

Further processing of eggs refers to removing the contents of eggs from their shells (Melange) and producing various products and products prepared from shell eggs which are ready to eat.

#### Shell Eggs.

1. *Salad Eggs (in two forms):*

I. Hard cooked peeled and chopped frozen egg – fast frozen – shelf life 2 to 3 months.

II. Hard cooked peeled eggs are packed in 1% solution which provides a refrigerated shelf life of five weeks.

2. Long or square hard cooked peeled eggs.

#### 3. *Eggs 'T' GO:*

Hard cooked eggs coated to give them shelf life upto 60 days.

– six egg packs with salt and pepper.

– two eggs packs enclosed.

4. *Poaching:-* Poached in water, milk or tomato juice.

5. *Pickled egg:* i) Vinegar based; and ii) Oil based.

6. *Boiled eggs:* Curry

7. *Fried eggs:* i) Fresh; and ii) Quick frozen.

8. Egg white rings.

9. Egg pakoda.

10. *Omlette:*

i) Fresh

ii) Frozen various sizes with an absorbent of fillings. Quick freezing of these omeletts provides optimum quality.

11. *Scrambled eggs:*

i) Fresh

ii) In a bag are widely used in hospitals. They are supplied frozen with or without milk and salt added. These products contain binders and preservative to prevent weeping or greening. Shelf life is 2 to 3 months.

**Products developed from egg melange:***Basic types:*

- (i) Liquid frozen or dried whole egg.
- (ii) Liquid frozen or dried egg white (albumen)
- (iii) Liquid frozen or dried yolk.

This liquid egg (melange) is then homogenised and filtered to remove any shell and chalazae.

(1) Liquid form (melange) is pasteurised at a temperature not lower than 64.4°C for at least 21/2 minutes and immediately cooled to a temperature below 3.3°C.

(2) Frozen form of melange: It is blast frozen after pasteurisation to -23°C or lower and then can be held at 18°C for long periods with minimum deterioration in quality.

(3) Dried form: It is given a special desugaring treatment in vats before pasteurization which helps to improve its stability and then:

(a) spray dried; and (b) pan dried - white only.

**4. Speciality egg products.**

Scrambled egg mix (dried, frozen and refrigerated).

Freeze - dried scrambled mix.

Frozen egg patties/burgers.

**Egg products at the research stage are:-**

Egg cheddar: Processed in a method similar to milk for making cheese. High sensory appeal and low cost.

— Egg yoghurt: using albumen in place of milk.

— Egg salad mix: in cans.

— Egg sandwich loaf: scrambled egg with soya protein.

— Egg sausage.

— Egg dips.

— High protein cookies: Made with dried albumen.

— Egg crust piza - pre-cooked, frozen piza with albumen crust.

**11. Poultry Processing:**

(a) *Primary Processing*:- It includes transportation of birds to processing plant, holding the birds 8 - 12 hours at the plant by providing water and processing them in a plant. All processing steps shall be carried out in a primary processing plant. Every day, over 8 lakh birds are being processed in the country.

India needs the following category of processing plants:

(1) Automatic poultry processing plants with a capacity to slaughter 4000 birds per hour for cosmopolitan cities.

(2) Semi automatic processing plants with:

(a) Capacity of 1000 birds/hour for district headquarters and cities.

(b) Capacity of 250 birds/hour for taluk headquarters and towns.

(3) Manually operated poultry slaughter houses 500 birds per day - for big villages and a cluster of villages. About 100 mechanical dressing plants may be set up to handle almost half of the output of broilers and culls in the country.

(b) *Further Processing of Poultry Meat*: The trend towards greater consumer acceptance of marinated, coated and other value added products will enhance the demand

**Poultry Products Developed From Poultry Meat**

<b>Whole chicken carcass</b>	<b>Half or quarters</b>	<b>cut - up - parts</b>	<b>Deboned meat MADM OR HDM</b>	<b>Minced meat</b>
Tandoori Barbecue Steamed Cured Smoked	Barbecue	Battered, breaded & friend. Butter Chicken; Ginger Chicken; chicken fry, chicken roast; chicken curry; chicken korma; chilly chicken.	Breast kiev chicken manchoori chicken - 65 chicken top overs chicken ham.	Burgers Patties Cutlets Tikkas Nuggats Kabab Fry meat balls

for poultry meat in urban and cosmopolitan cities in the country.

The range of products currently available and the equipment required to complete the critical stages of dusting, battering, breadding, frying and freezing are listed above.

**Ingredients for processed poultry products.**

The major types of ingredients used are classified below according to their function in the finished product.

1. Cures
2. Yield improves
3. Seasonings

4. Binders

5. Batters

6. Breadding and coaters

7. Speciality products - glazes, rubs, sauces, gravies and stuffings.

In order to develop the poultry products which are ready to eat, further processing plants are needed. Two units are in function and 6 more are under planning in the country.

**TYPES:**

(i) Automatic further processing plants.

(ii) Mini further processing poultry meat unit.

**KEY NOTE ADDRESS****Post Harvest Technology In  
Inland Fisheries**

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**F**isheries has been an important occupation in India and its significance is increasing. At present about 6 million people are engaged in fisheries. India has a vast water body which includes 6,800 km coast line, 27,600 km rivers including their main tributaries, 1,13,000 km canals and irrigation channels, 3 million hectares of reservoirs and lakes and 1.6 million hectares of freshwater ponds. Since large areas of fresh water systems are available, fresh water fish farming and production is of considerable importance. It is also necessary to increase production to bridge demand: supply gap and also to increase income of poor fishermen.

A survey conducted by Indian Institute of Management, Ahmedabad observed an increasing demand for fish in India. The demand for fish is expected to be between 12.5 to 20 million tonnes by 2000 AD (Srivastava, 1984). About half of this is expected to come from marine catch. The balance of 6 to 6.5 million tonnes as per the lowest estimate should come from inland waters. But the share from inland waters during 1987 - 88 is only 1.3 million tonnes (Anon, 1988). So to reduce the gap between demand and supply it is highly necessary to increase the inland water fish production at least 5

times. Aquaculture in an organised way can only increase the production and income of fish farmers.

Though there is considerable demand for fresh water fishes, the fishermen are getting very low price because most of the fish markets are far away from the capture localities. Since fish is highly perishable, it should be marketed immediately or preserved.

Most of the work on preservation has been conducted on marine fishes which are also applicable to fresh water fishes. Recently many research organisations have given importance for preservation of fresh water fishes envisaging the possibility of large scale fish farming and fish production in the near future.

**Iced and chill storage of fresh water fishes:**

The iced storage shelf life of some of the fresh water fishes have been studied and found a shelf life of 13 - 21 days in most cases (Bandhyopadhyay, 1985; Joseph *et al.*, 1988, 1990). The lowest shelf life of 8 - 9 days is observed for murrel (Perigreen *et al.*, 1987) and the longest shelf life of 36 days is observed for mrigal by Nair *et al.*, (1971). All other works on mrigal and major carps in India showed a

shelf life of 13 - 21 days. It has been noticed that size, location, season and spawning condition affect the quality and shelf life of fish. The small fishes have a shorter shelf life than the big ones of the same species. Location and season exert some influence on quality because of the variation in the bacterial population and bacterial flora. The condition of the fish has been found poor soon after spawning. Proper bleeding and gutting soon after catch has been found to enhance iced shelf life by 2 - 4 days.

The bacterial flora of the fresh water fishes are different from marine fishes. The bacterial flora of the fresh water fish *Catla catla* has been found to be 45% *Aeromonas*, 22% *Vibrio*, 22% *Acinetobacter* and 11% Enterobacteriaceae (Gopal *et al.*, 1990). While the oil sardine contains 20% *Pseudomonas*, 18% *Acinetobacter* 12%, *Moraxella*, 28% *Vibrio*, 7% *Micrococcus* and 6% *Flavobacterium* (Surendran *et al.*, 1989). On spoilage 88% of bacterial flora in *Catla* is *Aeromonas* while sardine has 75% *Pseudomonas*. Small amount (6%) of *Lactobacillus* is also found in *Catla*.

Clear difference is noticed in certain biochemical and organoleptic characteristics between fresh water and marine fishes. In marine fishes the spoiled or decayed odour characteristic of amines is noticed at the time of rejection while in the fresh water fish the decayed odour is not prominent. But the fresh water fishes become pasty as well as developed an odour associated with weeds. The volatile bases in marine fishes usually showed a sharp increase during iced storage and usually is about 25 - 30 mg% at the time of spoilage. But in the fresh water

fishes rohu, mrigal and catla the values were 16 - 20 mg% less same at the time of spoilage in melting ice, and a slight increase in volatile bases is observed during chill air storage at 0 - 2°C but it is below 25 mg% at the time of spoilage.

In all the fresh water fishes studied in India, 80 - 90% of the bacterial population is constituted by mesophiles. Of course, the amount of mesophiles decreased considerably during storage and was about 1% of total bacterial count at the time of rejection. When the total count of  $10^6/g$  muscle is attained in marine fishes incipient spoilage might have set in, but in the case of cultured rohu, mrigal and catla, even after the total count has reached  $10^6/g$  the fishes are organoleptically acceptable. Because of these variations in biochemical and bacterial characteristics, it is difficult to assess the shelf life in ice of fresh water fishes by the above parameters.

Detailed investigations were carried out to find the effect of modified atmospheric storage on the shelf life of catla fillets in chill storage (Gopal *et al.*, 1990). Significant extension in shelf life and changes in microflora is noticed on incorporation of different levels of CO<sub>2</sub> on the package of catla fillets. Proportional to the increase of CO<sub>2</sub> inside the package, a decrease in the percentage of *Aeromonas* and an increase in the *Lactobacillus* is observed. The shelf life of catla was 12 days in air, 20 days in 50% CO<sub>2</sub> and 50% O<sub>2</sub> and 28 days in 80% CO<sub>2</sub> on chill at 0 - 2°C. So packing in modified atmosphere is advantageous for marketing fresh water fish in chilled condition.

Irradiation also has been found effective to increase the shelf life of fishes. The shelf life of rohu at 0 - 2°C has been enhanced by about 7 - 10 days by 0.1 M rad irradiation and by 14 days by irradiation at 0.15 M rad (Bhattacharyya). Studies indicated that 98% of the bacteria is destroyed by 0.25M rad radiation and a proportional decrease in the total bacterial count with irradiation dose.

#### Freezing and frozen storage:

Fresh water fishes can be frozen and stored as whole, headed and gutted and fillets. Since large quantities of uniform sized fish can be made available in culture fisheries, adoption of any of the above methods depending on the marketability is economically viable. It is necessary to bleed the fish immediately after capture because it can improve the white colour and quality flesh. Gutting is also advantageous since this removes the major source of bacteria and digestive enzymes. Removal of head can reduce the bulk considerably and can save storage space. Filleting it can further reduce the bulk. Fillets can be handled easily, packed in attractive packets and conveniently frozen and stored.

Different methods of freezing are at present available. Whole and headed & gutted fish can be frozen in immersion freezers using sodium chloride and calcium chloride solutions. The salt absorption is low because of the skin. For individual quick frozen products belt freezers, fluidised bed freezers, spiral freezers and cryogenic freezers can also be used. When fillets are packed as blocks it is necessary to adopt plate freezing. Since most of the fresh

water fishes contain either low or medium fat it can be stored for 8 - 10 months at -20°C in good condition. The juiciness and tenderness of the meat can be retained for more than one year by storing at -30°C or below. Fluctuations in the frozen storage temperature affect the shelf life considerably. As a result, frequent energy failure or poor condition of the storage affect the shelf life of the product.

The development of rancidity is not a major problem in most fresh water fishes. During storage, the juiciness and tenderness of the flesh are lost and this loss is proportional to the storage temperature. The gradual development of tough texture is associated with changes in proteins and reduction in water holding capacity. During prolonged storage the white colour of the muscle fades. The spoilage due to bacteria is not a problem at frozen storage temperatures especially at -18°C or below. Freezing reduces the bacterial load considerably and there is not much variation in the bacterial reduction by adopting any of the modern freezing methods. The initial reduction of bacterial population immediately after freezing can range from only one to two percent up to 90 percent. The subsequent die - off is rapid at first, becoming slower as time passes.

Research work reported from India in the field of canning fresh water fish is scarce. Canning offers a method of processing fresh water fish in different forms and shape into a very stable product. Many fresh water fishes presented a texture problem on canning. An unpredictable over - soft texture is noticed in some fresh water fishes like rohu,

mrigal on canning. Balachandran & Vijayan (1988) found that the texture and other characteristics could be improved by cold blanching the rohu fillets in 15% brine containing 0.25%  $\text{CaCl}_2$  for 10 min. Fish can be packed as fillets or chunks in suitable containers and filling media (Bhattacharyya *et al.*, 1985) like its own juice, tomato sauce, brine, oil or curry medium can be used depending on the market acceptability. Good quality canned fish balls also can be produced from these fishes. It is reported that West Bengal Fisheries Corporation is already marketing some canned products from inland fishes.

#### **Cured, dried and smoked products:**

Drying of fresh water fishes is not very common in India since most of the fishes landed are sold immediately. Dried fresh water fish is not popular in the local markets. Some trials have been conducted on drying small varieties of fresh water fishes with and without brining. A dip for short time in hot brine before drying is advantageous to improve the quality of dried product. Further work is required in the field of drying to ensure a high quality dried product.

Brining and smoking has been reported to yield satisfactory product with good market potential from Africa and North America (Lantz, 1966). Cold smoke treated products have a limited shelf life, approximately the same as fresh fish

due to milk smoke and ambient temperature smoke treatment. The hot smoked product has a measure of preservation but this also has to be stored at low temperatures.

Many value added and speciality products can be prepared from fresh water fishes with suitable modifications in the processing techniques. Fish cutlet, patties, breaded fish fingers, fish balls, fish soup powder, fish wafers, fish sausage, fish pickle etc. are already developed from fresh water fishes.

At present the fresh water fishery is not in an organised sector. Most of the fresh water fishes are landed in remote rural areas where no infrastructure facilities are available for icing, storage and transportation. The ice/chill storage characteristics of most of the fresh water fishes are not screened. Special attention is required on the transportation of these fishes, especially the development of suitable and cheap containers. The changes in physical, chemical and bacteriological characteristics also should be studied to help preservation and storage. To ensure reasonable price for these fishes it is necessary to study the long term preservation techniques for these fishes in different forms and shapes. The development of soft texture of canned fresh water fishes deserved special attention. Research work also should be oriented to develop suitable value added products which satisfy the needs of the consumers.

## Profitability of Yeast Culture in Production Trends of Livestock and poultry

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### ABSTRACT

*Achievement of high yield from potentially high yielding livestock and poultry has its base, measures to ensure maximum feed conversion into absorbable nutrients. Modern technology has perfected yeast strains that act biochemically in a suitable fashion to ensure high production and growth of livestock and poultry.*

*The reports on the utilisation of Yeasacc 1026 and the consequent achievements in growth, milk yield, fat percentage of cattle, egg production and body weight gains in poultry are summarised and presented as an achievement in biotechnology.*

### Introduction

The use of yeast culture has been accepted in modern animal science. Much research work has been carried out in ruminants and less in single stomached animals such as pigs. The dairy's profitability is very much related to utilisation of forage in a better manner and thereby reduce the cost of feeding. Use of YEA-SACC has shown improvement in feed intake with increased milk production, accompanied with increase in fat and protein content. The general health and increase in live weight has been found. At the rumen level an increase of propionate and reduced lactic acid and soluble sugar levels have been found. These changes stabilise the rumen. The time taken for cellulolysis is reduced.

Broiler production in India is increasing by leaps and bounds as broiler meat is lean and processed chicken enables busy office goers to cook good, nutritious food quickly. Microcapsules containing lactic bacteria and yeast cultures have been found to selectively eliminate Salmonella and haemolytic coliforms, making the gut environment stable. The use of microcapsule containing lactic bacteria and yeasts makes the use of costly subtherapeutic levels of prophylactic pharmaceutical agents unnecessary. Some of these therapeutic agents have residual effect on the carcass quality. Use of YEA-SACC has been proved to improve the egg shell quality and hatchability of layers. *Phaffia rhodozyma*, a yeast produces a red coloured primary carotenoid, astaxanthin. This yeast when added to the

feed colours the flesh of Salmon and Trout and egg yolk of poultry.

Among the various firms in the world, Alltech Inc. USA Biotechnology Centre, Kentucky, U.S.A. are not only pioneers in the research work in Biotechnology in Feed Industry but also are leading manufacturers in the field of Animal Health and Nutrition. Recently "Vetcare", the young and one of the fastest growing Veterinary Pharmaceutical companies have joined hands with All tech Inc. U.S.A.

From time immemorial yeast has been used for fermentation like wine making, beer making, yogurt, curd making and bread making. There are 39 genera and 350 species of yeast. The commonest yeast used is *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* is aerobic and facultatively anaerobic. Since the pH of rumen is about 6.5 with low oxygen level, the yeast alters its metabolism. Further different strains have different end products. Yeasts usually excrete lipases, gluconases, proteases, amylases, B-vitamins, fatty acids, chelated minerals and aminoacids.

A yeast culture designed to afford the best, and evolved by Biotechnology techniques has (i) modified enzyme profile (ii) higher pH and temperature tolerance (iii) higher glutamic acid levels (iv) higher chelated mineral content (v) selected on the basis of amino acids (vi) increased Vitamins production (vii) altered lysis properties (viii) selected for stimulatory effect on specific bacteria (ix) selected levels of lactoferin (x) selected for hydrogen, ammonia and methane utilization and (xi) selected for com-

patibility with other micro organisms.

Alltech Inc. U.S.A., using Genetic manipulation techniques evolved a strain YEA-SACC 1026.

Addition of YEA-SACC 1026 has been shown to increase ruminal cellulose digestion and a reduction in loss from cellulose digestion.

YEA-SACC increases ruminal digestion, which brings about improved efficiency in feed utilization and gain. Stimulated feed intake increased availability of nutrients for production.

Increased microbial growth in the rumen, results in improved protein synthesis, increased microbial activities and decreased ammonia levels. Altered metabolic activities in the rumen results in improved efficiency in feed utilization and gain, stabilized digestive processes and increased fat and protein content in milk.

The possible mode of action in ruminal diet is shown in figures 1 & 2.

YEA-SACC brings about better utilization of cellulose. This will enable poor farmers to get better results after feeding their cattle with roughage and less concentrate.

In conclusion, I wish to say, that there should be greater collaboration of University with the trade, for bringing out quick improvement in overall Livestock and Poultry Production.

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Observed Rumen Effect	Animal Performance Effect
Increased ruminal cellulose	Improved efficiency in feed utilization and gain stimulated feed intake Increased nutrients for production.
Increased microbial growth in the rumen	Improved protein synthesis Increased microbial activities Decreased ammonia levels
Altered metabolic activities in the rumen	Improved efficiency in feed utilization and gain Stabilized digestive processes Altered milk composition

Figure -2, Mode of Action: Documented Beneficial Effects of Yea-Sacc<sup>1026</sup>/Bacteria Interaction.

YEA-SACC in ruminant diet interaction

Live yeast in rumen,

Cellulytic bacteria (others ?)

Observed effects of interactions:

1. Increased cellulose digestion Decreased lag time Increased initial rate of digestion
2. Increased concentrations of bacteria
3. Altered metabolic activities

Figure 3. Model for the Effects of Yea-Sacc<sup>1026</sup> in the Rumen.

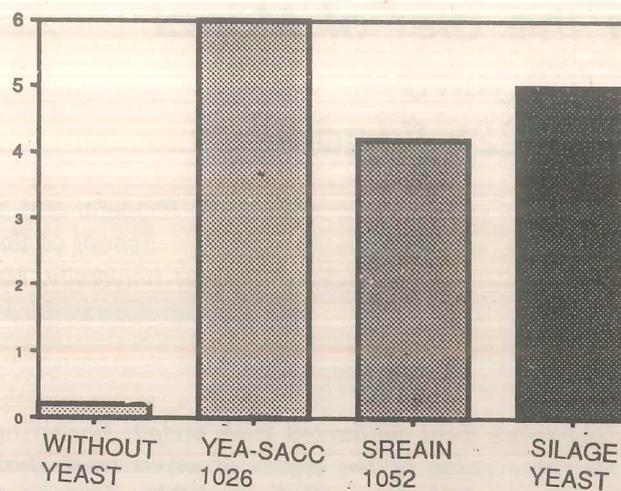


Fig. 1 mg of Cellulose digested by ruminal microbes in 48 hours with or without various strains of yeast

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## Evaluation of leaves of some wild plants as supplementary Source in the diet of Mrigal (*Cirrhinus mrigala*) (Hamilton 1822) fingerlings

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### ABSTRACT

Two feeding trials were conducted with mrigal fingerlings to ascertain the nutritive value of the leaves of seven wild species. Viz. *Amaranthus spinosus*, *Bidens* sp., *Sida cordifolia*, *Clitoria ternatea*, *Passiflora* sp. and *Coccinia cordifolia*. The isoproteinous (30% protein) pelleted feeds were prepared with the leaf powder at 30% incorporation level (W/V) with other ingredients such as groundnut oilcake, fishmeal and rice bran. In the control feed, cabbage leaf powder (30% W/V) was used instead of leaf powders. The biochemical composition and calorific value of the feeds were analysed.

The maximum consumption rate was recorded with *Clitoria* feed ( $32.3 \text{ mg}^{-1} \text{ gm}^{-1} \text{ live wt day}^{-1}$ ) followed by *Passiflora* feed (28.0). The *Polygonum* feed recorded the lowest consumption rate ( $20.7 \text{ mg}^{-1} \text{ gm}^{-1} \text{ live wt day}^{-1}$ ). The fish showed the assimilation rate in the range of  $16.2\text{-}24.75 \text{ mg}^{-1} \text{ gm}^{-1} \text{ live wt day}^{-1}$ . While the maximum production rate was obtained with *Clitoria* feed ( $9.9 \text{ mg}^{-1} \text{ gm}^{-1} \text{ live wt day}^{-1}$ ) the lowest production rate was obtained with *Bidens* feed ( $2.6 \text{ mg}^{-1} \text{ gm}^{-1} \text{ live wt day}^{-1}$ ). The conversion efficiency ranged from 10.87 - 32.5% in the feeds tested. The *Clitoria* feed showed the maximum conversion efficiency. From the results obtained it is concluded that mrigal feed could be prepared with *Clitoria*, *Coccinia*, *Polygonum* and *Sida* replacing the conventional ingredients such as fish meal and groundnut oil cake at 30% (W/V) inclusion level.

### Introduction

In intensive carp culture operations, the cost of artificial feed amounts to nearly 50% of the production cost. It is, therefore highly imperative to formulate low

cost feed without overlooking the nutritional requirements (Bardach *et al.*, 1972; Mac Gralh, 1976). In India, the artificial feed mainly used, is a mixture of Rice bran and groundnut oil cake at a ratio of 1:1. Both of

them have become costly commodities because of their use create a competition between animal husbandry and fish culture. Various types of materials are used as fish feed ingredients in previous years (Lakshmanan *et al.*, 1967; Singh and Bhanot, 1970; Jayachandran and Paulraj, 1977; Venkatesh and Shetty, 1978; Jayaram and Shetty, 1980; Christenson, 1981; Jackson *et al.*, 1982; Law *et al.*, 1983; Devaraj *et al.*, 1986; Hajra *et al.*, 1987).

In the present work an attempt is made to evaluate the nutritive value of the leaves of some wild plants available in Western Ghats.

#### Materials and Methods

**Diets:** Seven wild plants (only leaves) were selected on the basis of their availability and protein content. The shade dried leaves and the other ingredients such as groundnut oil-cake, fish meal, rice bran and wheat were pulverised and passed through 425 micron sieve. The protein content of the materials were analysed (microkjeldhal method). The feed composition is shown in Table 1. All the ingredients were weighed, mixed and the pelleted feed was prepared according to the method adopted by Paulraj and Kutty (1983).

Seven different feeds were prepared with 7 leaves and eighth one was control feed (without the

leaf). The feeds were dried in the oven at 60 – 80°C and stored in the refrigerator. The control feed was prepared with cabbage leaves instead of wild leaves. The feeds were analysed for protein (micro kjeldahl), crude fat (ether extraction), moisture (drying at 105° until get a constant weight), ash (incineration at 600° C for 8 hrs) and energy (bomb calorie meter) content.

**Fish:** The mrigal (*Cirrhinus mrigals*) fingerlings of around 1.000 gm size were obtained from FFDA (Fish Farmers Development Agencies) Madurai. They were acclimatized in the laboratory condition for 15 days. During this period they were fed with control feed.

**Experiment:** Feeding trials were conducted by stocking 10 fish in each plastic trough (945cm x 35cm x 15 cm) having 10 l capacity. Prior to stocking they were starved for one day and weighed individually. Another batch of five were killed and protein content was analysed. The experiment was conducted for 60 days. During the experimental period the animals were fed *ad libitum* at 9 a.m. & at 3 p.m. The feed was given in surplus and after one hour the excess feed collected dried and weighed and the actual consumption calculated. Faecal matter was collected every morning by simple siphoning dried and stored for fur-

Table 1 Feed Composition

No.	Component	Percentage (wt/wt)
1	Leaf powder	30
2	Fish meal	20
3	Groundnut oil cake	10
4	Rice bran	20
5	Wheat flour	20

Table 2 Protein content and calorific value of different leaves

No.	Species	Protein (%)	Energy value (j/g)
1	<i>Amaranthus spinosus</i>	27.2	11398.2
2	<i>Bidens</i> sp	26.9	11471.9
3	<i>Polygonum</i> sp	26.3	11549.8
4	<i>Sida rhombifolia</i>	25.9	12229.9
5	<i>Clitoria ternatea</i>	31.9	12493.7
6	<i>Coccidia cordifolia</i>	29.2	11191.8

Table 3 Biochemical Composition of the Feeds

(All the values are expressed in percentage)

Feed Name	feed No	Protein	crude	moisture	Ash	j/g
Amaranthus feed	1	30.1	7.3	8.8	7.3	13152.0
Bidens feed	2	29.1	10.1	8.5	16.2	13198.5
Polygonum feed	3	29.0	8.4	10.2	12.0	13402.4
Sida feed	4	30.0	7.0	9.1	14.0	12803.6
Clitoria feed	5	29.5	10.0	11.3	13.6	13381.5
Passiflora feed	6	28.5	8.0	8.9	12.5	12948.1
Coccidia feed	7	29.1	9.1	9.9	14.3	13031.8
Control feed	8	29.8	9.9	6.0	8.6	13240.8

## Energy value

ther analysis. 75% of the water was changed daily with least disturbance to the fish. At the end of the experiment the animals were weighed and 5 from each trough were sacrificed and stored for analysis.

The growth parameters were worked out by using the following formulae.

Initial average weight	- $W_1$
Final average weight	- $W_2$
Average weight gain	- $W_2 - W_1$
Average daily weight gain	- $W_2 - W_1 / \text{number of days of the experiment}$

Consumption rate	- Consumption/gm live wt./day
Assimilation rate	- Assimilation/gm live wt/day
Metabolic rate	- Metabolism/gm live wt/day
Gross conversion efficiency	- $P/C \times 100$
Net conversion efficiency	- $P/A \times 100$
Protein efficiency ratio	- gm weight gain/gm protein consumed

## Results and Discussion:

The protein content and calorific value of the different leaves are presented in Table 2. Maximum protein content 31.9% was seen in

Table 4 Growth Characteristics of the Mrigal Fingerlings Fed with 8 Different Diets

Parameters studied	Amar-anthus feed	Bidens feed	Polygonum feed	Sida feed	Clitoria feed	Pas-siflora feed	Coccidia feed	Control feed
Initial average weight (mg)	1009.6	1050.1	1010.8	1127.4	1000.3	954.4	1115.4	1133.2
Final average weight (mg)	1089.0	1129.0	1142.5	1260.5	1298.5	1063.0	1219.5	1227.5
Average weight gain (mg)	79.4	78.9	131.8	133.1	298.2	108.7	104.2	94.3
Average daily weight gain (mg)	2.7	2.6	4.4	4.4	9.9	3.6	3.5	3.1
Consumption rate (mg/gm live wt/day)	22.7	23.0	20.7	23.7	32.3	28.0	22.7	23.2
Assimilation rate (mg/gm live wt/day)	17.7	16.8	16.3	18.5	24.8	22.4	16.2	17.4
Metabolic rate (mg/gm live wt/day)	15.1	14.3	12.0	14.5	14.3	18.5	11.8	14.6
Gross conversion efficiency (%)	11.5	10.9	21.0	16.9	32.5	13.8	19.4	12.1
Net conversion efficiency (%)	14.7	14.9	26.7	21.6	42.4	17.2	27.2	16.1
Specific Growth Rate (SGR) (%)	0.1	0.1	0.2	0.2	0.4	0.2	0.1	0.1
PER	0.38	0.37	0.72	0.56	1.10	0.48	0.67	0.41

Specific Growth Rate (SGR) =  $100 (\log_e \text{ final wt} - \log_e \text{ initial wt}) / \text{No. of days of the experiment}$ .

PER - Protein Efficiency Ratio (gm weight gain/gm protein consumed)

*Clitoria ternatae* leaves. The other leaves have 26.3 - 29.2% protein. The calorific value ranged between 11398.2 - 12493.7 J/g. The biochemical composition of different feeds is shown in Table 3. The protein content of all the feeds is around 30%

(isoproteinous). The fat content is higher in the case Clitoria and Polygonum feed (10.1%). The ash content was maximum (16.2%) in Bidens feed. The calorific value of Polygonum (13402.4 J/g) and Clitoria feeds were higher (13381.5 J/g).

The experimental results of the feeding trial are indicated in Table 4. Among the test and control feeds, the highest daily weight gain is found in Clitoria feed (9.9 mg/g live wt./day). Polygonum and Sida feeds shows 4.4 mg/g/day as the production. The weight gain in control feed is 3.1 mg/g live wt/day. Passiflora, Amaranthus and Bidens contribute to the weight gain as 3.6, 2.7 and 2.6 mg/g live wt/day.

The consumption rate ranges between 20.7 to 32.3 mg/g live wt/day. The maximum consumption rate is found with fishes fed with Clitoria feed. The Polygonum feed showed the minimum consumption rate. This may be because of the non palatability of the feed by the fish. The Passiflora feed showed 28 mg/g live wt/day as the consumption rate.

The assimilation rate of mrigal ranged between 16.3 – 24.8 mg/g live wt/day. The maximum assimilation rate was obtained with the Clitoria feed. This result is similar with that of rohu fingerlings fed with *Amaranthus spinosus* leaf with fresh water mussel (25.9 mg/g live wt/day) and *A. spinosus* with frog thigh muscle (25.0 mg/g live wt/day). (Haniffa *et al.*, 1987). Passiflora showed 22.4 Sida 18.5 Polygonum 16.3, Amaranthus 17.7, Bidens 16.8, Coccidia 16.2, and control 17.4 mg/g live wt/day as the assimilation rates.

The metabolic rate varied from 11.82 – 18.5 mg/g live wt/day. The maximum metabolic rate is shown by the fishes fed with Passiflora diet (18.5 mg/g live wt/day). Previous workers attributed high metabolic demand as the reason for the negative conversion of the grass carp *Ctenopharyngodon idella* fed on goat

liver (Haniffa & Venkatachalam, 1980) and for the poor conversion of the freshwater snail *Pila global* fed on 100% plant food (Haniffa *et al.*, 1984). In mrigal also the fishes fed with Passiflora shows lesser growth rate (3.6 mg/g live wt/day) and conversion efficiency (13.8%) than the other feeds due to its higher metabolic rate. Clitoria & Bidens feeds shows 14.3, Sida 14.5, Amaranthus 15.1, Polygonum 12.0, Coccidia 11.8 and control 14.6 mg/g live wt/day as the metabolic rate.

The gross conversion efficiency (P/C x 100) ranged between 10.9 – 32.5%. The maximum efficiency is shown by the fishes fed with Clitoria feed (32.5%) and minimum efficiency in Bidens feed fed fishes. The control fish showed 12.1% conversion efficiency.

The Net conversion efficiency (P/A x 100) is maximum in mrigal fed with Clitoria feed (42.4%) and minimum with Amaranthus feed (14.7%). The control feed showed 16.1% as the conversion efficiency.

The specific growth rate (SGR) of the mrigal fingerlings fed with Clitoria feed (0.4) which is close to the value obtained with 100% soyabean meal (Jackson *et al.*, 1982) for Tilapia. All the other feeds show lesser SGR (0.1 – 0.2). The protein efficiency ratio (PER) is fairly good with Clitoria feed (1.1) which is much greater than the values obtained with *Cyprinus carpio* fed with 33 – 53% protein diets. (AL AsGah & Bedawi, 1984). This can be attributed to its excellent amino acid profile as well as their availability. The next best PER values are found with Polygonum feed (0.72) and Coccidia feed (0.6). The control feed

shows 0.41 as the PER value. The lowest PER value is found with Bidens (0.37) & Amaranths feed (0.38).

### Conclusion

From the results obtained it can be concluded that *Clitoria ternatea* leaf powder at 30% (W/V) inclusion level in the diet of (*Cirrhinus mrigala*) shows best production. By

improving the palatability the consumption of Polygonum feed can be increased and it will become one of the best protein source for mrigal. The reason for the higher metabolic rate in the Passiflora feed fed fishes is under investigation and if the cause is rectified it is foolproof that *Passiflora* will be a best feed for *Cirrhinus mrigala*.

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## Status of Shrimp Feeds : Formulation, Manufacture and Marketing

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### Abstract

*Nutrition is the backbone of the shrimp culture system. The major bottleneck in this area is the understanding of the nutritional requirement of a given species and formulating a balanced diet. Several commercial feeds are available in the market with the FCR ranging from 1.5:1 to over 2.6:1. Among the ingredients tested, there is a great diversity in the source of proteins, fat, vitamins etc., coupled with the divergent requirements of these components by various culturable shrimps. In general 40% protein and 10% fat were found to be satisfactory. The shrimp meat and molluscan meat were found to be more suitable than other sources for their amino acid and lipid profile. The role of growth promotors, attractants and binders in different shrimp feeds is also discussed.*

**S**hrimp nutrition, together with seed production and pond care, determines largely the viability of the shrimp farming industry. Although shrimp farming in India has developed rapidly during recent years, precise knowledge of the nutritional requirements of many of the local species of shrimps is limited. With the steady increase in the area under shrimp farming, the question of feed formulation, manufacture and marketing assumes greater significance.

Shrimp feed manufacturing industry is in existence in India for quite some time, but only very few manufacturers produce commercial quantities of shrimp feed. A recent survey of the animal feed manufacturing industry reveals that only few

companies are interested in commercial production of shrimp feed.

The general practice currently in vogue among shrimp farmers is to feed chopped or minced trash fish or pelleted feed prepared by them using local ingredients. Imported pelleted feeds which are quite expensive have been tried by a few farmers only. It is, therefore, imperative to develop suitable indigenous feeds to increase shrimp production per unit area in our farms.

### Feed Ingredients

The main objective in the preparation of fabricated feed is to combine several selected ingredients proportionately according to a formula in order to obtain the required amount of protein and various other nutrients. The feed ingredients (both

Table 1: Proximate analysis of feed ingredients

Feed stuff	% Dry matter	% Dry matter			
		C.Protein	C.Fat	C.Fibre	Ash
Bone Meal	96.3	7.4	3.4	8.8	84.8
Blood meals	87.1	87.4	1.3	1.2	4.3
Corn meal	86.5	9.5	4.0	4.0	1.5
Copra cake	91.6	20.3	11.4	16.2	6.2
Groundnut cake	91.7	46.6	7.7	6.5	7.7
Fish meal	90.7	54.7	5.3	4.1	29.9
Leaf meal	90.0	19.5	5.0	21.5	8.5
Palm oil	90.0	-	95.0	4.0	1.0
Palm kernel cake	91.1	12.2	4.9	25.6	2.6
Rice bran	89.9	12.6	11.3	19.3	10.2
Shrimp head meal	84.4	28.3	1.1	7.1	31.6
Sesame cake meal	91.7	41.9	9.2	6.18	14.8
Soy sauce refuse	88.0	13.5	8.2	5.80	5.3
Soy bean refust (fresh)	14.1	39.0	5.0	11.4	3.6
Soyabean meal	84.8	47.5	6.4	5.1	6.1
Wheat flour	87.7	18.1	94.3	9.4	4.0
Yeast brewary	90.3	47.1	0.3	6.6	5.3
Mussel meat	-	55 - 60	8 - 15	-	14 - 20
Prawn head	-	40 - 44	3 - 4	-	30 - 34;
Small tiny prawns	-	60 - 65	4 - 8	-	18 - 22
Squilla	-	40 - 45	2 - 4	-	36 - 40
Clam meat	-	38 - 42	12 - 14	-	36 - 44

animal or plant origin) include not only conventional materials (Table 1) but also unconventional products such as the wastes obtained from food processing plants.

The selection of ingredients is governed by three main factors: quality, price and availability. Considering these three factors together, soyabean waste or powder appears to

Table 2: Shrimp feed, manufacturing companies in India.

Name of firms Manufacturer feed	Protein	FCR	Cost
The Tata oil Mills Company TOMCO Ltd., extensive <i>P.indicus</i> intensive <i>P.monodon</i>	Premium feed; Crude protein-30-32% lipid 6-8% Fibre 3-5% High growth feed; Crude protein-35-38% lipid 8-10% Fibre 3-5%	3:1  3 mm Pellets 2:1	Rs.6.25/kg  Rs. 9.00/kg
M/s Mysore Snack foods Bangalore	Not available	(1.38)* 4.5:1**	-
M/s Vetcare Bangalore	upto 40%		-
M/s HIM feeds	Not available	2.12:1	Rs.12.00/kg
M/s Ruminant feeds Salem.	Protein - 14% Protein - 30%	2.13:1	Rs.2.25/kg Rs.5.25/kg
M/s Lakshmi Agro Products Vijayawada	Not available	5:1	Rs. 4.50/kg Rs. 6.0/kg
M/s Intersea Export Corporation Madras	Protein - 40%		-
M/s MA Laxmi Fertilizers Calcutta	-	-	-
M/s Omega feeds and Nutrients, Madras	Details not available		
M/s Lakshmi Feeds and Exports Pvt.ltd. Bombay	-	-	-
New Tirumala Giri Treaders, Gudivada, A.P. Hindustan Lever			

\* under laboratory trial      \*\* under farm trials

be one of the important protein sources (Table 1). There are a number of soyabean based industries in India, particularly in Tamil Nadu (M/s. Sakthi Soya at Udumalaipet produces large quantities of soyabean meal). The price of ingredient materials should necessarily be within the limits of economic

feasibility of the food manufacturing industry.

Animal protein is very important in shrimp diet. The principal sources of animal protein include shrimp meal, shrimp head meal, squid & cuttlefish waste mussel meat, clam meat and fish meal. Among these various animal ingredients, proteins

**Table 3: The companies in the process of setting up prawn feed manufacturing plants**

- Tayo Mataya Feeds, Calcutta
- Higershimares feeds (pvt) Ltd., Cochin
- George Maijo, Madras
- Hindustan Lever Ltd., Bombay  
(as its Sanderkali farm)

from invertebrate source (shrimps squids, cuttlefishes, mussels and clams) have been found to be more nutritive to shrimps than fish meal as the amino acid profile of these invertebrates especially the crustaceans is more compatible with that of penaeid prawns.

The quality of ingredients is important as it determines the quality of feed; however, price of raw materials has to be necessarily taken into consideration in ultimately deciding and prioritising the ingredients.

#### **Present status of feed manufacture**

If the price of the ingredients, and therefore, of the feed is too high, commercialisation of the production process might face initial setbacks, which, however, would minimise as intensive shrimp culture systems develop. At present, there are twelve Indian companies manufacturing shrimp feed at semicommercial scales (Table 2). Composition details of these feeds are not available. The crude protein in these feeds ranges from 30 to 40% (which is about the optimum) while their FCR varies from 1.38 to 5.0. These test results are preliminary, and hence, all commercial feeds need intensive testing under different farm conditions for determining their acceptability,

palatability and food conversion. Based on the information furnished by the companies themselves, Tomco feed (high protein feed) and HIM feed seem to yield the highest FCR values of 2:1 and 2.12:1 respectively under farm conditions. When tested in the laboratory using white prawns, the Mysore feed indicated a much better FCR value of 1.38:1, while in one farm at Tuticorin it resulted in FCR value of 5:1 for *P. indicus*. However M/s. George Maijo found the Mysore feed to be of extremely poor quality in terms of both FCR and stability in their Guntur shrimp farm. The feed marketed by M/s. Ruminant Feeds, Salem, is relatively cheap, but yields very poor FCR values owing to very poor protein content (14%). Among the available feeds, the one from M/s. Intersea Export Corporation, Madras, is of high protein (40%) followed by that from TOMCO (35 to 38%), but the cost of these feeds is over Rs.9/Kg. Besides the established companies like the Tatas (TOMCO feed), HIM, Mysore Snack Foods and M/s. Ruminant Feed, over half a dozen new companies including M/s. Hindustan Levers Limited and their Associates, Lipton, M/s. George Maijo and M/s. Vetcare are nearly poised to entering into large scale manufacture of shrimp feeds (Table 3).

As indicated by Paulraj<sup>1</sup> in his status paper, the feeds produced by TOMCO (Madras), Vetcare (Bangalore) and Mysore Snack Foods (Salem) are characterised by better nutritional profiles, and hence, these feeds could be considered for semi-intensive to intensive culture systems. However, intensive trials should be undertaken to test their performance under various farm conditions for different species of penaeid prawns before they are popularised. M/s. Vetcare is intending to produce shrimp feeds incorporated with antibiotics, which could be invaluable for intensive farming where stocking densities range from 200,000 to 600,000 post-larvae per hs.

As indicated by many of the feed manufacturers, poor market demand seems to be the major bottleneck to accelerate feed production to commercial heights. Shortage of good quality raw material such as shrimp meal, shrimp head meal, squid & cuttle fish waste and fish meal is yet another constraint to sustained production of feed. The farmers are of the view that the market price of feeds is too high to sustain wholly compounded feed based operations. It is, therefore, urgent to develop low cost, but efficient, feeds to accelerate and sustain the growth of the shrimp culture industry. As indicated by Paulraj, in some states like Orissa, the feed cost escalates because of sales tax and octroi. The MPEDA may take up such issues with the concerned governments to resolve them as quickly as possible.

1 In: Recommendations of the first meeting of the sub-committee on feeds, Department of Biotechnology, Government of India.

The next important issue in the manufacture of shrimp feed is the shortage of proteinous raw materials from the sea. Besides developing an organised system for the procurement of shrimp and squid wastes from processing plants for reduction into meal, large scale culture of clams and mussels may solve the problem of shortage of raw material to a great extent. The low cost mussel and clam culture technologies developed indigenously (by the CMFRI) would help bring down the production cost to be well within the reach of shrimp feed manufacturers.

### Feed Formulation

The main objective of feed formulation is to supply shrimp crops with nutritive feeds which yield optimum production. Only sporadic works have been carried out to assess the nutritional requirements of Indian prawns; precise knowledge on nutritional requirements is lacking for most cultivated species.

Formulation of feed is mainly based on the protein and energy levels required in the diet. Since protein nutrition is essentially amino acid nutrition, protein supplements should be so chosen so that they satisfy the essential amino acid requirements. Protein ingredients should be combined in such a way that each complements the amino acid profile of the other. One ingredient should not substitute for the other just because the level of protein is similar; the amino acid profile of each might be quite different. Details of essential amino

Table 4: FAO Growout feed formula for *Penaeus monodon*

Ingredients	in the diet	Crude Protein	FCR
Shrimp head meal	20		
Fish meal	30		
Soyabean meal	10		
Groundnut Cake	5		
Copra cake meal	10	40%	1.54
Corn meal	5		
Rice bran	12.5		
Wheat flour	7.0		
Basfin	0.5		

Formula for Starter mash for *P.monodon*

Shrimp head meal	30		
Fish meal	40		
Soyabean meal	10		
Rice bran	10	40%	1.40
Corn meal	5		
Corn oil	4		
Vit. mixture	1		

Table 5 Composition of Vitamin mix - Kanazawa, 1982

Vitamins	mg/kg of dry diet
Thiamic-Hcl	120.0
Riboflavin	40.0
Pyridoxine - Hcl	120.0
Nicotinic acid	150.0
Ca - Pantothenate	100.0
Folic acid	5.0
Biotin	1.0
Cyano coblamine	0.02
Inosital	4000
Choline chloride	1200
Na-Ascorbate	5000
Vitamin - A	5000 (IU)
Vitamin - D	1000 (IU)
Tocopherol	200
Menadione	40

acids required are furnished by Paulraj in his status paper. Vitamin en-

riched feeds are known to yield much better results (Tables 4 & 5).

### Major Issues and Strategies

1. There is no co-ordination or communication among the Indian shrimp feed manufacturers regarding formulations, ingredients, production rate, price and marketing. The MPEDA may initiate steps to bring these firms together to evolve guidelines for resolving the various issues (such as those listed above) constraining commercial production of feeds.

2. Though different formulations have been developed and adopted by different shrimp feed manufacturing companies, no details are available about feed composition and efficiency, necessary for identifying R & D needs of the feed industry. The MPEDA could consider subsidising the cost of feeds for testing in commercial farms in order to ascertain their conversion efficiencies.

3 The commercial feeds marketed do not have uniform levels of nutrient, particularly protein, which largely decides the growth and production of shrimps. Optimum levels of nutrient must be ensured in feeds specifically meant for semi-intensive to intensive culture systems.

4. In the case of the best feeds in the market, the MPEDA may assist in organising a marketing network for promoting market for such feeds.

5. The price of raw materials for feed, especially of smaller sergested shrimps, prawn head squid wastes, should be within reasonable limits. The northwest coast of India comprising the states of Gujarat and Maharashtra land annually about

100,000 tonnes of nonpenaeid prawns (mostly the smaller sergestids and pelaemonids) sold mostly for human consumption. Through organised procurement, a good deal of this prime raw material could be channelled for shrimp feed. A procurement network linking all sea food processing plants and the feed manufactures, is very essential for the supply of shrimp squid cuttlefish wastes. The MPEDA could assist the industry in organising such a network.

6. The cost of feed increases due to increase in sales tax, other taxes and octroi imposed by state governments. The concerned governments should be urged through organisations like the MPEDA to waive all kinds of taxes levied on shrimp feed as in the case of agricultural commodities.

7. Most shrimp farmers produce their own feeds with locally available low cost ingredients. Most of such improvised feeds are cheap, but are of poor nutritive value. Although they give low shrimp yields per unit area, they are rather cheap and hence, the profit margin is quite high. Demonstration farms may be set up in different locations of shrimp culture importance for the purpose of testing the performance of commercial feeds. Alternatively, the manufacturers may initially subsidise the feed cost for testing by farmers themselves in their own holdings.

8. Research and development on shrimp feed may be strengthened to bridge the gaps in our knowledge of nutrition, formulation, pelletizing, manufacture and sales.

## Karyological Studies on Toda Buffaloes in Comparison with Murrah and their Crosses

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### ABSTRACT:

Chromosomal banding techniques viz., Quinacrine mustard staining (Q.bands), Trypsin Giemsa staining (G.bands) and constitutive hetero-chromatin banding (C.bands) were applied to characterize the chromosomes of Toda buffaloes of Niligiri hills of Tamil Nadu. Fourteen males and 19 females were utilised for karyological studies. Their diploid chromosome number was found to be fifty ( $2n=50$ ) similar to that of river type. G-and Q-banding pattern permit unequivocal identification of individual chromosomes and the chromosome pairs were numbered 1-24 with description of X and Y in the karyotype. Centromeric or C-banding of Toda buffalo chromosomes revealed polymorphism in staining intensity of kinetochores of all acrocentric chromosomes. The largest acrocentric chromosome with intense C-band was identified as X - Chromosome and the Y - Chromosome was the smaller acrocentric without a C-band. Based on G and Q-banding patterns an idiogram of Toda buffalo was constructed.

### Introduction:

Among Indian breeds of buffaloes, Toda buffaloes are a genetically isolated group of animals found in and around Ootacomund, in the Nilagiri district of Tamil Nadu. These buffaloes are maintained by Toda, the tribal people of that locality for milk and milk by products. Their origin, habitat and

husbandry practices followed by the tribal owners have recently been reported (Nair *et al.*, 1986). Though they resemble swamp buffaloes in external appearance and habitat their diploid chromosome number was found to be fifty ( $2n=50$ ), similar to that of river buffaloes (Sethumadavan and Thiagarajan 1986; Nair *et al.*, 1986). However, detailed

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karyological features of Toda buffalo chromosomes are not available for comparative assessment with other breeds of buffaloes. Hence, an attempt has been made to characterize the chromosomes of Toda buffaloes by adopting Q, G and C banding techniques and described in comparison with Murrah and crossbreds.

#### Materials and Methods:

Ten ml. of heparinised blood was aseptically collected from Toda buffaloes comprising of 14 males and 19 females and transported to cytogenetics laboratory for karyological studies. Leucocyte culture technique of Bongso and Hilmi (1982) was adopted to display the metaphase chromosomes of buffalo.

Q bands were produced by staining the cytological preparations with 0.5% quinacrine dihydrochloride in phosphate buffer, (p<sup>H</sup> 6.8) for 20 min. as described by Caspersson *et al.* (1971), rinsed and mounted in the same buffer for examination under Carl Zeiss Fluorescent microscope.

The G-banding procedure adopted was a combination of the techniques of Sumner *et al.* (1971) and Seabright (1971). Slides were immersed in 2 x SSC solution for 60 min. at 60°C, rinsed with 0.9% NaCl and flooded in a 0.25% trypsin (Difco) in saline solution for 45 sec. Each slide was rinsed with saline, air-dried and stained in 10% Giemsa in phosphate buffer for 10 min.

The C-banding technique used for staining heterochromatin was that of Sumner (1972) with slight modification. The slides were treated with 0.2 N hydrochloric acid for 1h at room temp., rinsed with deionised water and placed in 1% aqueous solu-

tion of barium hydroxide at 50°C for 5-15 min. After thorough rinsing with several changes of deionised water, the slides were incubated for 1h. at 60°C in 2 x SSC, rinsed briefly with deionised water and stained for 90 min. in 4% Giemsa phosphate buffer. Finally the slides were again rinsed, dried and mounted in DPX.

Thirty good quality complete metaphase plates selected from each animal were photographed using Zeiss photomicroscope and karyotypes were prepared.

#### Results:

The diploid chromosome number was found to be fifty (2n=50) in Toda buffaloes, Murrah and crossbreds, thus confirming the earlier reports. (Chandra, 1968; Fisher and Ulbrich, 1968; Sethumadavan, 1978; Chakrabarthi and Benjamin, 1980; Nair *et al.*, 1986). The morphology of Toda buffalo chromosomes resemble that of the chromosomes of Murrah.

The karyotype presented in Fig.1 show the Q-banded Chromosomes of Toda buffalo male. A comparison was made based on the intensity of fluorescence and banding patterns. There were no observable differences either in the intensity of fluorescence or banding patterns among Toda, Murrah and crossbreds. The bright fluorescence observed on chromosomes 1, 2, 9, 10, 20 and 22 were found to be present in all the breeds. The X-chromosome in both sexes showed, a prominent kinetochore, with dull fluorescences in all the breeds studied.

The G-banded karyotype of Toda buffalo Male is presented in Fig.2. The karyotype was prepared in accordance with Reading Conference, 1976 (Ford *et al.*, 1980) and as described by Bongso and Hilmi

(1982). There were no observable differences between breeds in G-banding of chromosomes. On comparison the bright Q-bands corresponded well with the dark G-bands in all the chromosomes in Toda, Murrah and crosses. The bright fluorescence on the chromosomes 1, 2, 9, 10, 20 and 22 were seen intensely stained on G-banding.

The C-banding karyotype of Male Toda buffalo is presented in Fig.3. The chromosomes were arranged in the karyotype as demonstrated by Bongso and Hilmi (1982). All the acrocentric autosomes showed prominent dark centromeric bands while the centromeres of the sub-metacentric chromosomes were not deeply stained. The centromere of the one of the smaller acrocentric chromosomes in the male did not band. As it had no homologue, it was identified as the Y-chromosome. The X-chromosome, the largest acrocentric has the most distinct band on the heterochromatic region. In the females both X-chromosomes showed distinct C-bands. The C-banding technique thus helped to identify the sex chromosomes of buffaloes.

Pair(s)	Class	Description
1 to 5	Sub-metacentric autosomes	Small and very feeble C-band
6	acrocentric autosome	Large C-band
7		Medium C-band with telomeric bands
8		Large C-band
9 and 10		Medium C-band

11 - 13		Medium or small C-band
14 and 15		Medium C-band
16 to 18		Medium or small C-band
19		Small C-band
20		Medium C-band
21 to 23		Medium or small C-band
24		Small C-band
X	Sex Chromosome	Largest acrocentric, large and intensely stained C-band
Y	Sex Chromosome	Small acrocentric without a C-band

An idiogram of Toda buffalo was constructed based on Q and G-banding patterns to represent diagrammatically the position of the bands on the chromosomes. Actual measurement of the banded chromosomes were taken into account to construct the idiogram. The system adopted to number the regions was based on Toll and Halnan (1976) and ISCN (1978).

#### Discussion:

The Toda buffaloes of Nilagiri resemble the swamp buffaloes in their phenotypic appearance, behaviour and habitat. However, the normal diploid chromosome number was found to be fifty similar to that of river type. The classification of the Toda, as a south Indian river breed based on horn conformation (Cockrill, 1981) was now confirmed with the determination of the diploid number of

chromosomes (Sethumadavan and Thiagarajan, 1986; Nair *et al.*, 1976). It is probably that the Toda buffaloes are an isolated group of Indian river type animals and in course of time they would have got adapted to the marshy environment of Ootacamund and thus acquired the characters of swamp buffaloes with the retention of dairy characters.

Comparison of Q and G-banded karyotypes of Toda, Murrah and crossbreds revealed an identical banding pattern within the breeds. No discernible variations in Q-and G-banding pattern was encountered between the breeds. The general similarity of Q-and G-banding suggested that they represent the same underlying structures as suggested by Evans *et al.*, (1973).

The dense C-band on the acrocentrics excluding the Y chromosome suggested that heterochromatin was more resistant to denaturation by alkali treatment (Comings *et al.*, 1973). The description of the buffalo chromosome based on the features of the C-bands of the respective groups in the karyotype adopting the clas-

sification of swine chromosomes by Hansen (1982) has been attempted which was not hitherto taken. The evaluation of the length of the C-band in this classification as large, medium and small are relative. The occurrence of banding polymorphism with reference to the C-bands in the karyotype of buffaloes could be substantiated by this classification.

The Characterization of chromosomes of Toda buffaloes with particular reference to the disposition of the bands necessitated the construction of Idiogram for this breed. Their Chromosomal compatibility and banding homologies with river buffaloes revealed that the productivity of this unique breed could be improved by introducing superior germplasm such as Murrah.

#### Acknowledgement

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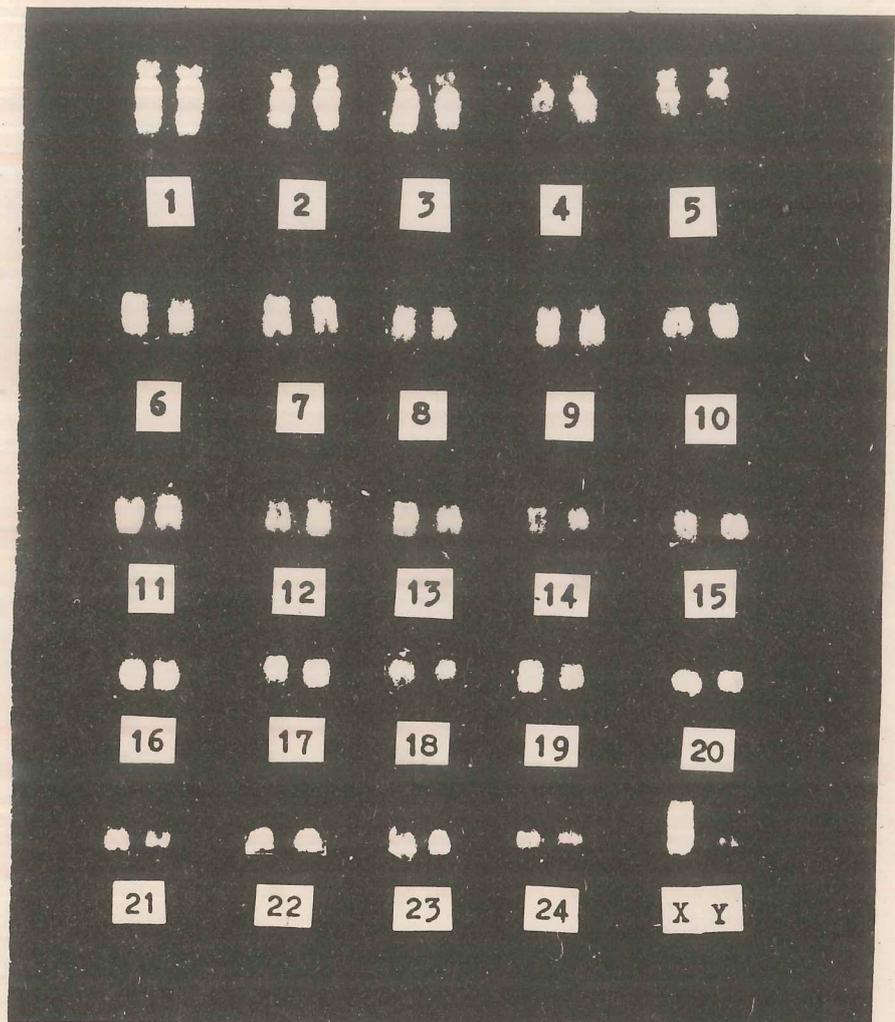


Fig. 1 Q-banded Chromosomes

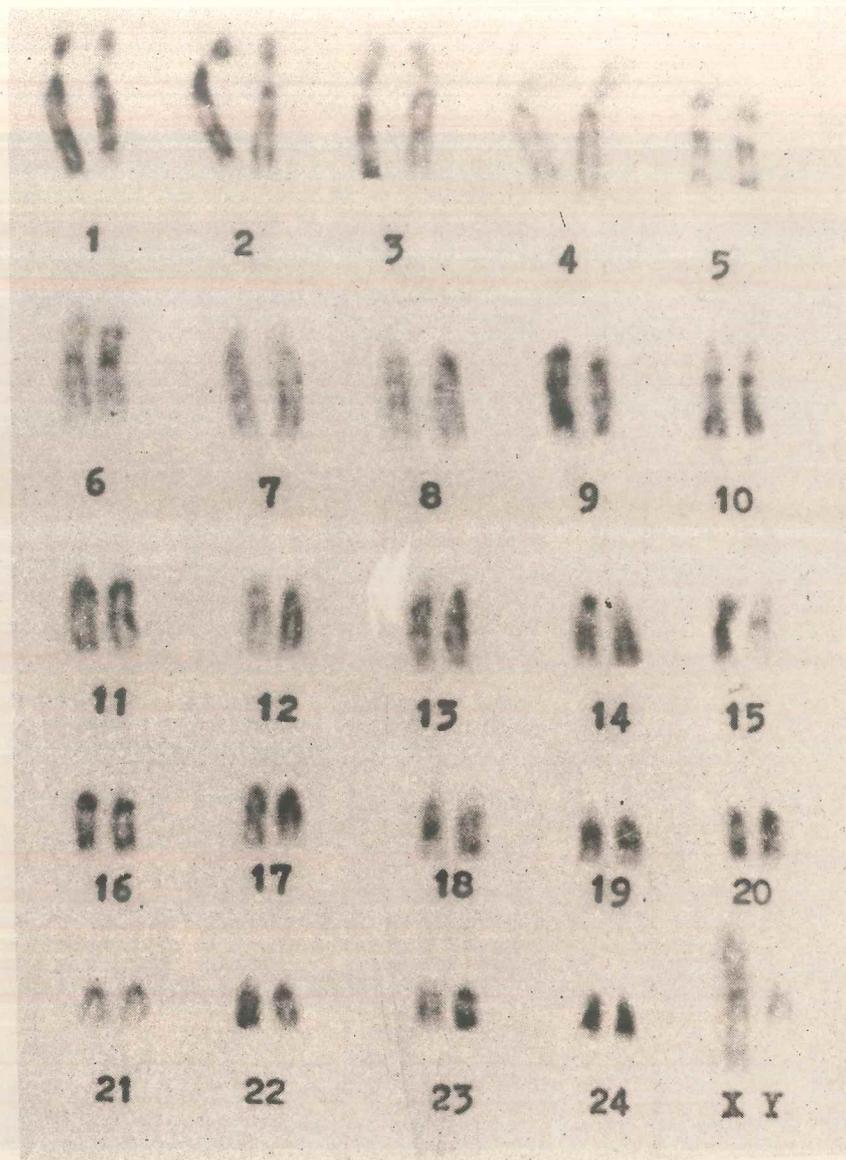


Fig. 2 G-banded Chromosomes

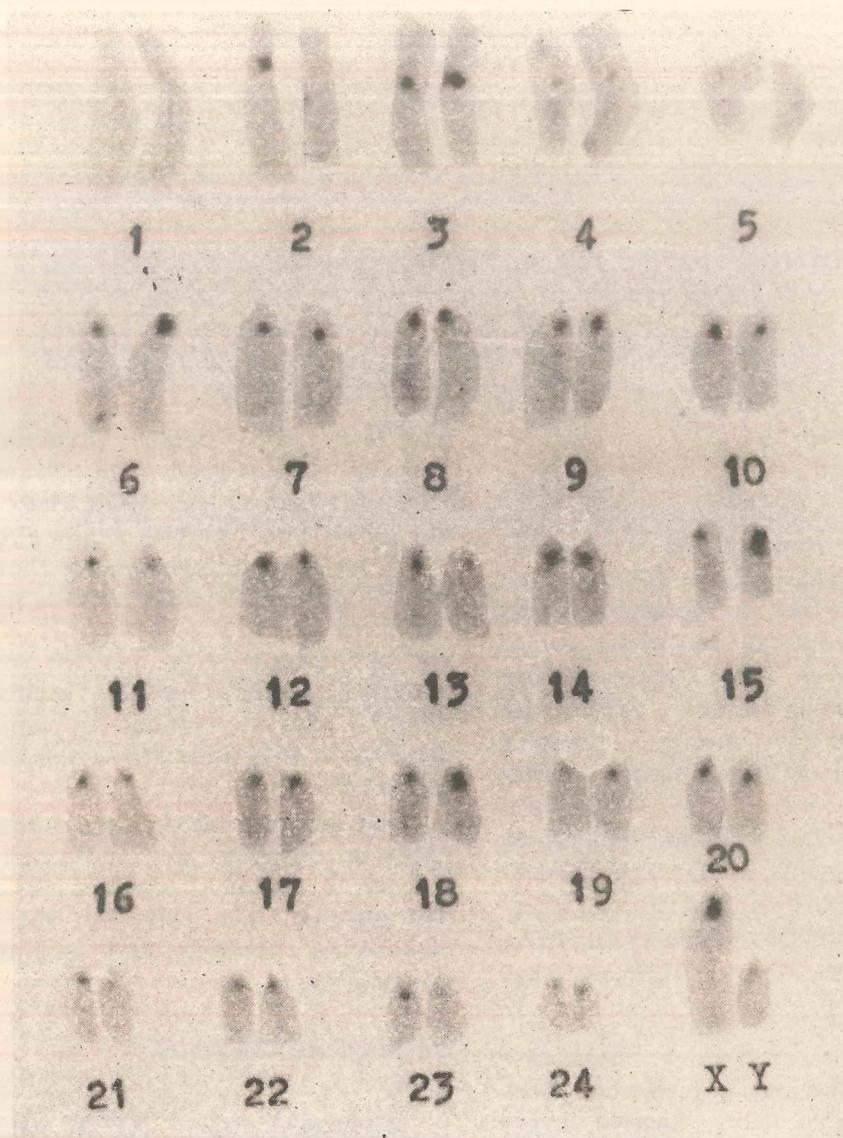


Fig. 3 C-banded Chromosomes

## Polypeptides of Rinderpest Virus

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### Abstract

Rinderpest virus specific proteins were labeled with  $^{35}\text{S}^{\text{m}}$  methionine and analysed on polyacrylamide gels. A 63 kDa protein (Nucleocapsid) was identified as virus specific upon direct analysis of radio-labeled lysates. Monoclonal antibodies against 'N' protein strongly precipitated this 63 kDa protein, thus confirming its identity. Immunoprecipitation with hyperimmune serum revealed the presence of other virus specific proteins such as L (200 kDa), H (71.6 kDa), Fo (56 kDa) and M (38 kDa). The nucleocapsid (N) protein was found in abundance compared to other virus proteins. The fusion (F) protein was inefficiently immunoprecipitated.

### Introduction

Rinderpest, a disease of livestock and wild animals, is known and feared as long as written records have been kept. It is still one of the major maladies of livestock in the developing world including India. The causative virus belongs to the genus Morbilli viruses within the Paramyxoviridae family. Measles virus (MV), canine distemper virus (CDV) and peste-des-petits ruminants virus (PPRV) belong to the same genus along with Rinderpest virus (RPV). Serologically they are closely related (Gibbs *et al.*, 1979). Among them MV and CDV are best known.

There have, however, been few studies in the area of molecular characterisation of RPV. Several investigators have purified RPV and

described the major structural proteins (Underwood and Brown, 1974; Prakash *et al.*, 1979; Sato *et al.*, 1981). More recently Diallo *et al.* (1987) analysed polypeptide synthesis in RPV and PPRV infected cells and identified five viral proteins P,H, N,F and M. Grubman *et al.*, (1988) have identified eight major induced proteins and a number of minor proteins in RPV infected bovine kidney cells.

The present study was undertaken to identify and characterise the viral proteins synthesised in RPV-infected vero cells by  $^{35}\text{S}$  - methionine labelling and immunoprecipitation.

### Materials and Methods

#### Virus:

Rinderpest virus - RBOK strain adapted to vero cells was used. Vero

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cells were propagated in Glasgow's modified Eagle's medium (Microlabs, India) supplemented with 5% colostrum deprived calf serum, 2mM L-glutamine and 5% tryptose phosphate broth.

#### Preparation of Radiolabeled Lysates

Subcultured vero cells were incubated with 1:50 dilution of the virus for 1 hr at 37° before seeding into 4 well plates. After the appearance of 50-60% cytopathic effect (CPE), infected cell monolayers were washed twice with methionine free medium and incubated in this medium for 1 hr. The cells were labeled for 4-6 hrs. by adding 50  $\mu$ Ci of 35S methionine (Amersham) in 1 ml methionine free medium. Actinomycin D (2.5  $\mu$ g/ml) (Pharmacia) was added to one 4-well dish to inhibit production of host cell mRNA.

The cells were lysed with Radioimmunoprecipitation assay (RIPA) buffer (10mM Tris, 150mM NaCl, 600mM KCl, 5mM EDTA, 2% Triton X 100) containing aprotinin (0.5%) and Phenyl methyl sufonyl fluoride (PMSF) (3mM) and microfuged for 20 min. at 4°C. The supernatant was stored at -20 °C for use in immunoprecipitation. Direct analysis of radiolabeled lysates by Sodium dodecyl Sulphate - Polyacrylamide gel electrophoresis (SDS - PAGE) was done after boiling the sample in Laemmli's loading buffer for 3 min.

#### Immunoprecipitation

Infected and mock infected cell lysates were immunoprecipitated with rinderpest hyperimmune sera and monoclonal antibody (MAb) against 'N' protein.

10  $\mu$ l of hyperimmune sera/MAb was added to 100  $\mu$ l of RIPA buffer lysate and kept on ice for 1 hr with periodic agitation. The protein - IgG complex was adsorbed onto protein-A sepharose for 1 hr. The protein A sepharose - IgG protein complex was pelleted and washed with RIPA buffer. The immune complexes were disrupted by boiling for 3 min in Laemmli's buffer. Supernatants were analysed by SDS - PAGE.

#### SDS - PAGE

Virus proteins were separated on a 12.5% polyacrylamide slab gel, according to the method of Laemmli (1970). Electrophoresis was performed at 60 volts overnight in tris - glycine buffer system. Gels were fixed in 12.5% acetic acid - 30% methanol for atleast 30 min, dried (LKB slab gel dryer) and exposed to Kodak/Indu X - ray film.

#### Fluorography

Fluorography of immunoprecipitated lysates was done according to the method of Laskey and Mills (1975). After fixation, the gels were washed twice with dimethyl sulfoxide (DMSO) for 30 min each followed by washing in DMSO containing diphenyl oxazole (PPO) for 3 hrs. The treated gel was washed in running water, dried and exposed to X - ray film at - 70 °C.

#### Molecular Weight Determination

The molecular weight (MW) of virus specific proteins were estimated by comparison with 14C - labeled protein markers (Amersham) having the molecular weights of 200, 100, 92.5, 69, 46, 30 and 12.5 KDa.

### Results

The proteins were labeled with  $^{35}\text{S}$  - methionine on day 3 when 50 - 60% CPE was observed. Radiolabeled lysates when analysed by PAGE revealed an additional protein with a MW of 63 KDa in virus infected cells (Fig.1, Lanes 2 and 4). This was presumed to be the

viral 'N' protein as this is the major protein found in morbilli virus infected cells. A number of cellular proteins were found both in control (Fig. 1, Lane 3) and infected lysates. Infected cells treated with actinomycin D showed lesser cellular protein bands but only one additional virus specific protein was distinct (Fig.1 Lane 4).

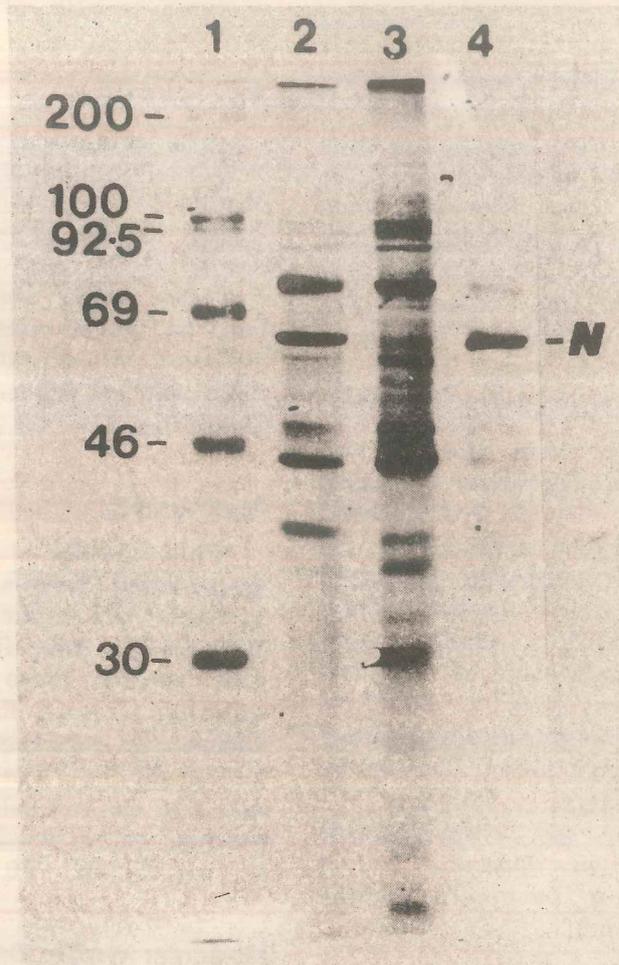


Fig. 1 Page of  $^{35}\text{S}$  labeled virus proteins

Lane 1 :  $^{14}\text{C}$  labeled protein markers

Lane 2 : Infected Cells without actinomycin D

Lane 3 : Control

Lane 4 : Infected Cells with actinomycin D

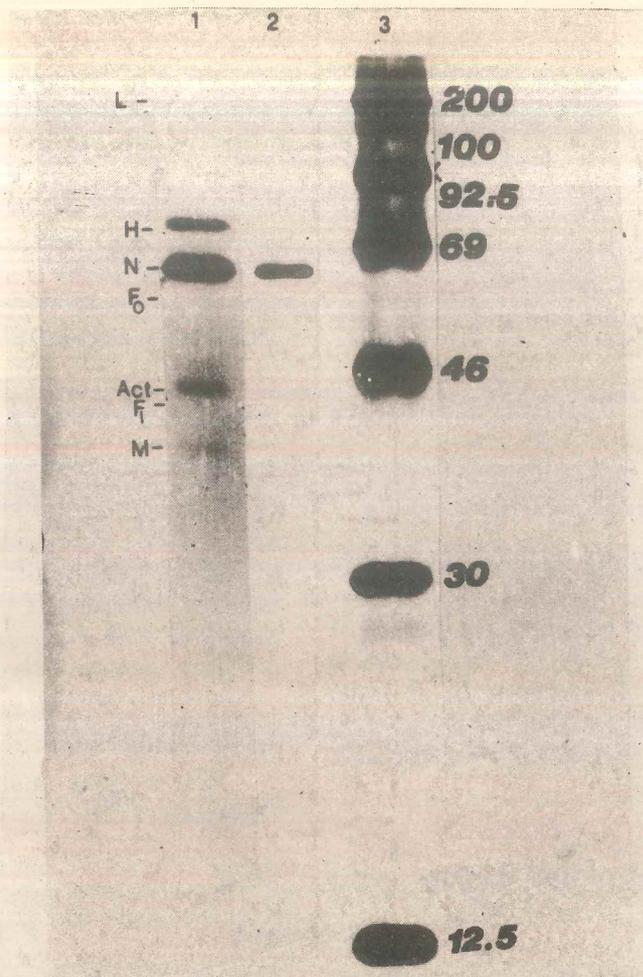


Fig 2. Page of Immunoprecipitated  $^{35}\text{S}$  labeled virus proteins  
 Lane 1: Immunoprecipitation with hyperimmune serum  
 Lane 2 : Immunoprecipitation with Mab to 'N' protein  
 Lane 3 :  $^{14}\text{C}$  labeled protein markers

To demonstrate the virus specific nature of additional proteins present in infected cell lysates, lysates were immuno precipitated with hyperimmune rabbit serum against RPV. As shown in Fig.2 protein of 200 KDa (L), 71.6 KDa (H), 63 KDa (N), 56 KDa (F<sub>0</sub>) and 38 KDa (M) were immunoprecipitated with polyclonal an-

tiserum. The 'N' protein gave a strong signal in comparison to other virus specific proteins. Virus induced polypeptides of 49 KDa (F<sub>1</sub>) and 92 KDa (P) were inefficiently immunoprecipitated. RPV MAb against 'N' protein precipitated the 63 KDa polypeptide, thus confirming its identity

### Discussion

Previous studies with RPV identified a number of proteins in purified virus by PAGE analysis (Prakash *et al.*, 1979; Underwood and Brown, 1974). Sato *et al.* (1981) and Diallo *et al.* (1987) used immunoprecipitation to identify 4 proteins in purified virus and 5 in RPV infected cells - P, H, N, F and M. The results in this study were in agreement with their findings. In addition, the RPV 'L' protein was also identified. Similar data has been presented by Barrett (1987) and Grubman *et al.* (1988), who identified 7 structural (L, P, H, N, F and M) and 1 non structural (C) proteins.

A few cellular protein bands were seen even in infected cell lysates treated with actinomycin D. This may be due to the low concentration (2.5 µg/ml) of actinomycin D used which probably might not have completely inhibited cellular mRNA synthesis.

The 'N' protein gave a strong signal in comparison to other virus

specific proteins on autoradiography. Rima (1983) has reported that the 'N' protein is the major viral protein both in the virion and infected cells. It has also been suggested that in unsegmented negative strand RNA viruses, the change from transcription of the input virus genome to replication of the virus genome is achieved by an accumulation of virus 'N' protein in the cell. This explains the abundance of the 'N' protein in the virus infected cells.

The 'F' protein was very inefficiently immunoprecipitated using hyperimmune sera against RPV. Similar results has been reported by Grubman *et al.*, (1988). Varsanyi *et al.*, (1984) observed that the 'F' protein of MV could be less readily isolated because of its tendency to associate with contaminating cellular proteins, particularly cellular actin.

### Acknowledgement

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## Strategies to Maximise Freezability and to Minimise Cryo - Injuries in Rooster Sperms

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### Abstract

*Chicken semen could be frozen and thawed with satisfactory sperm revival but the fertility of the frozen semen was found lowered. Developments in preservation of fowl semen by cryogenic treatments were slow.*

*The cryo injuries sustained by the spermatozoa on thawing were investigated and reported in terms of divergence of cell membrane destruction of cytoplasmic membrane, bent neck and acrosomal damage at the anterior and giving a bent or truncated appearance. The extent of total pre-freeze injuries varied from 0.60 percent to 3.45 percent and the total post-freeze injuries ranged from 39.44% to 83.99%. Dimethyl sulphoxide at two percent level revealed maximum injuries with either tris or sodium citrate buffers. Maximum protection appeared to be provided by dimethyl sulphoxide and glycerol with tris buffer as compared to sodium citrate buffer.*

### Introduction.

Freezing is a valuable method for preservation of semen and its utility for breeding. However, the problem of finding commercially feasible methods for the dilution and preservation of avian semen remains largely unsolved. To overcome the cryo injuries to spermatozoa, different cryophylactic agents such as glycerol, dimethyl sulphoxide, ethylene glycol, dimethyl formamide and others have been tried at different levels with various diluents viz. sodium citrate, glutamate

Beltville poultry semen extender, Tris (Hydroxy methyl) amino-methane, Lake's diluent, etc. (Sexton, 1977; Van Warnbeke, 1967; Lake, 1960; Brown, 1975; Bernon and Buckland, 1975).

In the spermatozoa of Muscovy drakes, acrosomal deterioration and deformity of spermatozoa induced by freezing and thawing have been examined, and it has been suggested that these characteristics might be a reliable indication of damage induced by freezing in avian spermatozoa (Maeda *et al.*, 1984).

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### Materials and Methods

Mature White Leghorn and New Hampshire Cocks five in each breed were utilized for semen collection and freezing. The method adopted by Burrows and Quinn (1937) was used. The experiments were conducted from 180 pooled samples of semen with the following ten diluents from the two breeds:

1. Sodium citrate + glycerol 2 percent
2. Sodium citrate + glycerol 4 percent
3. Sodium citrate + dimethyl sulphoxide 2 percent
4. Sodium citrate + dimethyl sulphoxide 4 percent
5. Sodium citrate + dimethyl sulphoxide 2 percent + glycerol 2 percent
6. Tris pH 6.75 + glycerol 2 percent
7. Tris pH 6.75 + glycerol 4 percent
8. Tris pH 6.75 + dimethyl sulphoxide 2 percent
9. Tris pH 6.75 + dimethyl sulphoxide 4 percent
10. Tris pH 6.75 + dimethyl sulphoxide 2 percent + glycerol 2 percent

The semen was extended on the above diluents and equilibrated. The processed semen was filled in straws and frozen over liquid nitrogen vapour.

The frozen semen was evaluated for its post - thaw revival rate and its acrosomal integrity after thawing. A storage period of three days was allowed in each case for assessment.

The thawed samples were examined for the freeze - thaw damages. The protection afforded by the cryoprotectives and their combination in the two diluents were compared. The acrosomal staining technique by Waston (1975) using Giemsa stain was adopted to determine the percent acrosomal damage in pre and post freezing.

The spermatozoa which have sustained cryo - injuries, are identified by the description given under the following classifications:

(i) *Divergence of the cell membrane:* The cytoplasmic membranes showed extensive divergence throughout their entire length. The sperm configuration is maintained.

(ii) *Destruction of the cytoplasmic membranes:* The cellular contents completely released but the acrosome remained intact. The tail could be seen attached which helped in the count (Fig.1).

(iii) *Bent:* The mid - piece was bent because of plasmolysis. The neck or the mid - piece allowed the head of the spermatozoa to fold back with the tail (Fig.2).

(iv) *Acrosome:* Destruction of the acrosomal region leading to the release of the contents of the acrosomal cap such that the anterior end became blunt or truncated.

The procedures outlined by Snedecor and Cochran (1967) were adopted for statistical analysis. The morphology of the frozen and thawed fowl spermatozoa were examined under the light microscope (LM). Normal spermatozoa are seen (Fig.3).

### Results and Discussion

The proportion of the types of injuries classified into cell membrane - divergence, bent, destroyed and acrosomal among the total sperms injured are presented in table 1. The pre-freeze and post-freeze damages, cryoprotective and bufferwise are presented in table 1. Statistical analysis are presented in Tables 3 and 4.

The total pre - freeze injuries ranged from 0.60 percent to 3.45 percent and the total post-freeze injuries ranged from 39.44 percent to 83.99 percent. High percentage of incidence of cryo injuries were seen in sodium citrate diluent in respect of breeds or cryoprotection (82.72%). Both divergency and bent sperms were also higher and higher incidence of bent spermatozoa (17.23%). Maximum protection appeared to be provided by DMSO and glycerol with tris buffer. The lesser incidence of cryo injuries with tris diluent would suggest bet-

ter balancing provided for the osmotic pressure gradient generation during the process. The higher incidence of acrosomal damage with sodium citrate would also suggest this view.

A maximum mean revival rate of  $23.96 \pm 0.03$  percent was obtained in the combination of tris, dimethyl sulphoxide and glycerol two percent each at two hours of equilibration with New Hampshire sperm. The sodium citrate buffer in all combinations of cryoprotectives studied and the equilibration periods adopted was not found to be effective in the maintenance of sperm motility upon revival. Nil revival rate was commonly observed with sodium citrate buffer used. The mean revival rate obtained over 15 samples tried per treatment were 18.40 in New Hampshire and 21.20 percent for White Leghorn when glycerol was used at 2 percent level and 24.0 percent in New Hampshire and 14.80

Table 1. Cryoinjuries during post-freeze (White Leghorn breed)

Diluent	Cryoprotective	Revival Rate %	Divergence %	Bent %	Destroyed %	Acrosome damage %	Total injured %	Normal	Percent
Tris	Glycerol 2%	14.30	20.79	0.30	18.02	0.33	39.45	60.56	61.59
Tris	DMSO 2%	9.67	32.37	0.25	22.76	0.58	55.96	44.04	45.00
Tris	Glycerol 2% + DMSO 2%	19.33	23.40	0.87	20.60	0.28	45.15	54.85	55.58
Sodium citrate	Glycerol 2%	1.33	53.93	16.07	13.53	0.46	83.99	16.01	16.11
Sodium citrate	DMSO 2%	2.33	50.20	16.50	14.70	1.53	82.97	22.03	21.08
Sodium citrate	Glycerol 2% + DMSO 2%	16.33	51.71	15.93	14.57	1.00	83.21	16.79	16.89

Table 2. Cryoinjuries to spermatozoa during post-freeze (New Hampshire breed)

Diluent	Cryo - protective	Revival Rate %	Divergency %	Bent %	Destroyed %	Acrosome damage%	Freeze damage	Normal	Percent
Tris	Glycerol 2%	5.33	30.68	0.95	22.34	1.40	55.37	44.63	45.00
Tris	DMSO 2%	13.00	29.05	0.21	18.53	1.27	49.06	50.94	51.00
Tris	DMSO 2% + Glycerol 2%	19.00	23.77	0.71	15.94	0.33	40.75	59.25	60.00
Sodium citrate	Glycerol 2%	1.67	35.91	20.46	24.94	1.68	82.99	17.01	19.00
Sodium citrate	DMSO 2%	2.92	39.23	17.20	19.24	2.30	77.97	22.03	23.00
Sodium citrate	DMSO 2% + Glycerol 2%	2.00	38.65	17.79	19.69	2.07	78.20	21.80	23.00

Table 3. Mean value and standard errors of revival rates of frozen semen with different diluents, cryoprotectives and equilibration periods

	Details	Sample size	Mean	S.E.
1.	Breeds:			
	White Leghorn	450	3.2938 <sup>a</sup>	0.0084
	New Hampshire	450	5.7678 <sup>b</sup>	0.0110
2.	Buffers:			
	Tris 6.75	450	10.6111 <sup>a</sup>	0.0145
	Sodium citrate	450	0.8125 <sup>b</sup>	0.0042
3.	Cryoprotectives:			
	Glycerol 2%	180	4.9462	0.0162
	Glycerol 4%	180	4.610	0.0156
	Dimethyl sulphoxide 2%	180	4.610	0.0156
	Dimethyl sulphoxide 4%	180	3.6827	0.0156
	Dimethyl sulphoxide and Glycerol 2%	180	4.2125	0.0140
4.	Equilibration periods:			
	Two hours	300	8.4500 <sup>a</sup>	0.0161
	Four hours	300	4.0077 <sup>b</sup>	0.0111
	Six hours	300	1.9952 <sup>c</sup>	0.0011
	Overall mean		4.4714	0.0069

**Table 4. Analysis of variance for comparing the revival rates of frozen semen in different diluents, cryoprotectives and equilibration periods**

Sources of variation	Degrees of freedom	M.S.	F value	R <sup>2</sup> value
Between breeds	1	2510.54	46.07**	2.35
Between buffers	1	43460.85	797.52**	60.64
Between cryoprotectives	4	57.85	1.01 <sup>NS</sup>	0.21
Between equilibration periods	2	6096.07	111.86**	11.40
Error	891	54.49		45.50

NS: Not significant

\*\* Highly significant (P < 0.01)

percent in White Leghorn when the two cryoprotection were used in combination. Maeda *et al.* (1984) suggested the inclusion of various cryoprotective as this may protect different parts of the spermatozoa. Further observations of morphology in future studies may help to identify mixtures which would protect the

whole spermatozoan during freezing and thawing.

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## Physical and Biochemical Aspects of Murrah Buffalo Bull Semen and their Relationship with Freezability

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### ABSTRACT

A total of 367 semen samples collected from 30 Murrah buffalo bulls were utilised to study the physical, biochemical and freezability aspects and their relationship. The mean values of physical and biochemical constituents in Murrah buffalo bull semen in this study agreed with the values already reported by many workers. The mean post-thaw motility (PTM) and percent intact acrosomes (PIA) after freezing were  $39.65 \pm 0.69$  percent and  $67.36 \pm 0.12$  percent respectively.

About 22 percent of ejaculates (82 out of 367) from buffalo bulls showed non - motile (static)/poor motility on initial evaluation and 60 percent of them gained motility on dilution. The static semen samples showed significant differences with respect to ejaculate volume, initial motility, fructose level, cholesterol, potassium and sodium level compared with normal motile semen. The static semen samples had  $37.93 \pm 1.03$  percent post-thaw motility and  $67.37 \pm 0.25$  percent intact acrosome on freezing and thawing.

The motility, live sperm percentage, fructose, fructolysis index and sodium in semen had positive significant correlations while cholesterol and potassium had negative significant correlations with freezability of semen. About 60 percent of static semen which gained motility on dilution freeze well with acceptable post-thaw motility and with intact acrosome.

### Introduction

India possess about 60 million buffaloes out of 130 million buffaloes in the world and more than 60 percent of total milk production is contributed by the buffaloes in our country. Although artificial insemination is now accepted as essential tool for extensive use of germplasm of selected males, in

buffaloes it has not been a complete success as it is with the zebu. The preservation of buffalo semen under the refrigeration temperature of  $5 \pm 1^\circ\text{C}$  or deep freezing in subzero temperature has been posing problems. Several striking differences in the physical and biochemical aspects of buffalo semen compared with cattle semen have been reported

by several workers (Cited by Ganguli, 1981).

#### Materials and Methods

The Murrah buffalo bulls at Livestock Research Station, Kattupakkam and Buffalo bulls at Frozen Semen Station, Erode were utilised for this study. A total of 367 semen samples collected from 30 Murrah bulls were used for this analysis. The physical characteristics like ejaculate volume, initial motility and live and dead spermatozoa were recorded immediately after collection. The concentration of spermatozoa was estimated by colorimetric method. The biochemical aspects viz. fructose and Fructolysis index were estimated as per Mann (1948), total cholesterol by the method of Ferro and Ham (1960) and sodium and potassium content by the method of Oser (1965). The acrosomal integrity was estimated by the method of Hancock (1952).

The semen samples were diluted in Tris egg yolk glycerol, filled in French medium straws, frozen in liquid nitrogen vapour and stored in liquid nitrogen at - 196°C.

#### Results

The mean values of physical, biochemical characteristics and freezability of Murrah buffalo semen with range and coefficient of variation are furnished in Table 1. The mean values of physical characters viz. ejaculate volume, initial motility, sperm concentration and live spermatozoa were 3.21 ml, 66.03%, 790.63 million/ml and 69.07% respectively.

The mean values of certain biochemical characters viz. Fructose, Fructolysis index, cholesterol, potassium and sodium in semen were 789.49 mg/100 ml, 1.85, 200.19 mg/100 ml, 96.29 mg/100 ml and 297.25 mg/100 ml respectively. The mean post - thaw motility and intact

TABLE 1 The mean values of physical, biochemical characteristics of Murrah buffalo semen and freezability

Sl. No.	Character	Mean	Range	COV %
1.	Ejaculate volume (ml)	3.21	0.50 - 8.50	45.04
2.	Initial motility (%)	66.03	0.00 - 80.00	24.26
3.	Sperm concentration (millions/ml)	790.63	400 - 1200	16.56
4.	Liver sperm (%)	69.07	15 - 91	20.64
5.	Initial Fructose level (mg/100 ml)	789.49	120 - 1440	36.00
6.	Fructolysis Index	1.85	0.50 - 3.57	33.17
7.	Cholestrol in semen (mg/100 ml)	200.19	133 - 333	23.99
8.	Potassium in semen (mg/100 ml)	96.29	64 - 200	22.21
9.	Sodium in semen (mg/100 ml)	297.25	260 - 358	8.19
10.	Post thaw motility (%)	39.65	0 - 60	33.51
11.	Intact Acrosome (%)	67.36	52 - 81	3.54

n = 367

acromore were 39.65% and 67.36% respectively.

The mean values of static semen samples with respect to physical, biochemical and freezability and its 't' value in comparison with that of normal semen samples are presented in the Table 2.

The values of correlation coefficient between physical and biochemical characters of semen with freezability of semen viz. post-thaw motility and percent acrosomal integrity after freezing are given in Table 3. The correlations ranged from 0.02 to 0.77.

### Discussion

The mean values of the physical and biochemical characteristics obtained in this study are comparable with the values reported by many workers (Naidu and Kanakaraj,

1975; Porwal and Karandikar, 1981; Chalapathy and Rao, 1981).

The mean initial fructose content of semen in the present study was found to be little higher than those reported by Roy *et al.* (1960), Singh and Sadhu (1978), Reddy and Raja (1979) and Porwal and Karandikar (*loc.ci.*). However, higher values to the present value have been reported by Pal (1957), Sengupta *et al.* (1963) and Tomar and Misra. (1971).

The mean fructolysis index was found to be 1.85 based on total sperm and 2.68 based on live sperm, similar values were reported by Sengupta *et al.* (*loc.ci.*) and lower values were reported by Sinha *et al.* (1966) and Kaker (1972). The total cholesterol in semen and in seminal plasma in buffalo semen were lower compared to that value of cattle semen. This low cholesterol in buf-

TABLE 2 The mean values of physical, biochemical and freezability of static (non - motile) semen

Sl. No.	Character	Mean $\pm$ S.E.	't' Value
1.	Ejaculate volume (ml)	2.51 $\pm$ 0.12	3.78**
2.	Initial motility on dilution (%)	69.02 $\pm$ 0.72	2.42*
3.	Sperm concentration (millions/ml)	813.54 $\pm$ 9.88	1.63NS
4.	Live sperm (%)	71.73 $\pm$ 0.63	1.44NS
5.	Initial fructose (mg/100 ml)	774.49 $\pm$ 24.36	5.86**
6.	Fructolysis Index	1.94 $\pm$ 0.52	0.30NS
7.	Total cholesterol (mg/100/ml)	183.45 $\pm$ 3.59	3.21**
8.	Potassium in semen (mg/100 ml)	107.34 $\pm$ 3.31	6.61**
9.	Sodium in semen (mg/100 ml)	288.21 $\pm$ 2.65	2.61**
10.	Post thaw motility (%)	37.93 $\pm$ 1.03	3.51**
11.	Intact Acrosome (%)	67.37 $\pm$ 0.25	2.65**

't' value in comparison with normal semen

\*Significant (P < 0.05)

\*\*Highly significant (P < 0.01)

NS: Not significant

Table 3 Correlation co-efficient between physical, biochemical characteristics of buffalo semen with PTM & PIA

Sl. No.	Characters	Post Thaw Motility	Percent Intact Acrosome
1.	Volume	0.09 ± 0.05	0.50 ± 0.04**
2.	Initial motility	0.77 ± 0.03**	0.44 ± 0.04**
3.	Live sperm	0.77 ± 0.03**	0.45 ± 0.04**
4.	Initial fructose	0.35 ± 0.04**	0.13 ± 0.05*
5.	Fructolysis Index	0.68 ± 0.03**	0.88 ± 0.02**
6.	Cholestrol	-0.21 ± 0.05**	-0.02 ± 0.05
7.	Pot - in semen	-0.23 ± 0.05**	-0.13 ± 0.05
8.	Sod - in semen	0.13 ± 0.05**	0.04 ± 0.05
9.	Post-thaw motility	-	0.67 ± 0.03**

\* Significant (P < 0.05)

\*\* Highly significant (P < 0.01)

falo semen may render it more susceptible to temperature shock according to Sharma and Venkata-subramanian (1975).

The bulk cations potassium and sodium are found in high concentration in the semen and are necessary for the normal functioning of the spermatozoa. The values of cations in buffalo semen obtained in this study were lower than that of bull semen.

The mean post-thaw motility and intact acrosome after freezing were 39.65% and 67.36% respectively, however, the post-thaw motility was low when compared to the cattle semen.

In this study 82 out of 367 semen samples showed non - motile (static) or poor motility on initial evaluation and 60 percent of them gained motility on dilution. There were significant differences with respect to volume, fructose, cholesterol, potassium and sodium content in static semen samples compared to normal motile semen.

The correlation coefficient between semen characteristics with post-thaw motility and intact acrosome are given in Table 3. Motility, live sperms, fructose content, fructolysis index and sodium showed significant positive correlations while cholesterol and potassium showed significant negative correlations with post-thaw motility. Volume, motility live sperms and fructolysis index showed significant positive correlations with intact acrosome. There was high positive significant correlation between post thaw motility and percent intact acrosome. About 60 percent of static semen which gained motility on dilution freeze well with acceptable post thaw motility and intact acrosomes.

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## Comparison of Superovulatory Response of Cross Bred Cows to FSH and PMSG Treatment

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### ABSTRACT

Nine Jersey X Sindhi cross bred cows were subjected to 28 mg FSH or 1500 IU PMSG superovulatory treatment adopting approved protocol for embryo transfer technology. The average number of ovulations, unovulatory follicles, total fertilized embryos and transferrable embryos collected in FSH treated group were  $12.0 \pm 2.46$ ,  $0.83 \pm 0.4$ ,  $4.5 \pm 1.61$  and  $3.3 \pm 1.0$  respectively. The respective figures for the PMSG treated group were  $8.0 \pm 2.36$ ,  $1.33 \pm 0.5$ ,  $3.5 \pm 0.81$  and  $2.0 \pm 0.5$ . The percentage of transferrable embryos to the total fertilized embryos collected was higher in FSH group (70.3%) than PMSG group (57.3%).

### INTRODUCTION

There have been many reports on multiple ovulation and embryo transfer programme in cattle. Both Follicle stimulating Hormone (FSH) and Pregnant Mare's Serum Gonadotrophin (PMSG) have been used for this purpose extensively in exotic breeds. Superovulatory response in terms of number of ovulations, fertilized embryos and viable embryos are extremely variable. Studies on the superovulatory response by cross bred cows to FSH and PMSG are less. Kathiresan *et al.*, (1989) reported that the PMSG for optimum superovulation is about 1,500 IU. Ramakrishna and Bose (1986) observed that luteal phase stimulations with 1,500 IU PMSG yielded greater number of ovulations than the follicular phase stimulations

in cross bred cows and heifers. Elsdon and Kessler (1983) reported that the minimum dose of FSH resulted in a more number of transferrable embryos than the higher doses in Nellore cows. This work was carried out with the view to compare the superovulatory effect of 28mg of FSH and 1,500 IU PMSG in cross bred cows.

### Materials and Methods

Nine jersey x Sindhi cross bred cows, aged between 4-8 years, body weight ranging from 250 to 310 kgs maintained in the non-surgical embryo transfer scheme, Department of Obstetrics and Gynaecology of Madras Veterinary College were used for this study. All the cows have calved atleast once and had exhibited oestrous at least twice after the last calving. The cows were maintained

in high plane of nutrition. Utilising these nine cows two superovulatory regimes consisting of 28mg FSH (Programme I) and 1,500 IU of PMSG (Programme II) were used. Of the nine cows, 3 were subjected to both programmes I and II at an interval of 6 months. In all 12 superovulations, consisting 6 of programme I and 6 of programme II were studied. The superovulatory treatment was started on day 10 or 11 of the oestrus cycle. PMSG was given as single dose and FSH was given as eight divided doses (5/5, 4/4, 3/3 and 2/2) at 12 hours interval. Forty eight hours after the initiation of superovulatory treatment each cow in both the groups received 25 mg PGF 2 alpha (Lutalyse, Unichem Laboratories) followed 8 hours later by 12.5 mg PGF 2 alpha intramuscularly. The cows were inseminated with double dose of frozen semen three times at 60, 72, and 84 hours after the first PGF 2 alpha injection. Seven days after the initial insemination the uteri were flushed and embryos were collected non-surgically. The total and average number of corpora lutea and un-ovulatory follicles were assessed by rectal examination of both ovaries of each cow prior to uterine flushing. Based on the morphological features observed through Zoom stereo microscope, the embryos were classified as fertilized and transferrable.

### Results and Discussion

Results of the ovarian response and embryos collected to superovulation with 28 mg FSH and 1,500 IU

of PMSG are summarised in the table. The findings regarding the average number of corpora lutea formed with FSH and PMSG were  $12.0 \pm 2.4$  (range 2-20) and  $8.0 \pm 2.36$  (range 3-21). Statistically there was no significant variation between two programmes. But the range suggests that the ovarian responses were quite variable in cross bred cows after superovulation either with FSH or with PMSG. Manickam *et al.*, (1990) and Battacharya *et al.* (1989) reported variation in cross bred cows after superovulation with PMSG and FSH. In this study, the individual variation may be due to differences in the age and body weight. Hauptat (1979) and Sreenan *et al.* (1980) reported that variability in superovulatory response was a consequence of the differences in the age, body weight and endogenous hormonal milieu among animals. In this study, unovulatory follicles were present in both PMSG and FSH treated cows at the time of embryo collection. Unovulatory follicles have also been reported by Nikoyan (1982) after PMSG treatment. Regarding embryos, the percentage of total transferrable embryos to the total fertilized embryos in FSH programme was significantly higher (70.3%) than the PMSG group (57.3%). Elsdon *et al.*, (1978) and Wright Jr (1988) reported that while PMSG was convenient to administer in a single injection, the percentage of transferrable embryos to the total fertilized embryos collected were higher when FSH was used in cows for superovulation.

TABLE

Treatment programme	Average number of corpora lutea formed	Average number of unovulatory follicles present	Average number of fertilized embryos collected	Average number of transferrable embryos collected	Percentage of transferable to the total fertilized embryos
I 28 mg FSH	12.0±2.46	0.83±0.40	4.5±1.61	3.5±0.81	70.3
II 1,500 IU PMSG	8.0±2.36	1.33±0.5	3.3±1.0	2.0±0.5	57.3

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## Effect of Two Different Routes of PGF<sub>2</sub> Alpha Administration in Crossbred Cows.

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### ABSTRACT

Twenty four normally cycling crossbred cows with a palpable 8 to 12 days old corpus luteum were selected for this study. Twelve cows selected at random were treated with 25mg of PGF<sub>2</sub> alpha intramucular and another 12 cows were treated with 12.5 mg of PGF<sub>2</sub> intra vulvar route. All the animals were observed for the onset, intensity and duration of oestrus after the treatment and at the onset of induced oestrus for serum progesterone assay.

The time taken for the onset of induced oestrus and duration of oestrus after intramuscular and intravulvar PGF<sub>2</sub> alpha injection were  $65.76 \pm 1.26$  hours,  $68.74 \pm 1.62$  hours, and  $18.58 \pm 0.29$  hours and  $16.85 \pm 0.40$  hours respectively. Correspondingly the percentage of normal oestrus and intense oestrus for 25 mg and 12.5 mg PGF<sub>2</sub> alpha were 58.33, 41.67 and 66.67, 33.33 respectively. The serum progesterone level at the time of induced oestrus were  $0.66 \pm 0.04$ ,  $0.69 \pm 0.01$  mg/ml in intramuscular and intravulvar route respectively. In this study, no animal exhibited suboestrus.

### Introduction

Since the report of Rowson *et al.* (1972) on PGF<sub>2</sub> alpha, the drug has been used on different routes for induction of luteolysis such as intra uterine (Shelton, 1973), intravaginal (Louis *et al.*, 1972), intramuscular (Roche, 1975), IVSM (Ono *et al.*, 1982) and intravulvar (Neduncheralathan and Kathiresan, 1986). Voh *et al.* (1987) confirmed the

luteolysis after PGF<sub>2</sub> alpha injection by studying the plasma progesterone level before the PGF<sub>2</sub> alpha injection and at the onset of induced oestrus. This study was undertaken to find out and compare the luteolytic effect of intramuscular and intravulvar administration of PGF<sub>2</sub> alpha

### Materials and Methods

Twenty four crossbred normally cycling cows of Livestock Research

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Station, Kattupakkam were divided at random into two groups namely Group I (n=12) and Group II (n=12). Eight to 12 days after natural oestrus, the animals in Group I were subjected to 25 mg of PGF<sub>2</sub> alpha injection by intramuscular route. Similarly animals in Group II were injected with 12.5 mg of PGF<sub>2</sub> alpha by intravulvar route. The intramuscular injection was given in gluteal muscle and the intravulvar injection was given in the labia majora. Forty eight hours after treatment they were examined for signs of oestrus by external observation and by rectal palpation of genitalia at 12 hourly interval. The time of onset of oestrus after PGF<sub>2</sub> alpha injection, duration and intensity of oestrus were observed based on method of Rao and Rao (1981).

Blood samples were collected from all the animals before giving PGF<sub>2</sub> alpha injection and at the time of induced oestrus. The progesterone level was assessed by RIA Technique at NDRI, Bangalore. The data was statistically analysed by paired 't' test (Snedecor and Cochran, 1968).

### Results and Discussion

All the animals in both the groups showed oestrous signs after the treatment and the results are summarised in Table 1. The onset of oestrus from the time of PGF<sub>2</sub> alpha injection was 65.76±1.26 hours and 68.74 ±1.62 hours and the duration of oestrus was 18.58 ± 0.29 hours and 16.85 ±0.40 hours in intramuscular and intravulvar routes respectively. In this study no animal exhibited sub-oestrus in both the groups. The serum progesterone

level at the time of treatment and at induced oestrus were 3.95±0.56, 3.92±0.68 and 0.66±0.04, 0.69±0.01 ng/ml for group I and group II respectively. The mean time taken from the time of PGF<sub>2</sub> alpha to onset of oestrus was 65.76±1.26 hours in group I and 68.74±1.62 hours in group II. The difference was not significant. But statistically, the duration of oestrus was significant and it was higher in intramuscular injection group (18.58±0.29 hours). The percentage of intense oestrus was also more in group I. The progesterone level at the time of (onset of induced) oestrus was low in group I (0.66±0.04 ng/ml) than group II (0.49±0.01ng/ml) and the difference was statistically significant (P<0.05). According to Nancarrow *et al.*(1974), Elamarimi *et al.*, 1983, Dahlan *et al.*, (1987), Voh. *et al.* , (1987) low progesterone level at the time of induced oestrus can be correlated with better conception rate. Eventhough the time taken for onset of oestrus after treatment appeared to be equal irrespective of route of administrations, there was significant variation in duration of oestrus and progesterone level at oestrus after intramuscular route, which may influence favourably the conception rate.

### Acknowledgement

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Table 1  
Effect on two different routes of PGF<sub>2</sub> alpha administration

Treatment route	No. of animals treated	Onset of oestrus in hours after PG injection Mean ± SE	Duration of oestrus in hours Mean ± SE	Intensity of oestrus (Number of animals)			Progesterone level (ng/ml) Mean ± S.E.	
				Sub-oestrus	Normal oestrus	Intense oestrus	Before PGF <sub>2</sub> alpha administration	At onset of oestrus
Intramuscular (25 mg PGF <sub>2</sub> )	12	65.76 1.26 <sup>NS</sup>	±18.58 0.295*	0	7 (58.33)	5 (41.67)	3.95 ± 0.56	0.66 ± 0.04*
Intravascular (12.5 mg PGF <sub>2</sub> )	12	±68.74 1.62	±16.85 0.40	0	8 (66.67)	4 (33.33)	3.92 ± 0.68	0.69 ± 0.01

Figures in parentheses are the percentage values

NS: Not significant (P > 0.05)

\* Significant (P < 0.05)

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## An Attempt to Separate X and Y Bearing Bovine Sperm

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### ABSTRACT

The desire to predetermine the sex before conception has led many to seek methods of separating the populations of X and Y bearing sperm. The sperm separation attempts were based on the differences in size and motility between the sperms carrying X and Y chromosomes.

The Y chromosome bearing sperms of human semen are identified under fluorescence after staining with Quinacrine. Attempts were made to separate the X and Y bearing sperms by filtration techniques.

Filtration of bovine semen using glasswool, BSA and sephadex gel columns was undertaken with 336 semen samples collected from eleven jersey bulls. The filtered and unfiltered samples were stained with Quinacrine and viewed under fluorescent microscope. The samples displayed fluorescence uniformly without any differentiation in brightness. Separation of X and Y bearing sperms through BSA and sephadex column filtration could not be confirmed in bovine semen.

### Introduction

The control of the sex ratio of the progeny still remains one of the most interesting unsolved problems in Biology. The ability to predetermine the sex of the offspring before conception could bring great economic benefit if applied to the breeding of livestock. The desire to predetermine the sex before conception has led many investigators to seek methods of separating the populations of X and Y bearing sperm. Attempts have been made by techniques of filtration to separate the X and Y bearing sperms and this separation was based on the differences in size and motility between them.

Successful separation of Y sperms in human semen by filtration with BSA was reported by Ericsson *et al.* (1973). Quinli Van *et al.* (1982) reported the separation of X sperms by filtration using sephadex column. But varying results were reported in bovine semen (Faust *et al.*, 1976; Beal *et al.*, 1984). Hence attempts were made through this study to separate X and Y bearing sperms of bovine semen with three different filtration techniques and the results reported.

### Materials and Methods

A total of 336 semen samples collected from eleven jersey bulls were used in this study. Filtration of

semen samples was done with Glasswool, BSA and sephadex columns.

The glasswool column was prepared by packing 40 - 60 mg of Borosilicate glasswool fibre in pasteur pipette (Chandrasahana *et al.*, 1982). BSA columns were prepared as per the method described by Faust *et al.* (1976). They were prepared with 2 ml of 2 percent BSA in Tris buffer in 13 X 125 mm tubes. Sephadex columns were made as suggested by Graham *et al.* (1976). They were prepared with 20 percent of sephadex gel of Grade 15 with 40 - 120 $\mu$  size.

The parameters of semen samples such as motility, live sperm percentage, abnormal sperm percentage, sperm concentration, acrosomal integrity and post thaw motility were studied before and after filtration.

The Quinacrine staining procedure described by Barlow and Vosa (1970) was followed to identify the Y bearing sperms under fluorescence microscope.

Spermatozoa were viewed under fluorescent microscope with ultraviolet illumination from a Zeiss Photomicroscope using exciter filters.

The modified procedure of Ogawa *et al.* (1968) was also tried. Semen smears were stained with 0.05 percent Quinacrine mustard for 24 hours and examined under fluorescence microscopy.

### Results and Discussion

The mean values of the semen characteristics recorded before and after filtration are presented in the table. The results showed differences in values. The filtration technique was observed to increase normal motile live sperms, exclude abnormal

density, improve post-thaw motility and show variable effects on acrosomal integrity of bovine semen.

The fluorescence microgram presented, revealed no differentiation between sperms.

Pearson and Bobrow (1970) observed that half of human spermatozoa contain a small fluorescent spot or F body while the remainder have no spot. They suggested that F body marks the Y chromosome in human sperm and that X and Y bearing sperms can thus be identified. The fluorescence microscopy of this study revealed no differentiation in light refraction of X and Y bearing sperms. The presence of F bodies could not be identified as the sperms displayed fluorescence uniformly. Similar observations were reported by Sunner *et al.* (1971) and Junginger (1987). Junginger (1987) stained the cattle sperms with both Quinacrine didhydrochloride and Quinacrine mustard but could not differentiate the X and Y sperms by fluorescence microscopy. Sunner *et al.* (1971) reported that F bodies do not occur in domestic animals.

Identification of Y bearing sperms was not obtained even with the modified procedure of Ogawa *et al.* (1988). Separation of X and Y bearing sperms through filtration



Fig. 1. Fluorescence microgram of Spermatozoa

techniques could not be confirmed with this staining procedure.

Professor of Animal Genetics for the facilities provided for this study.

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Table Mean values of the characteristics of bovine semen before and after filtration through glasswool, BSA and sephadex columns (Arcsin P)

Sl. No.	Semen Characteristics	No. of samples	Before filtration Mean $\pm$ S.E.	After filtration through		
				Glasswool Mean $\pm$ S.E.	BSA Mean $\pm$ S.E.	Sephadex Mean $\pm$ S.E.
1.	Motile sperms	200	54.62 <sup>a</sup> $\pm$ 0.93 (66.5)	60.81 <sup>b</sup> $\pm$ 0.50 (76.2)	61.62 <sup>b</sup> $\pm$ 0.94 (77.4)	62.17 <sup>b</sup> $\pm$ 1.41 (78.2)
2.	Live sperms	200	54.95 <sup>a</sup> $\pm$ 1.04 (67.0)	60.20 <sup>b</sup> $\pm$ 0.61 (75.3)	61.09 <sup>b</sup> $\pm$ 0.91 (76.6)	62.53 <sup>b</sup> $\pm$ 1.71 (78.7)
3.	Abnormal sperms	200	24.37 <sup>a</sup> $\pm$ 0.40 (17.0)	17.21 <sup>b</sup> $\pm$ 0.41 (8.8)	15.02 <sup>c</sup> $\pm$ 0.25 (6.7)	14.48 <sup>c</sup> $\pm$ 0.36 (6.2)
4.	Sperms concentration (millions)	198	209.93 <sup>a</sup> $\pm$ 4.12	123.78 <sup>b</sup> $\pm$ 5.18	91.84 <sup>c</sup> $\pm$ 6.18	95.66 <sup>b</sup> $\pm$ 5.06
5.	Acrosome damage	97	13.91 <sup>a</sup> $\pm$ 1.38 (5.8)	23.84 <sup>b</sup> $\pm$ 0.77 (16.3)	14.07 <sup>a</sup> $\pm$ 0.82 (5.9)	12.51 <sup>a</sup> $\pm$ 0.58 (4.7)
6.	Post thaw motility	73	35.59 <sup>a</sup> $\pm$ 1.00 (33.8)	35.62 <sup>a</sup> $\pm$ 0.39 (33.9)	37.35 <sup>a</sup> $\pm$ 0.43 (36.8)	41.12 <sup>b</sup> $\pm$ 0.36 (43.3)

Means bearing different superscripts in a row differ significantly

Figures in parenthesis are per cent values

## Reproductive Performance of Tellicherry Goats

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### ABSTRACT

Data on litter size, birth weight and percentage of sex in 124 kids, kidding interval and oestrous patterns of 24 does of the Tellicherry breed maintained under intensive conditions at the Department of Animal Husbandry were analysed. They are respectively  $1.99 \pm 0.09$ ,  $1.95 \pm 0.04$  kg, 49.2:50.8,  $339.9 \pm 27.78$  days and 68. Litter size was higher in second kidding. Highest numbers of oestrous cycles occurred in October (25.0%). Male kids were born with higher body weight but no difference in sex ratio was observed. Longer kidding interval indicated the reproductive efficiency of does.

### Introduction

Litter size, sex ratio, kidding interval and birth weight of goats are economic traits which largely determine their total productivity. These were studied in Tellicherry goats in Kerala - India (Mukundan and Rajagopalan, 1971; Raja and Mukundan, 1973; Sundarsanan and Raja, 1974; Mukundan, 1976). Observations on oestrous cycles were reported in other goat breeds (Mishra and Biswas, 1966; Sing *et al.*, 1978) under Indian condition. This report indicates the performance of Tellicherry goats.

### Materials and Methods

Data on litter size, birth weight, sex ratio of 124 kids and kidding interval of 20 does between 2 and 8

years of age and weighing 20 - 30 kg were analysed. The number born, sex of kid and birthweight were recorded within 12 hr of birth. Kidding interval (the period between two consecutive kiddings) incorporated the service and gestation periods. Goats were maintained in stalls with partition for kids with an open paddock and a covered portion that protects animals against drafts. Concentrate ration was fed twice daily (400 - 500 gm/day). Non - leguminous green grass was fed in addition to limited grazing on Hariyali grass for about two hours a day. The breeding pattern of 20 does in 68 kiddings were also analysed.

### Results and Discussion

Litter size ( $1.99 \pm 0.090$ , Table 1) was higher than reported by Mukun-

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Table 1 Average litter size, number and birth weight of kids

		KIDDING								
		I	II	III	IV	V	VI	VII	VIII	
I.	<i>Average litter size</i>									
	(a) Total Number of does	20	17	8	8	8	5	1	1	68
	(b) Total Number of kids born	30	36	16	15	13	10	2	2	124
	(c) Average litter size $\pm 0.11$	1.5 $\pm 0.11$	2.1 $\pm 0.11$	2.0 $\pm 0.26$	1.9 $\pm 0.12$	1.9 $\pm 0.26$	2.5 $\pm 0.57$	2.0	2.0	1.99 $\pm 0.09$
II.	<i>Number of kids:</i>									
	(a) Number of male kids (63.3%)	19 (63.3%)	13 (38.2%)	9 (56.2%)	9 (60.0%)	4 (30.8%)	5 (50.0%)	0	2 (100.0%)	61 (49.2%)
	(b) Number of female kids (36.7%)	11 (36.7%)	23 (61.8%)	7 (13.8%)	6 (40.0%)	9 (69.2%)	5 (50.0%)	2 (100.0%)	0	63 (50.8%)
III.	<i>Birth weight of kids in kg:</i>									
	(a) Male $\pm 0.05$	2.19 $\pm 0.05$	2.07 $\pm 0.09$	1.95 $\pm 0.07$	2.18 $\pm 0.10$	2.08 $\pm 0.05$	1.62 $\pm 0.78$	-	1.90 $\pm 0.07$	2.00 0.07,
	(b) Female $\pm 0.22$	2.00 $\pm 0.22$	1.93 $\pm 0.04$	1.80 $\pm 0.13$	2.28 $\pm 0.28$	1.85 $\pm 0.12$	1.54 $\pm 0.15$	2.00 $\pm 0.05$	-	1.90 $\pm 0.04$ 1.95 $\pm 0.04$

dan and Rajagopalan (1971) and Sudarsanan and Raja (1974). Increasing birth weight in the second and third kiddings was found similar to reports by Shanmugasundaram (1957). Singles were 26.47% of all kiddings, 64.71% twins and 8.82 triplets. Multiple births were higher than reported by Mukundan and Rajagopalan (1971) but lower than by Sudarsanan and Raja (1974), especially in triplets. Most first kiddings were single kids but had twins or triplets in subsequent kiddings.

Overall birth weight (Table 1) of  $1.95 \pm 0.04$  kg was comparable with other reports. Male kids weighed at birth ( $2.00 \pm 0.076$  kg) against female kids ( $1.90 \pm 0.070$  kg) were as observed by Naikarae and Jagtap (1989). Single kids were heavier at birth than twins or triplets in both sexes. Birth weight was almost constant upto the fourth kidding and thereafter it declined.

Sex ratio (Table 1) was not significantly different from 1:1 but ratio of females was 63.3% to 36.7% in

first kiddings and 38.2 to 61.8% in second kiddings. Kidding interval can be affected by breed of goat and environmental factors such as nutrition and physical condition of does due to illness (Devendra and Burns, 1983). In these Tellichery goats on average, kidding interval of  $339 \pm 27.78$  days was little higher than reported by Mukundan (1976), but lower than in reports of Raja and Mukundan (1973).

Analysis of breeding patterns in 68 kiddings showed that the highest number of oestrous cycles occurred in October (25.0%) and the lowest in February and March, and most occurring during June to November (70.6%) as observed by Mishra and Biswas (1966) in Desi goats, and by Singh *et al.* (1978) in other Indian goats. Higher percentage of oestrous cycles and conception rates in August to November are probably attributable to the availability of more adequate quantities of green fodder

and optimum climate factors. During these months, maximum and minimum air temperature range from  $30.33^{\circ}\text{C}$  to  $20.23^{\circ}\text{C}$ , respectively. Relative humidity ranges from 80 - 90% and wind velocity 2 - 10 km/hour. The total rainfall during these months from Southwest and Northeast monsoon ranges from 300 - 400 mm. This climate is conducive for green fodder production and good fertility rates in Tellicherry goats during the above months. High percentage of kidding occurred in March and April and lowest in August and September as reported by Vohradsky and Sada (1973).

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## Prospectives of Embryo Transfer in Goats

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### ABSTRACT

*The manipulation of reproduction through embryo transfer has had a major impact on genetic strategies in animal production during the last 20 years. The advent of estrous synchronization, embryo collection, transfer and embryo processing have allowed a move from the laboratory to the farm. Other aspects of embryo manipulation which have a major impact on breeding strategies include embryo splitting to produce monozygotic twins, in vitro fertilization, cross species fertilization, embryo sexing, production of tetraparental animal and nuclear transfer.*

### Introduction

India is an Agrarian country, where more than 70% of the population live in rural areas and most of the people depend on agriculture for their livelihood and contribute about 45% of the national income. To achieve a social justice in the rural areas there is a need for review of existing farming strategy and appropriate modification to generate income and employment potential for small holding through diversified goat production. Uncontrolled breeding in the periphery of the home tract of well known breed has resulted into percolation. This also suggest the presence of diverse genetic component with unknown interaction.

The threat of elimination of known meritorious breeds and types amongst the indigenous gene resources has therefore increased. Conser-

vation of all breeds have been essential in view of recent development in the field of biotechnology and genetic engineering. Sequencing of genes and its preservation in gene banks would be possible in the nearfuture. It would also be possible to identify the desirable genes or gene complex associated with economic traits viz. heat tolerance, disease resistance and production ability under specific ecological conditions. Indian breeds of goats that need very early conservation are Malabari, Jamnapari, Beetal, Surti etc. The disappearance or extinction of a breed can only be saved through the process of conservation which is difficult and time consuming. Technological means are available for conserving the germplasm in the form of gametes and embryos at subzero temperature whereby creating gene-banks to overcome the problem of extinction.

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### **Artificial Breeding in the Goat Industry**

Artificial breeding of goats falls into three headings viz. (a) control of oestrus and ovulation, (b) Artificial insemination and (c) embryo transfer. These controlled breeding techniques potentially offer a number of benefits.

Synchronisation of oestrus to facilitate artificial insemination and hand service helps for.

- Out of season breeding.
- increase litter size.
- synchronization of lambing to reduce supervision time.

### **Enhanced Reproductive rate of Superior females**

It would appear that embryo transfer offers tremendous potential for genetic improvement through enhanced reproduction of genetically superior females. Because artificial insemination provides a means of augmenting the number of offspring from genetically superior males and dramatic increase in production with artificial insemination has become a reality. Animal breeders have estimated the genetic progress possible with embryo transfer to a breeding scheme, improved anesthetic agents and success of embryo collection and transfer methodology have now made embryotransplantation in goats as realistic reproduction management tool for progressive goat breeders. Reports to date indicate that pregnancy rates following transfer of goat embryos to recipients should range from 45 - 80%. The variability in success rate appears to be related to quality of donors, nutritional status of does and transfer expertise.

### **In vitro Fertilization**

Follicular oocytes that had been collected from two sources, either from suitable follicles found in normal ovaries from abattoir materials or from mature follicles that have been stimulated in does that are cycling normally or have been artificially stimulated (females treated with gonadotropin). Taken together these factors promise that abattoir material may eventually provide a useful source of oocytes and embryos. This technique is employed to utilise oocytes from the slaughterhouse for cytogenetic analysis to find out chromosomal abnormalities and also for other embryo transfer work.

### **Micromanipulation of embryos**

It appears that animal reproduction is entering the era of embryo engineering - a part of the new biotechnology. With the development of embryo transfer technology, farm animal reproduction has already been progressing through, new age of technical advancement. In recent years viable offsprings have resulted from early stage embryos which have been separated using newly developed micromanipulation procedures.

With the real possibility of efficient embryo splitting methodology becoming available in the near future, number of offspring from valuable donor thus could theoretically be doubled at the time of embryo collection and transfer. This would be especially important for donor females who do not respond to superovulation treatment.

Embryo splitting can also be used to produce twin offspring in does that ovulate only one ovum per

cycle by use of an autotransfer procedure (split embryos returned to the uterus of the donor female). This would be one method of improving reproductive efficiency of the goat breeding herd. For does producing more than one ovum per cycle, one embryo could be split into halves and placed back into her uterus by autotransfer. The remaining embryos could be split into halves and transferred to other does or the embryos could be frozen for use by the producer at a later date.

In the future new embryo engineering techniques (intraspecies chimeric offspring), innercell mass transfer, sperm cell injection to fertilise ova, blastomere culture and others will likely be evaluated for efficacy in goat breeding management. Gene injection technique being developed for embryos may be used someday to improve the genes type of breeding goats and their offspring. With the recent advances now being made with genetic engineering of embryos, it is likely that the goat will become a benefactor of this new technology in the years to come.

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## Relationship of Serum Alkaline Phosphatase with Production Traits in Broiler Rabbits

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### ABSTRACT

*Alkaline phosphatase level was studied from 21 New Zealand White, 47 locals and 48 crosses. The mean level of Alkaline phosphatase was  $0.5638 \pm 0.023$  in New Zealand White,  $0.5250 \pm 0.022$  in local and  $0.4970 \pm 0.018$  mM/lt in crosses. The SAP levels more than 0.7 mM were classified as high level and the levels less than 0.7 mM were named as low level. The serum alkaline phosphatase exhibited a bimodel distribution in all the three genetic groups. It was also observed that the low level was predominant than high level in all the three genetic groups.*

*The production traits were studied in high and low levels of serum alkaline phosphatase irrespective of the genetic group. The analysis showed that alkaline phosphatase do not have any influence on litter size at birth, weaning and litter weight at weaning. However, the high serum alkaline phosphatase level was found to be associated with the heavier litter at birth. It is suggested that litter weight can be improved by selection of high alkaline phosphatase level rabbits for breeding broiler type.*

### Introduction

In many countries domesticated variety of rabbits, is recognised as an useful source of meat. The potential role of the broiler rabbits in meat production has transformed the rabbit production as a commercial industries adopting intensive system of rearing involving large number of rabbits. Exact knowledge and specialised experience of scientific breeding and management need to be framed to make the broiler rabbit production as a profitable industry.

Various breeds have been developed in temperate countries.

It will be a fertile field for cross breeding the natives with exotic breeds and evolve suitable strains for the tropical areas under rigid selection for economic traits. Economics of the rabbit farming obviously depended on the number of young ones born from each mother, their viability and ability for growth to adult hood. Selection based on performance are time consuming and normally inferior stocks may also

have to be maintained for quite some time before their production performances are recorded. This could be avoided, if selection at an early age after birth could be made, based on genetically determined biochemical traits which may have desirable association with production traits.

Not much work has been done in rabbits on the biochemical variants and their relationship with economic traits. An attempt was made to estimate the level of serum alkaline phosphatase in the rabbits reared for meat productivity at Sheep Breeding Research Station, Sandynallah, Livestock Research Station, Kattupakkam and Madras Veterinary College, Madras, and correlate its possible association with performance traits namely litter size and weight, weaning size and weight. It has been studied extensively in other domestic animals and birds. Wilcox (1963, 1966) reported positive correlation between serum alkaline phosphatase level and egg production. Katholm (1978) observed that high alkaline phosphatase activity was found to be associated with rapid growth rate in cattle. Rao *et al.* (1980) stated that serum alkaline phosphatase level was associated with early maturity, feed efficiency and eggs.

#### Materials and Methods

The materials for the study formed the population of rabbits maintained at Sheep Breeding Research Station, Sandynallah, Livestock Research Station, Kattupakkam and Madras Veterinary College, Madras. Materials and data were collected on the female population of adult female rabbits comprising of 21 New Zealand White, 47 Locals and 48 of their crosses. Three

to five ml of blood from the ear vein of rabbit was received in a sterile test tube previously rinsed with a drop of heparin (5000 IU/ml) before collection of blood. The plasma was separated and stored in deep freeze. Alkaline phosphatase activity was determined by the method described by Bessey *et al.* (1946). The estimation of organic phenolic group liberated by the activity of the enzyme on the substrate formed the basis of this method.

#### Results and Discussion

Mean level of the serum alkaline phosphatase was  $0.5638 \pm 0.023$  in New Zealand White,  $0.0525 \pm 0.022$  in local and  $0.4970 \pm 0.018$  mM/lit in crosses. The serum alkaline phosphatase exhibited bimodal distribution in all three genetic groups (Fig.). It was also observed that a low level was predominant rather than the high level in all the three genetic groups. Among the three locations, rabbits reared in hotter environments namely Livestock Research Station, Kattupakkam and Madras Veterinary College, Madras, were only of low serum alkaline phosphatase type while rabbits bred at Sheep Breeding Research Station, Sandynallah had both high (7) and low levels (35) serum alkaline phosphatase irrespective of the genetic group and the values are given in Table I.

This suggested the existence of distinct high and low levels of serum alkaline phosphatase in the population with a point of differentiation placed at 0.7 mM. Stray reports on serum alkaline phosphatase level are available in New Zealand White rabbits in the literature validating the present findings. Weisbroth *et*

*al.*(1974) reported a mean value of 9.9 King and Armstrong units of Serum alkaline phosphatase enzyme activity in adult New Zealand White rabbits.

The effects of high and low alkaline phosphatase phenotypes were examined for Sheep Breeding Research Station, Sandynallah data and the high type Doe's produced significantly heavier litter at birth. Since high levels were conspicuously absent in the hotter climate it is presumed that high alkaline phosphatase activity might confer the benefit only in the cooler climate. This presumption is further validated by a uniformly moderate though not significant positive correlation of litter weight at birth with alkaline phosphatase activity at Sheep Breed-

ing Research Station, Sandynallah, while the corresponding correlations at plains differed widely in the magnitude and direction. Although no literature is available in rabbits to validate the present findings, the large volume of literature available in other species indicates the positive association of high alkaline phosphatase activity with production traits such as egg yield in poultry (Wilcox, 1963, 1966; Rao *et al.*, 1980) and growth in cattle (Katholm, 1978).

#### Acknowledgement

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**Table I Production traits in rabbit with respect to high and low SAP level**

Traits	High Alkaline Phosphatase group (Mean $\pm$ S.E.)	Low Alkaline Phosphatase group (Mean $\pm$ S.E.)
Litter size at birth	6.12 $\pm$ 0.62 (7)	5.51 $\pm$ 0.28 (35)
Litter weight at birth	370.11 $\pm$ 34.61 <sup>a</sup> (7)	263.85 $\pm$ 15.45 <sup>b</sup> (35)
Litter size at weaning	4.49 $\pm$ 0.56 (7)	4.79 $\pm$ 0.25 (34)
Litter weight at weaning	3045.48 $\pm$ 120.35 (7)	3072.24 $\pm$ 54.61 (34)

\*Significant (P < 0.05)

Figures in parentheses are number of observations

FREQUENCY POLYGON OF SERUM ALKALINE PHOSPHATASE LEVEL IN RABBIT POPULATION

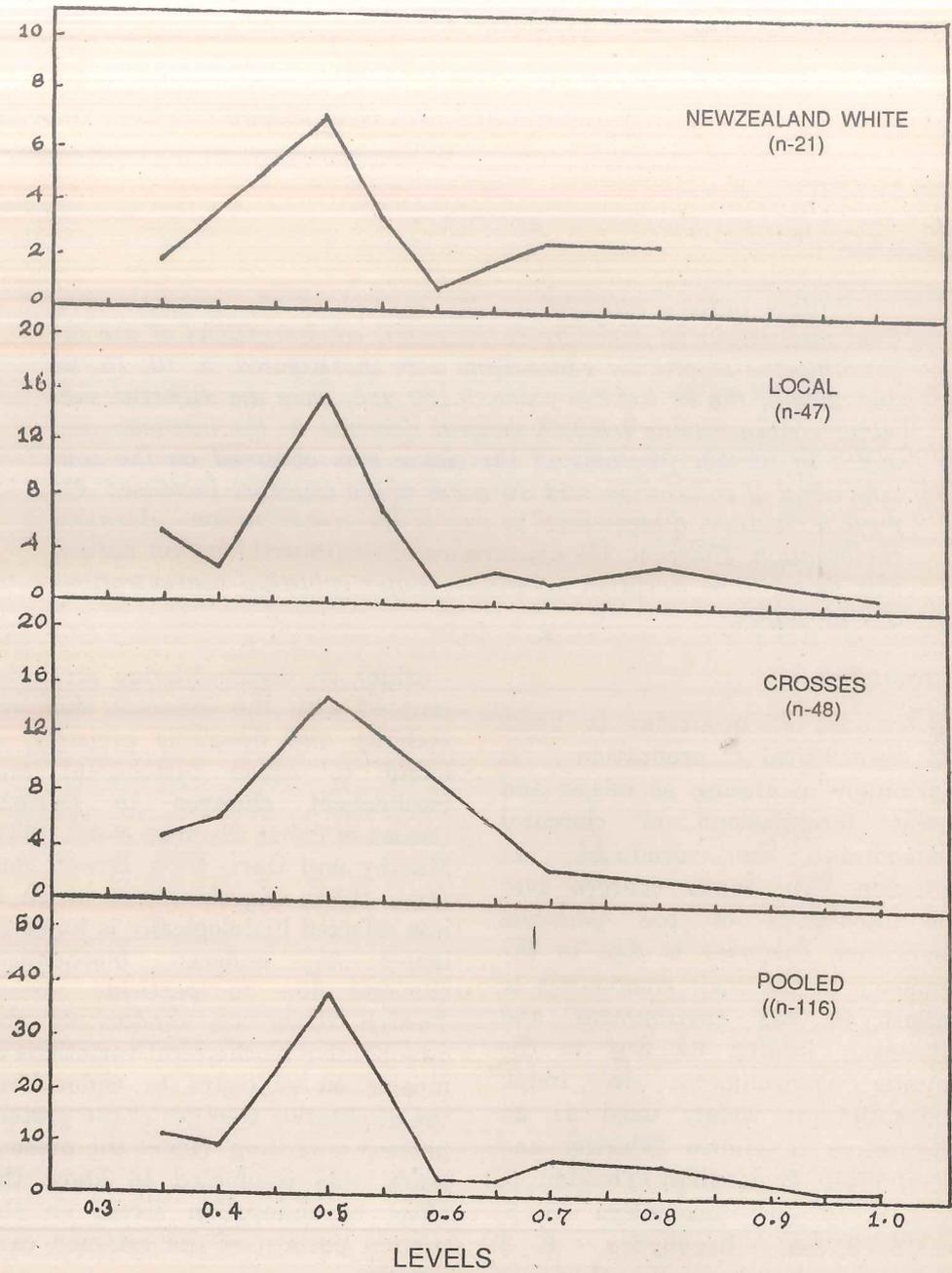


FIGURE-1

## Protein Pattern Variations in the *Cyprinus Carpio* Muscle on Exposure to Endosulfan

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### ABSTRACT

Protein pattern changes in the muscle of the common carp *Cyprinus carpio* on exposure to sublethal concentrations of the organochlorine insecticide endosulfan were investigated. 5, 10, 15, 20 and 25% of the 96 h LC<sub>50</sub> value, 5.199 ppb, were the different sublethal concentrations tried. A general decrease in the intensity and width of protein fractions of the tissue was observed as the concentration of endosulfan and duration of the exposure increased. No protein fractions disappeared in any of the concentration - duration combination. However, the appearance of additional fraction noticed was found to be duration dependent under sublethal concentrations of endosulfan.

### Introduction

The use of pesticides to boost agricultural production is alarmingly escalating as newer and newer formulations of chemical compounds are produced as pesticides. The major concern over the escalation of the pesticide application turnover is due to the tendency of the toxic compounds to persist in the environment and ultimately finding its way to the aquatic environment. In India, endosulfan is widely used as an alternative to endrin (Murthy and Devi, 1982). Endosulfan (Thiodan: 6, 7, 8, 9, 10, 10 - haxachloro - 1, 5, 5a, 6, 9, 9a - hexahydro - 6, 9 methano -2, 4, 3 benzodioxathiepin 3

- oxide), an organochlorine pesticide, coupled with the inherent chemical stability and lipophilic property, is found to cause histological and biochemical changes in various tissues of fishes (Swarup *et al.*, 1981; Murthy and Devi, 1982; Drewtt and Abel, 1983). Muscle tissue which is less affected histologically is however found to undergo biochemical changes due to pesticide stress. Though there are studies on the quantitative biochemical variations of muscle on exposure to endosulfan, the qualitative changes of the protein pattern is lacking. Hence the present study was conducted to know the effect of endosulfan stress on the protein pattern of the common carp muscle.

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### Materials and Methods

Fry ( $2.3 \pm 0.5\text{g}$ ) of *Cyprinus Carpio* var. *communis* obtained from Karnataka State Fisheries Farm at Gajanur were acclimated for 7 days in plastic pools during when they were fed with standard pelleted feed (Varghese *et al.*, 1976). Endosulfan 35 EC marketed by Bharat Pulverising Mills Pvt. Ltd., Bombay as Hexasulfan 35% EC was used for the experiment. The 96 h  $\text{LC}_{50}$  value (APHA, 1985) of endosulfan to *Cyprinus carpio* was found to be 5.199 ppb with a 95% confidence interval ranging between 4.69 and 5.76ppb. The fish were exposed to sublethal concentrations of 5, 10, 15, 20 and 25% of the 96 h  $\text{LC}_{50}$  value for 16 days besides control in glass troughs of 20 l capacity. All tests were performed in duplicate at room temperature ( $28.5 \pm 1^\circ\text{C}$ ) in filtered and aerated water. The pH of water was  $7.5 \pm 0.1$  and hardness  $52 \pm 2$  ppm (as  $\text{CaCO}_3$ ).

Fish were fed on alternate days and the medium was renewed once in 24h. At every 4 day interval, samples of 4 fish each were taken from the different sublethal concentrations of endosulfan and from control upto 16th day. After decapitating the fish, muscle was taken from the region between the dorsal fin and lateral line on the left side of the fish. The tissue was immediately subjected to disc electrophoresis (Davis, 1964) using 7% polyacrylamide gel as the separating medium. A current of 3 mA per tube was applied. Gels were stained with aqueous Coomassie brilliant blue after fixing the gels in 10% trichloroacetic acid. The protein bands, depending on their mobility were grouped as upper (cathodal),

middle and lower (anodal) as suggested by Maguire and Fielder (1975) and as dense, medium and light depending on their staining intensities (Khan *et al.*, 1977). Protein patterns are schematically represented along with control for different periods of exposure in various sublethal concentrations of endosulfan.

### Results and Discussion

The results obtained are presented in the figures 1 - 4. The muscle protein was fractionated into 13 components in the control fish. The bands 4, 7 and 9 stained lightly while 5, 8 and 12 were of medium intensity and rest 7 bands were stained densely. The protein pattern remained the same in the control fish on all the sampling period. A general decrease in the levels of the bands was noticed in most of the bands as the duration and concentration of the endosulfan exposure were increased. The fractions, 1, 4, 7, 9 and 13 did not show any staining intensity variation in any of the concentration - duration combination tried. One high molecular protein fraction (no.3) and 2 low molecular protein fractions (nos. 11 and 12) showed a decline in the staining intensity from the 1st sampling period till the last in the higher concentration. One densely stained middle fraction (no.6) which did not show any intensity change in 4th and 8th day exposure period declined in its intensity in the 2 subsequent exposure periods in 15,20 and 25% concentration.

Appearance of an additional protein fraction (3a) in the 8th, 12th and 16th day sampling period in all the concentrations except 5% and

regaining of the declined intensity of the bands 2, 5 and 10 in the last 2 exposure periods in the 20 and 25% concentration were also observed.

Pesticide stress has been reported to cause hyperglycemic condition in fish (Ito and Murata 1980); Srivastava and Singh, 1981; Mishra and Srivastava, 1984) indicated by a considerable rise in blood and serum glucose levels accompanied by a reduction in the tissue glycogen levels (Corbett, 1974). The general decrease in the levels of the protein fractions and reduction of the staining intensity of certain bands noticed may be due to the degradation of the tissue proteins to amino acids which in turn fed into tricarboxylic acid cycle (TCA) through amino transferase pathway (Harper *et al.*, 1977) to cope up with high energy demands for the detoxification and elimination of the pesticide like endosulfan (Swarup *et al.*, 1981) during the state of depleted glycogen reserves induced by stress (Ramalingam and Ramalingam 1982; Rao *et al.*, 1987).

The reduction in the staining intensity noticed in the higher concentration may be attributed to the impairment of protein synthesis due to the pesticide stress (Rao *et al.*, 1987; Reddy 1987). Further, Um-minger (1970) opined that while carbohydrates represent the principal

and immediate energy precursors for fishes exposed to stress, proteins are the energy sources to spare during chronic stress. The elevation in protein synthesis by the fish after acclimation period to the pesticide induced toxicity (Sahib *et al.*, 1984) may be the cause for the band 2, 5 and 10 have regained the intensity in the 12th day and 16th day exposure periods. Inferences for the additional fraction (3a) could not be drawn with the available literature. However, the augmented proteolysis in the tissue giving rise to an additional fraction due to the splitting up of high molecular weight protein may be reckoned with. Of the different changes observed, the appearance of additional fraction (3a) in the last 3 sampling periods and reduction in the staining intensity of a densely stained band (No.6) in the last 2 sampling periods implies that duration is more important while exposure to sublethal concentrations (Ramalingam and Ramalingam, 1982) of endosulfan.

#### Acknowledgement

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Fig. 1 FOURTH DAY

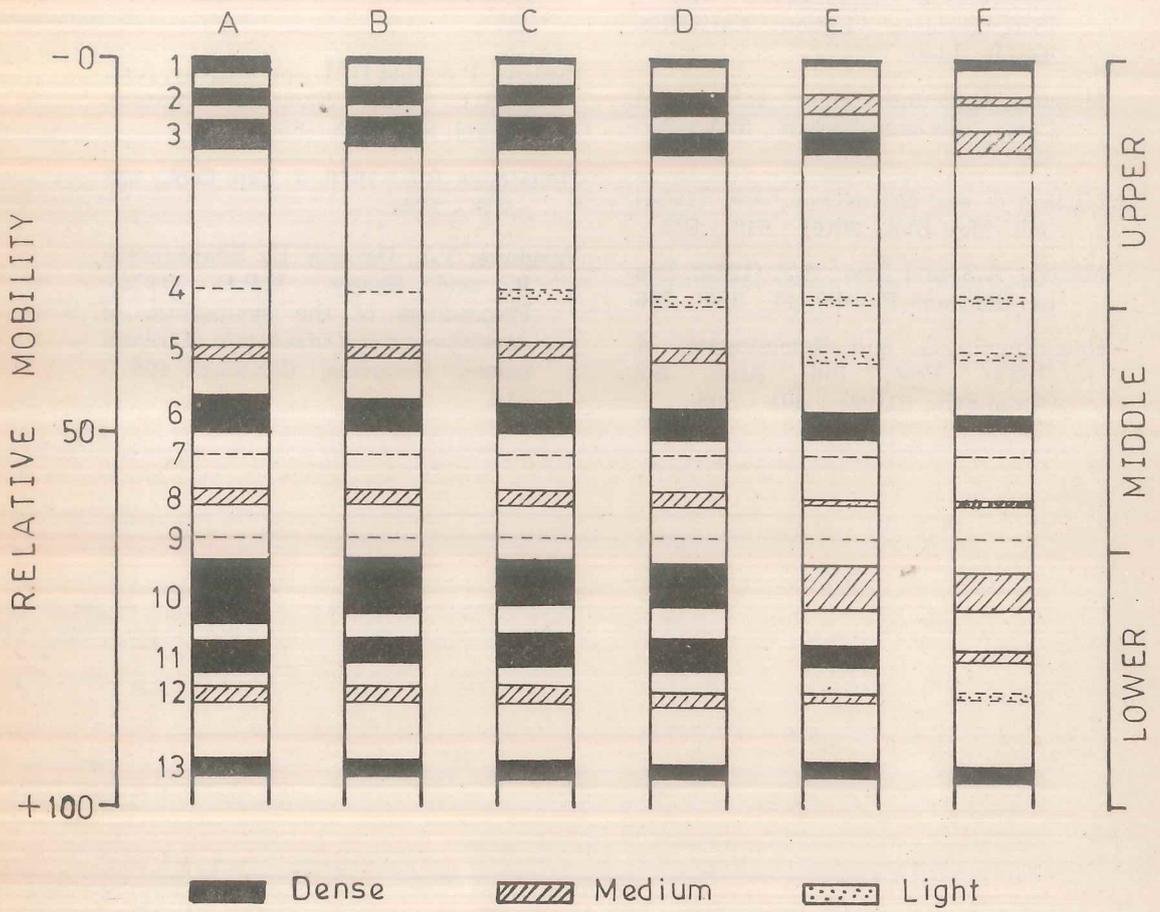


Fig. 2 EIGHTH DAY

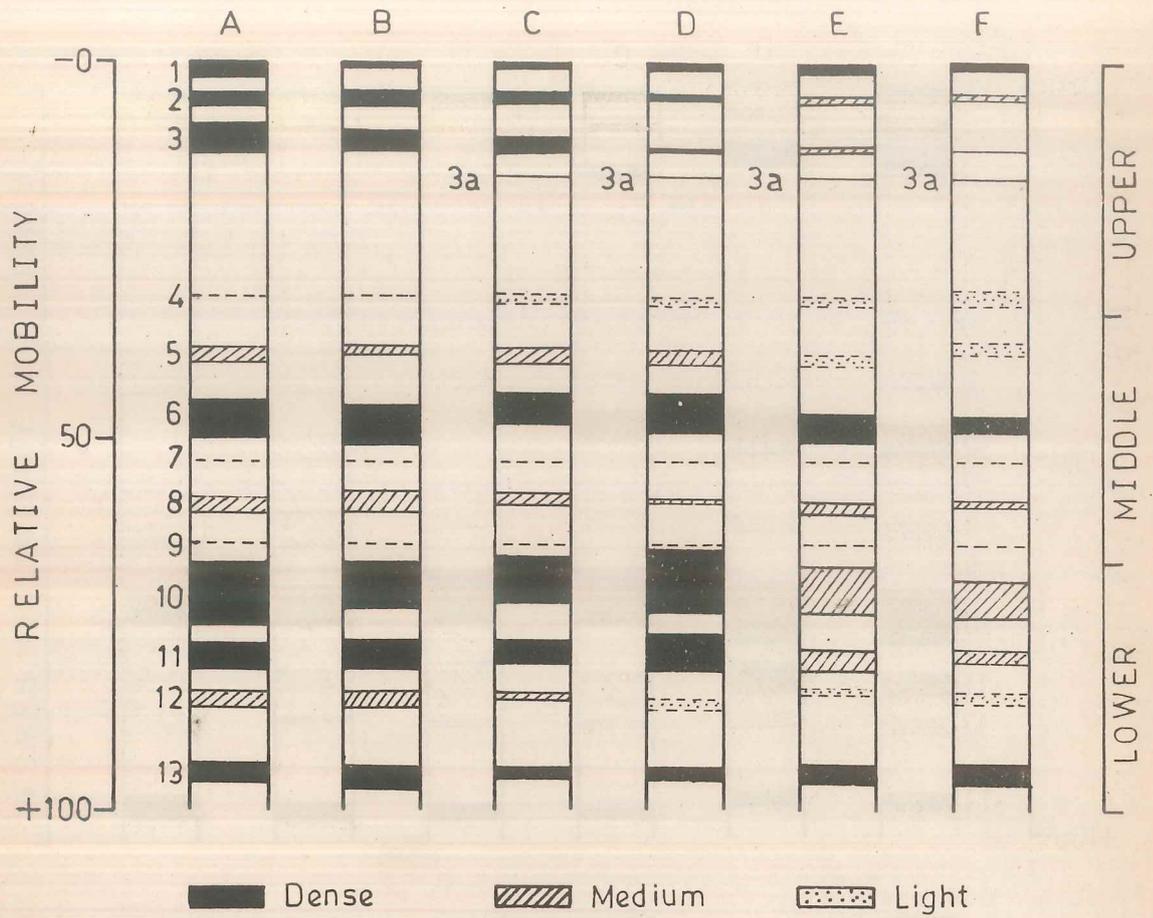


Fig. 3 TWELFTH DAY

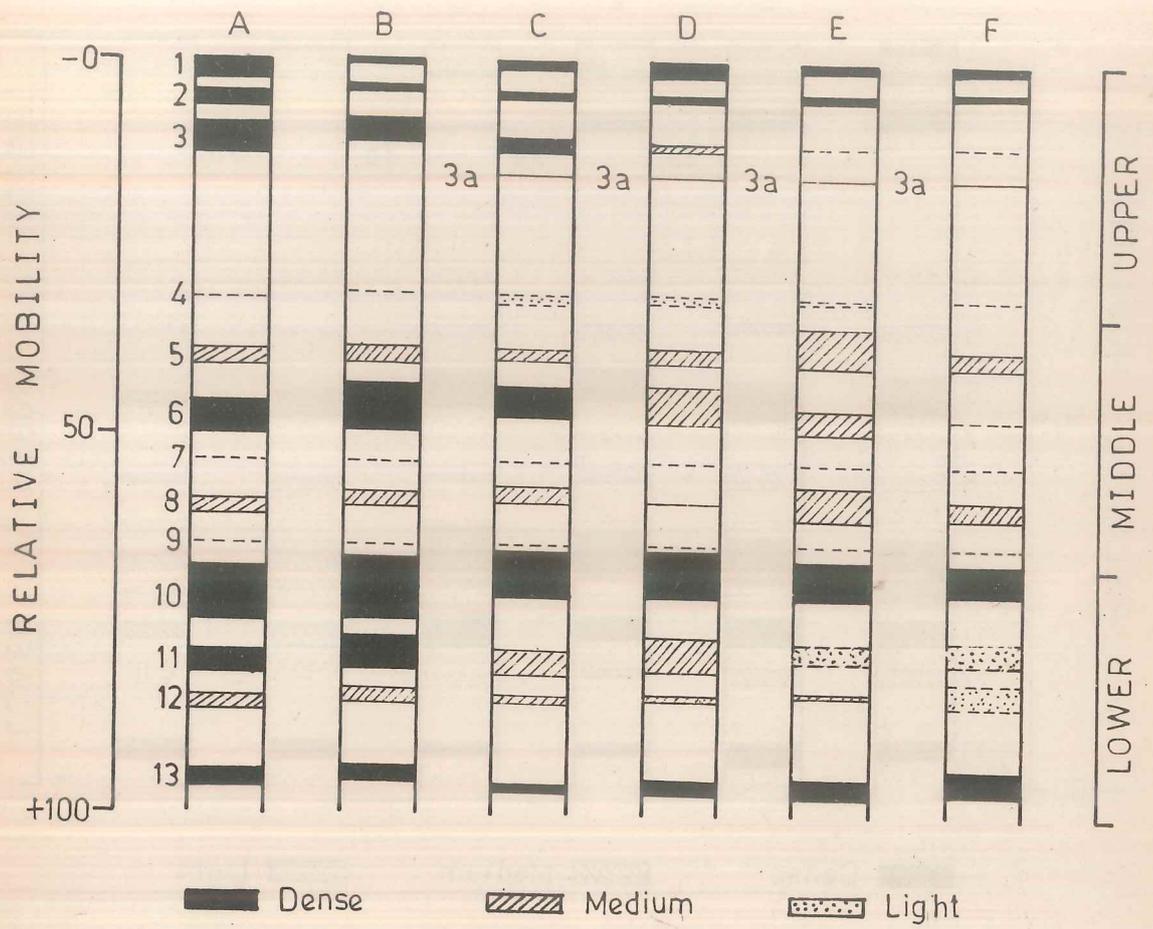
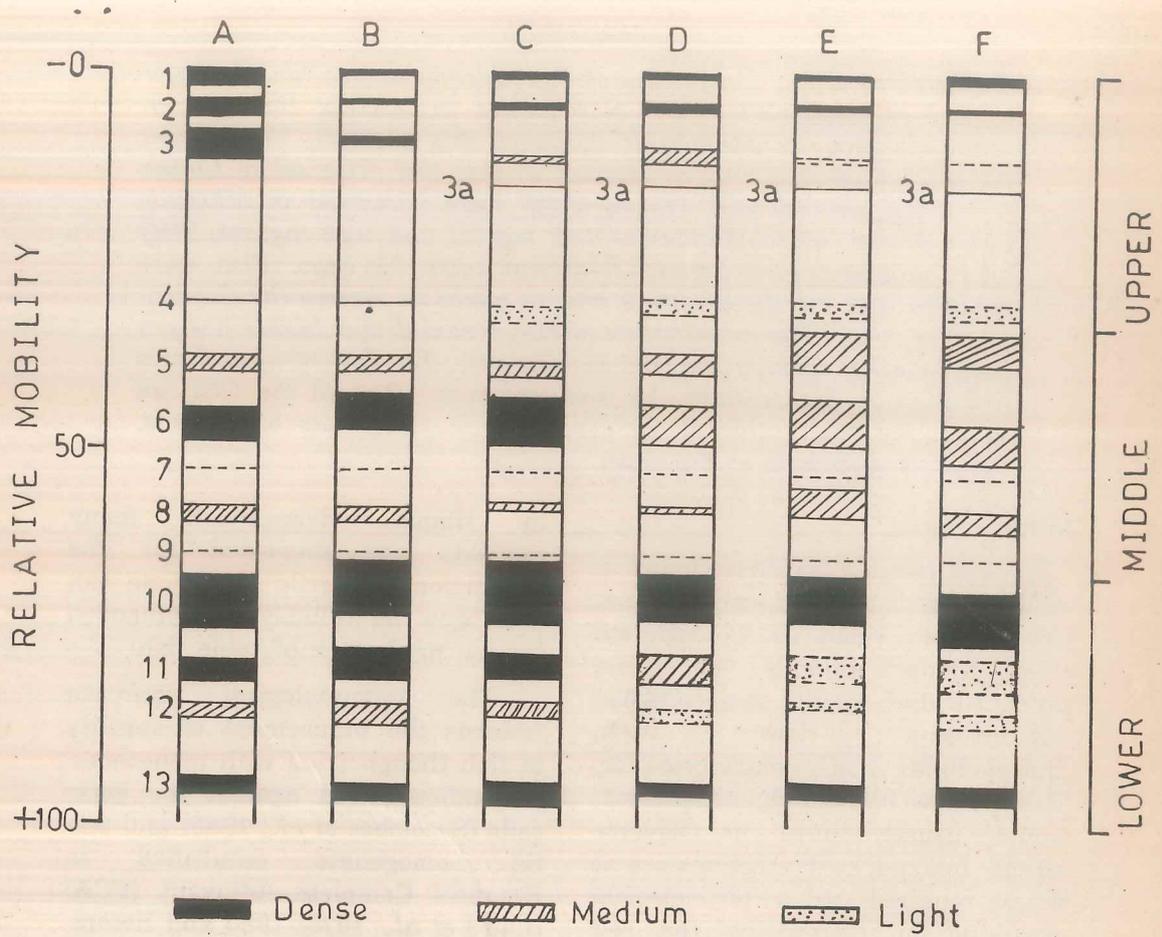


Fig. 4 SIXTEENTH DAY



## Studies on the Autoimmune Responses in Tilapia

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### ABSTRACT

Understanding and control of reproduction in fish have a major impact on aquaculture. The reproductive engineering through immunotechnology is one way to approach this problem. The present paper highlights a maiden attempt in this line. Two set of tilapia fry of 3.5 cm (1g) and 4.0 cm (1.3g) were immersed in allogenic ovary protein concentrations of 0.52 mg/ml and 0.26 mg/ml. They were subjected to a second treatment after 15 days. Fish were sampled and sacrificed after 9 and 13 weeks to observe the development of ovary. The examination of GSI revealed that higher antigen concentration reduced the GSI considerably. The GSI of control was found to be higher than the first treatment. Besides the GSI, an interesting observation of fat deposition in males has been made, which is discussed in the paper.

### Introduction

Automimmune responses found in normal healthy animals are not always deleterious. It is reported that many animals do have autoantibodies against some common autoantigens like DNA, phospholipids, RBC, lymphocytes etc. The reaction between these however, would usually have no adverse effects. Instead, such reactions are at times required as a physiological necessity for recognising the self antigen, regulating antibody responses, removal of senescent cells etc. Though autoimmunity studies in animals other than fish is frequent, in fish they are fragmentary. The present paper highlights the attempts made in the application of autoimmunity towards the control of maturation/sterilization of female sex

in tilapia. Eventhough many methods are suggested for the production of sterile fish, none can even now be reliably recommended for the production of table fish.

The immunological approach towards the inducement of sterility in fish though tried with monoclonal antibodies raised against the germ cells (Secombes *et al.*, 1986) and testis homogenate emulsified in Freund's Complete Adjuvant (FCA) (Laird *et al.*, 1978, 1980 and Secombes *et al.*, 1982) respectively in trout and salmonids, all came out with limited success. In this experiment, the scope of using either fish ovary proteins or hen's egg yolk proteins as antigens to induce an autoimmune response to suppress the development of ovarian tissue is investigated.

## Materials and Methods

### Animals

Different sets of tilapia young ones were collected from the farm ponds measuring 3.2 cm (0.8g), 3.5 cm (1g) and 4.0 cm (1.3g). They were acclimated to lab conditions before start of the experiment. The animals were maintained in cement cisterns (3 ft dia x 1 1/2 ft) at room temperature ( $29 \pm 1^\circ$ ).

### Antigens

Two types of antigens were tried in the experiment. The ovaries of an adult fish were collected, macerated and after centrifugation, the decanted solution was diluted to give a final concentration of 0.52 mg protein/ml as antigen for the first set of experiment. One part of the above solution was further diluted to 0.26 mg. protein/ml and used as antigen for the second set. For the 3rd set of experiment, egg yolk protein diluted 5,000 times was used.

### Antigen Administration

The antigen was administered to the 3 groups of fish by immersion treatment for 90 minutes duration. The treatments were given between 1400 - 1600 hrs. All the groups were given a second booster dose identical to the primary dose after 15 days.

The details of the experiments are given in the table No.1. The mortality of the fish were noted and dead ones were removed daily.

### GSI (Gonado Somatic Index)

Fish were sampled at the end of 9 weeks and later, at the end of the experiment at 13 weeks. The fish were, sacrificed and GSI of the females was calculated. The fish were depurated for 12 h before sacrifice to eliminate the error in GSI due to excessive feeding.

## Results and Discussion

The results are given in the table No.2. The GSI was plotted against the fish length (fig. 1 & 2). The mortality percentage varied from 36.36 to 57.7% while it was 20 % in control. However, the group received low concentration (Group II) showed very high GSI values. Yolk administered set (Group III) also showed low GSI values comparable to that of group I. A high amount of fat deposition on the gut and body tissues was noticed in the males. This may be due to the effect of hormones associated with crude ovary extract which may also be the reason for the enhanced GSI values noticed in the group II.

The mortality rate was comparably high in all the treatments. Whether the treatments bring about

Table I : No. of experimental animals and antigen concentrations

Group	I	II	III	control
No. of fishes	22	30	45	20
Antigen and Concentration	Ovary protein 0.52 mg/ml	Ovary protein 0.26 mg/ml	Egg Yolk dilution 5,000 times	Control
No. of females sacrificed	6	8	9	6
Males recovered	8	10	10	10

**Table 2 Average values of length, weight and GSI of female fishes and mortality percentage in each group**

Group (No. of female fishes sacrificed)	Length (mm)	Weight (g)	GSI	Percentage mortality
I (6)	58.50	3.0757	0.6589*	36.36
II (8)	59.50	3.5068	2.4574*	41.93
III (9)	45.89	1.5002	0.7911*	57.70
Control (6)	46.33	1.4131	1.7471	20.00

\* Significant at  $p < 0.05$

any lesions, granulomatous formation around the gonad or necrosis leading to death could not be ascertained. Secombes *et al.*, (1985) reported in the rainbow trout inflammation of tissue, necrotic fragments and grossly thickened ovaries when the fish were injected with testis homogenate.

Though the present study could not reveal the conclusive evidence for the lowering of GSI in the 1st test group, there is a possibility that autoimmune reaction which may develop following the immersion treatment with allogenic ovary material may cause the fish turning immune against the germ cells once they differentiate and begin vitellogenesis.

Experiments are on to delineate the effect of egg yolk, fish vitellogenin, ovary hormones, and related substances towards the mounting of an autoimmune response in tilapia against the differentiating and developing germ cells.

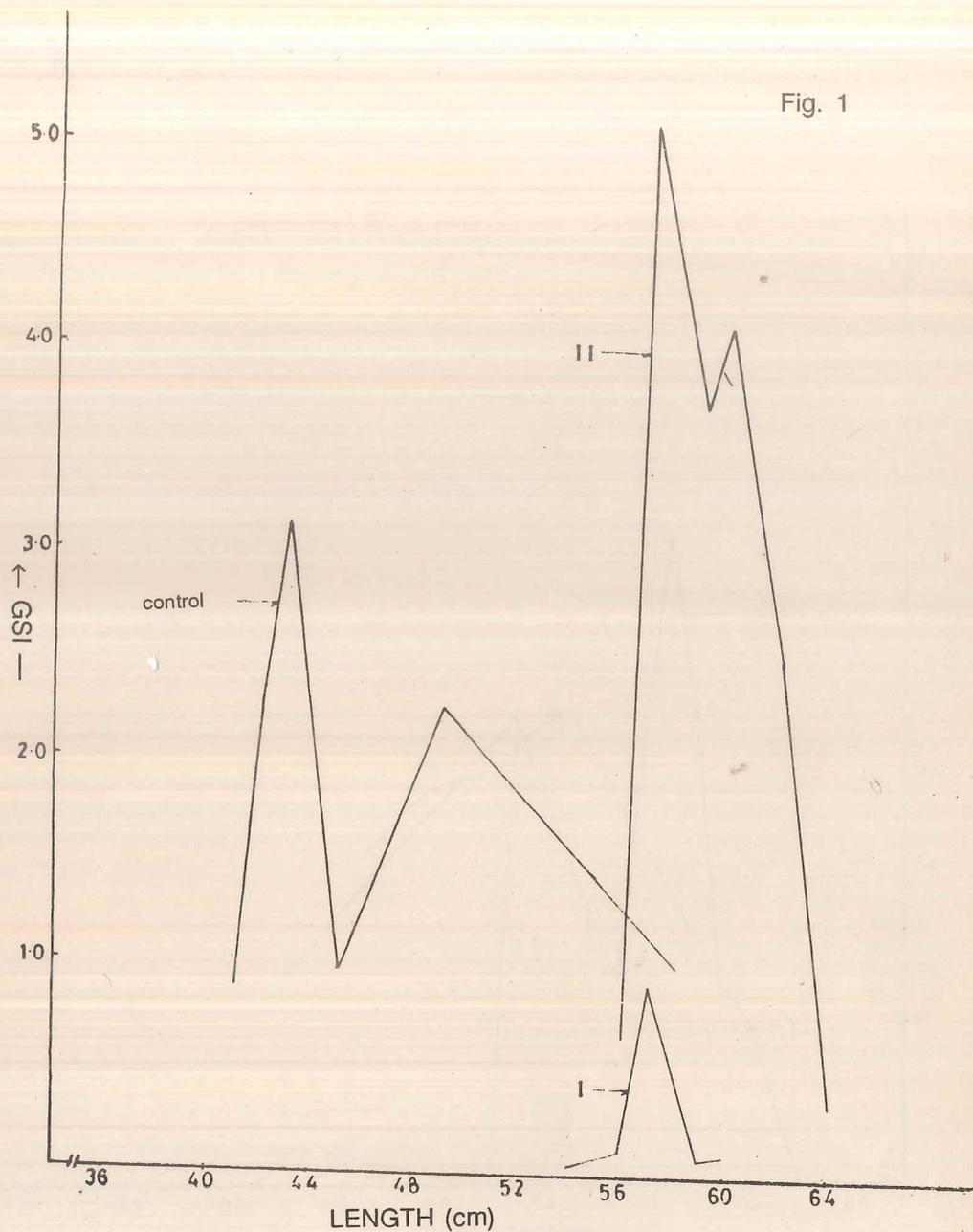
#### Acknowledgements

Authors are thankful to Dr. M. Devaraj, Dean, Fisheries College and Dr. V.Sundararaj, Professor and Head, Department of Aquaculture for the facilities provided for the experiment and Dr. N. Sukumaran for the interest and encouragement for the study.

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Fig. 1



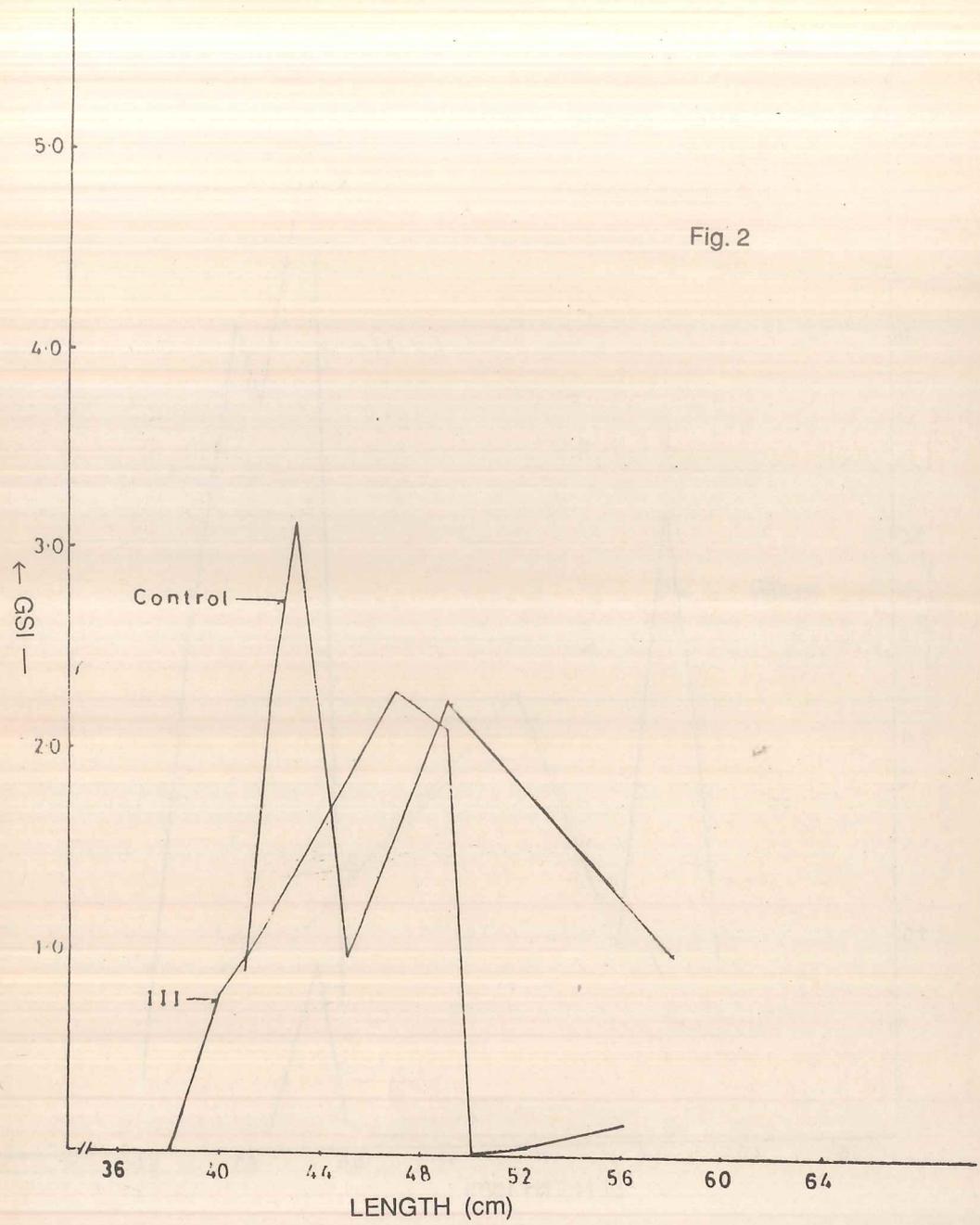


Fig. 2

## Antibiotics as Growth Promoters in Common CARP *Cyprinus carpio* Fry

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### ABSTRACT

*Antibiotics incorporated fish feeds are known to enhance growth and survival rate of carp fry. In the present study, the effect of tetracycline and chloromycetin added at 10, 25, 50, 75 and 100 mg/kg levels to the feed of common carp fry on growth and survival was followed for 30 days with a feeding ration at 10% of body weight/day. Among the two different antibiotics used, tetracycline (100 mg/kg) incorporated diet gave better growth and survival. Chloromycetin incorporated feed did not improve growth or survival significantly. The findings of the laboratory experiment, when tested in the field showed that tetracycline incorporated feed at 100 mg/kg significantly improved growth rate. By using antibiotics incorporated feed, the nursery rearing period could be subsequently reduced from 28 days to 21 days and this paves way for production of more batches of seeds during breeding season. Further, incidence of disease like blackspot that occurs in rearing phase of carp fry could also be controlled. Owing to the significant improvement in growth rate, survival rate and disease control, the incorporation of antibiotics in feed (at 100 mg/kg of tetracycline) is recommended for carp fry rearing.*

### Introduction

Malnutrition and bacterial attack result in poor growth and heavy loss of fishes during larval rearing. The growth of common carp declines sharply when the component of natural food in the daily ration is less than 17 - 20% (Sukhover Khov, 1967). To increase the growth rate, reduce the phase of nursery growth and also to have more batches of seeds reared in the breeding season, either the natural fish food production should be boosted through rational fertilisation or artificial feeding must be done. The use of non-hormonal growth promoters like

vitamins, amino acids such as Arginine and Lysine, micronutrients such as cobalt chloride, and yeast, and antibiotics such as tetracycline, terramycin, aureomycin, Virginiamycin, and chloromycetin have been found to enhance growth of fishes (Sen and Chatterjee, 1972). Apart from these, hormones like 17-methyl testosterone, thyroxin were also found to promote growth in fishes. But the higher dose and prolonged application of methyl testosterone is known to induce some structural aberrations in the testes accompanied by thickening of epidermis in certain species.

Androgens are probably actual anabolic hormones as they are known to affect the muscles directly. Moreover, in the case of steroids as growth promoters, the somatic indices and apparent general health are reported to be greater (Lone and Matty, 1980).

Owing to these harmful residual effects, the hormones have now been banned and the true substitute for hormones, which have neither side effects nor residual effects are the non-hormonal growth promoters. Based on the earlier report of Moore *et al.* (1946) the addition of antibiotics were recognised as growth promoting agents. However, initial attempts of testing antibiotics as growth promoting agents did not yield successful results. Later Mitia and Ghosh (1967) could evidence enhanced growth in fish while using some antibiotics and antibacterial agents.

In *Cyprinus carpio* with the addition of antibiotics such as Virginiamycin and terramycin to the diet, increased growth was achieved by Ahmad and Matty (1989).

The present study elucidates the growth enhancing potential of two antibiotics namely tetracycline and chloromycetin incorporated in the feed.

#### Materials and Methods

Fifteen days old fry of common carp, (*Cyprinus carpio*), were obtained from a homogenous healthy stock and acclimated to the lab conditions for a week. The fry were stocked in 15 l capacity plastic tanks at the rate of one fish/l in each tank. High protein diet with 40% protein was used and five different con-

centrations (viz. 10 mg, 25 mg, 50 mg, 75 mg and 100 mg/kg of feed) of tetracycline and chloromycetin were added separately and a control feed was prepared without any antibiotic incorporation.

For each concentration, three replicates were maintained and controls were run simultaneously. The mean initial weight of fry of each treatment was taken, and the fishes were fed at the rate of 7% of their body weight for 30 days. The sampling was done once in a week and feed ration was updated accordingly. The data on weight and survival of the fry recorded in each treatment were pooled and analysed and the results compared with the control. After the 30 days of the experiment, the mean final weight of fishes in each treatment was observed. Mean weight increment, percentage weight increment, Food Conversion Ratio (FCR), Specific Growth Rate (SGR) and survival rate were calculated and the data were subjected to statistical analysis. Finally, the best result obtained from laboratory experiment was tested in the field to assess the potency for practical use.

#### Results and Discussion

Tetracycline incorporated at concentrations of 10, 25, 50, 75 and 100 mg/kg in high protein diet (40% protein) gave mean weight increment of 105.33, 103.33, 103.23, 107.00 and 132.67 mg respectively whereas the mean weight increment in control group was 56.2 mg. The percentage increment weight was found to be better in the case of tetracycline used at 100 mg/kg concentration (236.03), when compared to other concentrations. In enterocycline incorporated

**Table 1. Effect of Tetracycline on growth and survival of common carp (*Cyprinus carpio*) fry**

Tetracycline level (mg/kg)	Mean initial weight (mg)	Mean final weight (mg)	Mean weight increment (mg)	Percentage increment (%)	Mean survival rate	FCR	SGR
0	172.00	228.20	56.20	100.00	92.00	6.25	1.87
10	136.67	242.00	105.33	187.42	83.33	3.33	3.51
25	170.00	273.33	103.33	186.83	76.66	3.40	3.44
50	146.66	250.00	103.33	183.86	76.66	3.40	3.44
75	155.00	262.00	107.00	190.39	93.33	3.28	3.56
100	190.00	322.67	132.67	236.07	96.66	2.65	4.42

feed, the mean weight increment over the control was only 6.66% (Sen and Chatterjee, 1979). The survival rate was also found to be better at 100 mg concentration and 25 mg concentration (96.66%) followed by others. The FCR values calculated showed a better value of 2.64 for 100 mg concentrations when compared to 6.25 for the control. For the other concentrations, the FCR values were above 3.25, similarly the SGR also showed better results with 100 mg/kg treatment (4.42 mg/day). The results shown in table I clearly indicate that

tetracycline incorporated at 100mg/kg can significantly increase the growth and survival of common carp fry.

Chloromycetin incorporated at 10, 25, 50, 75 and 100 mg/kg concentrations were observed to have mean weight increment of 124.33, 143.67, 140.66, 136.35 and 122.01 mg respectively, where the control group showed a mean weight increment of only 74.8 mg as shown in table 2. The percentage weight increment over control was higher at 25 mg/kg concentration of chloromycetin.

**Table 2: Effect of chloromycetin on the growth and survival of common carp (*Cyprinus carpio*) fry**

Chloromycetin level (mg/kg)	Mean initial weight (mg)	Mean final weight (mg)	Mean weight increment (mg)	Percentage increment (%)	Mean survival rate	FCR	SGR
0	75.80	154.20	78.40	100.00	88.00	2.90	3.61
10	75.00	199.33	124.33	158.58	86.66	2.05	4.14
25	81.33	225.00	143.67	183.25	93.33	1.77	4.79
50	73.34	214.00	140.66	179.41	86.66	1.81	4.68
75	77.32	213.67	136.35	173.92	70.00	1.87	4.54
100	76.66	198.67	122.01	155.63	70.00	2.09	4.07

Similarly the survival rate also was found to be better at that concentration. The FCR values calculated showed a minimum of 1.77 at 25mg/kg when compared to 2.90 in the case of control. The SGR value was found to be 4.79 mg/day at that concentration showing the better performance of chloromycetin at 25 mg/kg concentration than others.

The present study confirms the positive role of the antibiotics as growth promoters in common carp fry as reported by many authors (Sreenivasan, 1963; Sukhover khov, 1967; Mitra and Ghosh, 1967; Sharma and Kulshrestha, 1973, 1973; Mahajan and Sharma, 1976; Sen and Chatterjee, 1976; Ahmed and Matty, 1989). The antibiotics incorporated in low protein feed (25.30%) did not give significant effect on growth (Ahmed and Matty, 1989). In the present study, the 40% protein (rich) feed, incorporated with antibiotics resulted in protein (rich) feed, incorporated with antibiotics resulted in protein sparing effect for better growth of carps.

The apparent general health of fishes was not effected both with tetracycline and chloromycetin treatment. Whereas, in the case of steroids as growth promoters, the somatic indices and apparent general health were reported to be greatly ef-

ected (Lone and Matty, 1980). In the present study, the use of tetracycline at 100 mg/kg and chloromycetin at 25 mg/kg were found to have better effect on the growth and survival of common carp fry. Considering the maximum percentage weight increment over control and the increased survival rate, tetracycline at 100 mg/kg concentration was tried at farm level to confirm the laboratory results. From table 3, it is clear that, the average weight increment of the treated fish was 180.15 mg in 30 days of the experiment when compared to 140.75 mg for the control. There was also a considerable increase in the survival rate due to tetracycline treatment (78%) than the control (52%). It is also worth while to note that the treated fry attained the stockable size in 21 days when compared to the control fish that attained the same size in 30 days. This has much relevance in nursery rearing as the nursery phase is complete in 21 days than the usual period of 28 - 30 days, thus making it possible for more crops (or) batches during the fish feeding season.

### Conclusion

From the present study, it could be concluded that the use of antibiotic in the feed of common carp fry has significant effect on growth

Table 3. On farm experiment results showing the effect of tetracycline at 100 mg/kg feed on the growth and survival of common carp (*Cyprinus carpio*) fry .

Tetracycline level (mg/kg)	Mean initial (mg)	Mean final weight (mg)	Weight increment (mg)	Percentage increment (%)	Mean survival rate
100	4.00	184.20	180.20	127.99	78
0	4.00	144.80	140.80	100.00	52

and survival rate. Tetracycline at 100 mg/kg increased growth and survival rate of common carp fry. Application of antibiotics added to high protein feeds may be followed in nurseries for fast growth good survival,

many batches and more production of fish seeds. In Tamil Nadu where there is a great scarcity for fish seeds, the adoption of the present finding will solve the fish seed production considerably.

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## The Effect of 17 Alpha Methyl Testosterone on Growth in Black Molly, *Poecilia sphenops*

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### ABSTRACT

To assess the relative growth promoting efficiency of an androgenic hormone, 17 Alpha methyl testosterone on black molly, *Poecilia sphenops*, diets of different concentrations viz. 25, 35 & 50 ppm hormone incorporated and a control diet were fed to the newly born black mollies. The results indicated significant influence of the hormone 17 Alpha - MT on the feeding rate and the growth rate and food conversion efficiency of black molly. The maximum growth rate ( $5.38 \pm 0.36$  mg/day). Was obtained in 35 ppm concentration followed by 25 ppm ( $4.63 \pm 0.53$  mg/day) and at 50 ppm concentration, the growth performance was the poorest ( $2.24 \pm 0.38$  mg/day). The food conversion efficiency was also found to be the maximum at 35 ppm ( $62.2 \pm 3.62\%$ ) followed by 25 ppm. The increased feed intake coupled with better food conversion at 35 ppm clearly indicate the role of the hormone as appetite stimulant and growth promotor. The results are discussed in the light of the healthy maintenance of the stock of black molly.

### Introduction

Considerable information has been accumulated on the enhanced growth in fishes treated with anabolic steroid hormones (Mc Bride and Fagerlund, 1973; Fagerlund and Mc Bride, 1977; Yamazaki, 1976; Gurrero, 1976; Fagerlund *et al.*, 1978;1979; Lone and Matty, 1980; 1983; Schrack and Fowler, 1902; Mc Bride *et al.*, 1982; Fagerlund *et al.*, 1983; Nirmala and Pandian, 1983; Manzoor Ali, 1985). While there are studies on the enhancement of growth in fishes by the administration of 17 Alphamethyl testosterone (Yamazaki, 1976; Matty and Lone, 1989;

Manzoor Ali, 1985), studies on the combined influence of this hormone on the food intake and conversion efficiency of fish along with growth are limited (Pandian and Nirmala, 1983 and Arul, 1985). Hence the study on the effect of the steroid hormone, 17 alphamethyl testosterone on the growth parameters in a commonly available and commercially viable ornamental fish was undertaken in view of reducing the cost of production of this commercially important fish.

### Materials and Methods

The youngones of black molly of same brood were acclimated in the

laboratory in well aerated water prior to experimentation. The ambient temperature was kept at  $30 \pm 2^\circ\text{C}$  and the oxygen content of water was always maintained above 50% of air situation. The growth studies were conducted in plastic basins of uniform dimension and colour, of 15 litres capacity. Six numbers of fishes were released in 10 litres of water in each basin.

The steroid hormone used in this study was 17 alphamethyl testosterone (Organon Laboratories Ltd., London), which had been incorporated in feed at different concentration as suggested by Manzoor Ali (1985). The formulated feed were made into pellets and stored in a desicator. The experimental fishes were fed with the pelleted feed having three doses of hormone namely 25.0, 35.0 and 50.0 mg/kg feed. Replication for each treatments along with a control were also maintained. The control fish were fed with the

same formulated pellet without hormone. The growth study was continued for 40 days. The growth of fish was observed at 10 days interval. The data on feeding rate, feed conversion, feed efficiency, conversion ratio, conversion efficiency and growth rate were estimated and analysed statistically.

### Results

The treatment - wise mean values of feeding rate, food conversion and efficiency, conversion ratio and conversion efficiency and growth rate of black molly are given in Table 1. It is evident from Table 1 that the feeding rate showed a significant increase over control, whereas feed conversion values were generally lower in treated fish than in the control, indicating better feed conversion in the hormone fed fishes. It is also observed that the conversion rate and efficiency were also better in fish administered with hormone. To

Table 1 Effect of 17 Alpha MT on FCR and Growth rate of *Poecilia sphenops*

Parameter	Control	25ppm	35ppm	50ppm
Feeding rate (mg/day)	$30.8 \pm 2.87$	$48.3 \pm 2.72$	$49.4 \pm 3.15$	$28.6 \pm 3.63$
Feed conversion	$2.01 \pm 0.32$	$1.60 \pm 0.24$	$1.58 \pm 0.14$	$2.23 \pm 0.32$
Feed efficiency	$0.50 \pm 0.038$	$0.62 \pm 0.076$	$0.63 \pm 0.09$	$0.46 \pm 0.06$
Conversion efficiency	$29.5 \pm 3.71$	$59.7 \pm 5.43$	$62.2 \pm 3.62$	$26.3 \pm 2.69$
Growth rate (mg/day)	$2.38 \pm 0.47$	$4.63 \pm 0.53$	$5.38 \pm 0.36$	$2.24 \pm 0.38$

Table 2 The regression equations for the growthrate of *Poecilia sphenops* fed with normal 17 Alpha MT incorporated diets

17 Alpha MT Concentrations	Regression lines
control	$Y = 0.142 + 0.00268 X$
25 ppm	$Y = 0.156 + 0.00429 X$
35 ppm	$Y = 0.153 + 0.00456 X$
50 ppm	$Y = 0.152 + 0.00231 X$

depict the growth rate of the fish at different doses of hormone regression lines are fitted as per the equations given in Table 2. It is clear that the hormone - fed fish showed better growth, as indicated by the increased slope value. It is also evident from flesh production that the magnitude of wastage of food was maximum in control (66.7%), while in all hormone - fed fishes the values ranged from 39.3 to 45.8% suggesting better feed utilization in hormone treated fishes.

### Discussion

A perusal of literature revealed that 17 alphamethyl testosterone induces appetite and food consumption rate in gold fish, *Carassies auratus* (Yamazaki, 1976), *Oncorhynchus kisutch* (Fagerlund *et al.*, 1978, 1979), whereas it was found to depress appetite and feeding rate in *Salmo gairdneri* (Ghittino, 1970; Bulkley, 1972). Nirmala and Pandian (1983) observed a that the feeding rate in *Channa striatus* increases when the fish was injected with 10.0 ppm of methyl testosterone. It is likely that in molly the feeding rate was about 45% higher, suggesting that methyl testosterone could induce the appetite of the ornamental fish resulting in more food intake as observed in gold fish (Yomezaki, 1976)

and in *Channa striatus* (Nirmala and Pandian 1983).

Administration of androgen has been reported to increase the growth rate in fishes (Mc Bride and Fagerlund, 1973; Fagerlund *et al.*, 1978; Fagerlund *et al.*, 1979; Lone and Matty (1980 and 1983; Schreck and Fowler, 1982; Mc Bride *et al.*, 1982; Fagerlund *et al.*, 1983; Manzoor Ali, 1984; Nirmala and Pandian, 1983). Lone and Matty (1980) have obtained enhanced growthrate in common carp treated with 17 alphamethyl testosterone. This hormone would induce batter growth by acting probably in three different ways: (a) increased food conversion (b) activation on secretion of other androgenous anabolic hormones and (c) direct effect of 17 alpha MT on the gene expression in the muscle cells. The present study also revealed that the enhanced growthrate in molly may be due to the increased food intake coupled with better food conversion efficiency. It is evident from the present observations that the administration of 17 alphamethyl testosterone through feed increases growth rate and thus reducing the period of growth and cost of production which may be highly advantages from commercial aquarists point of view.

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## Application of Molecular Techniques in the Diagnosis and Control of Rabies.

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### ABSTRACT

*Recombinant DNA and monoclonal antibody techniques are being used to develop new diagnostic tests and new types of vaccines that will eventually supplement or supplant conventional whole - agent vaccines for animals and humans against rabies. Hybridoma produced monoclonal antibodies specific of antigenic determinations of the nucleocapsid and the glycoprotein of rabies virus showed differences between fixed and street strains of rabies virus.*

*Dot hybridization has been used to detect specific rabies RNA in the brain either from experimental infection in mouse or brain material to be processed for routine diagnosis. Detection of viral RNA was specific in the brain material left at room temperature even one week after the death of the animal. The ability to construct recombinant viruses expressing foreign genes clearly has great potential for vaccine development.*

### Introduction

**R**abies Virus (RV) is a bullet shaped virus containing a non - segmented negative standard RNA genome in Lyssavirus genus belongs to the Rhabdoviridae family. Rabies virion consists of a nucleocapsid core surrounded by a lipidic envelope. The nucleocapsid core is thought to contain all elements necessary for the viral transcription (Kawai, 1977). It is composed of the genomic RNA (about 12 kb) associated with the nucleoprotein N (MW = 58500), the polymerase L (MW = 170000) and the phosphoprotein M1 (MW = 39500) whose position has been reassigned from the envelope to the nucleocapsid core (Cox et al., 1981). Two membrane proteins are found in

the envelope the protein M2 (MW = 25000) occupies innerside and the transmembrane glycoprotein G (MW = 70500) which possesses the antigenic spikes outside (Dietzschold et al., 1978).

### Molecular cloning of Rabies virus Genome segments.

Recently the scientists from the Institut Pasteur, Paris have completed the rabies genome structure by the cloning and the sequencing of the entire virus (Tordo et al., 1986 a, 1986 b, 1988). Briefly, the Pasteur strain of rabies virus (PV) was grown on BHK - 21 cells. Virions were purified from culture supernatants. The viral genomic RNA was isolated by proteinase K treatment and fol-

lowed by the phenol - chloroform extraction and ethanol precipitation. The genomic RNA was 3' and labelled with (32p) C<sub>p</sub> and T4 RNA ligase. The purified genomic RNA was annealed to synthetic primer and transcribed into cDNA. Each class size was recovered and inserted in the *Pst* I site of P<sup>BR322</sup> plasmid vectors and transformed into the HB 101 strain of *E.Coli*. Rabies cDNA clones were selected either by colony hybridization with previously characterized probes or by Northern blots. Endonuclease fragments of selected inserts were subcloned in M13 vectors and sequenced by the chain terminating inhibitor method (Sanger et al., 1977). The complete determination of the sequence of the rabies genome will help in testing the differences observed in the Lyssa viruses namely rabies, Lagosbat virus, Mokola and Duvenhage viruses and to produce a library of complementary of DNA clones. This clones enable as to detect viral genes in infected cells or in infected animal tissues and to prepare antirabies vaccines.

#### Genetic Probe as Diagnostic Tools

Cloned cDNA copies of Rabies virus genome are exact replica of the original genomic RNA from which they had been cloned. As genetic strands with complementary nucleotide sequences will hybridize, the cloned cDNA can be used as a genetic probe to identify and hybridize to gene strands with similar nucleotide sequences.

#### Dot Blot Hybridization Assay

Advances in recombinant DNA technology have resulted in the availability of cloned DNA fragments,

from which DNA hybridization probes may be prepared without much difficulty. Ermine *et al.* (1988) from the Institut Pasteur, Paris, used dot hybridization and Northern transfer techniques to detect specific rabies RNA in the brains of experimentally infected mouse and from material processed for routine diagnosis. The test samples consisted of 1 gm of brain material. Nucleic acids were extracted with phenol and extraction fluid. Then the aqueous phase was precipitated with ethanol and pelleted by centrifugation. The RNA was electrophoresed on 1.2% formaldehyde gel in a horizontal agarose gel. Two types of Amersham nylon filters were prepared. For RNA filtered dots, 5 µl. of RNA solution was diluted in 100 µl. of dilution buffer, filtered on to nylon membrane with a multifiltration unit and then U.V. irradiated for RNA covalent fixation. For northern transfers, the agarose formaldehyde gel was blotted on nylon membrane which was then U.V. irradiated to covalently fix the molecules.

#### Hybridization

The cDNA probe used were from M13 clones complementary to the different rabies RNA genes and to all the rabies mRNA species. cDNA probes are <sup>32</sup>p labelled. The nylon membranes were incubated with labelled probes and autoradiographed. There was strong cross-hybridization between fixed rabies and street rabies virus RNA, which enable the detection of field strains for diagnostic purpose using a fixed rabies (PV strains) cDNA. A positive response was obtained with as little as 80 ng of brain RNA material. Detection of viral RNA was specific

even one week after the death and the brain material being left at room temperature.

#### **Protein Engineering and Rabies vaccination**

The use of inactivated rabies vaccine has the risk of infection through incomplete inactivation of the virus. One method to safer vaccines involves the elaboration of antigen material based upon purified G protein, subfragments of G, or anti-idiotypic antibodies raised against G. Certain subfragments of rabies G produced by chemical cleavage are able to induce the production of virus neutralizing antibodies in vivo and the proliferation of primed T cells in vitro.

With the advent of techniques for cleaving and reassociating DNA fragments much attention has been paid to the possibility of introducing sequences coding for foreign antigens into the genomes of large double stranded DNA viruses such as adenoviruses, herpes simplex virus and the pox viruses. In 1984 Kieny *et al* introduced the rabies ERA strain G coding sequence into the genome of the vaccinia virus Copenhagen strain. Vaccination of mice by scarification with Recombinant vaccinia virus expressing the glycoprotein of rabies virus induced high levels of rabies neutralizing antibodies, and these animals resisted challenge infection with street rabies virus.

#### **Use of Monoclonal Antibody Technology**

All viruses in the Lyssa virus serogroups of the rhabdovirus family were considered to be antigenically related. Only the virus neutralization test using the plaque reduction technique was able to demonstrate some differences in the antibodies produced by animals immunized with virions of viral components (Wiktor and Clark, 1973). Most of the rabies vaccines derived from a strain of virus isolated by Louis Pasteur in 1882. The potency of these vaccines are evaluated world wide using a challenge with the homologous virus strain. Antigenic differences among fixed and street rabies virus strains are detectable by using monoclonal antibodies obtained by fusion of mouse Myeloma cells with Spleen cells from BALB/C mice immunized with complete rabies virus or viral components (Wiktor and Koprowski, 1978).

Rollin and Sureau, (1984) from Institut Pasteur, Paris found that hybridoma produced monoclonal antibodies specific to antigenic determinants of the nucleocapsid and the glycoprotein of rabies virus showed differences between fixed and street strains of rabies virus. All France strains (23) gave a typical pattern of reactivity, confirming other reports (Blancou *et al.* 1982). Different patterns of reactivity were observed with African (168) and Asian isolates (110) of rabies viruses. Field strains originating from Madagascar, Thailand and Iran are the more diversified (Sureau *et al.*, 1983).

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## Studies on Immunisation Against *Schistosoma nasale* \*

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### ABSTRACT

Preliminary trials indicated that primary exposure of calf and sheep to un-irradiated *Schistosoma nasale* cercariae reduced the worm recovery of the challenge exposure with homologous species. Primary *Schistosoma incognitum* cercariae exposure to sheep was also found to confer partial immunity against heterologous challenge exposure of *Schistosoma nasale* cercariae. *Schistosoma nasale* cercariae irradiated at 5 K RAD level conferred immunity against homologous *Schistosoma nasale* infection and heterologous *Schistosoma spindale* infection in cattle, buffalo, sheep and goat.

### Introduction

Nasal schistosomiasis caused by *Schistosoma nasale* is widely prevalent in Tamilnadu. Though *S. nasale* affects cattle, buffaloes, sheep and goats, mainly cattle suffer clinical disease due to the development of granulomatous lesions in their nasal mucosa. Non-availability of effective drug and practical difficulty involved in its control, necessitates the need for alternate methods of control. Soulsby (1975) opines that prophylactic measures using immunological methods might offer the best means for long term control of helminthic disease of animals. Hence preliminary studies on the usefulness of homologous and heterologous cercariae and irradiated *S. nasale* cercariae as immunising agents in

reducing the development of worms of subsequent challenge exposure with *S. nasale* cercariae, were taken up.

### Materials and Methods

*Schistosoma nasale* cercariae released from naturally or laboratory infected *Indoplanorbis exustus* and *S. incognitum* cercariae shed by naturally infected *Lymnaea luteola* snails were used in the experiment. Young experimental animals from livestock farm and infection free areas were used. Animals were infected by percutaneous exposure of cercarial through shaved abdomen, for 30 minutes.

Irradiation of freshly shed *S. nasale* cercariae was carried out by exposing the cercarial suspension in water in a glass beaker to cobalt 60

\* Part of the thesis of the Senior author approved for the Ph.D. degree by the Tamil Nadu Agricultural University, Coimbatore

\*\*Since retired..

in Gamma Chamber 900 at the Regional Sophisticated Instrumentation Centre, I.I.T. Madras, at 5 KRAD Level. Cercariae were irradiated within 2 hours of their release and used for infection within an hour after irradiation.

Experimental animals were observed for the passage of *S. nasale* eggs and other symptoms. They were later challenged with large number of freshly shed *S. nasale* cercariae in the same manner, along with healthy controls.

Experimental animals were sacrificed and internal organs like nasal mucosa, liver, lungs, heart etc., were examined for the presence of worms of immunising and challenge infections. Recovered worms were counted and examined after intravital staining in wet glycerin preparations.

## Results

### Homologous Cercariae

A bull calf (No.1) which was exposed to 700 *S. nasale* cercariae showed no evidence of infection and had not passed eggs upto 217 days, when it was again exposed to 14,500 *S. nasale* cercariae. The calf passed eggs of the parasite in its nasal discharge from 89 to 510 days without showing clinical symptoms and on sacrifice on 534th day, did not reveal any worms in its nasal mucosa and other internal organs.

One sheep (No.2) showed patent infection, between 123 and 994 days, after initial exposure to 19,300 *S. nasale* cercariae. On challenge exposure to 10,700 homologous cercariae later, no eggs were passed upto 110th day after second ex-

posure, but on autopsy, mature *S. nasale* male and female worms with eggs were recovered from the nasal mucosa. Though worms were recovered from lungs, heart and liver, 57.50% of them were immature. Percentage of total worm recovery was 0.75 compared to 1.86 in the control sheep (No.3) which was exposed to 19,200 cercariae. The latter sheep had negligible numbers of immature worms.

### Heterologous Cercariae

One sheep (No.6) initially exposed to *S. incognitum* cercariae became patent on 80th day and was challenge exposed to 16,300 *S. nasale* cercariae on 150th day. After 119 days observation, the animal was sacrificed. Adult mature *S. nasale* worms were recovered from the nasal mucosa and lungs and few from heart and liver, while many immature worms were recovered from liver. Total challenge worm recovery was 4.58% of which 40.27% were immature.

Adult *S. incognitum* worms (1.27%) were recovered from liver and mesentery. This observation indicated that *S. incognitum* had conferred immunity against heterologous challenge exposure of *S. nasale* as evidenced by the presence of many immature worms of challenge infection in the liver after the prepatent period.

### Irradiated Cercariae

A white calf (No.20) and a buffalo calf (No.4) exposed to irradiated *S. nasale* cercariae were challenged 79 - 84 days later with double the number of *S. nasale* cercariae using an unexposed bull calf (No.22) as control. The calves sacrificed between

46 - 52 days after challenge exposure revealed 100% reduction of *S.nasale* recovery compared to the control calf.

Similar experiment with a ewe lamb (No.8) and a kid (No.3) using a sheep (No.7) and a Kid (No.4) as controls indicated, 100 per cent and 77.78 percent reduction of *S. nasale* worms, respectively compared to control animals. Reduction in *S.spindale* worm recovery in white calf (70%), buffalo calf (10%), sheep (88.64%) and goat (91.07%) compared to their control animals was also observed, apparently due to inadvertant mixing of *S.spindale* cercariae with *S. nasale* cercariae challenge exposure from the naturally infected *I. exustus* snails.

### Discussion

Reduction in the challenge worm recovery as well as retardation of worm growth in the previously exposed sheep and elimination of worms in the calf, suggested the operation of immunity to subsequent exposure of homologous cercariae, as resistance to reinfection can only be assessed by studying the fate of challenge infection. However, this immunity was partial in that neither all the worms were immature nor reproduction terminated in others. Resistance to re-infection with homologous cercariae has also been reported with *S.incognitum* (Borkakoty, 1980).

Under field conditions, continuous exposure to cercariae is likely to confer immunity resulting in maintenance of the infection at a certain level in animals and at the same time the adult worms in them evade the effects of immunity, suggesting the operation of concomitant immunity as was reported by Smithers

(1976) and Smithers and Terry (1976).

Heterologous *S.incognitum* cercariae conferred immunity against *S. nasale* in sheep. Similar observations were made with, *S.indicum* and *S.incognitum* in mice (Agarwal *et al.*, 1979) and *S. matthei* and *S.bovis* in sheep (Bickle *et al.*, 1979) and *S.spindale* and *S.incognitum* in mice (Varma *et al.*, 1983 and Agrawal *et al.*, 1983). Cattle and goat with concomitant infection with *S.spindale* and *S.indicum* had fewer liver granulomas than in those with single infection (Agrawal and Sahasrabudhe, 1982).

Development of irradiated *S.nasale* cercariae used for immunising exposure could not be observed in all animals sacrificed after 125 to 130 days, as against the development of mature worms from the un - irradiated cercariae observed in sheep and goat by that time.

Reduction in the challenge worm recovery of *S. nasale* by 77.78 - 100 per cent and *S.spindale* by 10.00 - 91.07 per cent in the immunised animals indicated that the irradiated *S.nasale* cercariae (5KRAD) conferred immunity in those animals against homologous *S. nasale* infection and also against heterologous *S.spindale* infection. However, more trials in this regard are needed to confirm the usefulness of irradiated cercariae as an immunising agent against *S. nasale* infection. Resistance to subsequent challenge conferred by immunisation with irradiated cercariae/schistosomula, was reported in respect of, *S. incognitum* in mice (Tewari and Biswas, 1972 and Sahasrabudhe 1977), *S. bovis* in calves (Bushara *et al.*, 1978)

and mice (Taylor *et al.*, 1979). James (1982) reported that highly irradiated schistosomula might be a suitable immunising agent against *S. japonicum*, *S. haematobium*, *S. matthei* and *S. bovis*.

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## DNA Hybridization in Veterinary Disease Diagnosis

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### ABSTRACT

Various methods have been used for identifying the microbes like direct smear examination, staining, culturing and serological methods like agglutination tests, complement fixation tests, neutralisation tests, fluorescent antibody tests, radio - immunoassay and enzyme linked immunosorbent assay. Recently, nucleic acid hybridization technique with restriction enzyme analysis has been included in this list for detecting the micro - organisms.

One of the advantages of hybridization technique is the detection sensitivity of fraction of a picogram (10 - 12g) or one millionth of a  $\mu$ g) without the loss of specificity. No other detection method has this degree of sensitivity. This hybridization technique has been successfully used for the detection of bacteria from clinical specimens in Leptospirosis, Tuberculosis, Campylobacteriosis and other bacterial diseases. Among viral infections, specific DNA/RNA probes have been prepared and used in the detection of Foot and Mouth disease virus, Parvovirus, Blue tongue, Rinderpest, Adenovirus, Bovine herpes virus and other viruses. Among protozoal infections, this method has been used in diagnosis of Leishmaniasis, Trypanosomiasis and Babesiosis.

Ready made hybridization probes are now available for diagnosis of Tuberculosis, Campylobacteriosis and Enteropathogenic *E.coli* infections, using both radioactive and non - radioactive biotinylated nucleotides. The nucleic acid hybridization method has proved to be a highly versatile method in diagnosis of diseases of animals as well as human beings. DNA hybridization methods have also been used in classification of micro - organisms.

### Introduction

The identification of micro - organisms is essential to arrive at a diagnosis of a disease. Only when the diagnosis can be achieved, the treatment and control of the disease can be initiated. Various methods have been used for

identifying the microbes like direct smear examination, staining, culturing and serological methods like agglutination tests, complement fixation test, neutralization test, fluorescent antibody test, radio - immuno assay and enzyme linked immunosorbent assay. Recently,

nucleic acid hybridization technique coupled with restriction endonuclease analysis has been included in this list for detecting the microbes.

One key advantage of hybridization technique is the detection sensitivity of fraction of picogram ( $10^{-12}$  g or one millionth of a  $\mu$ g). No other viral or bacterial detection method has this degree of sensitivity. DNA probe technology has helped in detection of virtually any organisms in food, clinical samples or environment. It is now possible to detect a wide variety of viral, bacterial and protozoal pathogens in urine, stools, tissues and sputum using traditional radio - labeled probes and newer non - isotopic assay systems.

### Methods

Nucleic acid hybridization is based on the ability of single stranded polynucleotide strands to form stable double stranded duplexes if complementary (homologous) base sequences are present. Hybridization can occur between two complementary molecules of DNA - DNA, DNA - RNA or RNA - RNA, and reactions can be carried out in solution, on a solid support or in cells (*in situ* hybridization).

Typically, a hybridization assay consists of the following steps:

1. The bacteria or other organisms are applied to a solid support such as a nitrocellulose membrane filter.

2. The bacteria are lysed to release their DNA and is denatured into separate strands.

3. The separated DNA strands are then fixed to the solid support so that they will not wash away later.

4. The filters are soaked in hybridization solution with probe. The probe DNA is more often a double standard DNA segment that is labeled by nick translation or random primer method. The double stranded probe molecule must be denatured before hybridization.

5. When the probe is mixed with test DNA on the nitrocellulose filter, the probe sequence finds its complementary sequence in the test DNA and forms hybrid molecule. These hybrids contain one radiolabeled strand that can then be detected by autoradiography.

### Use of DNA Hybridization in Disease Diagnosis

#### A. Bacterial Diseases

DNA hybridization has been used to detect leptospire in blood, urine and liver specimens using  $^{32}$ P and biotin labeled probes (Terpstra *et al.*, 1986). The smallest amount of leptospiral DNA that could be detected with  $^{32}$ P and biotin labeled probe was 1.5 pg and 5 pg, respectively, corresponding to about 750 and 2500 leptospire. *In situ* hybridization can be completed in 4 hours and it combines the advantage of visualization of the leptospiral morphology with the specificity of the hybridization reaction.

Specific DNA probes have been recently used for rapid identification of *Mycobacterium avium*, *M. intracellulare* and *M. tuberculosis* (Kiehn and Edwards, 1987). DNA hybridization techniques have also been used to characterize *M. paratuberculosis* organisms (Hurley *et al.*, 1988). Recently, a synthetic oligonucleotide probe labeled with alkaline phosphatase has been developed to detect

*Campylobacter jejuni* (Harasawa *et al.*, 1988).

#### B. Viral Diseases

The Foot and Mouth disease virus has been detected in oesophageal and pharyngeal fluids using dot blot hybridization (Rossi *et al.*, 1988). The probe used included the viral polymerase sequence which allows the detection of the 3 types of viruses (A,O,C). Viral concentration as low as 100 pg can be detected. A 3 kb DNA fragment of porcine parvovirus DNA was cloned and used for dot hybridization as a probe for detection of porcine parvovirus and this method was 100 times more sensitive than standard haemagglutination test (Krell *et al.*, 1988). Direct filter hybridization was used to detect Pseudorabies viral nucleic acid in nasal and tonsillar cells, lymphocytes and organ specimens (Linne, 1987).

A liquid phase hybridization method was adopted for the identification of blue tongue virus (Dangler *et al.*, 1988). Biotin - labeled DNA probes for bovine herpes virus type - 1 were used to detect virus in infected cell culture and clinical specimens (nasal swabs).

#### C. Protozoan Infections

Trypanosoma species - specific DNA probes had been used to detect *T.brucei* and *T.congolense* infections. Dot blot hybridization had been performed with DNA from *B.bovis* in diagnosis of babesiosis in cattle and ticks. A genomic DNA probe has been developed to detect *Anaplasma marginale* in blood (Eriks *et al.*, 1989). This probe can detect 0.01 ng of genomic DNA and 500 - 1000 infected erythrocytes in 0.5 ml of blood, which is equivalent to a parasitaemia of 0.000025%. This makes the probe at least 4000 times more sensitive than light microscopy.

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## Evaluation of Coagglutination Test for Rapid Detection of Salmonella Enterotoxin.

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### ABSTRACT

*Staphylococcal coagglutination (CoA) test by using antiserum against purified Salmonella enterotoxin (SE) was developed, standardized and carried out to screen salmonellae for their enterotoxigenicity. Of the 51 cell free culture supernatants (CFCS) from Salmonella strains belonging to 12 different serogroups isolated from foods of animal origin tested, 36(70.89%) were found to be enterotoxic. The CoA test correlated with the rabbit ligated ileal loop test completely for detection of enterotoxin producing salmonellae with 37 strains (24 enterotoxigenic and 13 non-enterotoxigenic) tested. As little as 7.5 ng of purified SE could be detected by this test. Besides, the test was standardized with antiserum to cholera toxin and could detect cholera toxin in 5.0 ng quantity. The usefulness of the test as routine, rapid and economical one for detection of SE is discussed.*

### Introduction

Salmonellae are widely distributed in nature, infecting man and animals alike (Kelterborn, 1979) and are major causes of diarrhoeal disease (Giannella, 1981). Salmonellae associated with diarrhoea have been shown to produce enterotoxic (Sedlock and Deibel, 1978 ; Baloda *et al.*, 1985). The enterotoxic activity has been demonstrated in a variety test systems including rabbit ligated ileal loops (Sedlock and Deibel, 1978) skin permeability test (Sandefur and

Peterson, 1976) and tissue cultures (Baloda *et al.*, 1983). Immunological nature of enterotoxins allows their detection by immunological tests like enzyme-linked immunosorbent assay (Houston *et al.*, 1981), counter immunoelectrophoresis (Shukla and Sharma, 1985) and immuno-dot-blot assay (Panigrahi *et al.*, 1987). But all these tests demand technical expertiseness and time consuming as well.

We attempted here a staphylococcal Coagglutination test using an-

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tiserum against purified SE and found that enterotoxin can be detected within few minutes after obtaining the cell free preparations from the test organisms.

#### Materials and Methods

**Bacterial strains:** A total of 51 strains of *Salmonella* belonging to 12 different serogroups isolated and maintained in this Department (Table 1) were used for this study. In addition, 24 enterotoxigenic and 13 non-enterotoxigenic strains of *Salmonella* as tested by the rabbit ligated illegal loop (RLIL) test (Rahman, 1989) were also included in this study.

**Enterotoxin Production:** Cell free culture supernatants (CFCS) of different salmonellae were prepared according to the procedure of Singh *et al.* (1983). Briefly, each *Salmonella* strain was in brain heart infusion (BHI) broth on a rotary shaker (200 rev/min) at 37°C for 18 hr and then the culture was centrifuged (6000 x g, 45 min at 4°C). The supernatant was collected, membrane filtered (Millipore, 0.22 µm) and stored at 4°C for further use.

**Purification of enterotoxin and antiserum production:** The enterotoxin moiety present in CFCS of *S. typhimurium* (p/536) was purified to homogeneity according to the procedure of Houston *et al.* (1981) with the help of computerised Fast Protein Liquid Chromatographic set (Pharmacia, Sweden) having automatic recording system and fraction collector. The homogeneity of purified enterotoxin was checked by polyacrylamide gel disc electrophoresis. The enterotoxicity of the purified enterotoxin determined by RLIL technique (De and Chatterjee,

1953) and protein concentration of enterotoxin was determined by Lowry's method (Lowry *et al.* 1951). The purified enterotoxin thus obtained gave a single band on polyacrylamide disc gel electrophoresis. The antiserum was raised in rabbits according to the procedure described by Rahman (1989). The immunisation schedule was same for *Salmonella* enterotoxin and cholera toxin (Sigma).

**Enterotoxin assay:** Coagglutination (CoA) test was performed as described by Rahman (1989). *Staphylococcus aureus* Cowan - 1 was grown in BHI broth at 37°C for 18 hr and was harvested by centrifugation. The sediment was washed twice with PBS (pH 7.2) and it was suspended in 0.5% formalin (BDH) in PBS and kept at room temperature for 3 hr. The formalin treated cells were washed thrice in PBS and heated at 80°C for 30 min in a water bath. One milliliter of a 10% of heat killed and formalin treated suspension of *Staphylococcus aureus* strains Cowan 1 in PBS (v/v) was sensitized with 0.1 ml of the undiluted antiserum against purified enterotoxin. The sensitized mixture was incubated at room temperature for 1 hr and finally, after washing with Pbs a 5% suspension of the cells, called CoA reagent, was made and stored at 4°C before use. The antibody coated cells were stained with 4.0% solution of methylene blue in PBS (Rahman, 1989.). The stained cells were washed as above and finally 5.0% (v/v) suspension was made as working solution. For Coagglutination test, one drop of the test preparation was mixed with equal volume of Coagglutination reagent on a clean glass slide. The result was

Table 1. Enterotoxigenicity in salmonellae as detected by Coagglutination test.

Sl. No.	Salmonella groups	No. tested	No. positive
1.	<i>S.bareilly</i>	12	9
2.	<i>S.saintpaul</i>	8	7
3.	<i>S.stanley</i>	8	8
4.	<i>S.newport</i>	5	3
5.	<i>S.weltevreden</i>	5	3
6.	<i>S.sneftenberg</i>	4	0
7.	<i>S.orienberg</i>	2	2
8.	<i>S.anatum</i>	2	1
9.	<i>S.indiana</i>	1	1
10.	<i>S.london</i>	1	1
11.	<i>S.agona</i>	1	0
12.	<i>S.havana</i>	2	1
	TOTAL	51	36(70.59%)

read within 2 min and agglutination was recorded as -, +, ++ and +++ which represent no, fine moderate and marked agglutination, respectively. A sample showing atleast (+) agglutination was recorded as positive. Ten fold dilution of enterotoxin were made in PBS (pH 7.2) so as to obtain 1.0, 0.5, 0.15, 0.1 and 0.01 µg of protein per ml. Each dilution was tested in order to determine the minimum detectable amount of enterotoxin.

The rabbit ligated ileal loop test was performed as per the surgical technique described by De and Chatterjee (1953) in young rabbits with cholera toxin as reference toxin.

### Results

The staphylococcal CoA test was standardized and evaluated for detection of *Salmonella* enterotoxin (SE). Details of the results are depicted in the Table 1 and 2 Fig. 1.

CFCS of as many as 51 strains of *Salmonella* belonging to 12 different serogroups were tested with CoA test standardized antiserum to purified SE for presence of enterotoxin (Table 1). A CFCS given a fine (+), moderate (++) or marked agglutination (+++) with

CoA reagent were taken as positive for enterotoxin (Fig 1). of the 51 CFCS tested, 36(70.89%) of 9 different serogroups were found to be enterotoxic. All the CFCS were also tested with CoA tested standardized with antiserum to cholera toxin. None was found to be positive for cholera related enterotoxin.

The CoA test was compared with rabbit ligated ileal loop test for detection of enterotoxin producing *Salmonella* with 37 strains (24 enterotoxigenic and 13 non-enterotoxigenic salmonellae) and was found that results by the two tests

Table - 2. Sensitivity and specificity of Coagglutination test for detection of purified *Salmonella enterotoxin* and cholera toxin.

Preparation	Concentration $\mu\text{g/ml}$	CoA test with	
		Anti - <i>Salmonella enterotoxin</i> .	Anti - cholera toxin.
Purified <i>Salmonella enterotoxin</i>	1.00	+++	-
	0.50	+	-
	0.15	+	-
	0.10	-	-
	0.01	-	-
Cholera toxin.	1.00	-	+++
	0.50	-	+
	0.25	-	+
	0.10	-	-
	0.01	-	-
PBS	-	-	-
BHI broth	-	-	-

+++ = marked agglutination, ++ = moderate agglutination, + = fine agglutination, - = no agglutination.

correlated completely. In staphylococcal CoA test, few strains gave fine granular agglutination, but this was clearly distinguishable from the negative control strains and most positive strains gave large clumps of agglutination.

Minimum detection levels of purified SE and cholera toxin by CoA test are given in the Table - 2. The lowest dilutions containing 0.15  $\mu\text{g/ml}$  and 0.10  $\mu\text{g/ml}$  of SE and cholera toxin, respectively, yielded positive reaction with CoA test with respective antisera. Since a drop of approximately 50  $\mu\text{l}$  was used for the test, the minimum amount calculated to be 7.5 ng and 5.0 ng of SE and cholera toxin, respectively. No cross

reactions were observed between the two toxin-antitoxin systems.

### Discussion

Many methods for detection of enterotoxins have been reported, but most of them have some disadvantage as well as advantages. Although RLIL technique is the oldest and widely used one for the detection of enterotoxin, it is quite expensive, cumbersome and time consuming technique. In the present investigation it was noted that in some rabbits all preparations, including known positive one, failed to induce fluid accumulation in the gut loops and in some case the animals died before 18 hr post-inoculation leading to inconclusive results. Such variability of intestinal loop reaction

to *Salmonella* and *E. coli*. enterotoxin has also been reported earlier (Clarke *et al.*, 1987). Thus, on the basis of the literature, the RLIL technique is liable to yield false negative results and eventually can not be considered to be a very reliable test for the detection of enterotoxin particularly *Salmonella* which are poor producer of enterotoxin (Baloda *et al.*, 1983).

The CoA test which was found to be completely correlated with RLIL and has advantages over other serological tests, like ELISA, counter immunoelectrophoresis and radioimmunoassay etc., as it is very simple, rapid and inexpensive requiring no sophisticated equipment or trained personnel. In addition, the quantity of antiserum required for CoA test is much less than that used in immunodiffusion test or Biken test. Further, antibodies coated to *S. aureus* cell have been reported to be very stable upon storage at 4°C and even at room temperature. Also there is no need to purify the specific antiserum for coating the bacteria, as killed staphylococci preferentially

bind the FC part of IgG antibodies to cell surface protein A (Kronvall, 1973). Although CoA test has been evaluated for epidemiological study for detection of heat - liable of *E. Coli* (Ronnberg and Wadstrom, 1983) the test has not earlier been used with respect to *Salmonella* enterotoxin. Thus keeping in view the sensitivity, economy and simplicity, Of all the tests used for enterotoxin detection, CoA test is considered as a test of choice for detection of enterotoxigenicity of *Salmonella* isolates, in public health laboratories, particularly, in developing countries including India where work has to be done with minimum facilities.

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Fig. 1 From left to right showing no (-), fine (+), moderate (+ +) and marked (+ + +) agglutination in coagglutination test with *Salmonella* enterotoxins and antienterotoxin system.

## Studies on the biological control of horse - flies by hymenopteran insect, *Telenomus* sp

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### Abstract

*Tabanid* flies are serious pests on livestock, particularly in cattle, buffaloes and horses. The female flies are blood suckers inflicting vicious bites leading to weals in soft skinned animals. Due to their intermittent feeding habits they are able to transmit mechanically *Trypanosoma evansi*, the causative agent of the disease 'Surra' affecting livestock.

*Tabanid* flies are so prevalent and widely distributed throughout Tamil Nadu that their eradication is not a practical proposition. Studies carried out in certain isolated pockets of Tamil Nadu revealed that the egg masses of these flies were being parasitized by a small wasp *Telenomus* sp. The larvae of these insects were endoparasitic on the egg masses. Moreover the life cycle of this parasitic wasp is so short that they destroy more or less the entire egg masses, thereby reducing the *tabanid* population in nature. Whether advantages could be taken of this wasp in artificial breeding for mass eradication of the *tabanid* flies, needs further investigation.

### Introduction

**H**orse - flies' of the genus *Tabanus* are among the most annoying of livestock pests. As many as 193 species and four subspecies have been recorded from India (Datta, 1985). They are the mechanical vectors of *Trypanosoma evansi* the cause of 'surra' in animals. They are also mechanical vectors in the transmission of bacterial disease like Anthrax, protozoan disease like Anaplasmosis and virus equine infectious anaemia (Roy and Brown, 1954). Bhatia

(1935) and Kapur (1941) have experimentally proved that these flies can successfully transmit Rinderpest virus. The bites of these flies are painful and irritating and may give rise to weals in soft skinned animals. Horses and cattle that are in harness become unmanageable when they are disturbed by these flies. Control of these flies is practically very difficult. The most simplest method of arresting this fly population is by the destruction of the egg masses of these flies which

are deposited on plants overhanging stagnated water. Patel (1920), Fletcher (1920, 1921) and Joseph *et al.* (1988) have observed certain hymenopteran insects of the families *Chalcididae* and *Scelionidae* playing a major role in the control of the tabanid flies by parasitizing the egg masses of these flies and reducing their population. Sen and Fletcher (1962) have also observed that the eggs of these flies were being eaten up by species of *Hapaloderus* (*Cantharidae* - *Coleoptera*) at Pusa.

In all cases they proved to be useful in arresting the tabanid population.

An intensive search was undertaken to study the role played by the hymenopteran insect in the control of tabanid fly population in Tamil Nadu.

#### Materials and Methods

A survey was carried out in certain places in the districts of Chingleput, Thanjavoor and Tiruchirappalli of Tamil Nadu to study the prevalence of these

Table : 1 Survey in Chingleput District

S.No.	Month of collection of egg mass	Number of egg in each egg mass in nearest ten	Average number of days spent by parasite inside tabanus egg	Male	Female	Total
1.	May'84	350	9	7	20	27
2.	May'84	230	9	3	8	11
3.	May'84	240	8	9	20	29
4.	May'84	220	10	6	13	19
5.	May'84	370	9	12	28	40
6.	May'84	440	9	15	38	53
7.	May'84	420	9	16	42	58
8.	May'84	450	8	21	50	71
9.	May'84	480	10	24	50	74
10.	May'84	560	9	28	74	102
11.	July'84	500	9	23	48	71
12.	July'84	380	9	16	38	54
13.	July'84	340	10	9	29	38
14.	July'84	480	10	16	36	52
15.	July'84	320	8	10	40	50
16.	July'84	340	9	15	55	70
	TOTAL	6120		230	589	819

Table : 2 Survey in Thanjavoor District

S.No.	Month of collection of egg mass	Number of egg in each egg mass in nearest ten	Average number of days spent by parasite inside tabanus egg	Male	Female	Total
1.	April 86	350	9	15	35	50
2.	April 86	370	8	27	52	79
3.	April 86	420	9	15	38	53
4.	May 86	560	9	35	80	115
5.	May 86	230	8	23	35	58
6.	May 86	500	10	40	85	125
7.	May 86	340	9	29	65	94
8.	May 86	510	9	45	95	140
9.	June 86	460	9	30	65	95
10.	June 86	320	8	20	45	65
	TOTAL	4060		279	595	874

Table : 3 Survey in Tiruchirapalli District

S.No.	Month of collection of egg mass	Number of egg in each egg mass in nearest ten	Average number of days spent by parasite inside Tabanus egg	Male	Female	Total
1.	May 86	350	9	25	40	65
2.	May 86	300	9	30	75	105
3.	May 86	400	8	40	85	125
4.	May 87	450	10	30	75	105
5.	May 87	400	9	38	65	103
6.	May 87	420	8	29	65	94
7.	May 87	410	9	25	55	80
8.	June 87	320	8	20	45	65
9.	June 87	330	10	30	60	90
10.	June 87	310	9	24	52	76
11.	June 87	410	9	28	60	88
12.	July 87	500	8	30	75	105
13.	July 87	420	9	30	70	100
14.	July 87	360	9	35	80	115
	Total	5380		414	902	1316

hymenopteran insects which were parasitic on the egg masses of tabanid flies.

Egg masses of the fly *Tabanus rubidus* Fig.1 deposited on the undersurface of the leaves of *Calotropis gigantea* Fig.2 over hanging the stagnated water in and around the cow sheds in certain localities of the above said districts were carefully collected and brought to the laboratory and kept under observation in individual petridishes. The emerged parasitic hymenopteran insects were carefully captured, studied and preserved in alcohol. Some of them were processed and made into permanent mounts for detailed microscopical study.

### Results

The tabanid egg masses collected from the three Districts on different months, the emergence of the male and female parasitic hymenopteran insects, their period of emergence etc., were shown in Tables 1 to 3.

It was observed that on an average *Tabanus rubidus* laid 380 eggs in an egg mass. The parasitic hymenopteran insects were able to complete their life cycle in about 9 days. The male, female ratio was found to be 1 : 2.

The entire length of the wasp ranged from 1.00 to 5.00 mm. Both sex posses a dark shining lusture on their body. The antennae were 12 segmented, elbowed. Wings were found to be astigmatic, but distinct stigmatic veins present. In both sexes, the oval shaped abdomen had a keeled margin. The female insect had a powerful and well developed ovipositor, protected by a pair of sheath on either side (fig.3). The tip

of the male abdomen was blunt. The wasps were identified as *Telenomus* sp. of the family *Scilionidae*, Order *Proctotrupoidea*.

Since the development of the larvae and nymphs of the wasps were of a very short duration, they were endoparasitic on the eggs of the horse fly. The parasite devour the contents of the tabanid egg and occupied the entire space inside the egg of the *Tabanus* (fig.4). On an average, on the 9th day, the wasp emerged out of the tabanid egg and hovered on the infected egg mass (fig.5).

The parasitized egg mass of the tabanus finally turns yellowish black in colour and appeared as fungus infected while the healthy egg mass from which tabanid larvae had emerged out were found to be cream coloured.

### Discussion

Generally parasitic hymenopteran insects of the Family *Scilionoidea* are parasitic on the eggs of Orthopteran insects, such as grass hoppers, katydids, crickets and mantids; some coleopteran insects and lepidopteran insects like moths and butterflies and certain ants of their own Order. Patton and Cragg (1984) observed *Phanurus tabanivorus* and *Telenomus benefactor* parasitizing the egg masses of *Tabanus* sp. in U.S.A. and Sudan respectively. This finding is substantiated with the present findings.

Parasitic hymenopteran insect, *Telenomus* sp. (*Scilionidae* - *Proctotrupoidea* - *Hymenoptera*), proved to be useful in reducing the tabanid fly population in nature.

**Acknowledgement**

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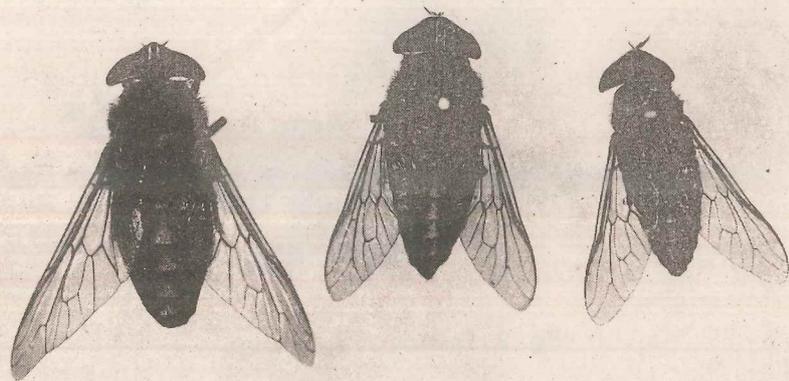


Fig. 1. *Tabanus rubidus*



Fig. 2. Egg mass of *Tabanus rubidus*

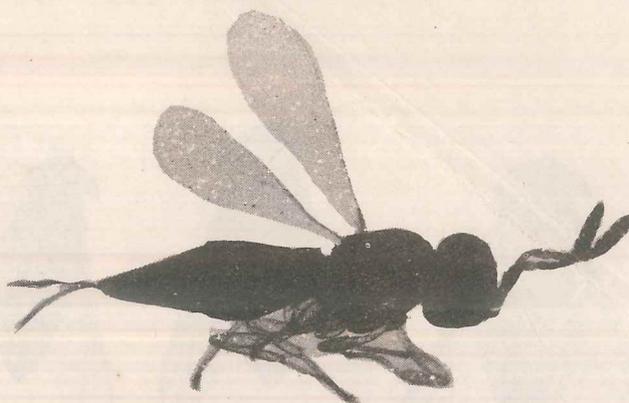


Fig. 3. *Telenomus* sp. Female

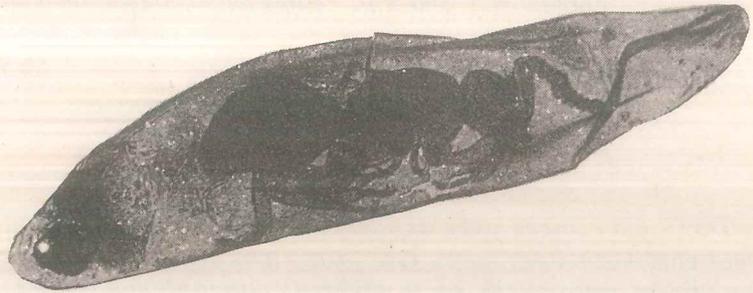


Fig. 4. Parasitic hymenoptera developing inside *Tabanus* sp.

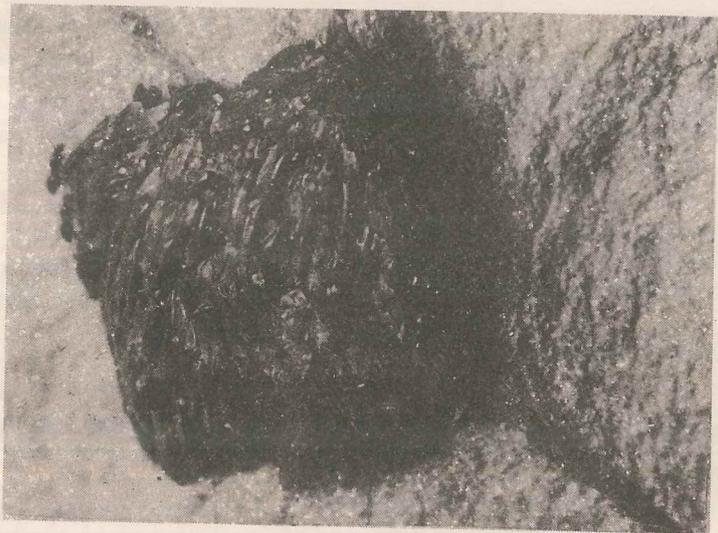


Fig. 5. Parasitic hymenoptera hovering on *Tabanus* egg mass.

## Micro Elisa for Detecting Newcastle Disease Virus Antibodies in Chicks

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### ABSTRACT

Day - old chicks were divided into five groups for adopting different vaccination regimens. One group was kept as control and other four groups were subjected to different vaccination schedules. Serum samples were collected at regular interval of 7 days. Antibody levels were assayed by ELISA technique. Residual antibody was detected by this technique; Primary response was detected by 14 days and secondary response by 7 days. ELISA technique was found to be a very sensitive technique to determine antibody level at various time points.

### Introduction

Antibodies to Newcastle disease virus (NDV) by ELISA have been identified by many workers (Snyder *et al.*, 1983 a & b; Mishra *et al.*, 1985). In this study ELISA technique has been used to monitor antibody levels in chicks subjected to different schedules of vaccination.

### Materials and Methods

Day old chicks numbering 350 were selected for experiment and

divided into five groups. The vaccination schedules were as given in the table below;

Group E served as unvaccinated control.

Serum samples were collected at regular intervals of 7 days and antibody level in each group was examined up to the 3rd week of last vaccination.

**ELISA:** The ELISA technique was performed as per Voller *et*

Group A	B	C	D	E
RDVF 5th day O/N	RDVF 5th day O/N	RDVF 5th day O/N	RDVF 5th day O/N	None
	RDVF 5th week after first vaccination O/N	RDVF 5th week after first vaccination O/N	RDVK 8th week after first vaccination I/M.	
	RDVK 11th week after first vaccination I/M			

Note: RDVF and RDVK vaccines were obtained from Institute of Veterinary Preventive Medicine, Ranipet.

*al.*(1979). The antigen used was RDVK strain of Newcastle disease raised in chicken embryo fibroblast. The antigen was coated on to microtitre ELISA plate (Dynatech, USA) following the procedure of Sukumar and Padmanaban (Personal Comm.) Immunoconjugate (anti - Chicken immunoglobulin raised in rabbits and conjugated with peroxidase - M/s Nordic Immunological Laboratories) was used at dilution of 1 : 800. Orthophenylene diamine was used as the indicator. For coating and washing standard method was followed.

The OD values were read in Biotech ELISA reader (Model 307) at a wave length of 405 nm. The mean negative OD value was 0.17 and twice the mean negative value was fixed as cut off value.

The standard curve to convert OD values into titre values was prepared as per Sukumar and Padmanaban (*loc. cit.*).

### Results and Discussion

The antibody titres detected in the serum samples of chicken subjected to different schedules of vaccination with New castle disease vaccines and unvaccinated controls as elicited by ELISA are presented in the table.

The group A chicks which received RDVF alone at 5th day of hatch, the antibody titre before vaccination was log 2.3. But after vaccination, the antibody titre rose up by second week of vaccination and the rise continued even during the third week of vaccination.

In group B chicks which received RDVF on 5th day of hatch and on 5th week of post vaccination, the an-

tibody titre gradually rose up upto 4th week and then there was slight reduction by 5th week. Second vaccination was done on 5th week. By sixth week the antibody level presented a sudden rise which was maintained thereafter upto 8th week.

In group C chicks which received RDVF at 5th day of hatch and 5th week of Post vaccination and RDVK at 11th week after first vaccination, the antibody level followed the same pattern as the group B upto 8th week. After 8th week antibody level gradually declined upto 11th week. After vaccination with RDVK, the antibody level suddenly rose up to a high level by 12th week and this level continued upto 14th week after first vaccination.

The group D chicks which received RDVF at 5th day of hatch and RDVK at 8th week after first vaccination, the antibody level presented a gradual rise from second week after first vaccination upto 4th week after first vaccination, then gradually declined upto 8th week after first vaccination. On revaccination the antibody level presented a rise by 9th week and continued upto 11th week.

In unvaccinated chicks (Group E) the antibody level at 5th day of hatch was log 2.3 and presented a decline thereafter. The lowest antibody titre detected in these chicks was log 1.50. The differences were statistically significant ( $P > 0.01$ ).

From these results it is clear that, in unvaccinated chicks, the level of maternal antibody could be monitored by Elisa.

In vaccinated chicks, the primary response could be detected after 14

days and the secondary response could be detected after 7 days.

The results of the present study correlated with those of Snyder *et al.* (1983 a), Mishra *et al.* (1985). IN positive samples, O.D. values were above the cut off values whereas in negative (Unvaccinated group) samples O.D. values were below the cut off value.

ELISA test is highly sensitive test, antibody level could be determined by this test at various time points with high degree of sensitivity. This test is easy to perform, Residual antibody, primary response and secondary response could be

determined by this test. This test could be used in field condition as absolute sterile condition is not necessary provided pre coated plates with suitable antigen are available. More number of samples could be screened within short period. So ELISA is one of the most adoptable technique.

#### Acknowledgement

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**Mean serum antibody titres of vaccinated and unvaccinated birds as measured by Micro ELISA.**

Week	Group				
	A	B	C	D	E
0 (5th day)	2.30 <sup>a</sup> 0.27				
1	2.30 <sup>a</sup> 0.27				
2	3.32 <sup>a</sup> 0.40	3.32 <sup>b</sup> 0.40	3.32 <sup>b</sup> 0.40	3.32 <sup>b</sup> 0.40	1.70 <sup>c</sup> 0.18
3	3.68 <sup>d</sup> 0.45	3.68 <sup>d</sup> 0.50	3.68 <sup>d</sup> 0.45	3.68 <sup>d</sup> 0.45	1.60 <sup>c</sup> 0.17
4		3.68 <sup>d</sup> 0.45	3.68 <sup>d</sup> 0.45	3.68 <sup>d</sup> 0.45	3.68 <sup>d</sup> 0.45
5		3.40 <sup>b</sup> 0.41	3.40 <sup>b</sup> 0.41	3.40 <sup>b</sup> 0.41	1.60 <sup>c</sup> 0.17
6		4.00 <sup>e</sup> 0.49	4.00 <sup>e</sup> 0.49	3.00 <sup>e</sup> 0.36	1.60 <sup>c</sup> 0.17
7		4.00 <sup>e</sup> 0.49	4.00 <sup>e</sup> 0.49	2.80 <sup>b</sup> 0.32	1.60 <sup>c</sup> 0.17
8		4.00 <sup>e</sup> 0.49	4.00 <sup>e</sup> 0.49	2.68 <sup>b</sup> 0.31	1.60 <sup>c</sup> 0.17
9			3.40 <sup>b</sup> 0.41	4.00 <sup>e</sup> 0.49	1.50 <sup>c</sup> 0.15
10			3.00 <sup>b</sup> 0.36	4.30 <sup>f</sup> 0.53	1.50 <sup>c</sup> 0.15
11			2.80 <sup>b</sup> 0.32	4.30 <sup>f</sup> 0.53	1.60 <sup>c</sup> 0.17
12			4.36 0.54		1.50 <sup>c</sup> 0.15
13			4.36 <sup>f</sup> 0.54		1.50 <sup>c</sup> 0.15
14			4.36 <sup>f</sup> 0.54		1.60 <sup>c</sup> 0.17

Note: Each set of entry denotes : Top - extrapolated log of end titre and bottom - OD reading.

Figures with same superscript do not differ significantly ( $P > 0.05$ ) O week = Day of vaccination.

## Detection of Infectious Bursal Disease Virus by Reversed Passive Haemagglutination Method

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### ABSTRACT

Reversed passive haemagglutination (RPHA) test was applied for the detection of Infectious Bursal Disease (IBD) antigen in the various organs of experimentally infected chicken, at different days post - inoculation (PI). This test could detect the antigen upto 14 days PI in bursa, kidney, liver and spleen and upto 21 days PI in bursa, kidney and liver.

### Introduction

RPHA, a simple and sensitive technique, has been applied for the detection of several viral antigens like Rinderpest virus (Bansal *et al.*, 1987), Rota virus (Sanekata *et al.*, 1979) and so on. Attempts have been made to detect IBD virus antigen by various tests like Agar - gel immunodiffusion (AGID), Fluorescent antibody technique (FAT), Immunoperoxidase test (IPT) and virus isolation with conflicting results (Okoye, 1984; Kulkarni *et al.*, 1983). In order to find out a more sensitive test for the detection of IBD antigen, RPHA was standardised and used to determine the duration of persistence of the antigen in the viscera of infected chicken.

### Materials and Methods

A local isolate of IBD virus serotype I was passaged thrice in White leghorn chicken before final inoculation into a group of 4 week

old susceptible lot, intra - ocularly. Bursa, Kidney, Liver and spleen were collected on different days PI and a 50% w/v suspension of these organs containing 2% Triton X 100 were employed for the detection of IBD antigen using RPHA.

Immunoglobulin (Ig) was prepared from hyperimmune serum against IBD virus, by precipitation with sodium sulphate at 34% saturation. Protein was estimated by the method of Lowry *et al.* (1951).

RPHA was performed following the method of Bansal *et al.*, (1987) using glutaraldehyde as the coupling agent. Nine volumes of washed, mature sheep red blood cells (SRBC, 2.5%) was mixed with 1 volume of 0.7% glutaraldehyde and kept at room temperature for 60 min. These cells were washed again thrice in PBS (pH 7.2) and resuspended at 2.5% concentration and mixed with equal volume of Ig (1 mg/ml) in PBS. The cells were incubated at 37 °C for

1 hr and washed with PBS containing 2% Bovine Serum Albumin (BSA). RPHA test was carried out in haemagglutination plates using PBS as diluent. Two fold serial dilutions of antigen (0.25 ml) was added to wells followed by 0.25 ml. of 1% coupled SRBC and incubated at 37 °C for 30 min. RPHA titre was expressed as the reciprocal of the highest dilution giving haemagglutination. A titre of 8 was taken as positive. Negative and Positive controls were also employed.

### Results

RPHA titres of different organs are shown in Table I. The titre was maximum in the bursa on the third day PI, which observation is in accordance with Wyeth and Chettle (1988). Kidney and spleen showed maximum titres on day 5 and 1 PI respectively. Liver showed consistent titres without much variation from day 1 to 14 PI.

RPHA could detect the IBD antigen in the bursa, kidney and liver

on day 21 PI while by 28 days PI the antigen could not be detected in any of these organs.

### Discussion

RPHA could detect the antigen in bursa, kidney and liver upto 21 days PI. Other tests like AGID, FAT and IPT could detect the antigen only up to about 7 - 10 days PI (Okoye, 1984).

Serological tests like Enzyme linked Immunosorbent assay, FAT, IPT require sophisticated equipment, costly chemicals and can only be performed in specialised labs. It has been found that RPHA is quite sensitive and has the added advantage of simplicity. It could be very useful for spot diagnosis.

### Acknowledgements

The authors wish to thank the Dean, Madras Veterinary College, for the facilities provided to carry out this work.

Table I RPHA Titre\* Of Different Organs from IBD Infected Chicken

Duration of persistence of IBD antigen (Days PI)	Organs employed to determine persistence of infection			
	Bursa	Liver	Kidney	Spleen
1	21.3	21.3	21.3	42.6
2	21.3	21.3	21.3	16.0
3	42.6	21.3	21.3	16.0
5	21.3	16.0	42.6	16.0
7	21.3	16.0	16.0	16.0
10	16.0	16.0	8.0	16.0
14	16.0	13.3	8.0	10.6
21	8.0	8.0	8.0	
28	4.0	2.0	2.0	

\* Mean of 3 samples.

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## Production and Evaluation of Anti - Idiotypes For Rinderpest\*

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### ABSTRACT

*Anti - idiotypic antibodies specific to anti - RP (rabbit) antibodies were produced in the same species (rabbits) using anti - RP IgG and F(ab)<sub>2</sub> as immunizing idiotypes. Two injections of IgG followed by an I/V booster of F(ab)<sub>2</sub> produced anti - idiotypic serum. This anti - idiotypic serum gave precipitation reaction with only rabbit HIS when tested after adsorption. It gave specific binding reaction with the interspecies (bovine) anti - RP IgG and F(ab)<sub>2</sub>. A titer of 400 was obtained in ELISA.*

*Rabbit anti - RP IgG (and F(ab)<sub>2</sub> booster) injections in guinea pigs did not raise any detectable anti - idiotypic activity in the system employed for testing. Rabbits and guinea pigs immunized with anti - RP bovine IgG (and F(ab)<sub>2</sub> booster) also did not produce any detectable anti - idiotypic activity. Booster injection also failed to induce anti - idiotypes in the above 3 sets of animals.*

### Introduction

**A**nti - idiotypes (a-id) are antibodies against the antigenic determinants present in the antigen combining site of immunoglobulins. A-id have been used to analyse antiinfluenza hemagglutinin antibodies (Liu et al., 1981; Nepom et al., 1982), Cell surface receptor for reovirus (Nepom et al; 1982) and receptors on reovirus specific cytolytic T cells (Ertl et al; 1982). As immunogens a-id were used to enhance anti - hepatitis B response (Kennedy et al; 1983), induce virus neutralizing response in mice against

rabies (Reagan et al; 1983) and induce anti - newcastle disease antibodies in mice (Tanaka et al., 1986).

In this study attempts were made to raise a-id against idiotypes against rinderpest (RP) allogenic and Xenogenic systems. IgG fractions from anti rinderpest bovine serum and rabbit serum were used as immunogens along with F (ab)<sub>2</sub> of respective IgG as booster. A set of rabbits and guinea pigs were immunized with bovine anti - RP IgG while another set of rabbits and guinea pigs were immunized with

\* Part of thesis submitted to IVRI Deemed University for the award of M.V.Sc. Degree.

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Table

Day	Dose injected	Route
0	Test bleeding and injection 500 µg of IgG/Rabbit 100 µg of IgG/Cuineapig	S/C in FCA
14	800 µg of IgG/Rabbit 100 µg of IgG/Guineapig	I/M in IFA
21	1000 µg of F(ab) <sub>2</sub> /Rabbit 200 µg of F(ab) <sub>2</sub> /Guineaping	I/V in PBS
28	Test bleeding	I/M in PBS

bit anti - RP IgG.

### Materials and Methods

**Virus:** Lapinized rinderpest virus (LRPV) used in this study is the freeze-dried Nakamura III strain of LRPV (Passage 990 to 991) maintained at Rinderpest Laboratory, IVRI, Mukteswar. Tissue culture rinderpest virus (TCRPV) used is the RBOK strain of rinderpest virus (99th passage) adopted in vero cell line (7th Passage).

**Anti - RP Hyperimmune Sera:** Hyperimmune serum (HIS) against LRPV was raised in rabbits according to the procedure followed by Bansal (1986). Bovine anti - RP HIS was received from Rinderpest Laboratory, Mukteswar.

**Preparation of anti - RP IgG and F (ab)<sub>2</sub>:** IgG fraction from bovine HIS was separated using ammonium sulphate precipitation at 40% saturation followed by DEAE ion exchange chromatography. IgG from rabbit HIS was affinity purified using protein A sepharose. F (ab)<sub>2</sub> fractions from respective IgG were made by digestion with Pepsin (Sigma, 2900 Units / mg) followed by gel filtration in ultrogel AcA 44 column.

**Immunizations I:** Four rabbits and four guinea pigs were immunized with bovine anti - RP IgG (F (ab)<sub>2</sub> booster). Another set of four rabbits and four guinea pigs were immunized with rabbit anti - RP IgG (F(ab)<sub>2</sub> booster). The Schedule adopted is shown in Table I

**Immunoabsorbents:** Immunoabsorbents were prepared by coupling respective anti - RP IgG to Sepharose 4B as per Hudson and Hay (1976) procedure. Immunoabsorbents were also prepared by coupling respective normal serum IgG to immobilized Bovine serum albumin (BSA) as per Avrameas and Ternynck (1969) procedure.

**Screening of antisera raised against anti - RP IgG:** Antisera raised against anti - RP IgG were tested in Agar gel precipitation test (AGPT) against respective normal and anti - RP IgG before and after absorption to normal serum IgG immunoabsorbents.

**Enzyme linked immunosorbent assay (ELISA)** was conducted to test respective absorbed pooled sera. For testing antibodies against rabbit anti - RP IgG, 0.2 ml of bovine HIS (1:5000 dilution) was coated on to wells of Nunc ELISA modules.

Likewise for testing antibodies against rabbit anti - RP IgG, rabbit HIS (1:5000) dilution) was coated on to the wells. Competition binding assay was conducted as per the following procedure. Wells of Nunc ELISA modules were coated with 0.2 µg/well of bovine anti - RP F(ab)<sub>2</sub> or IgG. After washing 0.1 ml of 1:25 dilution of antiserum raised against anti - RP rabbit IgG followed by 0.1 ml of serial log dilutions of purified TCRPV. After 1 hour incubation at 37 °C wells were washed followed by addition of conjugate. The test was also done after blocking with a single dilution of virus (1:10) followed by addition of serial dilutions of serum.

*Immunizations II:* Serum showing a-id activity was affinity purified using respective anti - RP IgG Sepharose 4B column. IgG and F(ab)<sub>2</sub> fractions from affinity isolated a - id were used for immunizing rabbits as per the procedure used in Immunizations I.

These rabbits were bled one week after the last injection and the sera were tested for virus neutralizing activity against Vero adapted TCRPV and for precipitation reaction in counter immuno- electrophoresis (CIE).

### Results

In AGPT, sera raised against both rabbit and bovine anti RP IgG fractions reacted with respective normal and anti - RP IgG before the after absorption to normal IgG immunoadsorbents. After absorption only antisera raised in rabbits, against rabbit anti - RP IgG reacted with respective anti - RP IgG.

In ELISA, rabbit serum against rabbit anti - RP IgG only was found

to show idotype a-id binding and gave a titre of 400. In competition binding assay the same a - id was competitively inhibited by increasing concentration of virus (Fig.1) while blocking with fixed dilution of virus (1:10) prevented a - id binding (Fig.2). Affinity isolation yielded 125 µg of proteins, from 1 ml of pooled a - id serum. Antisera raised against a - id gave a maximum SN index of 2.8. None of the sera showed precipitin activity in CIE against positive RP antigen.

### Discussion

In this study an attempt to raise a - id was made using unpurified IgG fractions separated from anti - RP HIS raised in cattle and rabbits. The unpurified a - id were found effective in eliciting an immune response against specific antigens by different studies (Sacks et al., 1982; Cell and Moss, 1985). Since HIS will contain more concentration of specific idiotypes compared to antibodies is an a-id serum which as stated earlier elicits an immune response, it was considered possible to generate an a-id response with IgG fraction and F(ab)<sub>2</sub> separated from anti - RP HIS. In this study a-id response was obtained in allogenic system. The failure encountered in raising Xenogenic a - id may be due to the low concentration of specific idiotypes and presence of other potential antigenic determinants such as isotypes and allotypes. Kennedy *et al.* (1983) showed interspecies idotype a - id reaction & used it to detect combining site specific a - id and confirmed it by using the ability of the antigen to inhibit the reaction. In the present report the allogenic a - id were found to bind to bovine anti - RP idiotypes

and the reaction was competitively inhibited by the virus. This proves the specificity of the a - id. Antisera raised against a - id showed virus neutralizing activity but did not show any precipitin activity in CIE. This could be due to lack of a - id against precipitins in the a - id used as immunogens. Further studies with specific affinity purified or

monoclonal anti - RP antibodies will be of help in analysing the usefulness of a - id in RP antigen system.

#### Acknowledgements:

The authors wish to thank the Director, IVRI for providing the necessary facilities to carry out this work.

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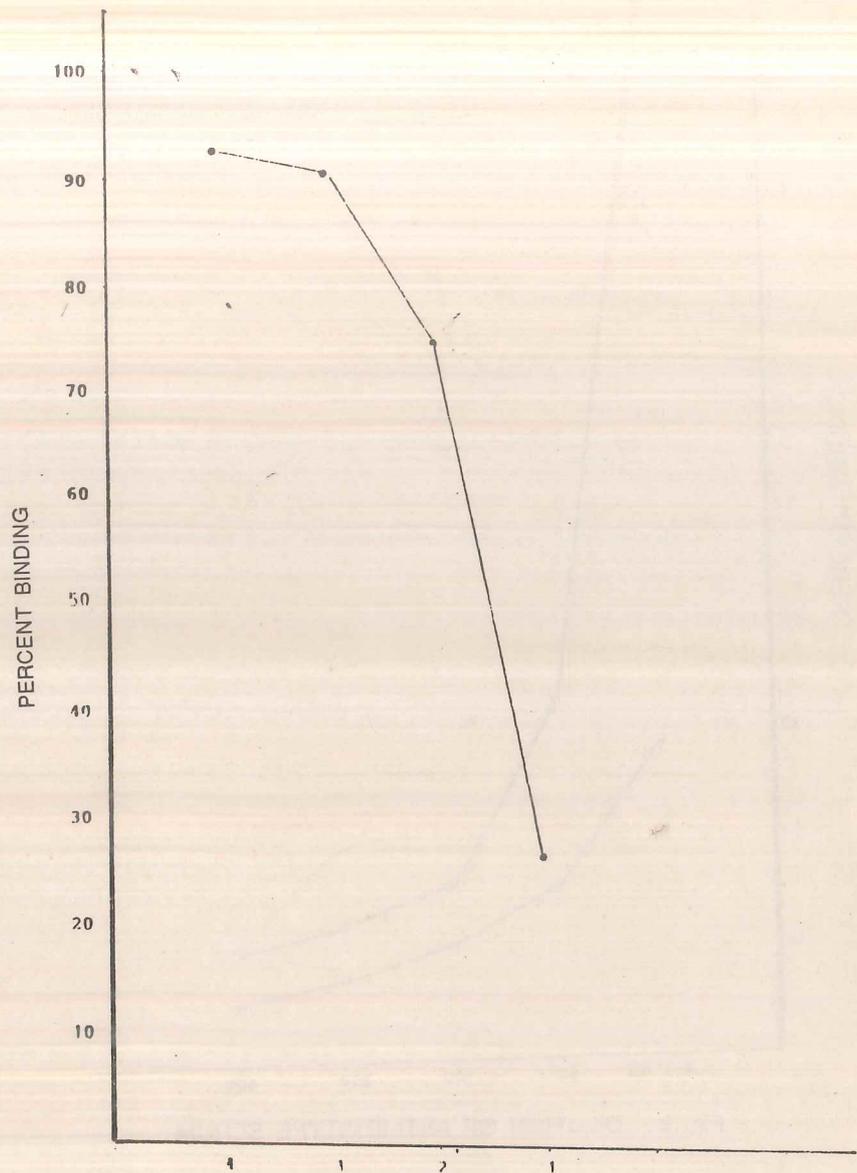


Fig. 1. PERCENT BINDING COMPETING ANTIGEN (TCRPV)  
Log dilution

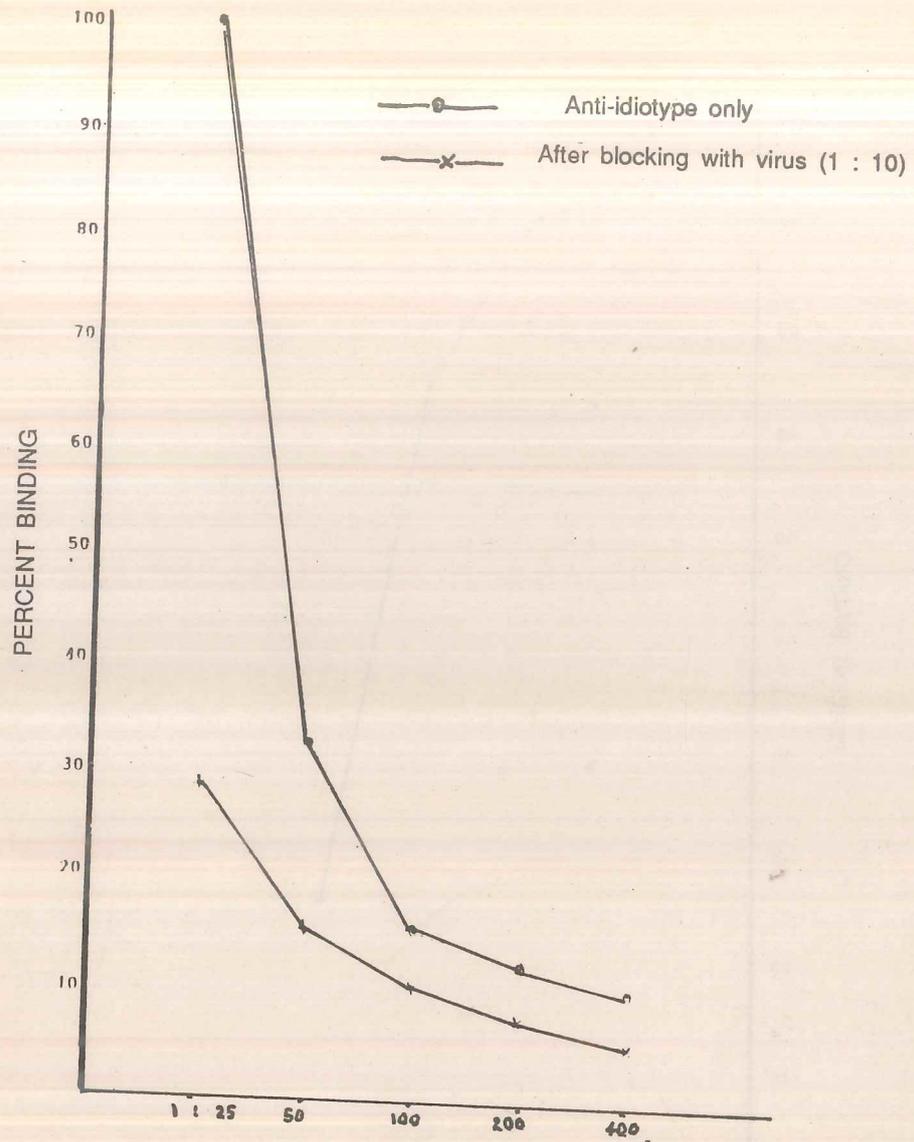


Fig. 2. DILUTION OF ANTI-IDIOTYPE SERUM

## Sub - Unit Vaccine for Poultry against Newcastle Disease Virus

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### ABSTRACT

Field strains of Newcastle disease virus were isolated and characterized. On characterization they were found to be of velogenic nature. The viruses were propagated in embryonated hen's eggs and further concentrated and purified. The purified virus isolates were subjected to Sodium dodecyl sulphate - Poly acrylamide gel electrophoresis for fractionation of viral proteins. Among the different viral proteins fractionated, those with a molecular weight of 74,000 d (VP<sub>74</sub>) and 64,000 d (VP<sub>64</sub>) were subjected to immunogenicity trials in chicks. Although both VP<sub>74</sub> and VP<sub>64</sub> were found to be immunogenic when given alone, better response was observed when they were administered together. The submit vaccine did not produce any side effects and was quite safe.

### Introduction

Although several types of live and inactivated vaccines were developed and used, Newcastle disease (ND) continues to plague the poultry industry to a great extent. In order to overcome the problems encountered with the existing vaccines, attempts are being made to find out the specific protective proteins of the virus. Immunity trials employing viral subunits have been carried out for several animal and poultry viruses (Bachrach *et al.*, 1975; Gough *et al.*, 1983; Rai *et al.*, 1986; Meulemans *et al.*, 1986; Umino *et al.*, 1987) including Newcastle disease Virus (NDV). The present work describes the immunogenic properties of the subunits of a field strain of NDV.

### Materials and Methods

#### Virus:

Three strains of Newcastle disease virus (NDV) were isolated from field outbreaks in embryonated hen's eggs and among them NDV 1 was employed in preparation of sub - unit vaccine.

#### Virus characterization tests:

Mean death time (MDT), Intra - cerebral Pathogenicity index (ICPI) and Intravenous pathogenicity index (IVPI) were conducted as described by Allan *et al.* (1978). Other tests like stability of haemagglutinins at 56°C (Tanwani, 1974), agglutination of mammalian erythrocytes (Winslow *et al.*, 1950) and adsorption of haemagglutinins by chicken brain cells (Han-

son *et al.*, 1967) were carried out as per standard procedures.

#### *Virus purification and preparation of viral sub units*

The virus was propagated in embryonated hen's eggs and the amnio allantoic fluid (AAF) was collected from several embryonated eggs. After testing for its haemagglutinating activity, the virus was purified as per the method of Haslam *et al.* (1969).

The virus sample was dissolved in 0.008M Sodium phosphate, 1% sodium dodecyl sulphate (SDS), 1% -mercaptoethanol and boiled at 100°C for two minutes and then dialysed in 0.008M Sodium phosphate, 0.1% SDS and 0.1% -mercaptoethanol.

#### *Polyacrylamide gel electrophoresis*

The gel system used contained 10% (W/V) acrylamide, 0.27% (W/V) N,N - ethylene bis acrylamide, 0.1% SDS, 3.37 M Tris - Hcl (pH 8.9), 0.05% V/V TEMED and 0.06% (W/V) Ammonium per sulphate. The electrophoresis buffer contained 0.05 M Tris, 0.38 M Glycine and 0.1% (W/V) SDS. Gels were prepared in 90 mm x 5 mm glass tubes and each gel tube was charged with 50 ul of the sample. Electrophoresis was performed initially at 2mA per gel tube for 10 minutes and then at 4 mA per gel tube till the end of the run.

#### *Elution of viral proteins and immunisation:*

The regions of several unstained gels corresponding to the location of various fractions of a stained reference gel run in parallel were collected by slicing and homogenizing in 0.1 ml of water per gel. This was allowed to stand at 4°C overnight and

the gel water mixture was diluted with a equal volume of phosphate buffered saline and clarified by centrifugation at 3000 rpm for 30 minutes. The supernatant containing the gel eluted protein was stored at -20°C.

Among the several viral protein fractions, two fractions namely VP<sub>74</sub> with a molecular weight of 74,000 d and VP<sub>64</sub> with a molecular weight of 64,000 d were employed for conducting immunity trials. Both the fractions were administered alone and in combination to 7 day old chicks and the immunity was assessed by haemagglutination inhibition (HI) test following the procedure of Cunningham (1966) and challenge experiments using 100 CMD<sub>50</sub> of virulent virus.

#### **Results**

Three strains were isolated from field outbreaks and their virulence and strain differentiating characters are given in Table 1. Characterisation results revealed that all the three strains were of velogenic nature.

On fractionation NDV 1 was found to contain a total of six fractions with approximate molecular weight of 89,000 d (VP<sub>89</sub>), 84,000 d (VP<sub>84</sub>), 74,000 d (VP<sub>74</sub>), 64,000 (VP<sub>64</sub>) 59,500 (VP<sub>59.5</sub>) and 48,500 (VP<sub>48.5</sub>). The results of immunogenicity trials conducted in chicks are presented in Table 2. In birds administered with VP<sub>74</sub>, the mean post - inoculation (PI) HI titres at 7, 14 and 21 days were 14.8 ± 3.16, 36.8 ± 4.8 and 67.2 ± 11.14 respectively. With regard to birds inoculated with VP<sub>64</sub>, the mean HI titres were found to be 15.6 ± 3.83, 41.6 ± 12.15 and 43.2 ± 10.94 on 7,

Table 1 Virulence and Strain Differentiating Characters of the Isolates

Isolate No.	Virulence characters			Strain differentiating characters			
	MDT	ICPI	IVPI	Stability of HA at 56°C	Agglutination of equine RBCs	% of HA adsorbed by chick-on brain cells	Inference
NDV 1	52.5	1.86	2.51	60	-	75.00	Velogenic
NDV 2	48.0	1.85	2.61	120	-	93.75	Velogenic
NDV 3	48.5	1.80	2.75	120	-	87.50	Velogenic

14 and 21 days respectively. When both VP<sub>74</sub> and VP<sub>64</sub> were administered together the titres were found to be  $9.6 \pm 1.81$  on 7th day PI,  $54.4 \pm 13.32$  on 14 days PI and  $86.4 \pm 11.73$  on 21 days PI. In un inoculated control birds, the HI antibody titres dwindled gradually and on 21st day PI no antibodies could be detected. The HI antibody response of different fractions of NDV is compared and presented graphically in Fig.1.

The results of challenge experiments are presented in Table 3. All the un inoculated control birds succumbed to the disease. The percentage of protection afforded by VP<sub>74</sub> VP<sub>64</sub> and VP<sub>74</sub> + 64 were found to be 70, 50 and 90 respectively. The post

- challenge, mean HI antibody titres were found to be 160, 112 and 224 for those inoculated with VP<sub>74</sub>, VP<sub>64</sub> and VP<sub>74</sub> + 64 respectively.

### Discussion

The main objective of the study is to assess the immunogenicity of NDV proteins and it is considered ideal to use local isolates for vaccine production. With this idea three strains of NDV were isolated from field outbreaks. From the results obtained (Table 1) all the three isolates could be characterized as Velogenic as it has been reported earlier (Lancaster, 1962; Hanson *et al.*, 1967; Allan *et al.*, 1978). The results of fractionation of NDV proteins cor-

Table 2 Mean HI Antibody Response of Different NDV Proteins

Fraction	Pre inoculation Titre	Post inoculation Titre		
		7 Days	14 Days	21 Days
VP <sub>74</sub>	$19.2 \pm 5.95$	14.80 $\pm 3.16$	36.80 $\pm 4.80$	67.20 $\pm 11.14$
VP <sub>64</sub>	$20.4 \pm 6.18$	15.60 $\pm 3.83$	41.60 $\pm 12.15$	43.20 $\pm 10.94$
VP <sub>74+64</sub>	$17.2 \pm 3.48$	9.60 $\pm 1.81$	54.40 $\pm 13.32$	86.40 $\pm 11.73$
Uninoculated controls	$24.0 \pm 6.18$	1.60 $\pm 0.89$	1.60 $\pm 0.89$	0.00

Table 3 Results of Challenge Experiments

Protein fraction inoculated	No. of birds survived/ challenged	% of survival	Post - challenge Mean HI titre
VP <sub>74</sub>	7/10	70	160.0
VP <sub>64</sub>	5/10	50	112.0
VP <sub>74+64</sub>	9/10	90	224.0
Uninoculated controls	0/10	0	

raborated with the studies of earlier workers (Evans and Kingsbury, 1969; Haslam *et al.*, 1969; Shapiro and Bratt, 1971).

Evaluation of antibody response to different sub units of NDV (Table 2) clearly indicates that these proteins could act as antigens and evolve antibody response as reported by earlier workers (Meulemans *et al.*, 1986; Umino *et al.*, 1987; Kumanan, 1989). However, it could be noticed that combination of subunits was more beneficial to one particular sub-unit or other. Moreover sub units VP<sub>74</sub> and VP<sub>64</sub> did not insight any untoward reaction in the vaccinates.

Results of challenge experiments indicate that neither VP<sub>74</sub> nor VP<sub>64</sub> could produce substantial protection when used alone. However, when these were used in combination substantial protection could be induced. The fact that the sub units VP<sub>74</sub> and VP<sub>64</sub> bear antigenic epitopes in common with whole NDV is exemplified by the anamnestic response obtained on challenge with virulent NDV (Table 3). However, further studies are required for improving the protection percentage and also to identify the genome responsible for the subunits which will lead to the production of recombinant vaccines.

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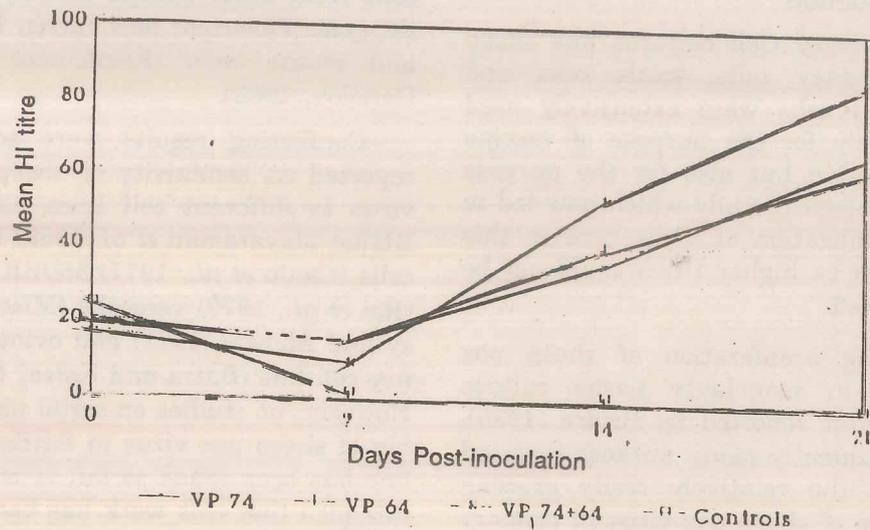


Fig. 1. MEAN HI ANTIBODY RESPONSE OF NDV PROTEINS

## Adaptation of Sheep Pox Virus to BHK<sub>21</sub> Cell Line

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### ABSTRACT

*An attempt was made to adapt the Ranipet strain of sheep pox virus to BHK<sub>21</sub> cell line. The virus was able to produce definite CPE from first passage itself. The titre of the sheep pox virus increased progressively and the titre value at 15th passage was 10<sup>6.5</sup>. Presence of sheep pox virus in the system was confirmed by Agar Gel Precipitation Test and Counter Immuno Electrophoresis. Kinetic studies revealed that peak titre value obtained at 60th hour was maintained till 72 hrs and thereafter no increase was noticed.*

### Introduction

Primary Cell cultures like sheep kidney cells, testis cells and thyroid cells were extensively used not only for the purpose of vaccine production but also for the purpose of serological study which now led to a stabilization of virus titre in this system as higher titres could not be achieved.

The proliferation of sheep pox virus in monolayer tissue culture was first reported by Bridre (1935). Subsequently many workers reported about the relatively ready growing nature of sheep pox virus in primary monolayers of epithelial or fibroblastic cells from many susceptible cell from sheep namely testis (Bridre, 1935) kidney (Boue *et al.*, 1957) thyroid cells (Nitzoche, *et al.*, 1967) embryonic cells (Aygun, 1950), and

cells from other species namely cattle, goat (Plowright and Ferris, 1958) and mouse cells (Romanenco and Gorrilov, 1963).

Conflicting results were so far reported on sensitivity of sheep pox virus to different cell lines namely BHK<sub>21</sub> (Jayaraman *et al.*, 1969) HeLa cells (Canju *et al.*, 1977) MDBK cells (Rai *et al.*, 1977) verocells (Mirchamisy and Ahowai, 1971) and ovine kidney cell line (Batra and Kalra, 1989). However, no studies on serial passaging of sheep pox virus in BHK<sub>21</sub> cell line has been made so far. It is with this idea that this work has been attempted.

### Materials and Methods

*Cell Line:* An established cell line BHK<sub>21</sub> (C13) obtained from Institute

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of Veterinary Preventive Medicine, Ranipet was used for this study.

*Virus:* The lyophilized sheep pox virus, (Ranipet Strain) adapted to sheep thyroid cells and to lamb kidney cells were used.

*Cell line Propagation:* Minimum Essential medium (Eagles Modification) with Earles Salts, L.Glutamine and non essential aminoacids without sodium bicarbonate was used as basal medium. Calf serum at 10% level and 2% level was used for growth and maintenance medium respectively. Trypsin (0.1%), versene (0.02%), glucose (0.05%) solution was used for monolayer dispersion. The subculturing interval was 5 to 7 days. The subculturing ratio was maintained at 1 : 4, the overlay medium replaced by maintenance medium after 48 hrs.

*Virus adaptation:* The start up virus was the virulent virus, the seed virus adapted to sheep thyroid and lamb kidney primary cell cultures. Simultaneous coverslip cultures were made and stained by Haematoxylin and Eosin Stain, Giemsa stain and Acridine Orange stain. The virus was released from infected monolayers by alternate freezing and thawing.

*Titration:* The method followed by Jayaraman *et al.*, (1969) was followed. BHK 21 Cells were uniformly dispersed after trypsinisation, the trypsin was removed using PBS. The cell suspension was suitably diluted with growth medium to have a cell concentration of  $6 \times 10^5$  cells per ml. One ml of cell suspension was added to tubes containing coverslips and incubated at 37°C for 24 to 48 hrs for the monolayer to become complete. The fifth, tenth and fifteenth passage sheep pox virus were serially diluted

with maintenance medium from  $10^{-1}$  to  $10^{-9}$ . For each dilution six tubes were used and an adsorption time of one hour was given. One ml of maintenance medium was added to all tubes. The tubes were observed for cytopathogenic effects at regular intervals (24,36,48,60 and 72 hrs) and the TCID<sub>50</sub> was calculated according to the method of Reed and Muench (1938).

*Agar Gel Precipitation Test:* The infected tissue culture fluid after freezing and thawing to which 50 percent glycerol and 10 percent foetal calf serum were added was used as test antigen, Ranikhet Disease Virus (Komorov) Strain was used as negative antigen. Hyper immune serum against sheep pox virus was raised as per the method of Williams and Chase (1974) in rabbits. Inactivated calf serum was used as negative serum control. A slight modification in the composition of gel prepared by Uppal and Nilakantan (1967) was followed. Aga-rose : 1.O.G. Sodium Chloride: 1.6 G Sodium thiomersalate to a final concentration of: 1 : 10,000, Triple Distilled water : 100 ml. Well of 6mm diameter with a central well and radial wells with an interwell distance of 6mm were cut. The bottom wells were sealed with Agarose, and wells were numbered clockwise.

*Counter immuno electro phoresis:* One per cent agarose was prepared in 0.04 M Barbitone buffer (Chand *et al.*, 1985). Molten agarose was poured on a glass slide, two rows of two wells were cut with an inter well distance of 3.5mm.

*Acridine Orange Staining:* The coverslip cultures were stained as per the methods of Anant Rai (1985).

**Kinetic Studies:** Kinetics of sheep pox virus multiplication in BHK<sub>21</sub> cell line was studied with fifteenth passage BHK<sub>21</sub> adapted sheep pox virus in BHK<sub>21</sub> cell line.

### Results and Discussion

Virulent sheep pox virus was able to cause cytopathogenic effects from the first passage itself whereas the sheep thyroid and lamb kidney adapted virus failed to grow in BHK<sub>21</sub> cell line, and the failure to grow may be due to delay in development of specific receptors for the host. On the contrary, the virulent virus being more invasive adapts immediately to the new surrounding.

The cell rounding which was noticed after 72hrs in the initial stages advanced to 48 hrs post - infection at 6th passage and at 24hrs post - infection at 15th passage. Angular separation of cytoplasm and spindle cells were noticed 24 hrs after rounding of cells. (Fig.2) The rounded cells often aggregate as dark brown foci and these heaped up and float. There was increasing tendency to peel off from glass surface. (Fig.3) Intracytoplasmic acidophilic inclusion bodies of various sizes were noticed (Fig.4 & 5) from 5th passage and these fluoresce as apple green mass in Acridine Orange staining. The findings were identical to those of Jayaraman *et al.* (1969).

The virulent sheep pox virus gave 2 precipitin lines whereas the BHK<sub>21</sub> adapted sheep pox virus gave only one line and this may be due to the difference in the antigenic composition and it could be changed depending upon the source of hyperimmune serum. (Fig.6) Uppal and Nilakanten (1968) obtained two precipitin lines and Bhambani and

Krishnamurthy (1963) obtained three precipitin lines using serum raised in rabbits. With hyperimmune serum raised in sheep, Sambyal and Singh (1980) obtained ten precipitin lines whereas Srivastava and Singh (1980) obtained only five lines.

Precipitin arcs were obtained against hyperimmune serum in rabbits with 4mA current per slide after 45 mts. This confirms the findings of Sarkar *et al.* (1976).

The titre of sheep pox virus at 5th passage was  $10^{4.4}/0.1\text{ml}$ , at 10th passage it was  $10^{5.16}/0.1\text{ ml}$  and at 15th passage it was  $10^{6.5}/0.1\text{ ml}$ . In the present study peak titre of  $10^{6.5}$  was obtained at fifteenth passage whereas a titre of  $10^{6.7}$  was obtained at seventh passage in lamb testis cells (Pandey *et al.*, 1969) but it was only  $10^{4.8}$  even at 11th passage in lamb kidney cells (Mateva and Stoitchev, 1976) and  $10^{5.5}$  in sheep thyroid cells (Jayaraman *et al.*, 1969) The titre values are as good as that from lamb kidney and sheep thyroid cells.

In the kinetic studies, cell rounding was noticed from 24 hrs which is a cellular reaction of host cell to ingestion of virus. The titre at 36th hour was  $10^{4.5}$ , then the titre gradually increased to  $10^{4.8}$  by 48th hour, the maximum titre of  $10^{6.5}$  was reached at 60th hour which was maintained till 72 hours.

Mathew (1987) while studying the kinetics of Cow pox, Buffalo pox and Vaccinia was able to record a reduction in the titre value after 120 hrs. Such a reduction could not be recorded in the present study; the reason being the use of cell line itself for titration whereas Mathew (1987) used embryonated eggs to assess the

titre of harvested fluid. The Ranipet strain of sheep pox virus which was used in the present study is not sen-

sitive to embryonated eggs according to Jayaraman *et al* (1969).

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Fig. 1. Uninfected BHK 21 cell line  $\times 80$



Fig. 2. Rounding of cells and stellate cells  $\times 80$

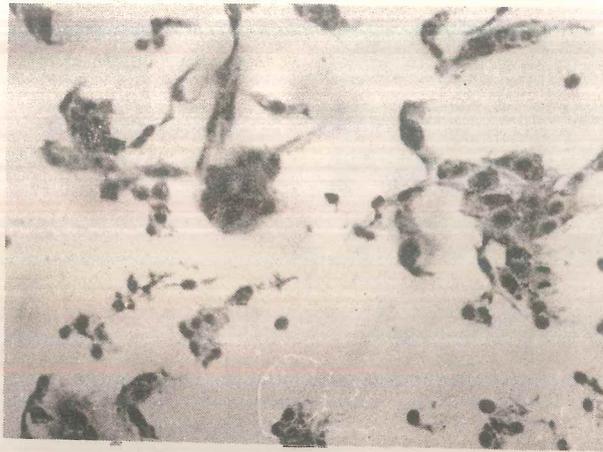


Fig. 3. Peeling of cells from glass surface  $\times 80$



Fig. 4. Intracytoplasmic acidophilic inclusion bodies  $\times 750$



Fig. 5. Intracytoplasmic acidophilic inclusion bodies of various size x 750

Fig. 6- Agar gel Precipitation test

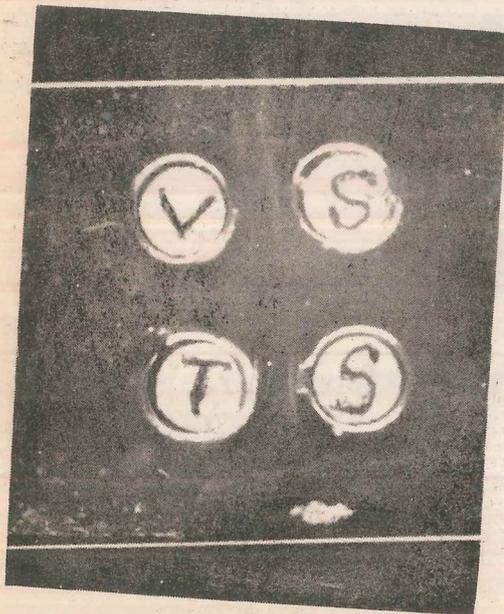
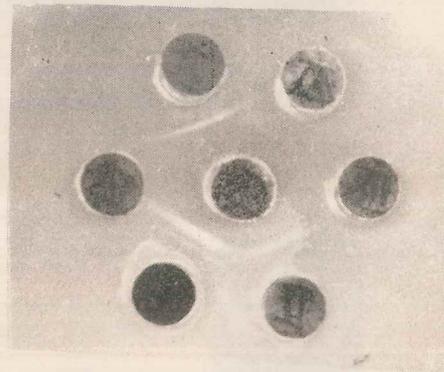


Fig. 7. Counter immuno electrophoresis

## Embryo Vaccination for Marek's Disease

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**M**arek's disease, a lymphoproliferative disease of chicks, caused heavy economic loss in poultry producing countries of the world. It was controlled by vaccinating the day old chicks with Herpesvirus of turkeys (HVT), an antigenically related virus to Marek's disease virus. Sharma and Burmester (1982) successfully vaccinated 18 day old embryos to maximise the gap between vaccination and challenge with virulent virus.

In this study, an attempt was made to vaccinate 18 day old embryonated eggs and the response was compared with the response to 'O' day old vaccination by serum neutralization test (SNT) and lymphocyte migration inhibition test (LMIT).

### Materials and methods

*Chicks and eggs:* Eggs and chicks obtained from vaccinated White leghorn hens were used for this study.

*Vaccine virus:* Cell free HVT FC 126 (Salsbury Laboratories, USA) diluted as per the manufacturer's instructions was used and the dose was 1000 plaque forming units (PFU) for both eggs and chicks.

*Virus for SNT:* Chick embryo fibroblast passaged HVT virus obtained from Institute of Veterinary

Preventive Medicine, Ranipet was used in SNT.

*Vaccination of eggs:* Embryonated eggs (18 day old) were vaccinated as per Sharma and Burmester (1982). Each embryonated egg received through its large end 0.1 ml of vaccine with one inch long 22G needle.

*Vaccination of chicks:* Each chick received 0.2 ml of vaccine, subcutaneously in the upper neck region on the day of hatch.

*SNT:* HVT was titrated as per the method of Calnek *et al.* (1972) and Sharma (1980). SNT was performed as per Churchill *et al.* (1973) and was carried out in test tubes with coverslip in CEF cultures.

*LMIT:* The test was conducted from blood collected from experimental birds, as per the methods of Timms (1974) and Timms and Cullen (1976).

*Serum samples:* Serum samples were collected from birds under treatment at different intervals and stored at -20°C until used.

### Results

*Embryo Vaccination:* The results of SNT on the serum samples of chicks, hatched from vaccinated eggs is presented in Table 1. The mean SN<sub>50</sub> titre of neutralizing antibodies on the day of hatch was 89.6 ± 23.3 as compared to SN<sub>50</sub> titre of 99.2 ±

**Table 1: Development of neutralizing antibody in chicks with maternal antibodies**

Sl. No.	Time of vaccination	*Serum samples tested in days of age						
		0	3	7	14	21	28	35
1.	18th day embryo	89.60	62.40	41.60	20.00	7.20	9.60	33.60
		± 23.30	± 14.56	± 13.90	± 7.17	± 1.77	± 3.15	± 15.80
2.	'O' day old chicks	99.20	67.20	36.40	18.40	6.40	12.00	36.80
		± 21.32	± 18.72	± 15.59	± 7.42	± 1.89	± 4.27	± 14.80

\* Mean of serum samples from 10 randomly selected chicks.

**Table 2: Leukocytes migration inhibition values in chicks with maternal antibodies**

Sl. No.	Time of vaccination	*Blood tested in days of age						
		0	3	7	14	21	28	35
1.	18th day embryo	20.49	26.57	33.12	38.56	44.36	48.74	53.73
		± 3.00	± 4.21	± 6.08	± 4.34	± 6.44	± 3.39	± 4.63
2.	'O' day chicks	10.14	21.46	30.57	35.78	42.44	50.22	54.48
		± 1.01	± 2.21	± 5.18	± 3.49	± 5.31	± 6.48	± 6.13

\* Mean of samples from 5 randomly selected chicks.

21.32 in 'O' day vaccinated chicks. The antibody level regressed from the day of hatch to a level of 7.2 ± 1.77 on 21 days. Thereafter the antibody level presented a rise by 28th day followed by a further rise by 35th day of hatch, when the SN<sub>50</sub> titre reached a level of 33.6 ± 15.8. The antibody profile of the 'O' day group was similar to that of embryo vaccinated group. There was also no significant difference between the groups on different days of test (P>0.05).

In chicks derived from eggs vaccinated on 18<sup>th</sup> day, the mean LMI on the day of hatch was 20.49 ± 3.00 and there was a steady rise in the LMI value which rose upto 53.73 ± 4.63 on 35th day of hatch. On the other hand, in the 'O' day vaccinated

chicks, the mean LMI on the day of hatch was 10.14 ± 1.01 and there was a steady rise in LMI value throughout thereafter and it was 54.48 ± 6.13 by 35 days of age. There was significant difference in the LMI values (P<0.05) of both the groups on days '0' and 3rd day of age.

### Discussion

In the present study embryonally vaccinated chicks had better immune responses than the other group of chicks. Sharma and Burmester (1982) reported that 18 day embryonally vaccinated chicks had much greater resistance to challenge than 'O' day vaccinated chicks.

It was also observed that though maternal antibodies in eggs could

reduce the vaccine efficacy, the 18 day embryo vaccinated chicks had better protection against early post-hatch challenge with virulent MDV when compared with chicks which have been vaccinated on the day of hatch (Sharma and Graham, 1982).

The observation that 18<sup>th</sup> day embryo vaccinated chicks had a reasonably good immune response even in the presence of maternal antibodies is in agreement with previous reports (Sharma and Graham, 1982; Sharma *et al.*, 1984).

In the present study both humoral and cell mediated responses of embryo vaccinated chicks were good nor the vaccination induced any lesions in the experimental chicks. Sharma and Witter (1983) observed that the administration of vaccines of serotype 1, 2, or 3 either singly or in combination in 18 day old embryos, afforded better protection than chicks vaccinated at hatch when they were

challenged at earlier days of life. Sharma *et al.* (1984) compared the viral, immunological and pathological responses of chicks inoculated with HVT on embryos or at hatch and observed that recoverable HVT in tissues of 18 day embryo vaccinated chicks were higher than hatch vaccinated chicks and embryo vaccination did not cause any lesions, reduction in body weight gain or impairment of humoral and cell mediated immune responses. They concluded that HVT could safely be used as an embryonal vaccine in chicks.

In contrast to these reports above, Werner *et al.* (1984) observed that embryo vaccinated chicks with Riem's vaccine in Germany had less protection than hatch vaccinated chicks. The differences of this finding with the present study might be due to the vaccine used and/or the strain of the chick.

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## Strategy of Split Dose Vaccination for Foot and Mouth Disease in Lambs

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**F**oot and Mouth disease is one of the worst feared animal viral disease as it caused a serious economic loss by way of reduction in milk yield, infertility, loss of calves, etc. In India, at least four major types namely O, A, C and Asia 1 have been recorded (Hickey, 1918, Kant *et al.*, 1986).

Multivalent vaccines comprising all the above major types have been used in cattle and sheep in India. Periodical revaccination once in 6 months is advocated for effective control program.

The study was undertaken to assess the age of primary vaccination without interference by maternal antibodies, and also to find out whether split vaccination could be successfully used in lambs.

### Materials and Methods

*Animals:* Mandya sheep obtained from the Livestock Research Station, Kattupakkam were used.

*Serum neutralization test:* Micro serum neutralization test (SNT) was done with serum samples collected from animals under experiment as per the method of Golding *et al.* (1976) with modifications (Francis and Black, 1983).

*Complement Fixation test:* Direct complement fixation test, (micro test)

was done with serum samples collected from lambs.

*Vaccine:* Inactivated polyvalent FMD vaccine brew No. 3/83 of Indian Veterinary Research Institute, Bangalore was used. The individual animal dose was 5 ml. for primary vaccination and after 21 days time, for secondary response 2.5 ml was inoculated.

*Design of experiment:* 8 lambs formed one group and the groups were vaccinated on 14, 30, 60, 90, 120, 150 or 180 days of age followed by revaccination after 21 days with half of the original dose. Blood was collected on the day of vaccination, 7 and 21 days after primary vaccination and 7 and 21 days after secondary vaccination for each group. The serum samples thus collected were subjected to micro SNT and micro CFT tests.

### Results

In all the first four groups namely 14, 30, 60 and 90 day old lambs, the maternal antibodies which were high upto 30 days of age, started a downward trend thereafter and got extinct by 140 days. In these groups the development of both neutralizing and complement fixing antibodies were very slow and this is the trend noticed with all the four types of FMDV (Tables 1 to 4).

Table 1 Ab response to FMDV 'O' type in sera of lambs

Sl. No.	Vaccination		Kind of test	Log Titre Value				
	I days	II days		Days of testing			Post vaccination	
				I			II	
				0	7	21	7	21
1.	14	35	SN50	1.76	1.22	1.11	1.07	0.80
			CF50	1.74	1.15	1.04	0.99	0.83
2.	30	51	SN50	1.72	1.24	0.95	0.87	1.06
			CF50	1.68	1.14	0.91	0.77	0.95
3.	60	80	SN50	1.37	1.03	0.72	0.79	1.11
			CF50	1.32	0.74	0.53	0.72	1.03
4.	90	111	SN50	1.13	0.94	0.60	0.75	1.47
			CF50	1.08	0.90	0.45	0.63	1.36
5.	120	141	SN50	0.26	1.04	1.24	1.50	1.93
			CF50	0.60	0.91	1.11	1.37	1.81
6.	150	171	SN50	0.60	0.71	1.40	1.66	2.00
			CF50	0.60	0.56	1.29	1.56	1.86
7.	180	201	SN50	0.60	0.73	1.55	1.73	2.02
			CF50	0.60	0.37	1.42	1.62	1.92

Table 2 Ab response to FMDV 'A' type in sera of lambs

Sl. No.	Vaccination		Kind of tests	Log Titre value				
	I days	II days		Days of testing			Post vaccination	
				I			II	
				0	7	21	7	21
1.	14	35	SN50	1.72	1.20	1.09	0.95	0.92
			CF50	1.73	1.15	1.06	0.92	0.71
2.	30	51	SN50	1.70	1.19	0.96	0.79	0.96
			CF50	1.65	1.14	0.83	0.67	0.82
3.	60	81	SN50	1.35	1.05	0.79	0.86	1.08
			CF50	1.30	0.93	0.64	0.75	1.01
4.	90	111	SN50	1.08	0.88	0.45	0.73	1.43
			CF50	1.04	0.71	0.37	0.60	1.31
5.	120	141	SN50	0.22	1.02	1.20	1.48	1.83
			CF50	0.60	0.91	1.06	1.36	1.72
6.	150	171	SN50	0.60	0.63	1.38	1.57	1.99
			CF50	0.60	0.52	1.25	1.42	1.83
7.	180	201	SN50	0.60	0.67	1.49	1.66	1.95
			CF50	0.60	0.48	1.38	1.55	1.83

Table 3 Ab response to FMDV 'C' type in sera of lambs

Sl. No.	Vaccination		Kind of tests	Log Titre value				
	I	II		Day of testing			Post Vaccination	
				I			II	
				0	7	21	7	21
days	days							
1.	14	35	SN50	1.69	1.20	1.07	0.99	0.76
			CF50	1.70	1.15	1.04	0.95	0.64
2.	30	51	SN50	1.70	1.24	0.69	0.92	0.90
			CF50	1.62	1.14	0.64	0.75	0.81
3.	60	81	SN50	1.34	1.06	0.70	0.79	0.96
			CF50	1.29	0.80	0.60	0.56	0.60
4.	90	111	SN50	1.02	0.81	0.32	0.84	1.38
			CF50	1.00	0.68	0.22	0.56	1.31
5.	120	141	SN50	0.15	0.88	1.14	1.46	1.79
			CF50	0.60	0.75	1.08	1.35	1.71
6.	150	171	SN50	0.06	0.56	1.37	1.61	1.86
			CF50	0.06	0.45	1.25	1.48	1.75
7.	180	201	SN50	0.26	1.04	1.23	1.50	1.93
			CF50	0.60	0.91	1.11	1.37	1.81

Table 4 Ab response to FMDV 'Asia - 1' type in sera of lambs

Sl. No.	Vaccination		Kind of tests	Log Titre value				
	I	II		Days of testing			Post vaccination	
				I			II	
				0	7	21	7	21
Days	Days							
1.	14	35	SN50	1.73	1.26	1.06	0.96	0.93
			CF50	1.71	1.13	0.93	0.92	0.59
2.	30	51	SN50	1.71	1.14	0.75	0.90	0.98
			CF50	1.67	1.10	0.63	0.76	0.93
3.	60	81	SN50	1.39	1.20	0.84	0.79	1.12
			CF50	1.35	1.10	0.67	0.68	0.98
4.	90	111	SN50	1.05	0.83	0.52	0.82	1.48
			CF50	1.00	0.73	0.30	0.63	1.39
5.	120	141	SN50	0.22	0.79	1.27	1.44	1.87
			CF50	0.60	0.75	1.20	1.36	1.77
6.	150	171	SN50	0.60	0.56	1.39	1.65	1.92
			CF50	0.60	0.45	1.29	1.53	1.80
7.	180	201	SN50	0.22	1.02	1.20	1.48	1.87
			CF50	0.60	0.91	1.06	1.36	1.75

The groups for which primary vaccination was done at 120, 150 or 180 days of age, the development of antibody response was on the increase from 7<sup>th</sup> day post-vaccination and continued to rise after secondary vaccination (Tables 1 - 4).

### Discussion

In the present study the maternal antibodies present in the lambs got extinct by 140 days. Laurenaudie *et al.* (1975) reported a similar observation with calves.

Double vaccinations in the four groups before 120 days of age evoked an unsatisfactory response, which is because of the interference by the maternal antibodies in the lambs of these groups. This observation is in agreement with the report of Nicholas *et al.* (1984), who observed that calves with maternal antibodies, not only failed to respond to vaccination but also presented a depressed serum titre and the depression was found to be proportional to the level of pre - existing maternal antibodies at the time of vaccination.

In the groups which received primary vaccination at 120 days of age or after, there was a good primary response and also a better secondary response after revaccination. The primary response induced is good because of non - interference by maternal antibodies the level of which are very low at the time of vaccination. Similar observations have been reported with calves and pigs by Nicholas *et al.* (1984) and Francis and Black (1986) respectively. The study also corroborates a similar study in lambs by Gopalan and Padmanaban (1985) with respect to tissue culture adapted rinderpest virus vaccine.

Shankar and Uppal (1982) hypothesised that split vaccination is best for FMD control irrespective of the presence or absence of maternal antibodies. However, in the present study, split vaccination schedule was found to be effective only when the maternal antibody level decreases to reasonable level. This has to be borne in mind while adopting split dose strategy of vaccination.

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## A Report on the Outbreak of Ranikhet Disease in Japanese Quail Layers

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### ABSTRACT

*An outbreak of Ranikhet in a commercial layer flock of 16 weeks old JAPANESE QUAILS (Coturnix coturnix japonica) is reported in Tamilnadu. The clinical picture in the affected flock was predominantly of nervous manifestations such as drooping of wings, extended legs, torticollis and paralysis with lateral recumbency. A mortality rate of 13% was recorded spread over a weeks time. A production drop of 20% was also noticed. No organisms of any aetiological significance could be isolated by bacteriological examination. Clarified tissue suspension of spleen, brain and trachea from dead and sacrificed birds caused deaths in embryonated hen's eggs in 48-72 hours and the HA titre of the harvested allantoic fluid was found to be  $2^6$ . Haemagglutination by the allantoic fluid was inhibited upto a dilution of  $2^6$  by known RDV antiserum. A four fold increase in HI titre of ailing and convalescent birds was noticed. A strain of RD virus in a commercial laying flock of Japanese quails was isolated and identified for the first time in Tamilnadu.*

**T**he present communication describes an outbreak of Ranikhet disease in Japanese Quail layers in Tamilnadu.

A commercial one thousand unit, 16 week old layer flock of Japanese quails exhibited symptoms of anorexia, diarrhoea and nervous manifestations such as drooping of wings, extended legs, torticollis, paralysis and lateral recumbency. The onset of mortality commenced 8-10 days after exhibition of clinical signs and went upto 13% within 7 days. A production drop from 70% to 50% was also noticed during that period.

Heartblood swabs, spleen, brain tissues and trachea were collected from dead birds and also by sacrificing moribund birds and subjected to microbiological examination.

Bacteriological examination by direct plating of heart blood swabs and plating after enrichment on standard media was attempted.

Conversely, the clarified spleen, brain and tracheal tissue suspension was inoculated in 0.10 ml. amounts into 9 day old embryonated hen's eggs through allantoic route and incubated at 37° and candled every 24 hr. Any death within 24 hr. was considered as non-specific. Allantoic

fluid was harvested from the dead embryos and haemagglutination (HA) test was carried out using chicken erythrocytes as per the method of Cunningham (1966). Against four HA units of the allantoic fluid in saline as antigen, hemagglutination inhibition (HI) test was conducted using a known positive chicken serum (Cunningham, 1966).

Paired sera samples were subjected to HI test from ailing and recovered birds after a lapse of 3 weeks.

Postmortem examination of the dead birds showed wasting of breast muscles, congestion of the intestines, lungs and meninges. Bacteriological examination did not reveal any pathogenic organism of aetiological significance. Embryo mortality was noticed between 48-72 hours, after inoculation with suspected material. The HA titre with chicken erythrocytes of the allantoic fluid collected from dead embryos was  $2^6$ . Haemagglutination by the allantoic fluid was inhibited upto a serum dilution of  $2^6$ .

No HI antibodies were detectable in ailing birds but convalescent sample mean HI antibody titre from 10 birds was  $2^5$ .

Japanese quails are generally considered to be resistant to many diseases including Ranikhet disease. However outbreaks of RD have been recorded on many occasions. [Higgins and Wong, 1968; Hashimoto *et al.*, 1969; Lu *et al.*, 1987; Kumanan *et al.*, 1989]. Kumanan *et al.* (1989) reported a mortality of 10% over a period of 4 months in 4 week old Japanese Quails but in this study it was 13% within one week of commencement of mortality in a layer flock coupled with a production drop of 20%.

Postmortem findings recorded in this study was also very similar to those observed by others [Higgins & Wong, 1968; Kumanan *et al.*, 1989]. Dead embryos showed haemorrhages on the cranium and the body. These results confirmed the findings of others (Jungherr *et al.*, 1946; Higgins and Wong, 1968; Kumanan *et al.*, 1989).

The haemagglutinating property of the allantoic fluid from dead embryos and inhibition of HA property by the known RDV antiserum confirmed the presence of RDV. Further the four fold increase in the HI titres of the paired sera samples reconfirmed the presence of the virus.

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## Characterization of A Newcastle Disease Virus from Japanese Quails in Lay

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### ABSTRACT

A highly virulent field strain of Newcastle disease virus was isolated from a commercial layer flock of 16 week old Japanese quails (*Coturnix coturnix japonica*). Virus isolation was through inoculation of embryonated hen's eggs and the isolate was purified by triple terminal dilution. Virus identification was done by standard Haemagglutination inhibition (HI) test. The isolate was characterised employing sever tests, namely mean death time, intra - cerebral pathogenecity index, intravenous pathogenecity index, thermostability of haemagglutination, haemagglutination (HA) of mammalian erythrocytes, adsorption of haemagglutinins by chicken brain cells and stability of infectivity at pH 3.0. The mean death time of chicken embryos given the minimum lethal dose, ICPI and IVPI values along with other tests employed indicated that the isolate was of the velogenic pathotype.

### Introduction:

Newcastle disease virus (NDV) is a Paramyxovirus that infects birds and cause the disease in poultry. Outbreaks of Newcastle disease (ND) have been reported among various avian species like pigeons (Hanson and Sinha, 1952), racing pigeons (Biancifiore and Fioroni, 1983), ducks and geese (Bush, 1954), turkeys (Gale *et al.*, 1961) and wild birds (Keymer, 1961). The severity of the symptoms of the disease varies from asymptomatic infection to near 100% mortality, depending on the virulence of the virus present (Waterson *et al.*, 1967). Though Japanese quails (*Coturnix*

*coturnix japonica*) are considered to be resistant to ND, they do succumb to ND under stress (Higgins and Wong, 1968; Hashimoto *et al.*, 1969). One such outbreak has already been reported in India in 4 weeks old Japanese Quails (Kumanan *et al.*, 1989). In this paper, ND virus (NDV) isolated from 16 weeks old laying Japanese quails from a commercial quailry has been characterized and presented.

### Materials and Methods

NDV isolated from 16 week old Japanese quails at peak of lay in embryonated hen's eggs was purified

by triple terminal dilution and employed for the study.

Mortality in the outbreak was about 13% spread over a one week period. The quails maintained in the farm have never received ND vaccination. The isolate was identified as NDV by haemagglutination inhibition (HI) using inactivated known NDV antiserum. All virus characterization tests were done with second egg passage of the isolate.

Seven tests were used to characterize the virus isolate: MDT, ICPI, IVPI, (Allan *et al.*, 1978), thermostability of haemagglutination (HA) (Tanwani, 1974), haemagglutination of mammalian erythrocytes (Winslow *et al.*, 1950), adsorption of haemagglutinins by chicken brain cells (Hanson *et al.*, 1967), stability of infectivity at pH 3.0 (Hanson *et al.*, were carried out as per standard procedures.

### Results

The HA titre of the ND virus isolate was found to be  $2^6$ . Known NDV antiserum was found to inhibit the HA activity of chicken erythrocytes by the virus and the HI titre was found to be  $2^6$ .

The MDT, ICPI and IVPI values were found to be 60 hour, 1.95 and 2.24 respectively. The haemag-

glutinins of the isolate was found to be stable upto a period of 90 minutes. The erythrocytes of cattle, sheep, goat, guineapig, monkey and human "O" were agglutinated by the isolate but not those of the horse. The HA titre of the isolate after adsorption with chicken brain cells was found to be  $2^4$  and the percentage of adsorption was 75.0. The infectivity of the isolate was found to be stable at pH 3.0.

The mean pre-infection HI titres of the susceptible chicken used for infectivity tests were  $<2$  and since all the birds succumbed to ND, post-infection titres could not be assessed. The clinical picture in the experimental birds was characterized by a short course, oedema of the head, haemorrhagic conjunctivitis, depression, greenish diarrhoea, torticollis and paralysis. No respiratory symptoms were recorded. Pathognomonic post-mortem lesions observed were the presence of diphtheritic ulcers in the intestine, petechial haemorrhages in the proventriculus and ileo-caecal junction.

### Discussion

Virus identification by standard HI test was considered as a valuable diagnostic tool (Hanson, 1975). In the present study also, the HA activity

#### Virulence and Strain Differentiating Characters

MDT	ICPI	IVPI	Thermostability of haemagglutinin at 56°X	Percentage of haemagglutinins absorbed by chicken brain cells.	Stability of infectivity at PH 3.0	HA of Equine RBCS.
60 hr.	1.96	2.24	90 min.	75%	YES	—

of the isolate was inhibited by known NDV antiserum indicating a serological relationship. Classification of NDV strains into velogenic, mesogenic and lentogenic was based on MDT, ICPI and IVPI values (Hanson *et al.*, 1949; Martone *et al.*, 1973, 1974). Strains having MDT values between 40 and 60 hours were considered velogenic (Hanson and Brandley, 1955) and this isolate with MDT values of 60 hour could be categorised under the velogenic group. The ICPI values ranged from 0.8 - 1.6 for mesogenic strains and 1.75 - 2.0 for velogenic strains (Hanson, 1956; Alexander and Allan, 1973; Bansal and Kumar, 1975) and this isolate with ICPI value of 1.96 and IVPI 2.24 confirms the velogenic nature of the strain (Allan *et al.*, 1978).

In establishing the velogenic nature of the strains, generally other tests like stability of haemagglutinins at 56°C, agglutination of mammalian erythrocytes and adsorption of haemagglutinins by chicken brain cells are not considered. However, it has been reported that velogenic strains do not agglutinate equine erythrocytes (Lancaster,

1967), the percentage of adsorption to chicken brain cells is higher (Piraino and Hanson, 1960) and the haemagglutinins have higher thermostability (Hanson, 1972). The present isolate therefore could firmly be classified as velogenic in view of its higher chicken brain cell adsorption percentage and thermostability for longer periods at 56°C.

An important aspect of ND in quails is the predominant manifestation of nervous signs in the field outbreak with absence of visceral lesions. The signs and lesion in experimental chickens were that of visceral and nervous involvement. It has been proposed that the physiological state of the host, mainly, the effect of residual immunity may be partly responsible for the changes observed in field outbreaks and experimental infection (El - Zein, 1986). The degree of predominance of signs and lesions in one system over the others may be a function of several epidemiological variables (El - Zein, 1986). Hence the differences in clinical picture and lesions between outbreak of ND in susceptible chicken populations and quails under stress needs further elucidation.

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## Screening of Enzootic Bovine Leucosis in Imported Exotic Bulls

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### ABSTRACT

One hundred and forty imported exotic purebred Jersey and Holstein Friesian bulls under quarantine were examined for enzootic bovine leucosis (EBL), employing haematological and serological keys. There is no recorded serological evidence of the disease in Tamilnadu. Since the disease is endemic in the exporting country, confirmation of absence of EBLV infection became mandatory. The circulating lymphocyte levels as indicated by total leucocyte count and increase in immature, atypical lymphocytes as indicated by differential leucocyte count was assessed on three occasions at an interval of 15 days. Serological testing for the presence of EBL antibodies was done using a glycoprotein antigen of the bovine leukaemia virus in an agar gel immuno diffusion test employing Leukassay B kit (Pitman - Moore, Inc., USA - Bovine leucosis - glycoprotein immuno diffusion test kit). Haematological keys and serological key employed did not reveal the presence of the infection in the 140 pure bred imported stock of bulls. For rapid diagnosis of EBL, Leukassay B test kit was found to be satisfactory, since it provided clear results in known positive and known negative cases.

**E**nzootic bovine leucosis (EBL) is a progressive and highly fatal disease of cattle characterised by aggregations of lymphocytes in a wide range of organs (Ferrer *et al.*, 1980), caused by a 'C' type oncornavirus (Tyler, 1978; Muller, 1980) and is widely distributed in most countries (Ferrer, 1980).

The procedure adopted to screen the 80 Jersey and 60 Friesian bulls imported by the Department of Animal Husbandry, Tamilnadu from Germany and Denmark for EBL is explained in this paper.

The bulls ranging in age from 9 months to 1 1/2 years were quarantined and tested thrice at an interval of 15 days by haematological keys comprising of total leucocyte count and differential count and once by agar gel immunodiffusion to detect EBL antibodies with the bovine leucosis virus Glycoprotein antigen obtained from Pitman moore Inc - USA ("Leukoassay B" test kit).

The total leucocyte counts were interpreted as per the method of Grimshaw *et al.* (1979). The mean total lymphocyte counts of the three

successive tests ranged from 3000 - 9000/Cumm. No blast cells or atypical lymphocytes characteristic of EBL were observed in the differential count examinations. The results of the AGID with the sera from the 140 bulls did not reveal the presence of antibodies to Bovine leucosis virus in contrast to the control wells where specific bands were observed with the known positive serum and no bands were seen with the known negative sera. No weak positive or nonspecific lines were observed in the tests, confirming the high sensitivity and specificity of diagnostic kit used in the screening.

The diagnosis of BLV infection is now based on two approaches; the haematological and serological (Doxey, 1983). The level of circulating lymphocytes in positive cases varies depending on the age of the animal, it has been observed (Grimshaw *et al.*, 1979). It has been pointed out that about 60% of the positive cases before the appearance

of clinical signs could be detected by an increase in circulating lymphocyte levels (Grimshaw *et al.*, 1979). In this study, none of the one hundred forty exotic bulls with an average age of 1 1/2 years showed an increase in circulating lymphocyte levels to 10,000/cmm. on repeated testing. The standard European method for detecting antibodies to BLV at present is being the agar gel immunodiffusion test using a viral envelope antigen (gp) and a viral core antigen (p24) (Tyler, 1978). However, owing to non - availability of viral core antigen, Leukassay B test kit (BLV glycoprotein envelop antigen) was employed. In strong positive cases, it has been observed that there was no significant differences in sensitivity between Immunodiffusion gp (IDgp) and the Radioimmunoassay (Ferrer *et al.*, 1980). The results of the IDgp test employing Leukassay B also were clear cut in known positive and known negative cases in this study.

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## Enzyme Linked Immunosorbent Assay for the Detection of Antibodies To Leptospirosis

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### ABSTRACT

Twenty seven sera samples from cattle with history of pyrexia, jaundice and abortion and 15 serum samples from dogs with history of pyrexia, vomition, diarrhoea and jaundice, from different parts of Madras city were screened with Microscopic Agglutination Test (MAT) and Enzyme Linked Immunosorbent Assay (ELISA) technique for leptospiral antibodies. It was observed that in 20 out of 27 cattle serum samples, MAT had shown insignificant titres whereas ELISA had shown significant reading in 25 out of 27 samples.

Similarly in the case of canine serum samples MAT had shown significant titres in 6 out of 15 samples whereas ELISA had given positive titres in 14 out of 15 serum samples. The ELISA technique was found to be more sensitive than MAT in detecting the leptospiral antibodies.

### Introduction

**L**eptospirosis, a common zoonotic disease of livestock and pet animals, is gaining importance at present in the field of both veterinary and human medicine. Besides causing acute illness in man and animals, it is responsible for considerable economic losses in livestock industry by way of abortion, still birth and infertility. Hence the diagnosis of this disease becomes very important.

The laboratory diagnosis can be established either by isolating leptospire from blood, urine and/or organs such as the liver and kidneys or by detecting specific circulating antibodies. The isolation and iden-

tification of leptospire is time consuming and will permit only a retrospective diagnosis.

The most commonly used procedure for the serologic diagnosis of leptospire, (i.e.) Microscopic Agglutination Test (MAT) is laborious to do, and necessitates the maintenance of live cultures of a large number of serovars for use as antigen.

The Enzyme Linked Immunosorbent Assay (ELISA) has been used for the serodiagnosis of a wide range of infectious diseases in man and animals including leptospirosis (Terpstra *et al.*, 1980; Adler *et al.*, 1981). ELISA is more sensitive and would not require live antigens. This

assay would permit rapid screening of large numbers of serum samples for leptospiral antibodies and make more definitive diagnosis.

Hence an attempt was made to compare the efficacy of MAT and ELISA in acute cases of clinical leptospirosis.

#### Materials and Methods

In the present study 27 serum samples from cattle with history of pyrexia, jaundice, abortion and 15 serum samples from dogs with history of pyrexia, vomiting, diarrhoea and jaundice from different parts of Madras city were collected during the monsoon period, Oct.89 to Dec.89.

The serum samples were subjected to MAT and ELISA to detect leptospiral antibodies.

The MAT was performed using live leptospiral serovars (Six serovars) as antigens as per the method described by Faine (1982). The reference strains (serovars *autumnalis*, *canicola*, *icterohaemorrhagiae*, *pomona*, *grippotyphosa*, *hebdomadis*) were obtained from Dr.R.B.Marshall, Massey University, Newzealand. A well grown 5 - 7 old live cultures in liquid EMJH medium was used as antigen. The density of the culture was so adjusted as to contain 200 organisms in 200X power of Dark Field Microscope (DFM). The serum was diluted in PBS pH 7.2 by doubling dilution with initial dilution of 1:10 upto 1:5120. Equal quantities of antigen was added to each dilution and incubated at room temperature for 2 hours. A drop of culture was examined under DFM. The titre of the serum was expressed as the reciprocal of the highest dilution of

serum at which at least 50% of leptospores were agglutinated. A titre of 80 and above was considered to be significant.

ELISA was performed as per the method of Terpstra *et al.*(1980) with slight modifications. Polystyrene microtitre plates (Laxbro, India) were coated for 24 hours with formalin killed heat extracted antigen of *Letospira interrogans* serovars *autumnalis* Akiyami A, *grippotyphosa* Moskva V, *pomona* leptospores (10 ug/ml of protein) to screen cattle sera samples and with serovars *canicola* Hond utrecht IV and *icterohaemorrhagiae* RGA (10 ug/ml of protein) to screen dog serum samples. The coated plates were washed three times with PBS pH 7.2 containing 0.05% Tween 20 (PBST). The cattle or dog serum samples were diluted with the diluent of PBS containing 0.05% Tween 20 and 1% of B.S.A. fraction V in 100ul quantities. After incubation at 37°C for an hour the plates were washed in PBST. Then 100 ul of goat anti - bovine peroxidase conjugated immunoglobulin for cattle serum samples and goat anti-dog peroxidase conjugated immunoglobulin for dog serum samples were added at 1 in 1000 dilution of the conjugate. After incubating for 1 hour at 37°C, the plates were washed; then 100 ul of substrate ABTS were added. Then the reaction was stopped with sodium fluoride 50 ul to each well and the optical density was read at 405 nm using Biotek ELISA reader.

Known negative, phosphate buffered saline, and substrate controls were included in the test. An absorbance reading of at least 2 times greater than the average value of negative reference sera was con-

Table Comparison of serologic test results of MAT and ELISA

Cattle			Dog		
Total sera screened	MAT Positive	ELISA Positive	Total sera screened	MAT Positive	ELISA Positive
27	7	25	15	6	14

sidered positive for leptospiral antibodies.

### Results

The results of the study are indicated in the table.

It was observed that 20 out of 27 cattle serum samples MAT had shown insignificant titre whereas ELISA had shown significant reading in 25 out of 27 samples screened.

Similarly in the case of canine serum samples MAT had shown significant titres in 6 out of 15 samples and whereas ELISA had given positive titres in 14 out of 15 serum samples. The ELISA had given 92.5% positive in cattle sera and in dog serum samples 93% were positive by ELISA. The comparison of the MAT and ELISA serological test for the detection of leptospiral antibodies in acute clinical cases indicated that ELISA was found to be a sensitive test in both cattle and dogs. No false positive results were obtained when testing known negative serum samples.

### Discussion

The MAT, though specific and continues to be the recognised standard reference test for the serodiagnosis of leptospirosis, is not very sensitive (Hartman *et al.*, 1984b). High titres are often not observed until second week after the onset of disease (Wolff, 1954; Turner, 1968). Low doubtful antibody titres, which are a common finding are a serious

problem in diagnosis in acute clinical cases of leptospirosis. Further administration of large doses of antibiotics at an early stage of the disease may cause the MAT titres to remain at a low level (Babudieri, 1961; Turner, 1968). ELISA has been used in cattle (Thiermann and Garrett, 1983), sheep (Adler *et al.*, 1981) and dog (Hartman *et al.*, 1984a) to detect leptospiral antibodies in experimental infection.

In the present study ELISA was used to detect leptospiral antibodies in acute cases which gave a definitive diagnosis compared to MAT. Many workers also viewed ELISA to be a sensitive test and could be used as a screening test to detect leptospirosis (Fairbrother, 1984; Hartman *et al.* 1986). The ELISA antigens can be prepared in large quantities and stored for several months (Terpstra *et al.*, 1980). However the ELISA technique will not distinguish between titres which are due to acute leptospirosis or result from previous infection or recent vaccination (Hartman *et al.*, 1984a). But in the present study only clinical cases have been taken in to criteria and in cattle there is no practice of vaccination against leptospirosis and only dogs which were not vaccinated against leptospirosis were included in the study. Hence ELISA test can be viewed as a reliable test for the detection of leptospiral antibodies in cattle and dogs.

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## Usefulness of Enzyme Linked Immunosorbent Assay in the Diagnosis of Brucellosis

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### ABSTRACT

A number of serological tests are available for the detection of Brucellosis. Enzyme linked immunosorbent assay (ELISA) is being used in many diseases with success. In the present study a total of 32 serum samples were screened with Standard tube agglutination test (STAT), Mercaptoethanol tube agglutination test (METAT) and ELISA. Of this, 12 were positive to STAT, 10 to METAT and 21 serum samples were positive to ELISA. All the samples positive to STAT and METAT were also positive to ELISA. ELISA was found to be more sensitive than the conventional tests.

### Introduction

Apart from the heavy, economical losses by way of loss of milk, infertility and abortion, Brucellosis contributes to occupational hazard in veterinarians, abattoir workers, farmers and laboratory workers. Hence early diagnosis is very essential to formulate suitable control measures.

The most common tests for the diagnosis of Brucellosis currently used are plate agglutination test, tube agglutination test and 2 - Mercaptoethanol test. Of late, ELISA is being used widely for the detection of many diseases in man and animals, which is quick, specific, sensitive and relatively inexpensive. In the present study an attempt was made to find out the usefulness of

ELISA for the diagnosis of bovine brucellosis.

### Materials and Methods

#### Antigens:

(i) *Tube Agglutination test:* *Brucella abortus* plain antigen strain 19 was obtained from IVPM, Ranipet, for standard tube agglutination test (STAT).

(ii) *Mercaptoethanol tube agglutination test (METAT):*

*Brucella abortus* antigen obtained from IVPM, Ranipet was used for this test.

(iii) *ELISA:* *Brucella abortus* LPS antigen supplied by CSIRO, Animal Health Laboratory, Australia was

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Table showing the results of STAT, METAT and ELISA

No. of samples Tested	No. positive in STAT	%	No. positive in METAT	%	No. positive in ELISA	%
32	12	37.5	10	31.3	21	65.6

used for coating the polysterene ELISA plates.

#### Serum samples:

Thirty two serum samples from white cattle were collected from different areas of Madras city.

#### Tests employed

##### 1. Standard tube agglutination test:

The method suggested by Alton *et al.* (1975) was employed for the test. The highest dilution of serum showing complete agglutination and sedimentation with 100% clearing was taken as the end point. A titre of 40 and above was taken as positive.

##### 2. 2 - Mercaptoethanol test:

The method suggested by Alton *et al.* (1975) was followed. The end point was determined as in the case of STAT.

##### 3. ELISA:

ELISA was performed by the methods recommended by Pope *et al.* (1982) with slight modifications. Rabbit antiovine immunoglobulin horse radish peroxidase conjugate (Kirke - gaard and Perry Lab. Inc. USA) in 1 in 1000 dilution was made. Ortho - Phenylene - diamine (OPD) (BDH, Germany) was used as a substrate.

The plates were read in Biotek (USA) ELISA reader model EL308

with a filter giving a wavelength of 490 nm.

PBS, substrate, known positive and negative controls were included in the test. An absorbance reading at least 2 times more than the average value of the negative reference serum was considered positive.

#### Results

Out of 32 serum samples screened 12 (37.5%) were positive with STAT, 10 (31.3%) were positive to METAT and 21 (65.60%) serum samples were positive to ELISA (Table). All the samples positive by STAT and METAT were also positive by ELISA. No false positive reactions were detected with known negative samples.

#### Discussion

Most serological tests for infectious disease are not completely accurate because of non - specific reactions. This limitation holds good for brucellosis also. Some workers (Kulshrestha and Ramachandran, 1970; Polan, 1977) have stated that STAT should not be taken as a sole basis for the diagnosis of brucellosis.

Evaluation of METAT for serological diagnosis of bovine brucellosis was studied by Wu *et al.* (1987). Their results showed that STAT alone could not be used in the diagnosis.

In this study STAT was found to be slightly more sensitive than the METAT.

Magee (1980) reported that ELISA may be of significant value in the diagnosis of brucella. Hunter *et al.* (1986) found ELISA to be more sensitive than the standard agglutination test. ELISA was precise, reliable and had a higher sensitivity than the conventional tests (Albert and Bohm, 1988).

The detection of anti - brucella antibodies by ELISA was compared with routinely used tests by Limit *et*

*al.* (1988) and the specificity of ELISA was 99.2%.

In the present study also ELISA detected more cases than STAT and METAT. ELISA is easy to perform with large number of samples and can also be adapted to detect different classes of antibodies separately or collectively unlike other serological tests. Further less serum is required for the test.

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## Rapid Diagnosis of Canine Coronaviral Enteritis

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### ABSTRACT

*Canine coronaviral infection has been diagnosed using counter immunoelectrophoresis for the first time in India. Twenty six percent of the diarrhoeic samples tested were found positive for canine coronaviral infection and 5% of mixed infection with canine parvovirus was also recorded. Pups with mixed infection showed severe enteric symptoms and the mixed infections were mostly seen in pups below 6 months of age. It is recommended to vaccinate pups against canine coronaviral infection at the age of 3 months and above.*

### Introduction

Canine parvovirus, canine coronavirus (ccv) and possibly rotavirus are considered as predominant enteropathogens of dogs (Carmichael and Binn 1981). Coronavirus infect man and a variety of domestic and laboratory animals (Holmes, 1979; Pensaert and Callebaut, 1978). Canine coronaviral enteritis, a systemic viral infection of dogs has been reported by many authors in various parts of the world (Binn *et al.*, 1975; Appel *et al.*, 1978; Cartwright, 1973; Schnagl & Holmes, 1978, Toma & Moraillon, 1980; Marchwicki and Binn, 1979). The disease in dogs is characterized clinically by gastrointestinal symptoms, many times misdiagnosed as canine parvoviral enteritis. Often dogs were brought to Madras Veterinary College Hospital Out Patient section with symptoms of yellow coloured diarrhoea, sometimes with temperature, lethargy, vomition

and inappetance, which are reported as main symptoms of coronaviral infection in dogs (Appel *et al.*, 1979). Coronaviral infection often occurs concurrently with other viruses, parasites and pathogenic bacteria. Dogs with multiple infection may develop a more severe and prolonged disease.

Some dogs showed severe forms of diarrhoea with mucus and blood and mortality was rare in these cases. However, most of the dogs recovered from the illness after a period of 8 - 10 days after supportive treatment with antibiotics, antihistamines and rehydration therapy (Keenan *et al.*, 1979). Such symptoms were also seen in dogs which were vaccinated for canine parvoviral infection. This lead us to investigate for viral etiology, other than canine parvovirus. In this present investigation, counter immunoelectrophoresis has been standardised for rapid diagnosis of canine coronaviral infection.

## Materials and Methods

1. *Collection of samples:* Faecal suspensions were prepared in normal saline (0.15M sodium chloride solution) from dogs attending Madras Veterinary College Hospital Out Patient section.

2. *Canine Coronaviral antiserum preparation:* Canine coronaviral hyperimmune serum was prepared in rabbits using killed coronaviral vaccine (Nordan, USA) incorporated with Freund's complete and incomplete adjuvants (Ramdass *et al.*, 1983).

3. *Counter immunoelectrophoresis (CIEP):* Counter immuno electrophoresis was performed on microscope slides covered with 1.2% Noble Agar in barbital buffer, pH 8.6. Parallel rows of wells, 4 mm diameter were cut, 6 mm apart in the agar. Each slide accommodated 4 pairs of wells. Serum samples to be tested were filled in the wells on the anode side and the antigen was filled in the wells on the cathode side. Electrophoresis was carried out at room temperature at 150 volts for 1½ to 2 hours. At the end of the run, precipitation lines were detected in gel between antigen and antibody cells in positive cases. The slides were then washed with 1.5% sodium chloride solution several times and final washing with distilled water. The plates were then dried and stained with 1% Amido Black stain and destained with 7% acetic acid. (Ramadass *et al.*, 1983).

## Results

Twenty six out of hundred faecal samples tested revealed the presence of coronaviral antigen. All of the faecal samples showed a single

precipitation line with hyperimmune serum. A typical counter immunoelectrophoresis pattern is shown in Fig.1. Many of these dogs had either one or more of the symptoms like inappetance, lethargy, vomition, mild to severe yellowish diarrhoea with fetid odour. Fifteen faecal samples out of twenty six positive samples (58%) were from dogs below 6 months of age. The ailing dogs recovered spontaneously after a period of 10 days which confirmed the report of Carmichael & Binn, 1981. Mixed infections with canine parvoviral infection to the extent of 5% were also recorded and the mortality in these cases were more than with single infection with canine coronaviral infection.

## Discussion

It is difficult to differentiate the disease caused by canine coronavirus and canine parvovirus in most cases. Counter immuno electrophoresis appears to be a good tool to differentiate it, as the viral antigen present in the stool is quickly identified by CIEP within 1½ hours, whereas with the use of Agar gel precipitation test it took 18 - 24 hours. Already CIEP had been used for diagnosis of Canine distemper (Ramadass *et al.*, 1983). In this study, CIEP was found to be sufficiently sensitive test in detection of CCV in faecal samples.

The symptoms observed in dogs at Madras Veterinary College Hospital were almost similar to the symptoms described by Carmichael & Binn, (1981). Mortality in pups were noted subsequently after infection with canine parvoviral infection. Faecal samples from these pups revealed mixed infections of corona and parvoviral antigens. Similar ob-

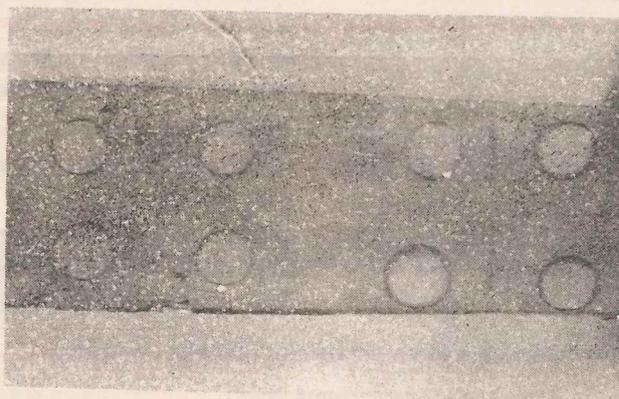
servations have been recorded by Carmichael & Binn (1981). They further found that dogs of all breeds and ages were equally susceptible to CCV infection. However, in this study more than 50% of cases occurred in dogs below 6 months of age.

Immunization of dogs to protect them from canine coronaviral infec-

tion is not practised in India yet as the disease has not been confirmed in India and the vaccine is also not commercially available. This being the first report of canine coronaviral infection in dogs in India, it is recommended that all the pups at the age of 3 - 4 months of age should be vaccinated against canine coronavirus infection.

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## Detection of Blue Tongue Virus Antigen in the Sera of Sheep by Agar Gel Immuno Diffusion Test

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### ABSTRACT

*Blue tongue virus antigen could be detected from sera of naturally infected sheep collected at the height of temperature (105° 106°C) using Ager Gel Immuno diffusion test (AGID).*

*As the results are usually obtained within 24 hrs, the test may be used as a routine diagnostic tool in Blue Tongue disease investigation work.*

### Introduction

**B**lue tongue (BT) is an arthropode borne orbi viral disease primarily of sheep affecting other ruminants also. Existence of Blue tongue disease of sheep in India has been recorded since 1964. There had been out break reports of BT like disease of sheep in various districts of southern part of India, since early 1980's.

Routine diagnostic procedure employed by United States Department of Agriculture (USDA), United States is the ager gel immuno diffusion (AGID) test (Pearson and Jochim, 1979). Presently diagnostic biologicals (BT virus positive antigen and BT positive antisera) and vaccines are not produced in India and

have to be imported. In the absence of detailed epidemiological data on the disease, the control of spread of disease could only be possible by segregating the animals to pastures at higher grounds or allowing the susceptible population to graze along with cattle (as the vector prefers the latter). This needs quick and reliable diagnosis. Detection of Blue Tongue Virus (BTV) antigen in the sera and plasma of camels has been reported by Abu Elzein, 1984, which is much earlier to the detection of BT antibodies.

Hence in the present work attempts were made to standardise a quick and economical test for laboratory diagnosis which will confirm the presence of the BT antigen

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in the sera of naturally infected sheep.

#### Materials and Methods

Reference soluble BTV type 1 antigen and reference sheep anti BT type 1 sera were, kindly supplied by Dr. Mahy, Director, Animal Virus Research Institute, Pirbright, Surrey, United Kingdom.

Blood samples of BT affected sheep from field outbreaks were collected, sera separated by centrifugation at 1500 rpm for 20 mts. inactivated at 56°C in water bath and stored at -20°C until used. (Henceforth will be indicated as field sera sample)

Paired sera samples were collected in the same manner from the same convalescing animals (1 month after 1st collection) and stored at -20°C until used. (Paired sera sample)

Blood samples were collected in equal volumes of OCG medium (Potassium oxalate 5G, Carbolic acid 5G and Glycerine 500ml made to IL with Distilled water.) from affected sheep at the height of temperature (105°F), stored at +4°C until used.

Chicken embryo antigen were prepared by inoculating 0.05 ml of sonicated Blood in OCG, intravenously into 11 - 12 days old embryonated chicken eggs. Inoculated eggs were incubated at 33.5° ± 1°C. in a BOD incubator. Embryo harvests were made from embryo that died after 48 hours post inoculation (stored at +4°C until used). A 10% solution of embryo harvest in phosphate buffer saline (PBS) with anti-biotics were prepared by triturating 1g. of embryo using sterile sand in a 5 cc dia porcelain pestle and mortar and making

the volume to 10 ml with PBS. The suspension was centrifuged at 2000 rpm. for 15 mts. and the supernatant aliquoted on the day of test and used.

Agar gel used were either 1.1% normal saline agar (Pearson and Jochim, 1979) or Borate buffer agar (Animal virus Research Institute, Pirbright, United Kingdom). The agar plates were made in 50 mm x 15 mm glass petridishes with 3 mm thickness of agar 5 mm dia templates were cut at 3 mm distance between the wells in a 7 - well pattern test. 3 mm dia templates at 3 mm distance between wells were also cut in a 7 well pattern by the side of the above test pattern for comparison.

#### *Trials were conducted as follows:*

1. AGID was conducted with reference soluble BT type - 1 antigen in the central well and reference anti-sheep BT type 1 antisera in the alternate wells of the peripheral wells. In between peripheral wells were loaded with the field sera samples.

2. Reference anti sheep BT type 1 antisera was loaded in the central well. Alternate peripheral wells were loaded with field sera samples which acted as antigen and the remaining wells were loaded with respective paired serum sample.

3. Reference anti sheep BT type 1 antisera was loaded in the central well. Alternate peripheral wells were loaded with field sera sample, which acted as antigen and the remaining wells were loaded with chicken embryo extract.

4. All the above tests were carried out with both Borate buffer agar and Normal Saline agar, along side.

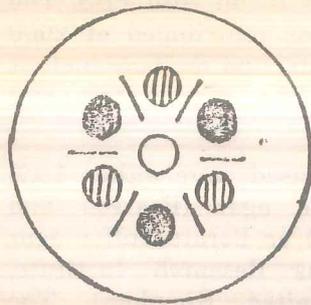


Fig. 1

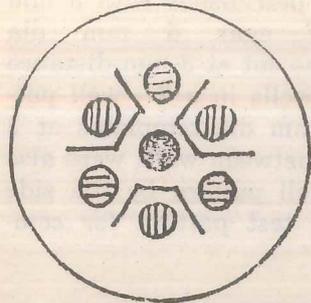


Fig. 2

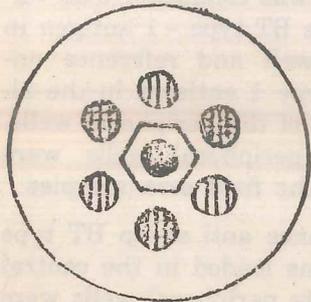


Fig. 3

KEY TO THE DIAGRAM

- - Reference soluble BT Type 1 antigen
- - Reference antish sheep BT Type 1 antisera
- ◐ - Field Sera Sample
- ◑ - Convalescent Sera Sample
- ◒ - Chicken embryo antigen.

5. The tests were conducted in both the template systems of 5 mm dia. pattern and 3 mm dia. pattern.

All the test plates were incubated in a humidified chamber and left undisturbed and results are read after 24 hours.

**Results**

Reference soluble BT type 1 antigen and reference antish sheep BT

type 1 antisera did not form a precipitating line between them, while sera samples collected from affected sheep at the height of temperature gave a precipitating line with reference antish sheep BT type 1 antisera. (Fig. 1)

Precipitation line was formed between the reference antish sheep BT type 1 antisera and the field sera samples (which acted as antigen) and

the above field sera samples and their respective paired sera samples (Fig. 2).

A continuous hexagonal pattern precipitation line was formed between reference antiserum BT type 1 antisera and the field sera samples and also the chicken embryo antigen (Fig. 3).

The results were the same when borate buffer agar and normal saline agar plates were used and the results were the same in both the template systems.

### Discussion

The results of trial 1 could be interpreted that the reference soluble BT type 1 antigen could have lost its potency, while the field sera samples, which gave precipitating line with reference antiserum BT Type 1 antisera, could have precipitating soluble Blue tongue antigen.

The precipitating line between the reference antiserum BT Type 1 antisera and the field sera samples and field sera samples with their respective paired sera samples indicated that the sera collected at the height of temperature has precipitating BT antigen and their respective convalescent serum contains the BT antibodies and that these BT antigen and its respective antibody hence could form lines between themselves.

The precipitating line formed by both chicken embryo antigen and the

field sera samples with the reference antiserum BT Type 1 antisera could prove the presence of soluble precipitating BT virus antigen in the serum.

Similar results have been recorded in Sudan (Abu Elzein, 1984) using camel sera collected at the height of temperature.

The holding capacity of 5 mm dia templates is 0.03 ml while that of 3 mm dia template is 0.01 ml. As the results are significantly correlating, by switching to 3 mm dia template system one third volume of reference antigen and antisera could be saved.

The results proved no significant variation with the usage of Borate buffer agar or normal saline agar in the AGID test.

Further as antigen could be detected much earlier to formation of specific antibodies, this could be used as a routine test reversing the test pattern of Pearson and Jochim 1979 for quick screening of sheep against BTV infection during outbreaks.

### Acknowledgement

The authors remember faithfully the belief and the encouragement offered by Dr. C.K. Kaliyanasundaram, former Director of Animal Husbandry, Madras and Dr. T.K. Govindarajan, present Director of Animal Husbandry, Madras.

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## Purification of Duck Hepatitis B Virus Surface Antigen by Poly Acrylamide Gel Electrophoresis

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### ABSTRACT

*An attempt has been made to purify the surface DHB - Ag using polyacrylamide gel electrophoresis (5%) from an Indian country duck which was found to be positive for the virus by both CIEP as well as by Dot - blot hybridization technique using DHBV DNA probe. The purity of the antigen was checked by CIEP using anti - DHB<sub>5</sub>Ag raised in Guinea pig. The purified antigen will be used to raise specific antibody against the DHB<sub>5</sub>Ag in order to screen the duck sera for the virus. The method employed here is a sensitive, specific and less time consuming without the involvement of sophisticated instruments like column chromatography, ultra - centrifugation and spectrophotometry.*

### Introduction

**D**uck hepatitis B virus (DHBV) is the only known avian hepadna virus which was discovered in 1980 first in sera of ducks from China and shortly thereafter in ducks of Chinese ancestry in commercial flocks in the United States. DHBV belongs to Hepadna viridae family which include human hepatitis B virus (HBV) woodchuck hepatitis virus (WHV), and ground squirrel hepatitis virus.

The DHB virion is of similar size of Hepadna viruses (40nm) and the virion DNA is partially single stranded with the length of 3000 base pairs compared with DNA of

3,182 to 3,300 base pairs in the mammalian viruses.

DHBV differs from the mammalian viruses in clearly replicating in the pancreas as well as liver. Where as GSHV and WSV appear to replicate in the liver only. While transmission of the mammalian members of this virus family, most often appears to be perinatal, transmission of DHBV occurs predominantly through the eggs. In ducks, the viraemia is associated with a broad spectrum of liver pathology, including chronic hepatitis, cirrhosis and Hepatocellular carcinoma but there are differences in the incidence of these lesions in ducks from different

countries. The present study is of considerable epidemiological and virological interest in which the possibility of DHBV infection in Indian country ducks is analysed for the first time in literature and a suitable and economical method of screening the DHBV infected Indian country ducks has been attempted

#### Materials and Methods

90 duck serum samples were collected from Indian country ducks (Desi and cross bred ducks) in and around Madras City. Which includes Cholavaram, Minjur, Chengalpattu and Vellore. The samples were tested for the presence of DHBV DNA by Dot - blot hybridization technique. Then these positive sera were tested by CIEP for DHB<sub>s</sub>Ag and the surface antigen was purified by polyacrylamide gel electrophoresis.

#### Dot - blot hybridization (Sun, et al):

About 20 µl of the duck sera was mixed with proteinase - K, SDS, TRIS and EDTA for digestion and incubated at 56° for 2 hours. Then the above preparation as mixed with equal volume of 2m NaOH and 2m NaCl and spotted on to the nylon membrane. The membrane was then exposed U-V for 5 minutes and prehybridization mixture was added and kept at 68°C overnight. Then hybridization was performed by adding prehybridization mixture with

32p labelled DHBV DHA (kindly supplied by Dr.Stephen Locarnini, Australia) to the membrane and kept for 48 hours at 68°C. Then the filter was washed and subjected to autoradiography (-70°/48 hours).

#### Counter immuno electrophoresis:

The duck sera which was positive by Dot - blot technique was again checked for the presence of surface antigen by using 1% Agarose and specific anti DHBV raised in Guinea Pig (kindly supplied by Dr.Stephen Locarnini Australia).

#### Poly acrylamide gel electrophoresis:

About 10 µl of the DHBV positive duck sera was loaded on the slab gel using stacking and separating gels of varying concentration. The gel was run at 100 volts and was stopped after a period of time till the marker reached the bottom of the gel.

#### Result

The 90 samples of duck sera when screened for DHBV infection by Dot - blot hybridization revealed 6 ducks to be seropositive for DHBV DHA and remaining were negative. By CIEP only 4 out of 6 positive sera showed DHB<sub>s</sub>Ag positivity. By using polyacrylamide gel electrophoresis the DHB<sub>s</sub>Ag from one of the positive ducks was purified successfully and utilised for immunization purpose (Table 1).

Table 1 Screening of Ducks

DHBV DNA (n = 90)	DHBs Ag (n = 6)
Dot - blot hybridization	CIEP
Positive 6 (6.6%)	Positive 4 (66.66%)
Negative 84 (93.33%)	Negative 2 (33.33%)

### Conclusion

As demonstrated by dot - blot hybridization 6 out 90 Duck sera were positive for DHBV DNA and out of these seropositive ducks 4 were positive for DHB<sub>2</sub>Ag as shown by CIEP. The method of employing PAGE for the purification of DHB<sub>s</sub>Ag is sensitive, easy to handle and less time consuming without the involve-

ment of sophisticated instruments like column chromatography, Ultracentrifugation and spectrophotometry. Further more the DHBV infected duck could be used as an effective animal model and might answer certain questions related to hepatocarcinogenesis within Hepadna viridae.

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## Evaluation of A Tissue Culture Strain of Newcastle Disease Virus for Vaccine Production

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### ABSTRACT

An attempt was made to find out the suitability of Baby Hamster Kidney - 21 (BHK - 21) cell line for evolving a tissue culture strain of Newcastle disease virus (NDV). A velogenic strain of NDV was passaged in BHK - 21 cell line. Characteristic cytopathogenic effects like rounding, cell fusion and syncytia were observed from third passage onwards. A total of 35 passages were carried out and the log<sub>10</sub> TCID<sub>50</sub> obtained at the end of 35th passage was 8.78 per 0.05 ml. Characterisation of tissue culture adapted virus revealed a reduction in its velogenicity and haemagglutination titre.

### Introduction

The chicken egg embryo adapted live Newcastle disease virus (NDV) vaccine, though in wide use has got few short comings, like transmission of egg borne avian viruses (Van Roekel, 1959; Yates *et al.*, 1960) and reverting back to virulent form on serial passage through in - contact birds (Thorne and Macleod, 1960). Virus strains adapted to cell culture, particularly cell lines of non - avian origin provide the best alternative to the egg adapted vaccine. Hence in the present study BHK - 21 cell line (Razi) which can be cultivated in suspension leading to large scale vaccine production, was used.

### Materials and Methods

#### Virus:

A velogenic challenge virus was obtained in freeze dried form from

I.V.P.M., Ranipet, and used for adaptation.

#### BHK - 21 cell line:

BHK - 21 (Razi) cell line received from FMD vaccine laboratory I.V.P.M., Ranipet at the passage level of 129 was used.

#### Maintenance of BHK - 21 cell line:

The cells were subcultured at an interval of 4 - 5 days using Trypsin - versene - glucose solution (0.1% trypsin, 0.02% versene and 0.2% glucose in calcium, magnesium free phosphate buffered saline). The cells were suspended in growth medium (Eagle (Glassgow) medium with 10% bovine serum and 0.3% (W/V) tryptose phosphate broth).

#### Adaptation of NDV:

The velogenic NDV strain was serially passaged in BHK - 21 cell

line for 35 times following regular procedures. At every passage level the virus was harvested when the cytopathic effect (CPE) extended to 80 per cent of the monolayer.

#### *Characterisation of BHK - 21 adapted NDV:*

The Haemagglutination (HA) titre and the infectivity titre were assessed at the end of every 5th passage as per the methods of Allan *et al.* (1978) and Ashok kumar (1984) respectively. Virulence and strain differentiating characters of the BHK - 21 adapted NDV was assessed at the end of 20th passage. Virulence characters like Mean death time (MDT), Intracerebral pathogenicity index (ICPI) and Intravenous pathogenicity index (IVPI) were performed as described by Allan *et al.* (1978). Strain differentiating characters like stability of HA at 56°C (Tanwani, 1974) and agglutination of equine erythrocytes (Winslow *et al.*, 1950) were carried out as per standard procedures.

#### **Results**

Initially it took two blind passages for the virus to produce any appreciable CPE. To begin with the CPE observed included rounding of cells and syncytia formation followed by extensive grouping and floating of cell aggregates. At 35th passage level, the cell monolayer was affected to an extent of 40 - 60% at 48 hours post - infection (PI) and 80 - 100% at 72 hours PI.

The HA and Infectivity titres of BHK - 21 passaged NDV are given in Table 1. The HA titre which was found to be 1024 before adaptation, decreased considerably during the initial five passages and continued to

decrease gradually thereafter. The infectivity titre increased gradually from 5th passage onwards and at the end of 35th passage it was found to be  $10^{8.78}$  TCID<sub>50</sub> per 0.05 ml.

The virulence and strain differentiating characters of the virus after 20 passages are furnished in Table 2. These results reveal that the virus has lost its virulence and has become a mesogenic strain.

#### **Discussion**

All the present day vaccines available in India for control of Newcastle disease are of chick embryo origin. The demerits of live vaccines of avian origin include the danger of transmission of avian pathogens and the interference of maternal antibodies. Since specific pathogen free eggs are costly and not available in sufficient numbers to meet out the demand of large scale vaccine production, tissue culture systems seem to provide an useful alternative. BHK - 21 cell line which is being widely used in the manufacture of Foot and mouth disease virus vaccine has been found to give reasonably good results when used for growing NDV (French and George, 1965; Alexander, 1973; Kumanan, 1989).

In the present study the virulent NDV strain produced changes like rounding, extensive grouping and syncytia formation in BHK - 21 cell line. These results concurs with the observations of earlier workers (French and George, 1965; Kumanan, 1989). Similarly, as observed by earlier workers (Rossi and Acciari, 1969; Kumanan, 1989) a reduction in HA titre was also observed after adaptation in BHK - 21 cell line. However, a gradual rise in infectivity titre was

observed in relation to the number of passages which conform the earlier findings of French and George (1965).

Based on the virulence characters such as MDT, ICPI and IVPI and strain differentiating characters like stability of haemagglutinin at 56°C and agglutination equine erythrocytes (Allan *et al.*, 1978) the virus has been characterised as mesogenic at 20th passage as against the velogenic character of the original virus. Further studies have got to be undertaken to find out whether by subsequent passages this mesogenic strain would get back its velogenicity or not.

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Table 1 HA and TCID<sub>50</sub> Titre of BHK<sub>21</sub> Passaged NDV

	PASSAGE LEVEL							
	0	5th	10	15th	20th	25th	30th	35th
HA titre	1024	32	16	16	16	8	8	8
log <sub>10</sub> TCID <sub>50</sub> /0.05 ml	-	3.24	4.50	6.50	7.50	8.24	8.75	8.78

**Table 2 Virulence and Strain Differentiating Characters of NDV after 20 Passages in BHK - 21 Cell Line**

<i>MDT in hours</i>	<i>EID<sub>50</sub> (log<sub>10</sub>)</i>	<i>ICPI</i>	<i>IVPI</i>	<i>Stability of HA at 56°C</i>	<i>Titre of Agglutination of equine RBCs</i>	<i>Inference</i>
82.4	8.5	1.42	1.40	30 min	2	Mesogenic

## Studies on the Screening of Fishes Grown in Sewage - Fed Water for Enterobacteriaceae (*Shigella* spp.) Group of Pathogens.

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### ABSTRACT

The gut, skin and muscle from the fishes viz., *Labeo rohita* and *L. fimbriatus* grown in three different habitats were screened for the enterobacteriaceae group of organisms. *Shigella flexnerii* was present in the samples of sewage - fed fishes. In 'reclaimed' fishes, it was isolated from the gut and skin and not in the muscle. This bacteria was completely absent in the steamed, fried and curry preparations of the fish 'rohu'. Higher counts of (i) total bacteria (ii) coliform group and (iii) faecal streptococci were observed in the water samples from sewage - fed ponds compared to other sources.

### Introduction

Biotechnology has relevance to countries like India which is confronted with ever expanding population, shortage of adequate amount of food and energy resources and accumulation of various types of wastes of various kinds. The utilisation of human and animal wastes to fertilize water for improving the growth of aquatic life has been well documented. (Mortimer and Hickling, 1954 and Allen, 1970). Usefulness of such ponds, for fish culture has been reported by Trimberger (1972) and Anon (1972). Experiments were conducted in sewage - fed as well as fresh water ponds in Tamil Nadu Agricultural University, by culturing Indian major carp 'rohu' (*Labeo rohita*) and fringe - lipped carp (*L.fimbriatus*). Though information available on the

bacteriology of sewage - fed fish is meagre, several workers have investigated the microflora of marine and estuarine fishes (Velankar and Kamasastri, 1955; Karthiani and Iyer, 1967; Fathima, 1973 and Mary *et al.*, 1975). Hence, an attempt is made in this study to screen the sewage - fed fishes for enterobacteriaceae group of organisms including the pathogens.

### Materials and Methods

The two fish varieties viz., *L.rohita* and *L.fimbriatus* were reared (i) completely in fresh water ponds with artificial feed (Group I) (ii) completely in ponds with stabilised domestic sewage without any supplementary feed (Group II) and (iii) partly in sewage - fed ponds and partly in fresh water (fish cultured for one year in sewage - fed

water without any supplementary feed and subsequently for one month in fresh water with artificial feed (Group III).

These fishes were screened for the presence of Enterobacteriaceae group of organisms such as *Shigella flexnerii*, *S. boydii*, *S. dysenteriae*, *S. sonnei*, *Salmonella spp.*; *Escherichia coli*, *Aerobacter spp.*, *Aeromonas spp.*, *protaus spp* and *Klebsiella spp.* The skin, muscle and gut samples from freshly caught fishes were collected in sterile 30 per cent glycerol buffer saline and from this transporting fluid, the specimens were inoculated on to desoxycholate

citrate agar (DCA), Mac Conkey agar (MA) and then into selenite 'F' enrichment broth (SF) as advocated by Bhat & Myers (1962); Edwards and Ewing (1972) and Rajasekaran *et al.* (1977). Following overnight incubation at 37°C, subcultures were made from SF to DCA. The plates were incubated at 37°C. Typical non-lactose fermenting colonies (NLF'S) were picked up and further characterised using (i) Triple sugar iron agar (TST) (ii) the mannitol motility medium (MM) and peptone water (PW) for indole production. The NLF'S suggestive of *Shigella* were confirmed, serologically.

Table 1 Enumeration of Enterobacteriaceae Group of Organisms in Rohu (*Labeo rohita*) and fimbriatus (*Labeo fimbriatus*) in different habitat

Organism	Rohu									Fimbriatus								
	Skin			Muscle			Gut			Skin			Muscle			Gut		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
<i>Shigella</i> spp.	+	-	+	+	-	-	+	-	+	+	-	+	-	-	-	+	-	+
<i>Enterobacter</i> sp.	-	-	-	+	+	-	-	+	-	-	-	-	+	-	-	-	-	-
<i>Klebsiella</i> sp.	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-
<i>Aerobacter</i> sp.	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
<i>Escherichia coli</i>	-	-	-	+	-	-	+	+	+	-	-	-	+	-	-	+	-	-
Non-fermenting rods	-	-	+	-	-	-	-	-	+	-	-	+	-	-	+	+	-	-
Others (A typical <i>E. coli</i> etc.)	+	-	+	+	-	-	+	-	+	+	+	-	-	-	-	-	+	+

I Cultured in Sewage - fed ponds

II Cultured in Freshwater ponds

III Cultured in freshwater ponds for 21 days after growing for one year in sewage - fed ponds.

**Table 2 Screening of fishes grown in sewage - fed ponds for the presence of Enterobacteriaceae group of organisms**

Organism	Uncooked (Fresh samples)						Cooked muscle (Rohu)		
	Lbaeo rohita (Rohu)			Labeo fimbriatus			Steamed	Fried	Curry
	Skin	Muscle	Gut	Skin	Muscle	Gut			
Shigella spp	+	+	+	+	-	+	-	-	-
Enterobacter sp.	-	+	-	-	+	-	-	-	-
Klebsiella sp	-	-	+	-	-	-	-	-	-
Aerobacter sp	-	-	+	-	-	-	-	-	-
Escherichia coli	-	+	+	-	+	+	-	-	-
Non - fermenting rods	-	-	-	-	-	+	-	-	-
Others (A typical E. coli etc.)	+	+	+	+	-	-	-	-	-

**Table 3 Microbial population in the Fish Samples cultured in sewage - fed and fresh-water ponds (Expressed in 10<sup>5</sup>/g of sample)**

Fish Variety	Part examined	Sewage - fed pond		Freshwater pond	
		Total Bacterial count	Coliform count	Total Bacterial count	Coliform count
Rohu	Skin	27.69	16.50	0.93	0.07
	Muscle	36.01	3.40	0.40	0.03
	Gut	400.10	26.69	2.47	0.13
Fimbriatus	Skin	18.87	8.12	0.82	0.04
	Muscle	11.16	3.05	0.29	0.01
	Gut	131.00	15.86	1.93	0.24

**Table 4 Bacteriological quality of different sources of water utilized for fish Culture**

Organism	Freshwater	Sewage water	Reclaimed water
Total Bacterial count (Express in 10 <sup>6</sup> /ml)	6.62	45.02	8.16
MPN Coliform count/100 ml	0.22 × 10 <sup>5</sup>	190 × 10 <sup>5</sup>	0.23 × 10 <sup>5</sup>
MPN Faecal Streptococcal count/100 ml	11.32 × 10 <sup>2</sup>	213.25 × 10 <sup>2</sup>	10.15 × 10 <sup>2</sup>

(Figures represent mean of four values)

The ambient water samples from fish ponds were collected and examined for coliform organisms and fecal streptococci as per procedures outlined in standard methods (Anon, 1971).

The sensory assessment of the fish rohu, grown in the three groups of ponds were carried out by (i) steaming for 10 minutes (ii) frying with oil and condiments and (iii) making curry as per normal kitchen practice and the muscle was screened for pathogens.

### Results

The various organisms enumerated from the fishes cultured in different ponds are presented in Table 1. The pathogens screened from the muscle of the steamed fried and fish curry are depicted in Table 2. The microbial load encountered in the skin, muscle and gut of both the fish varieties examined are indicated in Table 3. The bacteriological quality of different sources of water utilised for fish culture are presented in Table 4.

### Discussion

Among the organisms, *Shigella flexnerii* was present in the fish samples reared in sewage - fed ponds (Group I). In 'reclaimed' fishes (Group III) *S. flexnerii* was tested in all the tissues transferred within a week after transfer of the fish to fresh water; but tests done after 3 weeks revealed the presence of *Shigella* in the gut and skin and not in the muscle. (Vide Table 1): *Salmonella* spp. did not occur in any of the varieties of fishes tested. Species other than *S. flexnerii* namely *S. boydii*, *S. dysenteriae* and *S. sonnei* were not encountered in the tests

while *Escherichia coli* was obtained from the gut specimens of the fish *L. rohita* cultured in group I, II and III. The other organisms isolated viz., *Enterobacter* spp., *Klebsiella* spp. and non - fermenting rods encountered are represented in Table 1.

The muscle from the steamed, fried and fish curry were screened for the pathogens. It was significant to note that none of the enterobacteriaceae group of organisms could be observed in all the samples tested (Table 2). The cooking experiments with an exposure temperature of 160°C for 10 minutes in the present study, might have resulted in killing all the organisms belonging to Enterobacteriaceae. The normal cooking procedure followed in various houses could thus take care of the pathogens and as such the transmission of pathogens, if any, in the sewage - fed fishes is unlikely.

The microbial load of the skin, muscle and gut of both the fish varieties examined for (i) Total bacteria as well as (ii) Coliform count are presented in Table 3. Higher total as well as coliform count were encountered in the gut contents than that of the skin and muscle. Fathima (1973) and Mary *et al.* (1975) have reported highest bacterial population in the gut of Indian mackerel, *Restrelliger kanagurta* and three estuarine fishes respectively. The highest counts in the gut specimens might be due to the occurrence of microbes acting on the food substrates ingested when compared with the other tissues examined.

The bacteriological quality of different sources of water utilised for fish culture revealed higher (i) total bacterial count (ii) coliform and (iii)

fecal streptococci in sewage - fed ponds. (Table 4) The sewage - fed water pond was found to harbour maximum microbial population by virtue of higher organisms load.

### Conclusion

Successful utilization of sewage for fish production needs to be implemented with greater emphasis, though, at the moment it still remains to gain momentum. To make them pathogen - free, the fishes

grown in sewage water may be transferred from sewage - fed ponds to running water cisterns and maintained with normal feed for various periods of time. Even if they are contaminated, the procedure adopted for cooking would eliminate the possibility of any pathogen transmission. Therefore, apart from providing protein rich food for people, recycling of sewage water for culturing of fishes might add to human welfare and materials benefits.

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## Parasite Induced Changes on the Haematology of Freshwater Catfish, *Mystus guilto* (Hamilton - Buchanan)

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### ABSTRACT

Haematological studies are of immense use in understanding the aspects of fish prone to environmental hazards, nutritional deficiency, parasitic infestation and infectious disease. The parasite induced changes in the haematology of host fish could be a measure of rate of infestation. The haematological parameters used in the experiment were erythrocyte and leucocyte counts, haemoglobin content, haematocrit, erythrocyte sedimentation rate (ESR) along with MCV, MCH, and MCHC. These parameters were estimated for fish infested with parasites such as *Neomurraytrema tengra*, *Eragasilus*, *Philometra* and *Acanthosentis antiespinus* and also for the parasite free fish. Loss of haemoglobin and a significant drop in erythrocyte count ( $P < 0.01$ ) observed was due to the manifestation of secondary anemia caused by blood feeding gill parasites. Since the MCV level was also high due to parasitic infestation, the anemia could be classified as 'Macrocytic'. The gill infested fish, however, did not show any appreciable changes in leucocyte count because of the absence of inflammatory response in host fish.

The haematological changes in host fish due to diverse groups of parasites showed that except ESR all the other parameters increased at the onset of infestation followed by a drop at heavy infestation. The oscillating trend discernible in between these levels indicated the host's immune response to infestation.

### Introduction

**F**ish haematology, a subject which is identified immense practical application in aquaculture is recently attracting the attention of aquaculturists. Haematological studies are of great help in understanding the health of fish subjected to

diseases and parasitic infestation. Hickley (1976) stated that the effects of parasites on fish can be diagnosed haematologically rather than biochemically or physiologically, in the early stages of infection. The perusal of literature revealed that the parasitic attack on fish does

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cause enormous changes in the haematological pattern of the host fish (Ivasik, 1953; Uspenskaya, 1961; Bauer *et al.*, 1969; Bylund, 1972; Radhakrishnan, 1979; Nair *et al.*, 1983). An attempt has been made in this study to understand the extent to which changes occur in haematological parameters due to the attack of various kinds of parasites on the host fish.

#### Materials and Methods

The experimental fish, *Mystus guilo*, a freshwater catfish, were collected from the Korampallam freshwater reservoir of Chidambaranar District. After acclimated to the laboratory conditions for a week, they were subjected to various analysis. For the haematological studies, blood was drawn from the duct of cuvier' of fishes (Lied *et al.*, 1975). The blood thus drawn was used for the determination of total erythrocyte count, total leucocyte count, haemoglobin content (Hb), erythrocyte sedimentation rate (ESR) and haematocrit (Ht). The above haematological parameters were estimated through the methods suggested by Hesser (1960), Kolmer *et al.* (1969) and Davidsohn and Henry (1977). From the values of haemoglobin content, haematocrit and total erythrocyte count, factors like Mean Corpuscular Volume (MCV), Mean corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin concentration (MCHC) were estimated as follows.

$$(i) \text{ MCV} = \frac{\text{Ht}}{\text{RBC} \times 10^6 / \text{mm}^3} \times 10$$

$$(ii) \text{ MCH} = \frac{\text{Hb}}{\text{RBC} \times 10^6 / \text{mm}^3} \times 10$$

$$(iii) \text{ MCHC} = \frac{\text{Hb}}{\text{Ht}} \times 100$$

After drawing of blood samples for haematological studies, the fishes were sacrificed for the incidence of parasites. While the gills, opercular plates, branchial and mouth cavities were thoroughly examined for the evidence of ectoparasites all the internal organs and body cavities were examined for endoparasites and their distribution pattern and incidence were noted.

#### Results and Discussion

It was clear from the Table 1, that the infestation brought about conspicuous changes in the blood picture of the host fish. The various haematological parameters viz. the total erythrocyte count, total leucocyte count, haemoglobin content, erythrocyte sedimentation rate and haematocrit assessed both for uninfested and infested fishes revealed that the values were found to be reduced in infested fish.

A reduction of about 0.2 g% Hb content and a drop of  $0.19 \times 10^6 / \text{mm}^3$  of RBC count was evident in the present study. Similar observations of 10 - 18% reduction in Hb and a drop of  $0.05 \times 10^6 / \text{mm}^3$  in RBC count were reported earlier in *Merlangus merlangus*. Owing to the infestation due to *Lernaecera branchialis*, drop of 6 - 7% and 10% in Hb content in *Pimatuschistus* sp. and *Melanogrammus* sp. respectively were also reported. Further, Radhakrishnan *et al.* (1984) reported decreasing values of RBC, Hb and Ht in infested *Diodon hystrix* by the castode parasite,

#### *Gymnorhynchus gigas*

The factors such as mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean

corpuseular haemoglobin concentration (MCHC) on the other hand were found to increase in infested fish.

Haematological parameters in relation to different levels of infestation (Fig.1) revealed that ascending slopes for RBC were discernible during the onset of infestation. However, a steep downward slope due to heavy infestation was observed at heavy infestation level, suggesting that the drop in RBC count was maximum due to heavy infestation rate. Though an increase in WBC levels was observed during the onset of infestation a considerable reduction of 20.74% was noticed at increased infection level. Hb also showed a similar trend that of RBC and WBC. However, it was interesting to observe that thorough the ESR decreased at the onset of infestation, prominent increase was evident during increased level of infection. Thus all the factors except ESR showed an initial increase at the onset of infection and a final drop during heavy infection. The oscillating trend in between indicates the hosts reaction to withstand the attack.

The observations made on the haematological picture of the host fish due to the attack of specific parasitic group revealed interesting results (Table 2). Due to the heavy attack of *A. antepinus* and *Ergasilus* sp. a drastic reduction in RBC ob-

served in the order of 24.2% and 27.4% respectively, suggesting the occurrence of anaemia. The WBC count also showed a noticeable reduction, indicating the possible occurrence of the condition called as 'leucopenia'. The increase in Hb content due to *Ergasilus* sp. and *A. antepinus* infestation indicated a sort of compensatory factors to cope up with the ill effects of infection.

There is evident reduction in Ht & RBC owing to the infection of *Ergasilus* and *A. antepinus* suggesting the occurrence of anemia. Since MCV is high in these 2 cases the anemia may be classified as 'macrocytic'. Though increased Hb & MCH apparently speaks against the occurrence of anaemia, the anaemia is of the hyperchromic type. This observed hyperchromic is actually not a true over saturation of the RBC with Hb but a false manifestation resultant of 'microcytosis'. In short in *Ergasilus* sp. and *A. antepinus* infections, the haematological parameters suggested the occurrence of 'hyperchromic' macrocytic anaemia.

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Table 1 Mean (M) Standard Error (S.E) and Range (R) of Heamatological Parameters of Unifeted and Infeted Fish

Nature of infestation	Erythrocyte count (RBC) ( $\times 10^6/mm^3$ )	Leucocyte count (WBC) ( $\times 10^4/mm^3$ )	Haemoglobin in (Hb) (l g %)	Ery.sedi Rate (ESR) (mm/h)	Haematocrit (Ht) (%)	Mean Corpu Volume (MCV) (3)	Mean Corpu Haemo. (MCH) (g)	Mean Corpu. Hae. Conc. (MCHC) (%)
Uninfested Fish	M 1.83	5.88	7.55	2.25	43.75	245.89	42.41	17.28
	SC 0.17	0.77	0.26	0.10	0.85	19.45	4.10	0.65
	R 1.4 - 1.90	4.5 - 8.00	6.9 - 8.10	2.0 - 2.50	42.0 - 46.0	209.09 - 300.00	35.45 - 52.86	15.68 - 18.84
Infested Fish	M 0.92	4.66	7.35	1.23	42.58	303.01	49.48	18.47
	SC 0.11	0.60	0.28	0.10	2.21	25.95	4.62	1.45
	R 0.5 - 2.44	1.0 - 9.50	5.2 - 10.40	0.5 - 2.50	30.61 - 59.00	192.51 - 612.20	30.69 - 104.00	11.32 - 44.00

Table 2 Mean Haematological Values in Uninfested and Infested *Mystus Gulio*

Factors	Uninfested	<i>N. enora</i>	<i>Philometra sp.</i>	<i>Eraqasilus sp.</i>	<i>A.antespinus</i>
RBC ( $\times 10^6/\text{MM}^3$ )	1.83 $\pm$ 0.17	1.67 $\pm$ 0.10	1.63 $\pm$ 0.11	1.41 $\pm$ 0.49	1.33 $\pm$ 0.40
WBC ( $\times 10^4/\text{mm}^3$ )	5.88 $\pm$ 0.77	5.35 $\pm$ 0.58	5.62 $\pm$ 0.62	5.75 $\pm$ 3.75	3.00 $\pm$ 1.53
HB (g %)	7.55 $\pm$ 0.26	7.44 $\pm$ 0.28 **	7.33 $\pm$ 0.29 **	8.70 $\pm$ 0.10 **	8.73 $\pm$ 0.29 *
ESR (mm/h)	2.25 $\pm$ 0.10	1.27 $\pm$ 0.11	1.35 $\pm$ 0.12	1.25 $\pm$ 0.75	1.35 $\pm$ 0.33
Ht ( % )	43.75 $\pm$ 0.85	42.59 $\pm$ 2.16	43.01 $\pm$ 2.19	39.50 $\pm$ 19.50	37.98 $\pm$ 9.01
MCY ( $\mu^3$ )	244.89 $\pm$ 19.43	290.78 $\pm$ 25.36	298.56 $\pm$ 25.86	262.79 $\pm$ 47.74	315.53 $\pm$ 100.02
MCH (UU g)	42.41 $\pm$ 4.10	48.58 $\pm$ 0.57	48.51 $\pm$ 4.65	69.94 $\pm$ 24.68	75.33 $\pm$ 17.45
MCHC ( % )	17.28 $\pm$ 0.65	18.98 $\pm$ 1.45	18.37 $\pm$ 1.50	29.29 $\pm$ 14.71	27.03 $\pm$ 8.53

\*\* P &lt; 0.01; \* P &lt; .05

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## **Bottlenecks In Increasing the Production In Poultry Industry In Tamil Nadu**

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### ABSTRACT

*Increasing the production and productivity of animals (poultry) is very important in view of the continued population increase and limited potential for further quantum of increase in food output due to various factors like diminishing area for cultivation because of urbanization and expanding industries. It will be superfluous to emphasise the need for the availability of standard quality feed at reasonable price, vaccines and marketing facilities. As it is, there is escalation of cost of company feed and also there is fluctuation in prices of feed materials like fish meal, maize and rice bran depending upon the season. Notwithstanding the increase in price, the poultry farmers are not getting good quality feed materials. Fish meal with higher moisture and salt content, adulterated fish meal, substandard maize and adulterated rice bran are not uncommon in the market.*

*In case of vaccines, the preservation of vaccines by the traders is questionable and also till the vaccines reach the birds because of indiscriminate use of vaccines by quacks. As a result it is not uncommon to find disease outbreaks in the so called vaccinated birds. With respect to marketing of poultry products, the farmers find it difficult to meet both ends because of the following few important factors.*

*No minimum price is fixed for eggs as well as broilers, unlike agricultural products. Because of the fluctuation in prices of eggs and broilers and also the culled birds there used to be distress sales and farmers closing the farms incurring heavy losses. Finally, there is no way to control the traders from selling substandard poultry feed materials and sale of vaccines and also the vaccinators and this is also a very important matter for serious consideration.*

**I**ncreasing the production and productivity of animals including poultry is very important not only in Tamil Nadu but in India as a whole

in view of the continued population increase and limited potential for further quantum increase in food output due to various factors like

diminishing area for cultivation because of urbanisation and expanding industries.

Poultry production has registered an annual growth rate of 6.4% in Tamil Nadu during the sixth plan period. However, to maintain the growth rate, there are several bottlenecks like (a) Non - availability of good quality feed and feed ingredients at fixed prices, (b) absence of suitable Government agency to fix minimum support price for eggs and broilers, (c) lack of regular outlet for marketing poultry products and (d) Haphazard storage of vaccines and vaccination

At present, though the farmers are interested in rearing poultry, they are worried about the escalating feed cost; at the same time their inability to liquidate their poultry products (eggs and broiler) at reasonable price due to severe fluctuations in prices of the same. Sometimes the fluctuations are so drastic that the marginal farmers are unable to meet even the feed cost as a result distress sale and closure of farms are not uncommon.

*Quality chicks:* Farmers are not always sure of getting quality chicks from Hatcheries. Not in all hatcheries there is professional supervision; as a result there is apt to breakdown in Hatchery sanitation. Certification procedure for the quality of chicks is virtually lacking. Of late, there is no price stabilisation for sale of chicks due to lack of programming of hatch resulting in flooding of chicks in the market at low cost sometimes and increasing the price when there is demand.

*Feed:* Quality feed ingredients are not always available at fixed

price or atleast at reasonable price. There is steady escalation of cost of commercial feeds. Self compounded feeds by farmers are not of uniform quality resulting in fluctuations in production. There is no agency for controlling the quality of feed and feed ingredients. Besides, there is always the seasonal fluctuations in prices of feed ingredients and there is absolutely no price stabilisation or support price for poultry feed. There is virtually no way to check the sale of substandard feed causing economic loss to farmers. The need of the hour is to give a thrust in Research to replace fishmeal as an ingredient by an alternate cheap readily available ingredient, rich in protein. Meanwhile the Government or Government agencies like NAFED could come forward to provide subsidised feed or feed ingredients of good quality.

*Vaccination and Health Care:* At present effective disease surveillance is lacking due to various constraints. There is absolutely no restriction or control over the purchase, storage, reconstitution of vaccines and actual vaccination of birds because anybody can sell and anybody can vaccinate. There is no uniform vaccination schedule and different type of vaccines are available in the Market. The high incidence of Banikhet disease in vaccinated flocks is man-made. Therefore, there is immediate need for standardisation and quality control of vaccines. The need for a statutory body with cooperation from University need not be over emphasised. Besides, post - vaccination surveillance is a must and cooperation from the field Veterinarians could be sought in the respective areas. Besides, thrust should be given on the

Research side for the improvement of vaccines particularly Marek's vaccine since the present vaccine is ineffective. Therefore there is an increase in the incidence of Marek's disease since the middle of 1989 both in Parent and Commercial stock. Ranikhet Disease also is on the increase among vaccinated flock.

**Marketing:** Marketing of poultry products is really a problem for poultry farmers. No minimum price is fixed for eggs as well as for broilers, unlike agricultural products (support price). because of the drastic fluctuations in prices of eggs and broilers and also the culled birds there used to be distress sales, some-

times closing the farms incurring heavy losses. With respect to Tamil Nadu, the main outlet is Kerala and the demand for eggs, broilers and culled birds depends upon the seasonal crop of fish and it is inversely proportional. In Namakkal area, the price is fixed weekly by the National Egg Coordination Committee (NECC) in which the farmers, traders and the committee members deliberate and fix the price every THURSDAY effective from Friday for a week though in other centres like Madras, Hyderabad, Vijayawada the price of egg is fixed daily like 'bullion' price. Price always depends upon the demand and supply. How-

**Table 1 Comparison of Feed Cost per Egg and NECC Price at Namakkal with Percent Returns Over Feed Cost**

	1987 - 88			1988 - 89			1989 - 90		
	Feed cost per egg	Egg price	Per-cent returns over feed cost	Feed cost per egg	Egg price	Per-cent returns over feed cost	Feed cost per egg	Egg price	Per-cent returns over feed cost
April	34.51	45.23	31.06	41.30	48.13	16.54	43.37	47.40	9.29
May	34.51	53.64	55.43	44.11	57.83	40.87	43.37	43.77	12.45
July	35.07	56.00	59.68	42.24	63.84	51.11	43.75	56.06	28.14
August	36.21	50.96	40.73	41.67	57.41	37.77	43.75	54.51	24.59
September	36.21	53.76	48.47	39.22	53.53	36.49	44.31	55.63	25.55
October	35.83	54.00	50.71	39.22	55.93	42.61	43.37	48.38	11.55
November	36.77	63.30	72.15	37.90	61.93	63.40	44.31	56.66	27.87
December	37.34	58.67	57.12	39.60	55.45	40.02	44.31	56.65	27.85
January	37.90	48.41	27.73	39.60	58.16	46.87	43.75	55.84	27.63
February	38.66	56.48	46.09	41.49	59.10	42.44	45.26	49.03	8.33
March	39.61	56.35	42.26	41.49	46.52	12.12	45.26	53.45	18.10
Average	36.47	54.14	48.45	40.59	56.75	39.81	43.94	53.71	22.23

(Price in paise)

**Table 2 Escalation of Feed Cost to Produce One Kilo Live Weight of Broiler**

	1987 - 88 Rs.	1988 - 89 Rs.	1989 - 90 Rs.
April	7.20	8.64	9.44
May	7.20	8.64	9.44
June	7.30	8.83	9.28
July	7.30	8.83	9.50
August	7.52	8.77	9.50
September	7.52	8.64	9.60
October	7.52	8.64	9.28
November	7.68	8.48	9.60
December	7.78	8.80	9.60
January	8.00	8.80	9.44
February	8.16	9.12	9.79
March	8.32	9.12	9.79
Percent increase over the year	+15.56	+5.56	+3/71

**Table 3 Comparative Egg Price Fixed by 'NECC' at Different Centres**

	1987 - 88	1988 - 89	1989 - 90
Namakkal	54.13	56.75	53.71
Madras	55.57	60.68	55.89
Nellore	50.54	54.84	52.32
Hyderabad	48.89	54.68	49.49
Vijayawada	49.55	54.73	50.85
Average	51.74	56.34	52.45

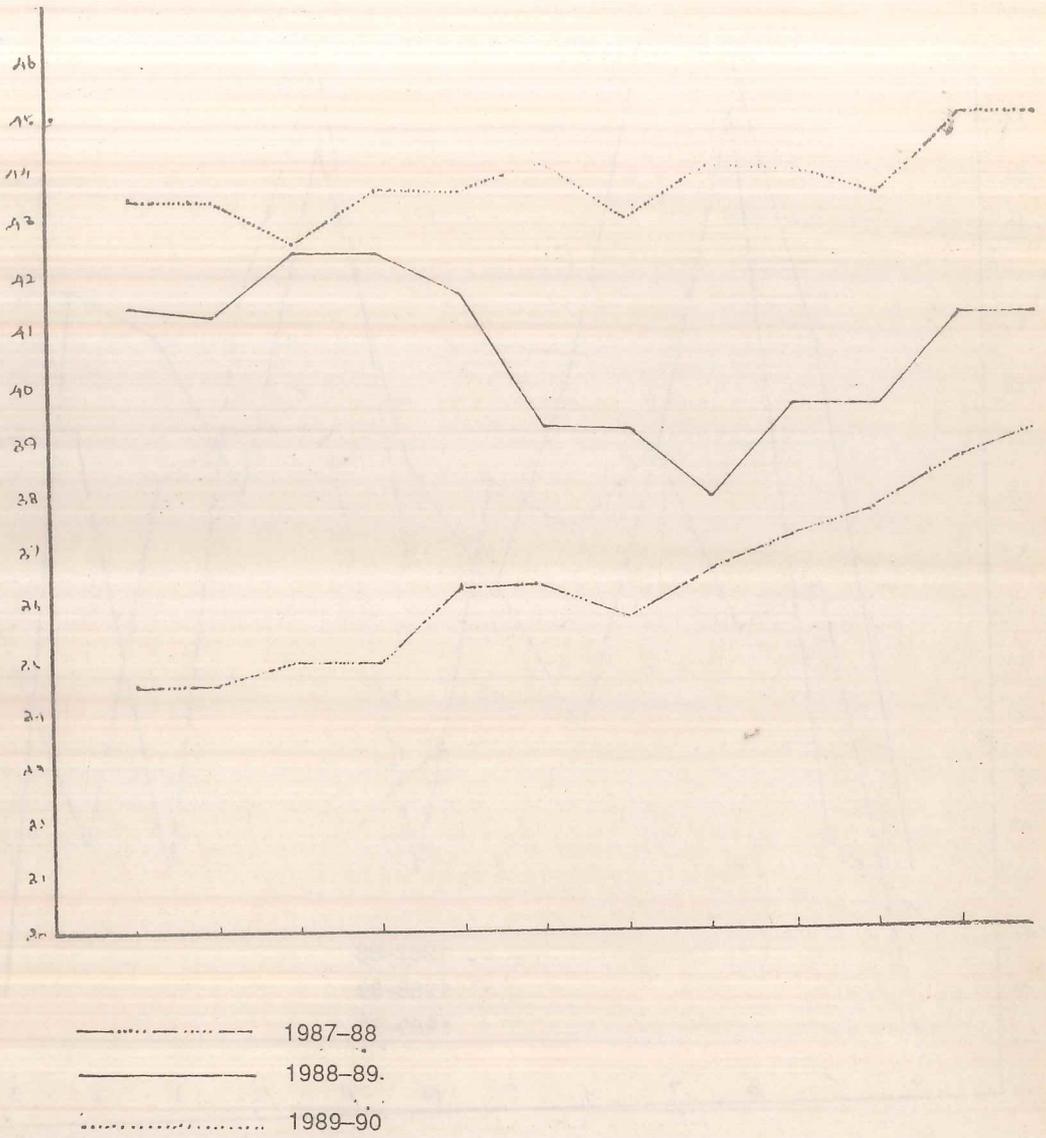
Price in rupees for 100 eggs

ever, southern part of Tamil Nadu particularly Namakkal, Erode and Palladam belts are affected because to Madras, the supply is mostly from Andhra Pradesh and transport cost is less. Generally the production cost is also less in Andhra Pradesh. Sometimes the farmers have to sell the eggs below the price fixed by the NECC.

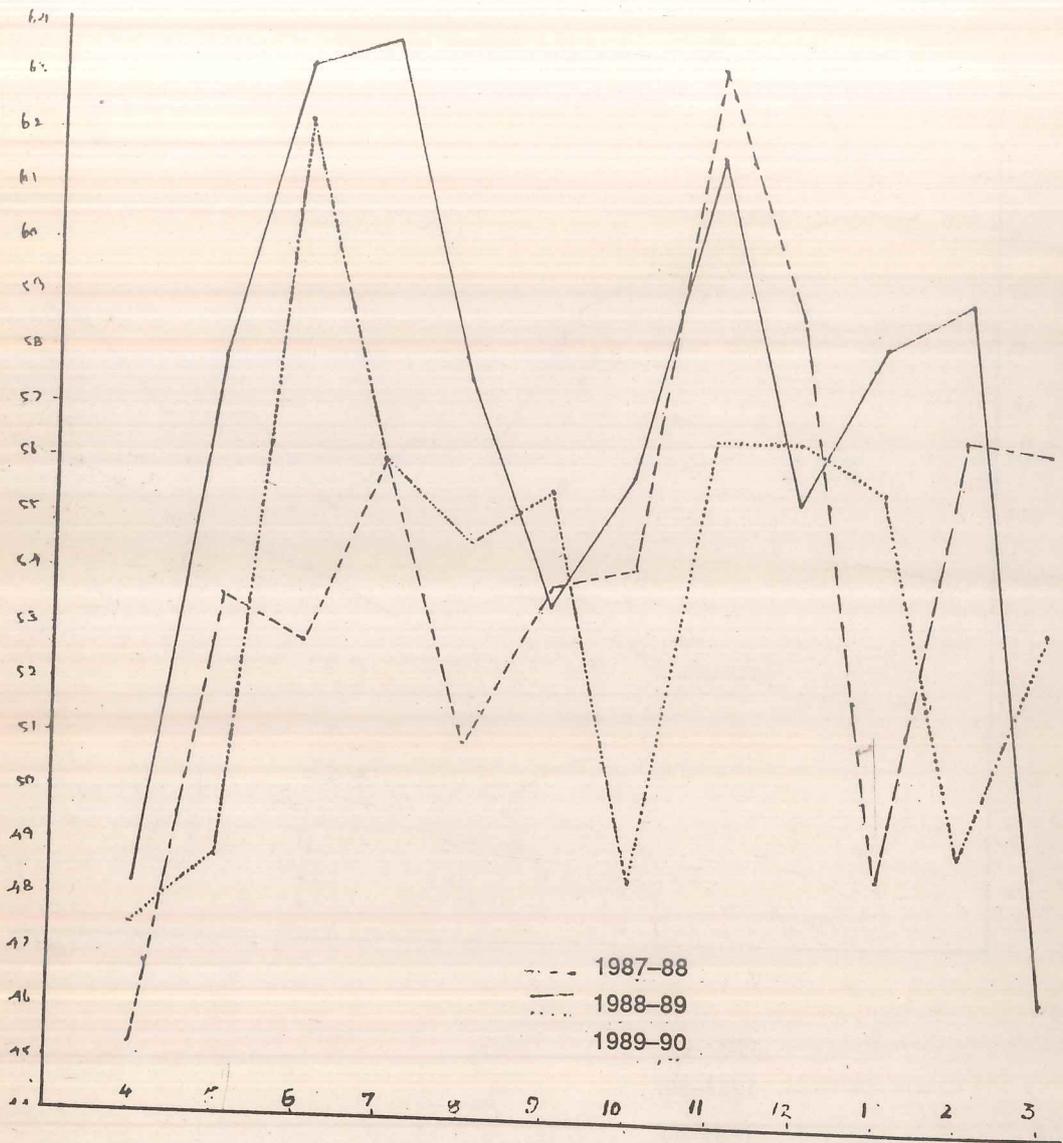
Therefore it is evident that there is a necessity for coordinated market-

ing agencies in addition to the need for basic price assurance (support price) which should not fall below the production cost. Post-harvest technology should be improved to avoid pressure on producers. Marketing research on poultry products is essential so that the constraints in the field of marketing of poultry products could be identified and proper solutions could be found to help the poultry farmers.

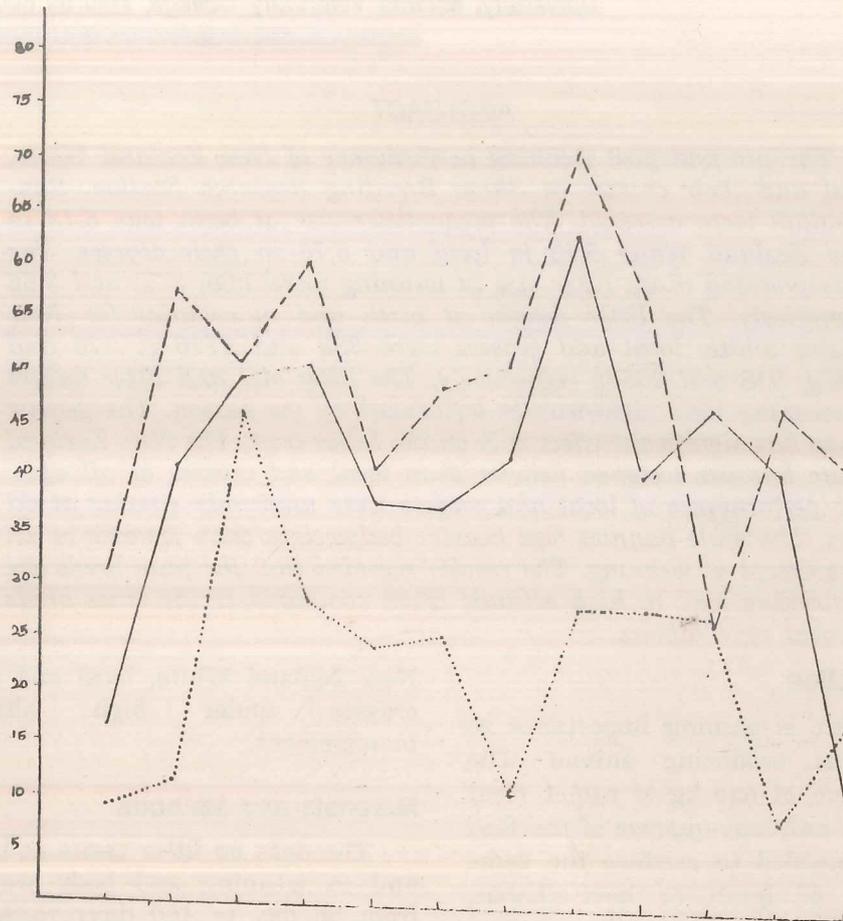
1. FEED COST PER EGG



2.EGG PRICE (NECC)



3. PERCENT RETURNS OVER FEED COST



--- 1987-88  
— 1988-89  
... 1989-90

## Pre and Post Weaning Performance of Broiler Rabbits

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### ABSTRACT

*The pre and post weaning performance of New Zealand White, local and their crosses at Sheep Breeding Research Station, Sandynallah were assessed. The mean litter size at birth was 5.12 in New Zealand White 5.13 in local and 5.76 in their crosses. The corresponding mean litter size at weaning were 3.99, 3.72 and 3.65 respectively. The litter weight at birth and at weaning for New Zealand white, local and crosses were 333 and 2716 g, 276 and 2317g; 318 and 2458g respectively. The litter size and litter weight at weaning were significantly influenced by the season. The genetic group has significant effect only on the latter trait. The New Zealand White bunnies weighed heavier than local and crosses at all ages. The performance of local and crosses were uniformly similar at all ages. The male bunnies had heavier bodyweight than females at all ages except at weaning. The results revealed that the pure bred are performing well in high altitude when compared to the cross bred of meat type rabbits.*

### Introduction

Rabbit is gaining importance as meat producing animal. The production of one kg of rabbit meat requires only one-quarter of the feed energy needed to produce the same amount of lamb or beef (Lebas, 1981). An FAO Expert Consultation on Rural Poultry and Rabbits, 1981, emphasized that if the high rate of meat consumption in future years was to be met, the increase in meat production would have to come from short-cycle animals like rabbits kept by small scale farmers (Lebas, 1983). This calls for intensive research on rabbit production and to evaluate the pre and post weaning performance of

New Zealand White, local and their crosses under high altitude management.

### Materials and Methods

The data on litter traits at birth, and at weaning and body weights from 56 day to 180 days were collected from the Rabbit Breeding Unit of SBRS, Sandynallah, Nilgiris for a period of one year. The influence of season and the genetic group namely the New Zealand White, local and their crosses on litter trait at birth and at weaning were analysed by Least-square analyses as per Harvey (1975). Similarly the post weaning body weights were also analysed for the effect of genetic groups and sex.

**Results**

The Least-squares mean ( $\pm$ SE) for the litter traits at birth and at weaning are presented in Table 1.

The mean litter size at birth was 5.12 in New Zealand White, 5.13 in local and 5.76 in their crosses. The

corresponding mean litter size at weaning were 3.99, 3.72 and 3.65 respectively. The litter weight at birth and at weaning for the New Zealand White, local and their crosses were 333 and 2716 g; 276 and 2317 g; and 318 and 2458 g respectively. The litter size and weight at

**Table 1 Least-square means for litter traits**

Factors	No of records	At birth		No. of records	At weaning	
		Litter size	Litter wt. (g)		Litter size	Litter wt. (g)
<b>Seasons</b>						
South West monsoon	49	6.1	321	46	5.1 <sup>a</sup>	4179 <sup>a</sup>
North east monsoon	27	5.4	292	23	4.6 <sup>ab</sup>	2796 <sup>b</sup>
Winter	14	5.1	317	11	2.9 <sup>b</sup>	1778 <sup>c</sup>
Summer	19	4.8	305	8	2.5 <sup>b</sup>	1236 <sup>d</sup>
<b>Breeds</b>						
New Zealand White	33	5.1	333	24	4.0	2716 <sup>a</sup>
Local	40	5.1	276	32	3.7	2317 <sup>b</sup>
Crosses	36	5.8	317	32	3.7	2458 <sup>c</sup>
Overall	109	5.3	309	88	3.8	2497

(Means bearing same superscript within the sub-class do not differ significantly)

**Table 2 Least-square means for post weaning body weight (g)**

Factors	56 days	90 days	120 days	150 days	180 days
<b>Breed</b>					
New Zealand White	907 <sup>a</sup>	1383 <sup>a</sup>	1634 <sup>a</sup>	1722 <sup>a</sup>	1772
Local	782 <sup>b</sup>	1078 <sup>b</sup>	1312 <sup>b</sup>	1490 <sup>b</sup>	1626
Crosses	758 <sup>b</sup>	1116 <sup>b</sup>	1277 <sup>b</sup>	1456 <sup>b</sup>	1505
<b>Sex</b>					
Male	809	1235 <sup>a</sup>	1457	1603 <sup>a</sup>	1664
Female	822	1150 <sup>b</sup>	1359	1508 <sup>b</sup>	1604
Overall	816	1192	1408	1556	1634
(No. of records)	(295)	(222)	(188)	(158)	(137)

(Means bearing same superscript within the sub-class do not differ significantly)

weaning were significantly influenced by the season. The genetic group had significant effect only on the latter trait. The highest litter weight at weaning was observed in New Zealand White breed.

#### *Post weaning Performance*

The least squares mean ( $\pm$  SE) for the post weaning body weights are given in Table 2.

The New Zealand White bunnies weighed significantly heavier than local and their crosses at weaning (56 days) to 150 days of age. At 180 days, eventhough the New Zealand White weighed heavier (1772 g) than local (1626 g) and crosses (1505 g), the differences in body weight were not significant. The performance of local and crosses were uniformly similar at all ages. The male bunnies had significantly heavier body weight at 90 and 150 days only.

#### **Discussion**

The pre and post weaning traits were studied and discussed. The overall mean litter size at birth and at weaning were 5.3 and 3.8 respectively. Jayarama Krishna *et al.* (1989) reported an average of 5.2 and 3.8 for these two traits which are very much similar to those obtained in this study. Lebas *et al.* (1986) reported 6.9 and 5.7 for the litter size at birth and at weaning in New Zealand White breed. These values were higher than the values reported in this study. However, Easwaran *et al.* (1989) reported similar values for these traits in New Zealand White, locals and their crosses in high altitude as well as in plains. The overall mean litter weight at birth and at weaning were 309 g and 2497 g respectively. Lebas *et al.* (1989)

reported higher value of 520g and 3195 g for these traits in New Zealand White breed. The values reported by Eswaran *et al.* (1989) for these traits were in close agreement with the values obtained in this study.

The litter size at birth was not influenced either by season or genetic group. However, the litter size was higher in South West monsoon when compared to other seasons. Similarly the cross bred had slightly higher litter size than the pure bred. The litter size and litter weight at weaning were significantly influenced by the season; whereas the genetic group had significant effect only on the latter trait. The highest litter weight at weaning was reported in the South west monsoon season and the lowest in the summer season. Similarly in genetic groups, the New Zealand White showed the highest litter weight at weaning.

The heterosis obtained for the litter size at birth and at weaning were 12.28% and - 5.44% respectively. The corresponding values for litter weight at birth and at weaning were 4.43 and -2.38 respectively. A higher positive heterosis value of 17.9 and 27.3% were reported by Jayarama Krishna *et al.* (1989) between New Zealand White and Chinchilla crosses. The litter traits upto weaning is primarily a function of the dam and the dams being pure bred in single crosses, no heterosis were obtained for litter traits upto weaning.

#### *Post weaning*

The body weights were recorded sexwise from 56 days to 180 days. The New Zealand White breed weighed heavier than local and their crosses at all age. Similarly the male

bunnies were heavier than females in all ages except in weaning weight. The breed and sex had significant influence on most of the post weaning traits. Viroji Rao *et al.* (1989) reported similar findings in New Zealand White at 6, 8, 10 and 12 weeks of age. The results revealed that the pure breds are performing well in high altitude when compared

to the cross breds of meat type rabbits.

#### Acknowledgement

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## Association of Milk Yield, Major Milk Constituents and Mineral Contents in Crossbred Cows and Buffaloes Milk

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### ABSTRACT

*The inter-relationships of milk yield, major milk constituents and mineral contents in crossbred cows and Murrah buffalo's milk were investigated. The interrelationship between milk yield and major milk constituents were similar in both the species. Milk yield and lactose were inversely related with fat and protein percentages. The inter-relationships of mineral contents amongst themselves and with milk yield and major milk constituents indicated that the mineral metabolism in cows and buffalo's milk are likely to be different atleast partially as far as calcium and phosphorus are concerned. The milk yield significantly affected the calcium content inversely in cows milk and it had no effect on calcium in buffalo's milk. Butter fat percentage was negatively associated with phosphorus in cow's milk and positively in buffalo's milk. Phosphorus was found to be significantly associated with magnesium and sodium in cow's milk but with calcium only in buffaloes milk.*

**T**he milk salts are not only important from nutritional point of view but also they determine the physico-chemical status of the milk serum. It has been well established that these salts have a controlling influence on heat stability of milk, feathering of cream in coffee, age thickening of sweetened condensed milk, clumping of fat globules on homogenization, coagulation of milk by rennin etc. Therefore a study on the association of major milk constituents with mineral contents of milk will help development of appropriate product technology and

newer milk standards. The present study is to find out such associations in milk from crossbred cows and buffaloes in organised dairies.

### Materials and Methods

Twelve crossbred cows from Loyola College Dairy Farm, Madras and 12 Murrah buffaloes from Central Cattle Breeding Farm, Alamadhi were followed from the initiation to the end of lactation by sampling milk at monthly intervals. To minimise the effect of season, the animals calved during October/November '85' were only con-

sidered. Samples from morning and evening milkings of individual animals were mixed and the composite samples were analysed for fat, protein, lactose, ash and the minerals Ca, Mg, P, Na and K. The milk yield on the days of sampling were recorded. The correlation coefficients of milk yield and major milk constituents with mineral contents were estimated and tested for significance using the conventional methods given by Snedecor and Cochran (1967).

### Results and Discussion

The correlation coefficients of milk yield and major milk constituents are tabulated in Tables 1 & 2. All the correlations were highly significant (P). Milk yield was found to be negatively correlated with fat and protein percentages and positively with lactose percentage. Correlations between major milk constituents were positive with the exception of those with lactose percentage. There was general agreement in the magnitude and the direction of correlation coefficients in both the species. The reverse relationship of milk yield with fat and protein percentages and those of lactose with fat and protein are well validated by the reports available in the literature (Ghosh and Anantakrishnan, 1965, Pal *et al.*, 1972, Akinosyinu, 1981 and Sbodio *et al.*, 1985).

The correlation coefficients of mineral contents with milk yield and major milk constituents are tabulated in Tables 3 & 4. Significant (P) inverse relationships of milk yield and lactose percentage with magnesium and sodium content of the milk and positive association with

potassium content were encountered in both the species. Milk yield and calcium content was reciprocally related in cows milk while they were not associated in buffaloes milk. The relationship of fat and protein percentage with the above minerals were also significant in both the species, but in opposite direction. Phosphorus was unaffected except by fat percentage in both the species and lactose percentages in buffaloes. Fat percentage was negatively associated with phosphorus in cow's milk and positively in buffalo's milk. The correlation coefficients of mineral contents with milk yield and major milk constituents reported in the literature are too fragmentary to authenticate the present findings in full. Rook and Wood (1958), Konar *et al.*, (1971) and Cambrell and Smith (1970) showed reciprocal relationship between sodium and lactose. Roberston and Dixon (1969) reported positive association of fat with calcium and protein with calcium and phosphorus. These results are in agreement with the present findings.

The interrelationship between mineral contents are tabulated in Tables 5 & 6. The relationship of phosphorus with other minerals differed between the species. Phosphorus was positively associated with magnesium in cows and calcium in buffaloes. There was also negative association of phosphorus with sodium in cow's milk. The reciprocal relationship between sodium and potassium encountered in both the species has been widely reported (Rook and Wood, 1958 and Konar *et al.*, 1971).

The inter-relationship between major milk constituents and minerals and among the minerals indicate

that the mineral metabolism in cows and buffaloes are likely to be different atleast partially as far as calcium and phosphorus are concerned. Fat was found to be negatively associated with phosphorus in cows milk and positively in buffaloes milk. Further phosphorus was found to be significantly associated with calcium

in buffaloes but not in cows. Correlation of phosphorus with magnesium and sodium were significant in cows but not in buffaloes. The milk yield and calcium were inversely related in cows milk while in buffaloe's the association was not in significant.

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**Table - 1 Correlation Among the Major Milk Constituent and, Milk Yield in Crossbred Cows**

Constituents	Milk yield kg	Fat %	Protein %
Fat	- 0.6541**		
Protein	- 0.6915**	0.6616**	
Lactose	0.7504**	- 0.4142**	- 0.5889**

\*\* Significant 1% level.

**Table 2 Correlation Among the Major Milk Constituents and Milk Yield in Murrah Buffaloes**

Constituents	Milk yield kg	Fat %	Protein %
Fat	-0.8138**		
Protein	- 0.6168**	0.6242**	
Lactose	0.6683**	- 0.6068**	- 0.3979**

\*\* Significant 1 % level.

**Table 3 Correlation Coefficient of Mineral Contents with Milk Yield and Major Milk Constituents in Crossbred Cows**

Constituents mg/100 ml	Milk Yield kg	Fat %	Protein %	Lactose %
Calcium	-0.7432**	0.5857**	0.6636**	- 0.6475**
Magnesium	- 0.2755*	0.1999*	0.2999**	- 0.3558**
Phosphorus	0.1384	- 0.3901**	- 0.0621**	- 0.1678**
Sodium	- 0.6647**	0.7530**	0.6804**	- 0.3993**
Potassium	0.8100**	- 0.6429**	- 0.6924**	0.8043**

\* Significant at 5% level

\*\* Significant at 1% level.

**Table 4 Correlation Coefficients of Mineral Contents with Milk Yield and Major Milk Constituents in Murrah Buffaloes**

Constituents mg/100 ml	Milk Yield Kg	Fat %	Protein %	Lactose %
Calcium	- 0.0769	0.8476**	0.4368**	- 0.6619**
Magnesium	- 0.3797**	0.3289**	0.3105**	- 0.1600
Phosphorus	- 0.1239**	0.2088**	0.1636	- 0.3514**
Sodium	- 0.8099**	0.8728**	0.5189**	- 0.7112**
Potassium	0.8161**	0.8831**	- 0.6820**	0.5912**

\* Significant at 5% level

\*\* Significant at 1% level

**Table 5 Correlation Among the Mineral Constituents in Crossbred Cows**

Constituents mg/100 ml	Calcium mg/100 ml	Magnesium mg/100 ml	Phosphorus mg/100 ml	Sodium mg/100 ml
Magnesium	0.1983*			
Phosphorus	0.1140	0.2536*		
Sodium	0.6970**	0.1419	-0.3113**	
Potassium	-0.7727*	-0.1111	0.0980	-0.6938**

\* Significant at 5% level

\*\* Significant at 1% level

Table 6 Correlation Among the Mineral Constituents in Murrah Buffaloes

Constituents mg/100 ml	Calcium mg/100 ml	Magnesium mg/100ml	Phosphorus mg/100 ml	Sodium mg/100 ml
Magnesium	0.4008**			
Phosphorus	0.4146	0.1423*		
Sodium	0.7629**	0.3596**	0.0492	
Potassium	- 0.7427**	- 0.3367**	- 0.00778	- 0.8727**

\* Significant at 5% level

\*\* Significant at 1% level

## Possibilities of Fish Rearing in Sewage water

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### ABSTRACT

*The protein of fish has very high digestibility, nutritive and biological value and is growth promoting.*

*In India, where rivers and lakes dry up in summer, recycling of waste water for fish culture could be a solution. Hence the scope for expansion of sewage fed fish culture in various places like municipalities, town ships and hostels where there are organised sewage disposal systems were examined. The fish species *Tilapia mossabica* was selected for the study. The fish were classified on the basis of weight into five groups from 10g. to 120g. Comparing the edible muscle portion, devoid of bones, the sewage fish was found to be superior in its calcium, phosphorus and content iron to fresh water fish. The higher calcium and iron content of sewage water might have contributed to the increase in these minerals in the fish reared in sewage water.*

*Two types of recipes, fish fry and fish molli were tried to compare sewage grown and fresh water fish. Regarding acceptability there was no difference in the scores for taste. To assess the biochemical impact of textile effluents on aquatic life, fish collected from wells around the dyeing units in Tiruppur and were compared with similar species from a pond in Coimbatore city.*

*Acetylcholinesterase activity of fish found in the well water of Tiruppur was 22% less then compared with the activity of fish grown a Coimbatore pond.*

### Introduction

The sea is a potential source of food for the thickly populated world of today. The cost is relatively low compared to other flesh foods and the waste is comparatively less and yet the use of fish is not

widespread. Consumption of fish in India is still low, being of the order of two kilogram for per head per annum.

The protein content of fish varies from 14 to 25 g. per cent, has very high digestibility, nutritive and

biological value and is growth promoting. The calcium content is in the range of 250 mgm., iron 1-3 mgm. per 100g. Fish is an excellent source of Vitamin A, thaimin and riboflavin (Gopalan *et al.*, 1989)<sup>†</sup>.

Fish culture in Indian Subcontinent is undoubtedly several, hundreds of years old. where, rivers and lakes dry up in summer recycling of waste water for fish culture could be a solution. It not only prevents pollution problem but could also facilitate a more rational conservation of natural resources. The steamed, fried and curry preparations of fish when examined revealed complete absence of any of the bacteria including the pathogens.

#### Materials and Methods

Studies were undertaken at Sri Avinashilingam Education Trust Institutions to explore possibilities of fish in sewage water. The scope for expansion of sewage fed fish culture in various places like municipalities, town ships and hostels where there are organised sewage disposal systems was examined. Samples were drawn for an experimental Oxidation pond of a sewage collection. For this study of fish from sewage the selected species was *Tilapia mosambica* (Sarathorodon - Mossambicus).

The fish were classified on the basis of weight into five groups from 10g. to 120g. Biochemical estimations such as moisture, ash, calcium, phosphorus, sodium, potassium, manganese, iron, copper and zinc were carried out with edible muscle portion, devoid of bones of both sewage and fresh water fishes.

#### Results and Discussion

The mean moisture contents of fresh water and sewage fish were  $81.474 \pm 1.9158\%$  and  $8.462 \pm 1.8609\%$  respectively. The difference between the moisture content of sewage and freshwater fishes was not statistically significant in all weight groups.

The calcium content varied with each sample. The mean calcium content of fresh water and sewage fish were  $145.6 \pm 30.91$  mg. per cent and  $171.91 \pm 31.39$  mg. per cent respectively. The increase in the content of calcium was noticed with increase in weight, in both the types. The difference was statistically significant at 1 per cent level. This may be due to the higher amount of calcium in sewage water (132 p.p.m.) as compared to fresh water (95 p.p.m.).

The phosphorus content was also higher in sewage fish. The increase probably indicates that the presence of phosphorus in the muscle is associated with lipids presumably in form of phospholipids. The increase in phosphorus content between sewage and freshwater fish in groups weighing above 50g. was statistically significant at 1 per cent level.

Iron content was also higher in sewage fish ( $9.62 \pm 7.26$  mg. per 100g.) compared to fresh water fish ( $7.2 \pm 3.1$  mg. per 100g.). The iron content increased with weight in both the fishes. The iron content of sewage (0.25 p.p.m.) might have contributed to the increase in sewage fish.

#### Acceptability Test

Fish cooked by two methods (frying and stewing) were subjected to acceptability tests. Identical por-

Table 1 Scores for the Fish Preparations(maximum score 20)

Criteria	Fish Fry		Fish Moli	
	fresh fish	sewage fish	fresh fish	sewage fish
Appearance	4.0	4.0	4.0	4.0
Flavour	4.2	4.2	3.0	3.0
Taste	3.8	4.6	3.8	4.8
Testure	5.0	5.0	5.0	5.0
	17.0	17.8	15.8	16.8

tions were selected and a panel of trained experts assessed these preparation and awarded scores. The scores are given in Table I.

From the Table is seen that there was no difference in the scores.

#### Effect of Textile Effluents

Very few studies have been done on the effect of textile effluents on aquatic biots, crops and fish. Hence to assess the biochemical impact of textile effluents on aquatic life, fish were collected from wells around the dyeing units in Tiruppur and from a pond in Coimbatore city.

Results indicated that Acetylcholinesterase activity of the fresh fish reared in the well water of Tiruppur was 21.7 per cent less when compared with the activity of fish grown a Coimbatore (control). The reduction of acetylcholinesterase activity may have been due to the textiles effluents in the well water.

The brain tissue had 0.01 mg/g. of lead and mercury, 0.2mg. of cadmium, chromium and antimony, 0.4 mg/g. of nickel and aluminum while the corresponding controls did not

contain any of these metals. The heart tissue of the experimental fishes had 0.4 mg/g. of cadmium and chromium, 0.03mg/g. of antimony and aluminium while the fishes in fresh water pond contained no metals in their heart tissue. The muscle tissue of experimental fishes had 0.01mg/g or lead, mercury and cadmium, 0.03 mg/g. of chromium and 0.01 mg./g of antimony and 0.6mg./g. of aluminum. Accumulation of cadmium and chromium is highest in heart while that of aluminum is highest in muscle. In spite of the coloured nature of the well water around the industrial area, its higher dissolved oxygen content made the fishes survive. The possibility of recycling sewage and textile effluent and use for fish cultivation, needs further investigation.

#### Acknowledgement

Grateful thanks are accorded to Dr. (Mrs.) Rajammal P.Devadas, (Madras), Vice Chancellor Avinashilingam Deemed University for her deep interest and facilities provided for these studies.

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Narasinga Rao B.S. Deoftihale and Pant K.C.

## Sewagefed Fishculture with Reference to Different BOD Levels

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### ABSTRACT

*Sewage was used in 5 BOD levels viz., control (well water alone), 30, 55, 87 and 113 in sewage fed fish culture. Physico-chemical characteristics, bacterial analysis (total count & enterobacterial count) were carried out every 15 days. The primary productivity studies recorded, maximum primary production in 55 BOD level.*

*Experiments carried out in the sewage fed ponds showed a considerable increase in fish production than the control (Well water). The maximum total fish production was recorded from 55 BOD level. Among the different fish species cultured, catla showed the highest growth (1690 gm/210 days).*

### Introduction

The utilization of domestic sewage as fertilizer for fish culture in freshwater ponds is known in many countries (Sarig 1956, Wolny 1962). Experiments carried out in India (Gopalakrishnan and Srinath 1963, Banerjee *et al.*, 1969, Natarajan and Varghese, 1980) have proved that fertilization of fish pond with oxidised domestic sewage can substantially increase the fish production. Allen and Hepler, (1976) summarised methods to utilise waste waters for pond fish culture. These include dilution of sewage (Vaas, 1948 and Bose, 1944) and partial treatment of waste water, making it acceptable for fish culture. In general these systems aim to reducing the level of BOD at waters and recycling of nutrients. In our experiment we

have maintained domestic sewage at 5 different BOD levels in the fish ponds to study the optimum BOD level for high fish production.

### Materials and Methods

Sewage from the hostel of the University is drawn through a series of stabilization ponds. The oxidised sewage (Average BOD 113) is pumped to the earthen ponds of 200 square meter each. Fresh water also was added to get the required dilution. Thus the BOD of the inlet water for different ponds was maintained as 0 (freshwater), 30,55,87 and 113 mg/l. The daily inlet of BOD through sewage was decided based on the dissolved organic matter and the corresponding BOD was calculated through a standard curve for

	Species	%
Silver Carp	<i>Hypothalmichthys molitrix</i>	25
Common carp	<i>Cyprinus carpio var communis</i>	20
Grass carp	<i>Ctenopharyngodon idella</i>	5
Catla	<i>Catla catla</i>	15
Rohu	<i>Labeo rohita</i>	15
Mrigal	<i>Cirrhinus mrigala</i>	20

BOD plotted against the corresponding dissolved organic matter.

The stocking density adopted was 10,000 fingerlings/ha. The above species were stocked.

Fingerlings (1.8 -2.8 g) were introduced in each pond (200 Nos./pond). Observation on the growth of the fish was made fortnightly by catching about 5-10 fish in each species at random by drag netting and were released back after measurement.

The physico-chemical parameters of collection pond, sump and different experimental ponds were analysed every fortnightly as per methods of APHA (1985).

The primary productivity and, microbial enumeration of different experimental pond water were made once in 15 days as per methods of APHA (1985).

### Results and Discussion

The physico-chemical characteristics of raw sewage for every 15 days for seven months are given in Table 1. The dissolved oxygen in the 113 BOD level pond was low on some days as 2-4.5 mg/l between 4-6 a.m. (Fig.1) mainly due to enormous decomposition of organic matter. Muthusamy *et al.*, (1978) reported

that low dissolved oxygen in sewage pond prevailed for about 1-2 h. in early morning. But in other ponds the level was always above 4mg/l.

The temperature during the period of investigation varied from 28-33° C. The high pH values recorded in different sewage concentrated ponds than the raw sewage, collection pond and oxidation pond may be due to high photosynthetic activity (Govindan, 1989). BOD level was maintained based on organic matter present in pond.

Primary productivity studies shows high gross primary productivity in the BOD 55 level pond than the other concentrated ponds (Fig.2). In all ponds surface area has higher production than column and bottom. This is mainly due to higher level of sunlight and oxygen in the surface than in bottom and column. Sometimes very low primary productivity occurs in the BOD 113 level pond at bottom. It may be due to the absence of sunlight and also high utility of oxygen for organic decomposition.

The microbial enumeration of sewage in the collection and sedimentation tanks and various experimental ponds are presented in Table 2. The maximum total bacterial count is found in the raw

sewage ( $5.62 \times 10^6$ /ml) than in sedimentation ( $5.0 \times 10^6$ /ml). Coliforms, salmonella and Streptococci counts are higher in collection pond than in sedimentation tank. The population of enteric bacteria decreases with decreasing concentration of sewage in different ponds. The above results indicates that the enteric bacterial population of original sewage depletes conspicuously in cultural ponds (Govindan, 1989). Nawaz Tariq & Ahmed (1985) stated that longer detention time resulted in better bacterial removal. Presumably the high pH resulting from photosynthetic activity of algae rather than detention time, reduces the bacterial load. Parhad and Rao (1974) reported that *Escherichia coli* not only failed to grow but was eliminated at a pH of 9.4.

Fish production and survival details of the different varieties of fish in different ponds (control, 30, 55, 87 and 113 BOD level) are presented in Table 3.

The average initial weight of silver carp was 1.6 g which in 210 days in control, 30, 55, 87 and 113 BOD level showed a weight of 223.3, 669.2, 1120.5, 910.8, 895.5 g respectively. The total silver carp production after 210 days in control 30, 55, 87, 113 BOD level ponds were 10.71, 28.78, 48.18, 38.24, 35.82 Kg respectively. The survival percentage was high in control (88%) and low in 113 BOD level pond (80%).

The average initial weight of common carp was 1.7 g. The average final weight in control, 30, 55, 87 and 113 BOD level ponds were 431.5, 713, 800.1, 712.2, 710.2 g/210 days respectively. The total yield of common carp was high in 55 BOD level

pond (28.8 kg) and low in control pond (16.4 Kg). The survival percentages was high in control and 55 BOD level pond (95%). Low survival percentage was noted in 87 and 113 BOD ponds (87.5%).

The average initial weight of grass carp was 1.4 g. The average final weight in control, 30, 55, 87 and 113 BOD level ponds were 819, 712.2, 711, 812, 716 g/210 days respectively. When we compare all the ponds one another control pond showed higher production of grass carp (8.19 Kg/210 days) than the other ponds. The survival percentage is high in control, 30 and 55 BOD level pond (100%) and low in 87 and 113 BOD level pond (90%).

The average initial weight of catla was 1 g in all ponds. The average final weight in control, 30, 55, 87 and 113 BOD level ponds were 262.3, 1212.5, 1690.2, 1400.5, 1450.2 g/210 days respectively. The total yield of catla was high in 55 BOD level pond (45.64 Kg/210 days) and low in control pond (7.34 Kg). The survival percentage is high in control, 30 and 87 BOD level pond (93.3%). Low survival percentage is observed in 55 and 113 BOD level ponds (90%).

The average initial weight of rohu was 3.8 g. The average final weight in control, 30, 55, 87 and 113 BOD level ponds were 190.2, 609.3, 905.5, 612.3 and 605.5 g respectively. The total yield of rohu was high in 55 BOD level pond (27.17 Kg/210 days) and low in control pond (5.71 Kg). The survival percentage is high in control 30, 55 and 87 BOD level ponds (100%) and low survival percentage was found in 113 BOD level pond (93.3%)

Initial average weight was 2.4 g in all ponds. Average final weight was high in 55 BOD level pond (512 g) and low in control pond (195.5 g). The total yield of mrigal was high in 55 BOD level pond (19.46 Kg/210 days). The survival percentage was high in control (110%) and low in 113 BOD level pond (87.5%).

Total fish yield/ha/yr in different BOD level ponds are graphically expressed (Fig. 3). The total fish yield obtained in control, 30, 55, 87, 113 BOD level ponds were estimated as 4.88, 11.57, 15.23, 12.66, 12.18/t/ha/year respectively. In West Bengal fish production utilizing Calcutta city sewage have been reported to be 3000-4000 Kg/ha/yr (Saigal

1972). Ghose *et al.* (1974) reported fish production of 7676 Kg/ha/yr utilizing the domestic waste as organic fertilizer. Muthuswamy *et al.* (1978) reported fish production of 11,545 kg/ha/yr. But in our study in BOD 55 level pond the fish yield is estimated as 15.23 t/ha/yr, which is considerably higher than that reported elsewhere. It is concluded that 55 mg/l - 87 mg/l BOD level of domestic sewage water is optimum for high fish production.

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TABLE 1 Physico-Chemical parameters of raw Sewage (mg/l)

Parameter	Mean Value _ S.D
pH	7.3+ 0.2
Temperature (C°)	30+2.3
Conductivity (m mhos)	1014.5+110.6
Total solids	870+98.2
Total alkalinity	429+19.5
EDTA hardness	296+19.5
Dissolved oxygen	0.5+0.3
Dissolved free Co <sub>2</sub>	77.3+17.4
BOD	196.5+63.8
Chloride	77.2+2.6
Total nitrogen	32.8+6.1
Total phosphorus	25.2+5.6
Potassium	11.7+0.1

Table 2 Microbial Analysis

BOD Level	Total count no. x 10 <sup>6</sup> /ml	Coliforms no. x 10 <sup>6</sup> /ml	Salmonella no. x 10 <sup>3</sup> /ml	Streptococci no. x 10 <sup>3</sup> ml.
Well water	0.23 - 0.31	0.008 - 0.03	0 - 0.011	-
30	0.35 - 0.65	0.008 - 0.47	0 - 0.018	-
55	0.10 - 0.70	0.006 - 0.59	0 - 0.105	-
87	0.70 - 3.27	0.02 - 0.40	0 - 0.35	0 - 1.00
113	2.17 - 4.65	0.03 - 2.67	0.16 - 0.59	0.20-2.0
Oxidation pond	2.72 - 5.00	0.1 - 4.4	1.87 - 4.58	0.55 - 2.06
Raw sewage	5.3 - 5.62	5.0 - 11.0	3.66 - 8.22	2.65 - 14.0

Table 3 Fish Production and Survival details

Sewage Concentration	Fish species	Average initial wt./(g)	Average final wt./(g)	Individual speles production kg/pond	Survival percentage	Total net production ton/ha/year
Control (Well Water)	Silver Carp	1.6	223.2	10.71	88.0	4.88
	Common Carp	1.7	431.5	16.40	95.0	
	Grass Carp	1.4	819.0	8.19	100.0	
	Catla	1.0	262.3	7.34	93.3	
	Rohu	3.8	190.2	5.71	100.0	
	Mrigal	2.4	195.5	7.82	100.0	
30 BOD level pond	Silver Carp	1.6	669.2	28.78	86.0	11.57
	Common Carp	1.7	713.0	27.09	95.0	
	Gras Carp	1.4	712.2	7.12	100.0	
	Catla	1.0	1212.5	33.95	93.3	
	Rohu	3.8	609.3	18.28	100.0	
	Mrigal	2.4	459.0	17.90	97.8	
55 BOD level pond	Silver Carp	1.6	1120.5	48.18	86.0	15.23
	Common Carp	1.7	800.1	28.8	90.0	
	Grass Carp	1.4	711.0	7.11	100.0	
	Catla	1.0	1690.2	45.64	90.0	
	Rohu	3.8	905.5	27.17	100.0	
	Mrigal	2.4	512.0	19.46	95.0	
87 BOD level pond	Silver Carp	1.6	910.5	38.24	84.0	12.66
	Common Carp	1.7	712.2	24.93	87.5	
	Grass Carp	1.4	812.0	7.31	90.0	
	Catla	1.0	1400.5	39.21	93.3	
	Rohu	3.8	612.3	18.37	100.0	
	Mrigal	2.4	490.0	17.64	90.0	
113 BOD level pond	Silver Carp	1.6	895.5	35.82	80.0	12.18
	Common Carp	1.7	710.2	24.86	87.5	
	Grass Carp	1.4	716.0	6.44	90.0	
	Catla	1.0	1450.2	39.16	90.0	
	Rohu	3.8	605.5	16.95	93.3	
	Mrigal	2.4	490.0	16.98	90.0	

Fig. 1.

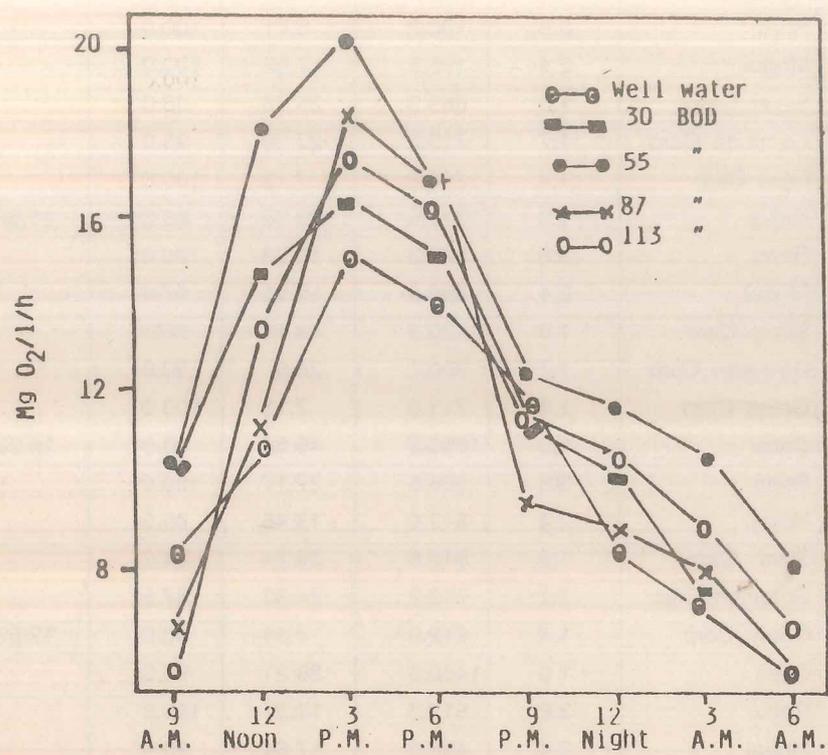


Fig. 2.

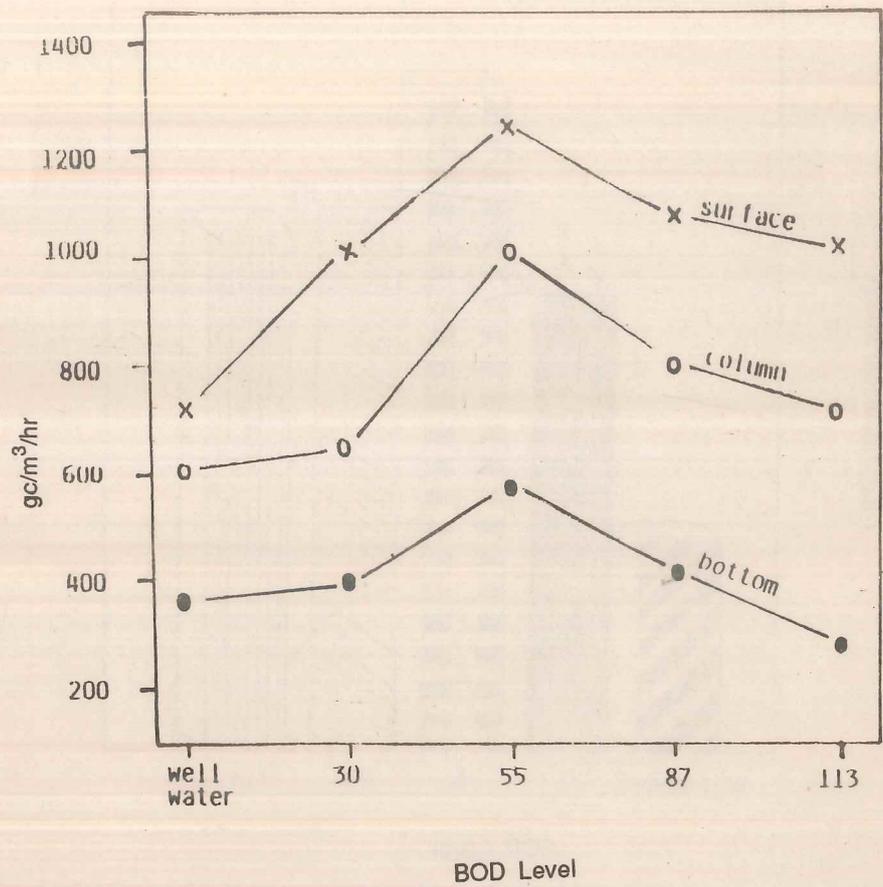
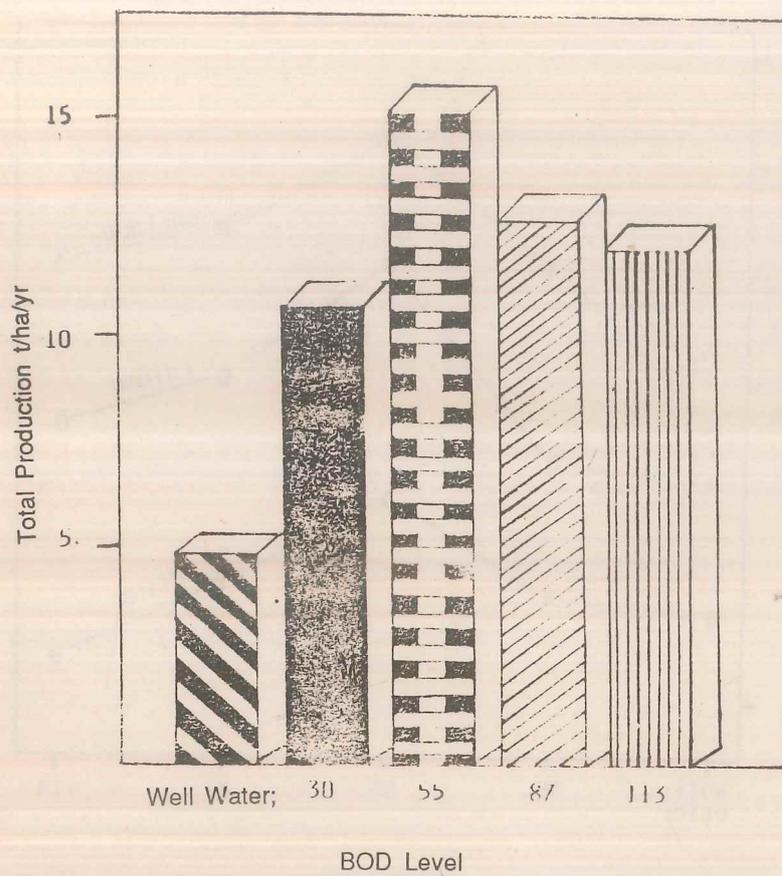


Fig. 3



## Treatment of Waste Water from Seafood Processing Plant for Fish Production

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### ABSTRACT

Seafood processing plants are known to release large quantities of suspended and soluble organic matter through waste waters. High concentration of  $H_2S$  ( $>2\text{ml/l}$ ),  $BOD_5$  ( $>30\text{ mg/l}$ ),  $COD$  ( $>100\text{ mg/l}$ ) and low pH (6.5) of these effluents make them unsuitable for direct aquaculture practices and hence they warrant suitable biological and chemical treatments. In the present investigation, effluents from a cuttlefish processing factory were subjected to treatment procedures. In the first step, decomposition of organic matter (mostly anaerobic) was undertaken in large tanks. Following this,  $BOD$  load of the water decreased with commendable increment in  $H_2S$  and trigger flocculation and precipitation of the soluble organic matter that imparted yellow colour in the oxidation tanks and rose colour in the waste receiving terminal tank. Due to this chemical treatment, the pH of the water remained between 9 and 10. In order to bring it to alkaline pH condition conducive for fish growth (pH around 8), aerobically decomposed cowdung rich with essential plant nutrients and large quantity of  $CO_2$  was used. The resulting mixed water had physico-chemical characteristics quite conducive for the better growth of fishes. Laboratory bioassay proved that such treated waste water supported 100% survival of test fishes like tilapia and common carp. It was also suggestive that murrels can be grown in these waste waters feeding them with the rejected wastes of the cuttlefish processing factory.

### Introduction

Seafood processing plants discharge waste waters which are known to contain large quantity of suspended and soluble organic matter. Among the seafood processing plants, cuttlefish processing plants receive much attention owing to the release of cuttlefish ink which

possesses antiseptic properties (Mochizuki, 1979). Knowledge on the treatment of waste waters from seafood processing plants for possible utilisation in fish farming is meagre. Therefore, the present investigation was carried out to study the various stages of treatment of waste waters of cuttlefish processing plants and

their physico-chemical characteristics.

### Materials and Methods

Waste waters were collected from the different waste stabilizing tanks of a cuttlefish processing plant situated at the outskirts of Tuticorin. Salinity, pH, Oxygen, BOD<sub>5</sub>, COD, H<sub>2</sub>S and NH<sub>3</sub> were estimated adopting standard procedures of FAO (1975). In the laboratory, biochemical oxidation was carried out by effecting sustained aeration round the clock. In factory the waste water was decomposed in a cement tank fitted with a water agitator. Chemical treatment of the waste water was effected by applying Ca(OH)<sub>2</sub> followed by the addition of very small quantity (17 mg) of FeCl<sub>3</sub>. Treated waste water was tested for toxicity conducting laboratory bioassay using common carp and tilapia as test animals.

### Results

#### 1. Biochemical oxidation and water quality:

The physico chemical characteristics of the raw waste water present in the suspended matter set-

tlement tank of the cuttlefish processing plant are shown in Table 1. The raw waste waters stabilized in the suspended matter settlement tank was pumped to the primary waste processing tank where water agitators were used to homogenise and aerate the waste water. The characteristics of the processed waste water of the waste stabilizing tank are shown in Table 1. In the secondary and tertiary treatment tanks, the water attained a faint green colour first, which turned to a stable yellow colour. The pH of the water was in the acidic range (6-6.5) in both treatment tanks. The very slow rate of organic matter loss observed in these tanks (BOD<sub>5</sub> = 29 mg/l) could be attributed to the anaerobic oxidation prevalent in these tanks. In the terminal tank, the bottom is earthen, so that the waste water may percolate through the bottom. In this tank, the waste water attained rose colour and became translucent also. The characteristic features of the waste water accumulated in the terminal tank are shown in Table 1.

The waste water of the terminal tank which was rose red in colour

Table I. Physico-chemical characteristics (average values) of waste water

Parameters	Raw waste water	Primary treatment tank	Terminal tank
Colour	Black	Black	Rose Red
Salinity%	0.001	0.001	0.001
PH	7.1	6.5	6.4
Oxygen (ml/l)	2.3	Nil	Nil
H <sub>2</sub> S (ml/l)	Nil	2.5	1.8
BOD <sub>5</sub> (ml/l)	37	32	28.5
COD (mg/l)	142	128	107.0
Suspended solids	470	Nil	Nil
NH <sub>3</sub> (NH <sub>3</sub> -mgN/l)		Not measured	5.6

was analysed for its chemical characteristics/ When diluted HCl was added to this aerated waste water (aerated in the laboratory) at all pH ranges (1 to 6), no precipitation could be observed. Though this waste water had very low salt content (0.001%), addition of sodium chloride did not trigger the flocculation of organic matter. Thus the stability of the dissolved organic matter in high ionic strength was established. When the waste water (not aerated) collected from the terminal tank was treated with dil.  $H_2SO_4$  slight turbidity occurred and this might be attributed to the acidic oxidation of  $H_2S$  to elemental sulphur. When the waste water from the terminal tank was filtered using 0.45  $\mu$  membrane filter paper, the rose red coloured organic matter was not retained by the filter paper. This observation confirmed the fact that the rose red coloured organic matter was present either as water soluble fraction or as small (0.45  $\mu$ ) colloidal particles which were stable even in increased electrolytic strength.

Hydrated CaO ( $Ca(OH)_2$ ) was added to the waste water all the soluble organic matter got flocculated, precipitated and sedimented. In the pH range of 9-10, precipitation was effective.  $H_2S$ , present in the waste water reacted with  $Ca(OH)_2$  to produce insoluble CaS. Treatment with ferric chloride (17 mg/l) after liming was observed to accelerate precipitation in aerated water collected from the stabilising tanks. Waste water, thus settled, biochemically oxidised and chemically treated had low COD (<15 mg/l) and  $BOD_5$  (<10 mg/l) but high pH (10.0 - 11.0). In the laboratory, sustained aeration of the water for 3-4 days resulted in

the total precipitation of  $Ca(OH)_2$  as  $CaCO_3$  and drastic reduction in pH from 10.5 to 8.1. In such recarbonated waste water, 100 % survival was recorded for the test animals viz., tilapia and common carp. Instead of aerating the water, cowdung decomposed in water for 10 days could be used to bring down the pH immediately. Since the decomposed cowdung-water mixture attained the pH 5.0 after decomposition for ten days, addition of the decomposed cowdung suppressed the pH by triggering the preprecipitation of  $Ca(OH)_2$  as  $CaCO_3$ .

### Discussion

In the raw waste water receiving suspended matter settlement tank, large quantity of suspended solids may get settled. Primary sedimentation of waste water usually results in 30 to 45% removal of  $BOD_5$  and 45 to 60% removal of suspended solids depending on the concentration and characteristics of solids in suspension (CPH & EEO, India., 1980). However, in the present study,  $BOD_5$  removal in settling tank was not observed to be very effective. In the primary water processing tank of the factory, water agitators were used to keep the waste in aerobic condition. However, this type of oxidation did not seem to satisfy the oxygen demand of the waste water and hence following denitrification, sulphate reduction happened as evidenced by the presence of  $H_2S$ . In the secondary treatment tank, the pH was acidic and the water attained faint green colour. In the tertiary tank,  $H_2S$  rich waste water attained rose colour. Hence chemical precipitation was adopted to improve water quality.

Chemical precipitation of waste water is analogous to coagulation in water purification process. Chemical treatment by adding cationic coagulants (calcium hydroxide and ferric chloride) is very effective (EPA, 1980).

CaO, hydrated as  $\text{Ca}(\text{OH})_2$  is the cheapest chemical used in chemical precipitation (CPH & EEO, India., 1980). In the present investigation, the amount of CaO (commercial grade) required to treat one liter of waste water was found to be 3 g. Treatment with  $\text{FeCl}_3$  was observed to enhance the rate and extent of precipitation of organic matter present in the waste water. Treatment with  $\text{Ca}(\text{OH})_2$  eliminated the free ammonia (due to high pH) as well as  $\text{H}_2\text{S}$  (0.5 mg/l) to cultivable fishes (Jorgensen, 1979) were observed to lie below the absolute concentration of these two toxicants in the biochemically and chemically treated waste water of the factory.

High pH of the chemically treated waste water could be lowered by adding sulphuric acid or by recarbonation, since sulphuric acid treat-

ment may lead to increased water hardness, recarbonation was effected either by passing  $\text{CO}_2$  or ordinary air for a prolonged period. In the present study, recarbonation was effected by adding decomposed cowdung to the chemically treated waste water. Since cowdung could also function as organic manure, addition of decomposed cowdung to the medium supported the multiplication of microalga like *chlorella* sp. Thus, after biological and chemical treatments, the waste water seemed to support the growth of cultivable fishes like tilapia and common carp. The alternative way for the better utilisation of this waste water is to grow tilapia and murels which could also be fed with the discarded protein rich wastes of the cuttlefish processing factory.

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## Utilization of Sewage Nutrients for Mass Culture of Algae for Fish Feed

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### ABSTRACT

Sewage effluent containing high concentration of phosphorus (6 to 7 mg P/l) and ammonia (1.5 to 2.3 mg N/l) was processed in sewage stabilizing tanks to remove the very high level of  $H_2S$  (10.5 mg/l) and biochemically degradable organic matter. Air stripping of the waste water resulted in the transformation of  $H_2S$  to sulphate and drastic decrement in BOD. Effluents from these oxidation tanks were utilized for mass culture of *Chlorella vulgaris*. Experiments were conducted using oxidised sewage effluents and sewage effluents enriched with different levels of ammonia (as Diammonium phosphate) which occurred in comparatively lower concentrations than phosphorus. In oxidised sewage at the end of 22 days the algal production, recorded to the tune of 8.25 g/m<sup>2</sup>. From the dried chlorella powder fish feed was prepared and feeding trials were conducted in common carp seed. Higher growth rate and better food conversion ratios were observed in this feed than that of control feed.

### Introduction

Micro-algae rich in protein and vitamin are cultivated in waste waters containing large quantity of plant nutrients. Though micro-algae can not find applications in human diet owing to their high nuclei acid content, their utilization as a component of compounded feed for many aquatic organisms has been well established (Becker, 1978). Feeding experiments conducted by Meske and Pfeffer (1977) proved that such unicellular algae can replace 30-80% of commercial fish meal in carp rations. Among the waste nutrients phosphate plays significant role in most algae especially in energy transformation reactions. The

significant difference between the phosphate metabolism in higher vascular plants and algae is the formation of large amounts of inorganic polyphosphate in algae. In the case of different species of chlorella the optimum phosphate requirement ranges from 100-2000  $\mu$ g P/l (Chu, 1943). Hence *Chlorella* sp. is the most suitable genus of micro-algae for the efficient utilization of phosphorus enriched waste waters.

### Materials and Methods

The waste water meant for the culture of *chlorella vulgaris* was aerated for two days to remove  $H_2S$ . Cement cisterns of 1 m diameter and 0.5 m depth were used for cultivatin

green algae and for the simultaneous reduction of BOD of the waste water and the other trials were simultaneous run by adding different initial doses of Diammonium phosphate (DAP). To this effluent, *Chlorella vulgaris* was inoculated (5 mg wet/tank). Sampling was done frequently by analysing the algal content of 10 ml of samples taken from the different cement cisterns. After the completion of the algal culture (22 days), the water with algae was dried in the sun and then in an incubator (60 ° C) to remove the moisture and obtain dry algal powder. The nitrogen and phosphorus content of the water and algae were assessed following the standard procedures of FAO (1975). In order to conduct feed trials two types of feed viz., control and experimental algal feeds with 40% protein were prepared. The feeds were prepared adopting the box model of Santhanam *et al.* (1987). Feed trials were conducted using common carp seed (0.14 g; 0.26 g - Av.Wt) and food conversion efficiency was calculated on dry weight basis. Feeding experiments were carried out by using both the control (conventional) and experimental algal feed. The test animals were fed *ad libitum*.

### Results

Δ N:P ratio of the sewage effluents was 0.73 : 1 and that of sewage with DAP was around 4:5 :1. On applying DAP at different doses i.e., 10, 20, 30 and 40g/100 l, the  $\text{NH}_3\text{-N}$  levels expected were 0.0212, 0.0424, 0.0636 and 0.0848 g of  $\text{NH}_3\text{-N/l}$  respectively. After the inoculation of *Chlorella vulgaris*, the data on growth of algae (g dry wt/m<sup>2</sup>) recorded during the different stages

of culture period are shown in the following table.

### Discussion

The main principle of high rate algal ponds (HRAP) is that the waste nutrients are efficiently utilized by the microalgae in dense population which subsequently produce large quantity of oxygen that is needed to biochemically decompose or mineralize the organic matter. For the past 20 years only small scale pilot installations of HRAP have been established and large scale production remains yet to be achieved. The highest per day yield recorded in the sewage during the present study on 22 day was 8.25 g/m<sup>2</sup>/day. Saxena *et al.* (1986) obtained a maximum yield of 8.8 g/m<sup>2</sup>/day (dry weight) for *Spirulina* sp grown in sewage. In the present study, the DAP addition was observed to accelerate the per day production of *Chlorella vulgaris* and the highest value recorded was 36 g/m<sup>2</sup>/day (dry weight). Very high figures for algal biomass production under outdoor conditions i.e., upto 50-60 g/m<sup>2</sup>/day have already been reported (Laws *et al.*, 1983). Thus it was apparent from the present study that by boosting the ammonia and phosphorus contents of the sewage the final yield of microalgae could be enhanced. Feeding experiments conducted with control feed and algal feed showed interesting results. It was quite striking that despite the lower assimilation efficiency observed in the algal feed than the control feed, animals fed with algal feed exhibited higher gross (9.6%) and net (10.6%) growth efficiencies and relative growth rate (0.027) than that fed with control feed. Similarly FCR was better (1.98) when the animal were

fed with algal feed than in the control feed (2.5). Thus the observation made in the present investigation confirmed that large scale production of micro - algae in waste water will be a profitable venture towards the

production of low cost protein rich fish feeds.

#### Acknowledgement

We thank Dr. M. Devaraj, Dean, Fisheries College, Tuticorin for encouragements.

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Table 1

<i>Time in weeks</i>	<i>I Raw Sewage</i>	<i>II Sewage + 10 g DAP</i>	<i>III Sewage + 20 g DAP</i>	<i>IV Sewage + 40 g Dap</i>	<i>V Sewage + 40 g DAP</i>
I week	0.12	0.20	0.22	0.27	1.54
II Week	0.40	1.82	2.85	3.60	11.65
III week 8.25	8.58	20.80	22.44	36.00	

The growth parameters observed in common carp seed after the feeding experiments with control and experimental algal feed were as given below.

Table 2

	Control feed	Experimental algal feed
Initial dry wt. (g; w1)	0.14	0.26
Final dry wt. (g; W2)	0.21	0.42
Weighted mean (g;w)	0.175	0.34
Production (P=W2-W1)	0.07	0.16
Consumption (c)	0.92	1.74
Faecal output (F)	0.09	0.23
Assimilation (A=C-F)	0.83	1.51
Metabolism (R=A-P)	0.84	1.35
Assimilation efficiency (A/C X 100)	90.22	86.78
Gross growth efficiency (P/C X 100 or kl)	7.61	9.20
Net growth efficiency (P/A x 100 or k2)	8.43	10.60
Relative growth rate (P/W/No. of days)	0.02	0.027
FCR $\left( \frac{C}{\text{live wt.increase}} \right)$	2.50	1.98

## New Vista for Economic Utility of Coastal fallow Land by Prawn Culture with Saline Ground Water

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### ABSTRACT

*Sub soil saline water at 4-5 km. away from Coastal area was used for culture of the tiger prawn *Penaeus monodon*. The ponds were manured with raw cow dung at the rate of 3000kg/ha and DAP at the rate of 100 kg/ha. The ponds were then stocked with post-larvae (15 to 20 mm) of *P. monodon* at the rate of 25,000/per ha. The average weight of individual prawn at the time of harvest was 30 gms. in 113 days and the rate of production was 416 kg/ha. The result opened new avenue for utilization of Coastal saline soil area for commercial production of shrimps.*

### Introduction

India is one of the leading producers and exporters of prawns in the world. However, inspite of increased fishing efforts, prawn production in India has remained in a more or less stagnant state during the last few years. In order to increase the production of prawns, encouragement is given to coastal aquaculture.

Due to the high value and ever increasing demand of shrimp in the world market coupled with breakthroughs and new developments, shrimp culture at present is economically viable. As coastal aquaculture is also recognised as an important source for generating employment and income in rural areas, both union and state governments make concerted efforts to bring more and

more area under prawn culture. The Marine Products Export Development Authority in recent years has taken up steps in different levels through extension services to improve the existing aquaculture practices in the country with prawn farming techniques on modern scientific lines. As a result, a number of entrepreneurs are paying greater attention in shrimp all over India.

In the Southeast coast of Tamil Nadu, particularly in the District of V.O.Chidamaranar, a district phenomenon exists. This district is a leading salt producer in the country. Major portion of the salt is produced by the high saline borewells even at a distance of 4-5 km. away from the sea. In some places, the borewells yielded low saline water in the course of time making salt production practically unprofitable.

In some areas neither agriculture nor salt production is possible due to the salinity of the soil and the borewell water. Unable to tackle such a situation, at the same time trying to put such a land under effective use to earn for the living, a young farmer Mr. D.Baskaran approached us for suggestions. Our attempt to bring such area under prawn farming to grow the fast growing economic variety of shrimp *Penaeus monodon* was a success. The present contribution is based on such a work at Meelavittan village in Tuticorin. In other words, it is an attempt to use the abandoned borewells in that area. This result has created an awareness along the southeast coast of Tamil Nadu for the utilisation of saline fallow coastal lands including salt pan area for culture practices with the low saline ground water.

#### *Location and construction of ponds:*

Quite a good extent of salt pan area is available in Meelavittan village of Tuticorin in Chidambaranar District. The site is a flat land without any vegetation and was used as salt pan years ago. The soil is composed mainly of clay and suitable for prawn farming. The site is situated at a distance of 9 km away from the sea without any water body connecting to the sea. Hence, water had to be drawn from below the ground. Two borewells of 3 inches dia. were present at the site. These borewell were abandoned as unsuitable for salt manufacture because they were supplying low saline water. Salinity of the water samples from these borewells were 15 and 25 ppt. Bunds of 5 feet height with a slope of 1:1:5 was made with the

mud excavated from the peripheral canal, draining trenches and catching pits. The top fertile soil was undisturbed. The pond had a gradient slope towards the catching pit and draining pipe. Inlet canal was provided on the top of the bund. The 30 cm. dia polyvinyl chloride pipes with wooden plugs, fitted in the width side of the ponds formed the outlets. The pond was rectangular in shape. The outlets were provided with velon screen to prevent escape of prawns. The pond area was 0.36 ha. The pond could hold 1 m water in the peripheral canal and 70-80 cm. water in other area. A 5 hp motor with pump with 2.5" diameter delivery pipe was sufficient to deliver the required water.

#### *Preparation of Pond:*

Initial dose of 100 kgs of lime was applied. After completing the tilling work, organic manure (R.D.D.) at the rate of 3,000 kg/ha was applied to the pond bottom. Water level was gradually raised to 3 inches. On the third day DAP at the rate of 100 kgs/ha was applied during the bright light day and the water column was raised by 3 inches further. Four days after fertilisation, the water colour was light straw, indicating the phytoplankton abundance. At the same time a mat of lab lab had also formed at the bottom.

#### *Stocking:*

Since the bore was having an ideal salinity of 15 ppt initially, the pond was stocked with 8,000 numbers of *P. monodon* post-larvae brought from M/s. Hindustan Level Hatchery, Madras. The stocking density works out to 25,000 per ha. The seeds brought from the hatcheries

Table-1 Results of Culture for *P.monodon*

Particulars	Pond-I
Area of Pond (ha)	0.36
Average water depth (m)	0.7
Date of stocking	28/5/88
Date of harvest	17/9/88
Days of culture	113
Size of stocking (mm)	20
size at harvest (mm/g)	190/30
Rate of growth (mm/g/month)	52.7/7.4
No. of prawn seeds stocked	8,,000
Rate of stocking (per ha)	25,,000
Percentage of survival	62.5
No. of prawn harvested	5,,000
Total production (Kg)	150
Rate of Production (Kg/ha/crop)	416.6
Total food supplied (Kg)	1,,350

Table-2 Hydrology of Pond

Period	Water temp at 08.00 hrs.(°C)		Salinity (ppt)		Dissolved Oxygen (ml/l)		pH	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
June to September 1988	26.5 to 32.5	29.5	15.0 to 25.0	20.0	2.5 to 3.8	3.75	7.5 to 8.8	8.15

were properly acclimatised to the conditions prevailing in the pond. The seeds were in the size range of 15-20 mm. The particulars of stocking are given in Table-1.

#### Farm Management:

Water samples for salinity, pH and dissolved oxygen from the culture ponds were analysed once in a fortnight. About one third of the volume of pond water was flushed once in three days to maintain water quality and salinity in the range of 15-25 ppt. Draining of the ponds were carried out during the early hours of the day while pumping in

was adlib to maintain 70 cm. water in the pond throughout the culture period. The hydrological parameters recorded are shown in Table-2. Water temperature, recorded was in the range of 26.5 to 32.5°C during the culture period. The dissolved oxygen content varied from 2.5 to 3.8ml/l and the pH measured around 8.0. The lab developed in the pond bottom was sufficient to meet the food requirements for first 30 days after stocking. Thereafter supplementary feed consisting of fresh fish, rice bran, groundnut oil cake were given at 10% of the body weight. Food requirement was determined based on

Table-3 Sampling of *P. mondon*

No. of days after stocking	Average Size	
	mm	g
0	20	0.3
15	65	4.5
30	85	8.5
45	105	11.0
60	125	15.0
75	145	18.0
90	155	23.0
105	170	27.0
119	190	30.0

the weight of standing crop by periodic sampling. Supplementary feed in earthen plates were kept uniformly at an interval of 15 mts. The feed was supplied in the morning at 8.00 hrs. and in the evening at 18.00 hrs.

#### Growth Assessment:

Growth was assessed by taking random sampling with cast net fortnightly at different points of the pond as presented in Table-3. The rate of growth throughout the period was good probably due to the lower stocking density and ideal salinity range throughout the crop method.

After ascertaining the rates from different buyers on the basis of samples given, arrangements, were made for harvesting. Slope of the pond bottom and the central trench facilitated quick draining of pond water during the night hours. As the water receded, bulk of the prawns were caught by spreading a net exactly on the floor of the catching it. The catching it was of the size 8 × 2 mts. The left over stock were caught with cast net and finally by hand picking. Harvesting operations were completed before 09.00 hrs. to

avoid spoilage due to heat. At the survival rate of 62.5%, 150 kgs of prawn with an average size of 190mm length and 30 g weight each were harvested. The rate of production was 416 kg/ha/113 days.

#### Marketing and Economics:

M/s. Baby Marine of Mandapam a leading shrimp exporter offered the best price for the cultured prawns. The catches were disposed off on the spot at the rate of Rs. 110/kg. This works to an average income of Rs. 45,833/ha/113 days. The Recurring expenditure such as wages for watchman, cost of supplementary feed, fertilizer, fuel, tilling of pond, harvesting and marketing works out approximately 40% of the income. The capital expenditure involved in the construction of the pond, pump etc., can be recovered in 5 to 6 crops at the rate of 25% of the profit. The income can be substantially increased by proper management practice, procuring quality seeds and also by curtailing expenditure on excess feeding. The result has encouraged the entrepreneur to extend the area of operation to 3 ha. in the next crop.

## Effect of The Plant Enzyme (papain) on the Acceleration of Fermentation Process in Fish Sauce Preparation

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### ABSTRACT

A process for the preparation of fermented fish sauce using commercially available proteolytic enzyme (papain) is described. Study was conducted at 0.5% papain level using anchovy (*Stolephorus indicus*), chemical analysis included volatile bases (as nitrogen) total nitrogen, alpha-amino nitrogen, volatile acids, salt content, pH and free amino acids as tyrosine. Papain was found to completely disintegrate the fish within 30 days. The  $\alpha$ -amino nitrogen and free amino nitrogen were high in papain added sample indicating enhanced proteolysis. Papain was found to improve the colour of final sauce.

The bacterial counts including halophilic bacterial count were estimated during different stages of fermentation. At the end of nine months, filtered fish-salt mixture was evaluated for organoleptic quality. The use of papain had beneficial effect on hastening fermentation process.

### Introduction

The fish sauce manufacture plays a major role in fish preservation in Southeast Asia. Fish sauce is rich in amino acids and other protein breakdown products. Fermentation process is a time consuming process in fish sauce industry. A variety of rapid methods have been investigated in an attempt to shorten the time required to produce fish sauce. The use of added enzymes in the production of fermented fish products has been reported by several authors. (Beddows *et al.*,

1976; 1979, Sripathy, 1975; Orejana, 1982).

### Materials and Methods

Anchovies (*Stolephorus indicus*) Brought by the purse seine boats at old mangalore port were used for this study. Commercially available common salt (white crystalline) was used. Wide mouth glass jars of 2-3l capacity were used for keeping the fish-salt mixture throughout the period of fermentation. Enzyme papain obtained from Enzo Chem Laboratory, India was used. Fish

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Table I

Storage Period (months)	VBN mg%		$\alpha$ -amino N mg%		Volatile acids as acetic acid g% (w/w)	
	C	P	C	P	C	P
0.5	71.82	117.04	306.60	654.10	0.020	0.020
1.0	75.40	135.79	367.90	490.56	0.020	0.020
1.5	105.38	172.93	551.88	838.10	0.022	0.022
2.0	113.48	194.54	633.64	848.26	0.024	0.024
2.5	119.70	119.50	643.86	838.10	0.024	0.029
3.09	116.20	205.36	644.84	850.64	0.029	0.039
6.0	149.58	247.56	602.57	727.32	0.194	0.269
9.0	219.24	343.47	665.28	640.64	0.070	0.070

were mixed with salt in 3.5:1 ratio (Fish to salt) and minced. To the fish-salt mixture, 0.5% by weight of papain was added and mixed thoroughly. Papain was suspended in small amount of water before the addition. The mixture was packed in glass jars and top surface was covered with polyethylene sheet and allowed to ferment for 9 months.

Fish-salt mixtures kept for fermentation were analysed at regular intervals of 15 days for a period upto 3 months by mixing the contents thoroughly and drawing samples of about 30g. After 3 months the samples were drawn at an interval of 3 months upto 9 months. Protein content of fish-salt mixture (N x 6.25) was determined by macrokjeldahl method as per, A.O.A.C (1970). method described by FAO (1981) was adopted for salt content (NaCl) estimation. Total volatile bases was determined by Conway's micro-diffusion analysis method as described by Beatt and Gibbons (1937). Method described by Plummer (1971) was adopted for Free amino nitrogen.  $\alpha$ -

amino nitrogen was estimated by using the method described by Pope and Stevens (1939). Volatile acid content of fermented fish sauce was determined by the method described in A.O.A.C. (1975). The pH of the fish-salt mixture was directly recorded using a combined electrode pH meter (Horiba, Japan). All media and broths were prepared according to "Compendium of methods for the microbiological examination of foods" (APHA, 1976).

After 9 months of fermentation, the filtered fish sauce was subjected to sensory evaluation. 't' test was adopted for comparing the quality characteristics of finished fish sauce sample.

## Results

After the preparation of fish-salt mixture using white crystalline salt, it was divided into two batches. To one of the batches the enzyme papain was added. The other batch served as control. These two batches were kept for fermentation and analysed for various biochemical

Table II

Storage Period (months)	Total N g%		Salt (NaCl) %		P <sup>H</sup>		Free amino acid as Tyrosine mg/g	
	C	P	C	P	C	P	C	P
0.5	2.32	2.32	24.80	20.12	5.80	5.60	29.16	48.60
1.0	2.23	2.55	21.06	19.19	4.70	4.80	65.94	106.48
1.5	2.20	2.20	2.23	23.40	19.66	4.70	4.75	116.32
2.0	2.19	2.19	21.29	19.19	5.00	5.15	92.40	122.43
2.5	2.17	2.17	21.06	18.72	5.20	5.60	57.12	102.48
3.0	2.16	2.19	21.53	19.19	5.36	5.75	41.60	79.04
6.0	2.20	2.21	21.35	19.19	5.38	5.85	38.85	67.95
9.0	2.40	2.25	22.99	18.74	5.82	5.96	262.10	245.70

Table III Microbiological Analysis of Fish and White Crystalline Salt Mixture

Storage Period (month)	Total plate count (No./g)	Halophilic count (No./g)
1	est. $3 \times 10^3$	est. $1.2 \times 10^2$
3	est. $5 \times 10^2$	est. $9.5 \times 10^2$
6	est. $2 \times 10^2$	est. $3 \times 10^3$
9	est. $2.1 \times 10^1$	est. $4.5 \times 10^3$

Table IV Results of the mean scores of organoleptic evaluation of fermented fish sauce

Sample	Characteristics		
	Colour	Odour	Taste
Control	2.91	3.58	.08
Papain	3.50	4.00	3.92

Note: All scores are out of a possible maximum of 5.0

parameters and the result are presented in table I & II and also in figure 1 and 2. It is clearly evident from the figures and tables that the papain added samples generally contained more volatile acids and free amino nitrogen than the control. The results of microbiological analysis are presented in table III. The results of organoleptic evaluation are presented in table IV which gives mean scores

for sensory qualities, such as colour, odour and taste.

### Discussion

It was observed that papain added samples contained more volatile bases than the control from the very beginning of fermentation. This could be attributed to higher degree of hydrolysis of proteins in papain added samples resulting in

more amino acid nitrogen which in turn get converted to volatile bases. Uyenco *et al.* (1953) have explained the above mentioned conversion of amino acid nitrogen to volatile bases by bacterial action.

$\alpha$ -amino nitrogen was found to increase upto a period of 3 months with fluctuation thereafter in both sample (treatments). Similarly free amino acid content increased upto 1.5 months and decreased thereafter. However, in the ninth month, free amino acid values were unusually high in both samples. As  $\alpha$ -amino nitrogen content and also free amino acids are more in papain added samples, it can be said that more protein hydrolysis has taken place in the papain added fish-salt mixture.

From the results of analysis of total nitrogen and salt content, a slight variation in value were observed in fish-salt mixture analysed at different intervals of time. Similar variations in salt content were detected by Orehana and Liston (1979) during fermentation of patis. Amano (1962) has reported the total nitrogen value of 2.5% in the pickle for fishes like anchovy. Volatile acids increased slowly but steadily upto period of six months and then in the ninth month, the values decreased in the control and papain added sample. This probably was due to the reaction between volatile acids and the organic base present in the fish salt mixture. The volatile acid content of the prime quality was reported to be

5g/l (Nieto, 1982). Hiremath *et al.*, (1985) have reported volatile acid values of 1.4g/l and 2.69g/l in fish sauce prepared from *Anchoviella* and *Caran* respectively.

The slight variation in pH during fermentation period may be due to the production of organic bases, volatile acids and probably due to the chemical reaction between these bases and acids. Orejana and Liston (1979) have reported a slight decrease in pH during fermentation. total plate count shows a slight decreasing trend from est.  $3 \times 10^3$  per gram at sixth month to est.  $2.1 \times 10^1$  per gram at ninth month. Whereas halophilic count shows a slight increasing trend from est.  $1.2 \times 10^2$  per gram at first month to est.  $4.5 \times 10^3$  per gram at ninth month. The same trend was observed by several workers. (Saisithi *et al.*, 1966).

Statistical analyses (t - test) have revealed that there was no significant difference in the organoleptic scores of fish sauce sample prepared by adding papain from the control.

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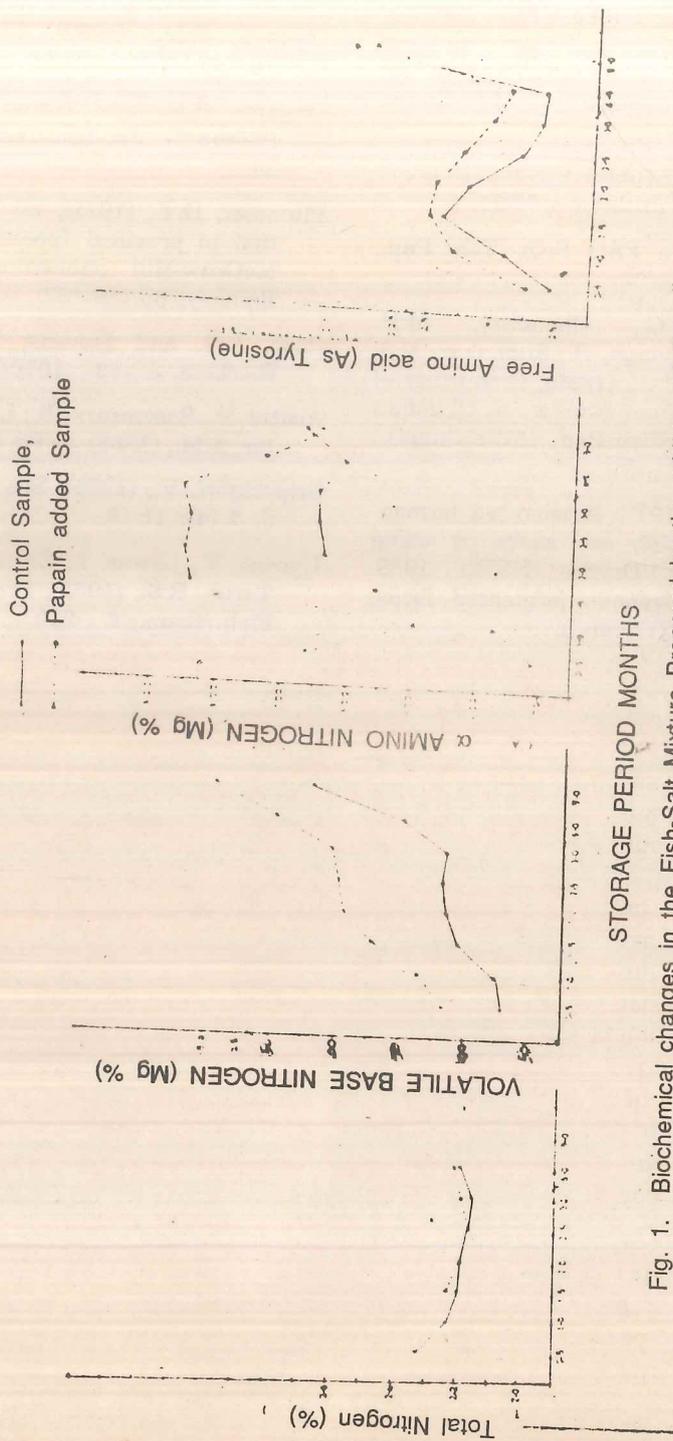


Fig. 1. Biochemical changes in the Fish-Salt Mixture Prepared by the addition of Papain

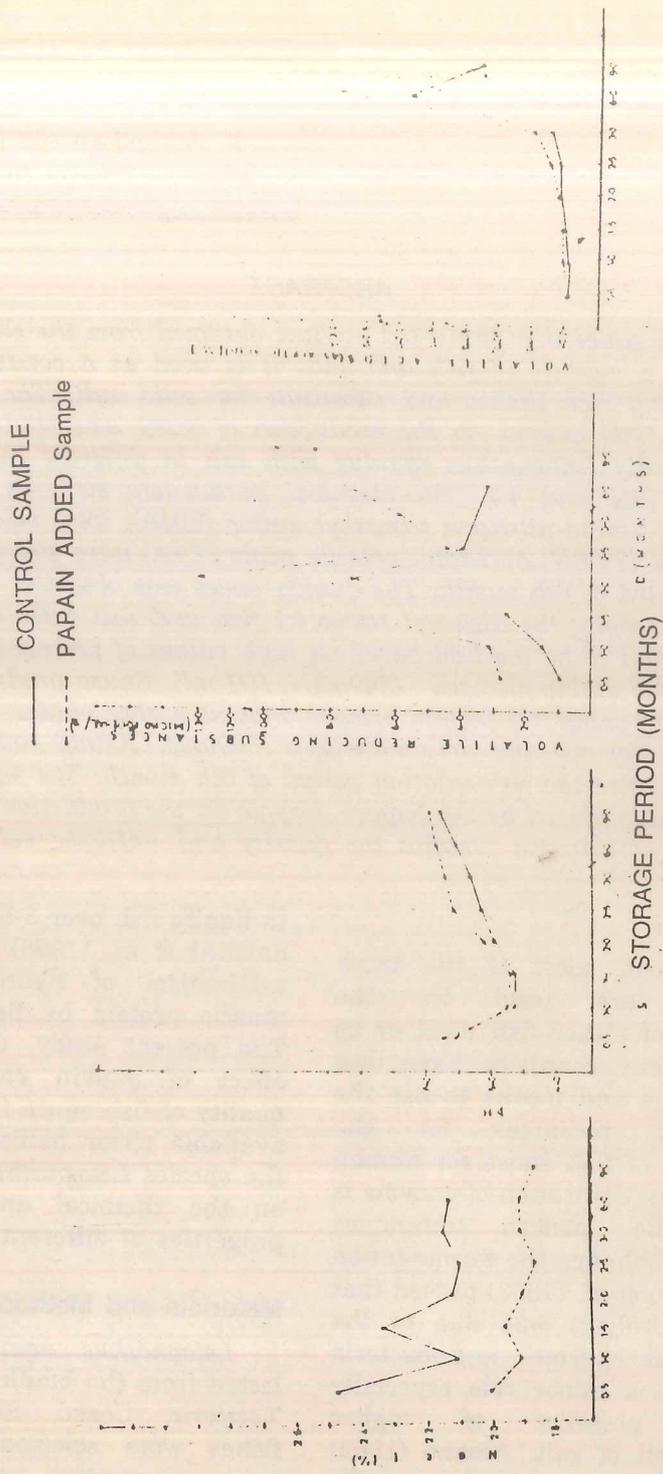


Fig. 2. Biochemical changes in the Fish-Salt Mixture Prepared by the addition of Papain

## Effect of Enzyme on the Quality of Fish Sauce

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### ABSTRACT

Fish sauce is a fermented product obtained from the slow digestion of a mixture of fish and salt. It is used as a condiment for flavouring rich dishes and substitute for solid salt. The effect of papain (1%) enzyme on the production of sauce was studied using silver belly *Leiognathus equulus* with salt in different ratios such as 2:1, 3:1 and 4:1. The chemical parameters such as pH, salt protein, amino-nitrogen, trimethyl amine (TMA), total volatile base nitrogen (TVBN) and total volatile acids (TVA) were studied at the end of 3rd & 6th month. The quality sauce was obtained in all the ratios. Among the different ratios, 4:1 fish and salt ratio in control was found to be the best based on high values of protein (8.2g/100 ml) and amino-nitrogen (540mgN/100 ml). Sauce produced from 4:1 ratio using 1% papain enzyme showed higher values of protein (1450g/100 ml) and amino-nitrogen (780mgN/100ml) compared to control after the fermentation period of 6th month. The significance of the role played by the papain enzyme on the production of higher values of nitrogen justifies the quality and nutritive value of the sauce.

### Introduction

At present, most of the trash fishes are used for the production of either fish meal or as manure. Many countries have now devised ways and means to use the trash fish resources for the preparation of fish sauce for human consumption. Utilization of enzyme is one of the modern techniques adopted to enhance the fermentation rate. Uyenco *et al.* (1953) proved that protein hydrolysis was due to the fish visceral enzymes and bacteria played only a minor role especially in the presence of higher concentration of salt. Amano (1962) used enzymes from *Bacillus subtilis*

to liquify fish over 3-5 hours at 50°C. Saisithi *et al.* (1966) elucidated the mechanism of hydrolysis of fish muscle protein by fish proteinases. The present study, deals with the effect of papain enzyme on the quality of fish sauce from the locally available silver bellies belonging to the species *Leiognathus equulus* and on the chemical and organoleptic properties of different fish sauces.

### Materials and Methods

*Leiognathus equulus* was collected from the landing centre of the Tuticorin Coast. Slightly spoiled fishes were selected for the fish sauce preparation as suggested by

Mciver *et al.* (1982). The fish and salt were mixed in different ratios viz. 2:1, 3:1, and 4:1. One set of samples with three ratios was taken as a control and another set of samples was treated with 1% papain enzyme. Fermentation of fish samples was carried out in plastic containers of 5l capacity. The sauce was prepared following the method of Baens - Arcega (1970). Crude filter paper or cotton was used to filter the clear liquid from the fermented samples. The fish sauce samples were collected at the end of 3rd and 6th month for studying the chemical, and organoleptic properties. Chemical evaluation of the sauce included analysis of various parameters such as pH, salt, total protein, amino - nitrogen, trimethylamine (TMA), total volatile base nitrogen (TVBN) and total volatile acids (TVA). Protein content of the sauce was assessed by estimating the total nitrogen content by microkjeldahl method (Oser, 1971) followed by multiplying the total nitrogen content by the factor 6.25. Trimethylamine and total Volatile base nitrogen were determined by conway's micro diffusion techniques (Beatty and Gibbon 1937). Sodium chloride content of fish sauce was analysed by following the Volhard's titration method (Pearson, 1977). Total volatile acid was determined by the steam distillation method of Chandrasekhar and Manisseri (1976). Amino nitrogen was determined adopting the procedure of Pope and Stevens (1939).

### Results

The values of salt of fish sauce obtained from *L. equulus* showed a decreasing trend in all the ratios both in control and enzyme treated

samples at the end of the 6th month. The protein, amino-nitrogen, TMA, TVBN, and TVA contents were on the otherhand, increasing in all the ratios. The results pertaining to chemicals and organoleptic properties of the different fish sauce obtained from control and enzyme treated samples are given in the Table I and II.

### Discussion

Nonaka *et al.* (1975) observed a pH range of 5.6 - 6.1 for various fish sauces. Fujii and Sakai (1984a) reported a maximum pH value of 6.2 for quality fish sauce. During the present study also, the values of pH of different fish sauces produced were well within the pH range for quality fish sauces. The values of salt content, TMA, TVBN, and TVA of fish sauces observed were also agreeing with that of Fujii and Sakai (1984b). The 4:1 ratio showed higher values of protein and amino-nitrogen among the different fish - salt ratios in control and enzyme treated samples at the end of the 6th month. The sauce produced from 4:1 ratio using 1% papain enzyme showed higher values of protein (14.50g/100ml) and amino-nitrogen (780mgN/100ml) compared to control at the end of the fermentation period of the 6th month. The Food and Drug Administration (1977) fixed a minimum protein level of 6 to 8% in fish sauce meant for domestic trade and export. The significance of the role played by the papain enzyme on the production of higher values of nitrogen justified the quality and nutritive value of the sauce.

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Table 1: Chemical and organoleptic properties of fish sauce produced from *Leiognathus equulus* using different ratios without Papain enzyme.

Ratio	Fermentation period in months	pH	Salt g/100 ml	Total Protein g/100ml	Amino N mg N/100ml	TMA mgN/100ml	TVBN mgN/100ml	TVA (ml of N/100 NaOH/100ml)	Organoleptic Properties		
									Color	Odour	Taste
2:1		5.8	35.50	2.75	220.50	9.25	33.00	18.25	Turbid White	Fishy	Good and more salty
3:1	3	5.8	34.00	3.60	290.00	9.50	36.00	29.50	Turbid Pale yellow	Fishy	Good and salty
4:1		5.9	33.00	4.40	310.00	9.80	42.50	33.00	Pale yellow	Cheesy	Good and less salty
2:1		5.8	32.50	4.80	340.00	11.00	73.50	49.00	Yellow	Acetic Acid	Good and more salty
3:1	6	5.9	29.00	6.75	410.50	11.70	80.50	74.00	Clear yellow	Butyric Acid	Good and salty
4:1		6.1	28.50	8.20	540.00	11.50	80.00	72.50	Clear Yellow	Acetic Acid	Good and less salty

Each value is the mean of three replicates.

Table 2 : Chemical and organoleptic properties of fish sauce produced from *Leiognathus equulus* using different ratios with Papain (1% enzyme),

Ratio	Fermentation period in (months)	pH	Salt g/100 ml	Total Protein g/10.0 ml	Amino N mg N/100ml	TMA mgN/10ml	TVN mgN/100ml	TVA (ml of N/100 NaOH/ 100 ml)	Organoleptic Properties, +		
									Color	Odour	Taste
2:1		5.7	36.00	3.90	320.50	9.00	32.00	19.00	Turbid white	Fishy	Good and more salty,
3:1	3	5.8	34.00	5.00	380.00	9.50	35.00	29.00	Pale Yellow	Fishy	Good and more salty
4:1		5.8	32.00	6.90	420.50	9.50	37.00	34.00	Turbid Yellow	Cheesy	Good and less salty
2:1		5.8	32.00	8.60	550.00	11.00	75.00	51.00	Clear Yellow	Malty	Good and more salty
3:1	6	5.9	29.50	12.50	660.50	11.25	76.25	76.00	Dark Yellow	Butyric Acid	Good and salty
4:1		6.0	28.00	14.50	780.00	11.50	79.00	75.50	Yellow	Butyric Acid	Good and less salty

Each value is the mean of three replicates.

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## Biotechnology As a Tool in Aquaculture Its Relevance in Indian Context

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### ABSTRACT

*The biotechnological tools are intensively used for food production programmes in many developed countries. Developing countries like India also involve much in this area by utilising the talented human resources. There are diverse areas spread over for biotechnological approach in the production oriented field of aquaculture. Among them certain areas at molecular level (like transgenic fish) need lot of systematic and intensive research with great deal of investment and foreign collaboration for clear visibility of the target itself. Any breakthrough in these lines will bring international recognition and appreciation besides academic contribution in the areas of basic research. But in developing countries, the mandate will press the scientists involved in the area to come out with wonderful findings because of allocation of relatively more funds. Attaining a great leap in certain biotechnological areas like genetic engineering is not common in global arena. Scientists all round the world are working to produce transgenic fish but yet to attain success for commercial application. In certain areas, biotechnological inventions smother at laboratory level and don't reach the need farmers. However, certain breakthroughs can be harvested immediately by farmers without much escalation of cost. A dozen of such promising fields in aquacultural biotechnology under Indian conditions are identified among many and discussed.*

### INTRODUCTION

**B**iotecnology offers sophisticated tools to improve animal protein production through aquaculture. It provides scope for integration of beneficial contribution from the fields of molecular biology, immunology, biochemistry, biophysics, cell biology, genetics, microbiology, computer science and information technology. This holistic approach gives greater thrust in

compelling the human society at scientific and political levels to ponder more on biotechnological tools to alleviate the problems associated with food and protein facing the exploding population. At this juncture, evaluation of certain biotechnological research in aquaculture may help the future generation of scientific community to choose the best to attain short term and long term benefits productive to

Indian conditions under various levels of socio-economic status.

### Materials and Methods

The materials for reviewing the biotechnological aspects of aquaculture were gathered from different geographical regions of the world and their relevance were assessed based on immediate needs to Indian aquaculture. Short term and long term objectives of various aspects of aquaculture were critically analysed based on their merits and demerits.

### Results & Discussion

#### *Plants of importance in Aquacultural Biotechnology*

*Algal culture.* Algal culture is one of the important components in hatchery production of aquacultural organisms especially the shellfishes. The importance of single cell protein is well recognized in larval rearing of aquacultural species and certain entrepreneurs in western countries produce algal micro feed commercially for instant use. In India, for fish and shellfish seed production, there are two options namely: 1) to go in a big way by establishing various components of hatchery systems with heavy investment and of course with environmental, social and political risks and 2) the Government can encourage small backyard hatcheries with limited investment in diverse locations to meet the environmental and hydrobiological needs of individual systems. In the latter case, small farmers may not be in a position to establish sub units like algal culture unit. So, biotechniques involved in the preparation of cost effective micro algal feeds and nutritionally enriched encapsulated feeds for ready use by back-yard

hatchery units/kitchen pond units may help Indian farmers in a big way to solve the problem of seed shortage in aquaculture. Micro algae produced out of sewage effluents can also be purified and processed through biotechnological tools (to keep free from the toxic substances and pathogens) to minimise the cost of production and to recover the resources from waste. There are thousands of species of micro algae existing in planktonic and benthic environments of fresh, brackish, marine and hyperhaline waters in the world. But only certain specific micro algae are preferred for hatchery production of specific species. Biotechnological tools can aid in a big way in the preparation of 'seed materials' of selected nutritious and highly preferred micro algal strains for instant usage by hatchery units of diverse species amenable for aquaculture and artificial propagation.

*Sea Weed Culture.* Sea weeds offer excellent products of nutrition of diverse nature for human beings, livestock and even agricultural crops. they serve as valuable sources for many organic and inorganic compounds such as glycerol, polysaccharide, agar agar, algin, phytal, steroids, aminoacids, vitamins, essential fatty acids, carbohydrates, iodine etc. Bioactive compounds are also extracted from sea weeds. However, in India, these resources are over exploited from nature and are diverted only to least meaningful ways of 'utilization' while considering their actual values and their appreciation for use for better purposes in other countries like Japan or China. We have not yet thought about the

production of value added materials from these valuable resources.

There are immense scope for introduction of biotechniques like tissue culture, selective reproduction and hybridization for improving the sea weed strain to achieve efficacy in production of powerful bioactive organic and inorganic compounds. But as for India is concerned, though the aquacultural techniques of certain selected sea weeds like *Gracilaria* and *Gelidiella* species are standardized by national level laboratories (CMFRI & CSMCRI) a decade before, it is unfortunate that commercial production has not yet been undertaken by any entrepreneurs. When the simple sea weed culture techniques did not reach the 'land' from the lab due to lack of extension machineries, cultural, socio-political, economic and climatological constraints pertaining to mariculture, it is unworthy to concentrate our effort in seeking biotechnological tools for further advancement because we do not find a 'field' to 'plant' our findings.

### 3.3. *Azolla* cum Fish culture

*Azolla* is an ideal biofertilizer for rice crop. *Anabaena azollae*, a blue green alga showing symbiotic relationship with *Azolla* spp. fixes atmospheric nitrogen and aids in reduction of nitrogenous fertilizer of chemical nature required for crop, there by reducing the cost of production. *Azolla* grows well in pond waters too. Grass carp also feeds voraciously on *Azolla* and hence there is a biotic control. *Azolla* provides on dry weight basis 27% crude protein to fish. A balanced stocking of *Azolla* and grass carp helps in flourishing of both the or-

ganisms. It is worth pointing out here that upto 800% body weight of green matter can be consumed by grass carp per day and the pulp rejected in the form of faeces serves as food for other cultivable fishes, particularly the common carp, a bottom feeder. If any more remains left unutilized by the common carp it can be utilised as green manure. Voracious grazing habit of grass carp thus strongly supports enhancement of nutrient recycling, green manuring, microbial enrichment of nutrients through beneficial gut microflora of fish and ready food for other carps.

*Azolla* spp. also serve as a powerful genetic resources in integrated fish-rice culture system and integrated fishlivestock system. There are more than half a dozen species of *Azolla* available in the gene bank of our Agricultural Universities and usage and biotechnological tools to identify appropriate species, inter specific crosses and strains to suit different climatological zones and nutritional requirements of herbivorous fish will be of immense use in food production programmes.

*Weed utilization in Aquaculture* *Eichhornia crassipes* and *Ipcmoea fistulosa* are the top ranking weeds found in freshwater ponds of India. The former one is an aquatic weed causing menace to culture system, whereas the latter species is a terrestrial plant dwelling in various agroclimatic condition, occupying shallow areas of ponds and channels. These weeds are noted for their genetic vigour and proliferation. Biotechnological tools can be geared up to identify the genes responsible for effective photosynthesis, proliferation and resistance to wide

amplitude of environmental variables in these plants. these plants can serve as basic plants in our area of fundamental research. Moreover they have tremendous potential in bio-energy production, nutrient enrichment through microbial decomposition and utilisation as organic manure. Sophisticated biotechniques can bring lot of benefits to the farming community in general and fish farming community in particular by intensive research in the area of microbiology, enzymology and nutritional biochemistry. Moreover, aquatic weed utilization cum control can also be initiated by converting the raw weeds of water hyacinth into nutritious cattle feed (especially as buffalo feed by addition of probiotics, additives, vitamins, minerals etc.) through biotechnological research for accruing multiple benefits in integrated farming.

#### Areas of animal research in Aquacultural Biotechnology

Important areas of biotechnological research in aquaculture of finfish and shellfish in freshwater, brackish-water and marine environment are listed in Table I with comments on their relevance from Indian context. The reasons behind such comments are listed with basic data (whenever

necessary), of diverse nature such as economic, demographic, biological and sociocultural background. About 45 percent of the techniques listed in the table were categorised under non-priority area, under Indian condition at this stage. There is a tendency among the scientists at national level to go ahead with research problems of academic interest in their own fields and ultimately the general need of fish farmers are not met with. Subordination of individual scientists' own interest for the general interest of farming community will be the need of the hour when selecting appropriate biotechniques for the welfare of the nation. Accountability of tax payers 'money' long term and timebound benefits to the fish farmers through biotechnological research, adaptability of findings in field level trials and cost benefit analyses of a system when biotechnological tools are introduced under Indian economic condition should be critically considered.

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## Priority and Non-priority Areas of Aquacultural Biotechnology

Sl. No.	Area of re-search	Organisms	Priority or Non-Priority area	Remarks,
1.	Artificial in-semination	Penaeid prawns	Priority area	Scarcity of berried female prawns from sea to hatchery units – Severe seed shortage – Export orientated large scale shrimp farming activities – Area of long term benefit.
2.	Artificial removal of spermatophores through electric stimuli	Male penaeid shrimps	Priority area	Scarcity of berried female prawns from sea to hatchery units – Severe seed shortage – Export oriented large scale shrimp farming activities – Area of long term benefit – For artificial in-semination of female prawns, spermatophores should have to be taken from the males.
3.	Cryopreserva-tion of sperms and embryos	Fishes and Shell Fishes	Priority area	To over ripe male spectmens shortage during peak season of spawning in fish breeding centres To facilitate hybridization of fish – In the case of embryos, seasonal tanks can be benefitted if the technology is successful – In certain fish groups (like Tilapia) males can be produced more by providing methyl testosterone or ethyl testosterone along with feed in the early stages.
4.	Electro-cauterization	Shrimps & Other Crus-taceans	Priority area	To understand basic facts about X organ, Y organ, Sinus gland, VSOH and Vitallogenin – To hasten faster growth through moult inducement and to attain brood stock of penaeid shrimps and other crustaceans in confinement through faster gonadal development.
5.	Feminization of fish through hormone (Diethyl stil-bestrol)	Fish	Priority area	May be useful in induced spawning of fish – Proved as useful in super male production of <i>Tilapia</i> .
6.	Growth promoters of hormonal origin	Fish	Non-Priority area	Ethyltestosterone, Methyl testosterone, S.T.H., etc. were used to promote growth in fish – Due to ill effects in human beings, certain countries ban such approach.

Sl. No.	Area of research	Organisms	Priority or Non-Priority area	Remarks
7.	Growth promoters of non-hormonal origin	Fish	Priority area	To meet the protein requirement of India's growing population, such research by using various micro nutrients (Cobalt, Boron, Manganese and Molybdenum) and antibiotics (Eg. Virginamycin).
8.	Growth promoters of microbial origin	Fish and shell fish	Priority area	Certain microbes like yeast can act as 'probiotics' in fishes to increase efficiency of feed.
9.	Heat shock in reproduction of sea cucumber	Sea cucumbers	Priority area	High price of the commodity in South East Asian countries – Export value – Sea ranching of larval stages to minimise total destruction of feeble moving population because of over exploitation and undersized exploitation.
10.	Heat shock in reproduction of molluscs	Bivalves such as oysters mussels & clams	Non-Priority area	Though the potential production is very high, there is no organised commercial production from India because of Indian food habit – Natural resources were exploited mainly for lime industry. If export market is established for these bivalves, if the resource is utilized for shrimp farming and if the food habits of Indians are changed through proper extension machineries and media, this non-priority area may become priority area in near future.
11.	Hybrid fish	Fish	Non-Priority area	The gap between experimental fish culture production and fish farmers production is very high. Such gap could be narrowed by proper extension machineries and by improving the quality of the inputs like seed & feed. The purity of certain genetic major carps are severely affected by hybridization in 'open waters'.
12.	Masculinization through hormones	Fish	Priority area	Could be tried in I C S C to avoid shortage of male specimens.

Sl. No.	Area of research	Organisms	Priority or Non-Priority area	Remarks
13.	Micro encapsulated feed	Fish & Shell fish	Non-Priority Area	To avoid escalation of cost in seed production, indigeneous techniques like 'processed stomatopod meal in suspension' could be attempted taking advantage of the tropical condition— Underutilized bycatch of shell fish in trawl landings could be used for this purpose, thus converting waste into wealth.
14.	Pelletized feed of superior nutritional quality for shrimps.	Shrimp	Priority area	Ideal feed with good FCR will help the shrimp culture industry to flourish in India – Foreign exchange earning can be boosted up.
15.	Semi moist feed for Sea Bass	Sea Bass	Priority area	To fulfil the nutritional requirements of this carnivorous perch, feed formulation by using biotechnological tools will be very useful.
16.	Sex related Chromosome manipulation,			
	a) Haploidy	Tilapia (Fish)	Non-Priority area	It is of academic interest
	b) Triploidy	Tilapia (Fish)	Priority area	To attain sexually non-functional fish with more fish flesh production, triploidy is useful. Among heat shock, cold shock, pressure shock and chemical shock to produce ploidy, heat shock is cost effective and hence be commercialised/popularised after the results are proved in field level.
	c) Tetraploidy	Tilapia (Fish)	Non-Priority area	It is of academic interest
	d) Allotriploidy	Tilapia (Fish)	Non-priority area	Hybrid fishes are also involved in this programme along with shock treatment – Therefore these chromosome manipulated animals should have to be reared in confined (protective) environment until the superiority is proved in the field. protection of such animals in confined environment is very difficult in Indian condition due to environmental calamities and social problems.

Sl. No.	Area of re-search	Organisms	Priority or Non-Priority area	Remarks
	e) Andro-genesis	Tilapia	Non-Priority area	Irradiation is involved and hence very difficult for adoption by ordinary fish farmers.
	f) Gyno-genesis	tilapia	Non-Priority area	-do-
17.	spermatozoa release in shrimp spawning tanks	Shrimp	Priority area	To increase fertilization rate of eggs and ultimately to boost hatchery production of seeds.
18.	Synthetic hormones for reproduction	Fish	Priority area	Hypophysation techniques face problem because of shortage of pituitary gland from right size and type of fish. LHRH analogue and dopamine antagonist when mixed together and administered in fish showed excellent spawning in fish. Such synthetic hormones can revolutionise induced breeding and seed production in fish.
19.	Tissue culture (Mantle tissue culture)	Pearl Oysters	Non-Priority area	Pearl culture itself is not catchy among the entrepreneurs in India due to lack of extension machinery, trained personnel and socioeconomic constraints. So, advanced biotechniques in these fields don't have the prospect of flourishing because of lack of foundation.
20.	Transgenic fish	Fish	Non-priority area	We need heavy initial investment to visualise the objective itself. The genome of fish is more complicated than that of human being and only 8 genes were studied so far from the fish when comparing 600 genes from man. So it is too early to burn our fingers so much. However, from academic point of view and to go on par with global scientific calibre, we need not miss the bus to utilize when right time comes.

Sources of Information: Anon (1990), Caillouet (1972), Chaudhuri and Singh (1984), Choudary, (1990), Cognie *et al.* (1989), Lin and Ting (1985), Meusy (1980), Ozato *et al.* (1989), Pandian and Muthukrishnan (1990), Primavera (1985), Scura *et al.* (1985) & Varadaraj and Pandian (1989) (a) and (b).

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## Author Index

Albert, A.	189
Anandan, R.	156
Appaji Rao, V.N.	208, 210, 214
Austin, A.J.S.	101
Balagopal, R.	120
Balasubramanian, R.	247
Balasubramanian, S.	114
Balasubramanian, S.	266
Bhandary, M.H.	287
Bhat, P.P.	96
Bojan, J.	283
Cecilia Christopher	114
Das, K.M.	6
Devanathan, T.G.	111
Devaraj, M.	55, 82
Dewan Muthu Mohammed, M.S.	123
Dhinakar Raj, G.	96, 180
Dorairajan, N.	201, 204
Doraiswamy, J.	226
Eswaran, B.M.	106, 117, 254
Ebenezer Raja, E.	156
Elankumaran, S.	208, 210, 214
Ezakial Napolian, R.	111
Felix, S.	148, 242
Ganesan, P.I.	223
Godavari Kamalanathan	263
Govindarajan, R.	226
Guneseelan, L.	223
Jagatheesan, G.	294
Jameson, J.D.	299

Janabai .Giri	263
Jayabalan, N.	130
Jayakumar, R.	152
Jhonson Rajeswar	226
John Edwin, M.	106, 117
John Francis Gnanabaranam	208, 210, 214
John Kirubaharan, J.	194
Joseph, S.A.	169
Kandaswamy, N.	254
Kanakaraj, P.	117
Karunamoorthy, G.	169
Kathiresan, D.	111, 114, 123
Kothandaraman, P.	47
Krishnan, A.R.	89
Kumanan, K.	189, 233
Mahalinga Nainar, A.	106
Manickam, R.	120
Manimaran, B.	279
Md. Kaleemur Rahman	82, 138
Mohan, M.	120
Mohanty, S.N.	6
Moses, J.S.	72
Mukherjee, S.C.	33
Mukhopadhyay, P.K.	6
Mustaq Ahmed	126
Nachimuthu, K.	180
Nedunchellyan, S.	152
Nageswara Rao, K.	64
Natanam, R.	1
Natarajan, C.	29
Natarajan, N.	101
Natarajan, P.	242

Padmanaban, V.D.	123, 176, 189, 194, 201, 204, 233
Padmanabhan, P.N.	258
Padmaraj, A.	233
Palaniswami, K.S.	208, 210, 214
Palaniswamy, A.	114
Pappathi, R.	76
Parimal Roy	176
Pattabiraman, S.R.	111, 114
Paul raj, R.	15
Prabhu, P.V.	68
Prince Jeyaseelan, M.J.	299
Quayam, S.A.	111
Rachel, I.	148
Raghavan, N.	216, 220
Rahman, H.	163
Rahmathulla Khan, G.A.	208, 210, 214
Rajan, M.R.	266
Rajasekaran, P.	237
Ramadass, P.	160
Ramadhas, V.	275, 279
Ramaswamy, V.	201, 204
Ramesh Babu, K.R.	283
Ramkrishna, J.	216, 220, 230
Rathnakumar, K.	287
Riji John, K.	130, 138
Rosalind, G.M.	130, 138
Sadasivam Pillai, K.G.	120
Samuel Paul Raj	76, 266
Santhanam, R.	275, 279
Sharma, B.	183
Sharma, V.D.	163
Shetty, T.M.R.	287

Singh, V.B.	163
Sridhar, G.	230
Srinivasan, A.	275
Srinivasan, V.K.	220
Sukumaran, N.	82, 143, 148
Sundararaj, V.	143, 148, 299
Suresh, R.	143
Thanappa Pillai, M.	223
Thangaraju, P.	126, 254
Thangavelu, A.	180, 183
Thiagarajan, S.P.	230
Thiagarajan, V.	89
Thilagar, S.	111, 120, 123
Ulaganathan, V.	26, 89, 106, 126, 254, 258
Vajravelu Jayakumar	226
Valliammal, T.	230
Vedavyasa Rao, P.	40
Velayutham, P.	294
Venkataraman, K.S.	216
Venkatesan, R.A.	180
Venkoba Rao, K.	126, 254
Vijayan, S.	143
Vijayarani, K.	189



Calf through embryo transfer  
Left: Original mother (Donor) Right: Surrogate mother (Recipient)



Common carp (*Cyprinus carpio*)



Calf through embryo transfer  
Left: Original mother (Donor) Right: Surrogate mother (Recipient)



Common carp (*Cyprinus carpio*)