Fishery Technology
2004, Vol. 41(1) pp : 49 - 56

Biochemical Changes in Fish Feeds and Ingredients during Storage

O. Kavitha, R. Anandan, Suseela Mathew and P.G. Viswanathan Nair
Biochemistry and Nutrition Division
Central Institute of Fisheries Technology, Cochin - 682 029, India

The changes in major biochemical components of three fish feeds (1000 Grower 15+, 3000 Grower and 6000 Grower A) and three feed ingredients (Indian fish meal, Danish fish meal and wheat flour) during storage for six months are reported. There was no significant change in total protein content either in fish feeds or their ingredients during the storage period. But a significant reduction in crude fat content was noticed in both feeds and ingredients upon storage. Histamine content of feeds and their ingredients was found to be increasing till the fourth month of storage. Significant decline in the levels of various amino acids was also observed. Unsaturated fatty acids and most of the saturated fatty acids decreased in stored ingredients. But in feeds only a marginal decrease in the levels of unsaturated fatty acids was observed. The results indicate that storage considerably reduces the nutritive value of the feeds and at the same time produces toxic compounds such as biogenic amines.

Key words : Fish feed, feed ingredients, amino acid composition, fatty acid profile, storage

Feed is the most important input for a successful and sustainable aquaculture. The feed used in aquaculture should be nutritionally adequate, environmentally friendly and economically viable (Rath, 1993). All feeds are susceptible to degradation when stored for a considerable period. This may cause the loss of nutrients and may affect the quality (Gadient *et al.*, 1992). Enzymes, microbes and insects are the major cause for storage degradation (Branen, 1993). The biological molecules in the feed are susceptible to oxidation and the products of oxidation in turn interact with other biomolecules and affect their bioavailability. Moreover, interactions between the various chemical entities in the ingredients lead to the formation of substances, which affect the quality of the feed (Chow, 2000). Physico-chemical interactions are the major factors making some nutrients unavailable under biological conditions (Garcia-Ortega *et al.*, 2000). In addition to this some of the compounds formed by biological or chemical activities during storage are extremely toxic to the living organisms (Edmunds and Eitenmiller, 1975). Hence it is important to determine the changes taking place to feeds and ingredients during storage. In the present study, an attempt has been made to study the changes in the major nutrients of common fish feeds (1000 Grower 15+, 3000 Grower and 6000 Grower A) and feed ingredients (Indian fish meal, Danish fish meal and wheat flour) during storage.

Materials and Methods

Three pelleted fish feeds (1000 Grower 15+, 3000 Grower and 6000 Grower A) and three ingredients (Indian fish meal, Danish

Corresponding author : Dr. P.G.Viswanathan Nair, Central Institute of Fisheries Technology, Cochin - 682 029, INDIA, Email: pgv@cift.ker.nic.in

The work reported was carried out under World Bank funded National Agricultural Technology Project Scheme

fish meal and wheat flour) obtained from one of the leading fish feed manufacturers in Cochin were selected for the study. Equal amounts (approximately 500 g) of these feeds and ingredients were packed separately in polyethylene bags and kept at room temperature and analyzed periodically for a period of six months. Moisture content, crude protein, crude fat and ash content were determined according to standard AOAC procedures (1975).The amino acid composition of feed and ingredient samples was determined by hydrolyzing the samples in 6N HCl for 24 h at 110^{0}C. The acid was removed by vacuum evaporation, made up to a known volume with 0.05N HCl and then analyzed using HPLC (Ammu *et al.*, 1994). Tryptophan content of the samples was determined after alkali hydrolysis (Sastry & Tummuru, 1985). Lipid extraction was done using Bligh and Dyer method (1959) and the fatty acid profile of the extracted lipid of the feed and ingredient samples was determined by GLC (Metcalfe *et al.*, 1966), using packed column (2mx1/8", 15% OV 275), flame ionization detector and carrier nitrogen. Identification and quantification of fatty acids were done using Sigma standards. Histamine content was determined according to the procedure of Hardy and Smith (1976).

Results and Discussion

In the present study, an increase in moisture content was observed in the samples of ingredients and the feeds except in wheat flour (Fig. 1). Since small variations in the moisture content largely influence the quality of the low moisture feed during storage, it is important to bring the moisture content as minimum as possible to ensure storage stability (Jain, 1998; Gomez *et al.*, 1997). Increased moisture content has an

Table 1. Changes in the amino acids (% of protein) of the ingredients during storage

	Indian fish meal				Fish meal Danish				Wheat flour			
	Initial	After 2 months	After 4 months	After 6 months	Initial	After 2 months	After 4 months	After 6 months	Initial	After 2 months	After 4 months	After 6 months
Asp	12.5	10.9	8.6	8.5	11.4	9.4	8.0	6.2	7.8	7.5	7.1	6.8
Thr	3.6	3.4	3.2	3.2	3.7	3.3	3.3	3.3	3.2	3.0	3.0	3.0
Ser	4.0	3.8	3.6	2.9	5.5	5.2	3.5	1.9	4.0	3.8	3.5	3.5
Glu	17.9	17.8	14.3	14.1	15.6	15.3	14.2	13.9	22.4	20.9	20.8	20.8
Pro	4.3	3.9	1.1	0.6	3.7	2.2	2.1	2.0	7.4	6.5	6.5	6.5
Gly	8.7	6.9	6.8	6.8	6.5	6.3	4.2	2.1	6.6	6.2	6.1	6.0
Ala	11.0	9.7	7.4	7.4	8.8	8.7	8.4	8.1	5.8	5.4	5.4	5.3
Cys	-	-	-	-	1.4	0.7	0.3	-	1.2	0.8	0.4	-
Val	2.9	2.6	2.4	2.3	5.8	5.3	5.2	5.2	5.8	5.8	5.7	5.6
Met	3.3	3.1	2.8	2.7	4.5	4.1	3.2	2.2	1.4	0.9	0.7	0.4
Ile	4.3	4.2	4.1	3.9	5.0	4.4	4.0	3.9	5.8	5.5	5.4	5.3
Leu	8.7	7.3	7.2	7.2	9.3	8.7	8.2	7.2	7.8	6.9	6.8	6.7
Tyr	2.9	2.5	1.6	1.5	4.5	3.6	3.5	3.4	2.2	2.2	2.1	1.7
Phe	4.3	4.0	3.8	3.6	5.2	4.9	4.5	3.8	4.6	4.2	4.2	4.2
His	2.5	2.1	1.4	1.4	4.4	3.7	2.7	1.7	2.6	2.7	2.4	2.1
Lys	2.7	2.6	2.4	2.3	2.5	2.1	2.1	2.1	1.6	1.6	1.5	1.5
Arg	5.6	5.4	4.5	4.3	5.4	5.2	4.5	3.8	6.1	6.0	5.1	4.3
Trp	1.6	0.6	1.6	1.2	2.0	1.4	1.1	0.9	3.1	3.0	2.9	2.7

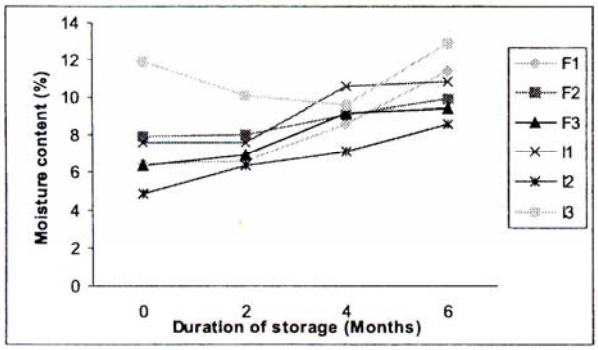

Fig. 1. Changes in the moisture content of the feeds and ingredients during storage

adverse effect on the feeds as it favors the growth of microorganisms, especially fungi that produce mycotoxins (Gomez *et al.*, 1997). Wheat flour was found to have the highest moisture content (11.9%), and was exhibiting a different pattern during storage. In this case an initial decrease in the moisture content was followed by a significant increase at the end of the storage period.

Proteins serve both structural and functional roles in living organisms. Although proteins may be metabolized for energy, one goal in aquaculture is to utilize as much of dietary protein as possible for growth, allowing the carbohydrates and lipids to provide metabolic energy (Agrawal, 1999). Fishes have a high requirement of proteins (Al Hafedh, 1999; El-Sayed and Teshima, 1991). During storage, various changes occur to the proteins including the availability of the amino acids (Mavromichalis & Baker, 2000). Since the quality of the protein is determined by the presence and availability of essential amino acids, feed quality is negatively affected by these changes. But in the present study significant changes in the protein content was not observed in any of the feeds or ingredients (Fig. 2).

Table 2. Changes in the amino acids (% of protein) of the feeds during storage

	1000 grower 15+				3000 grower				6000 grower A			
	Initial	After 2 months	After 4 months	After 6 months	Initial	After 2 months	After 4 months	After 6 months	Initial	After 2 months	After 4 months	After 6 months
Asp	10.4	9.1	7.6	5.1	9.3	7.8	6.8	6.3	10.4	9.4	6.6	6.1
Thr	3.5	3.2	2.8	1.9	3.0	2.6	2.6	2.7	3.5	2.9	2.6	2.3
Ser	5.2	4.6	3.9	1.5	4.9	4.0	2.2	2.3	5.2	4.5	2.0	1.8
Glu	20.5	17.8	15.3	10.3	18.5	16.8	14.5	13.6	20.5	19.0	13.4	12.0
Pro	5.2	4.6	4.5	3.5	5.2	4.9	4.5	4.5	5.2	3.6	4.3	4.0
Gly	6.7	6.5	5.5	3.6	6.7	5.7	4.7	4.4	7.4	6.6	4.9	4.1
Ala	6.6	6.2	5.4	2.7	6.5	5.5	3.5	3.4	7.4	6.8	3.6	3.2
Cys	0.8	0.6	0.7	-	0.8	0.6	0.4	0.2	0.8	-	-	0.2
Val	4.9	4.3	3.9	2.8	5.0	4.0	3.4	3.4	5.3	5.3	3.3	3.3
Met	8.4	5.9	5.4	5.1	8.4	6.5	6.5	6.7	9.3	7.9	6.4	5.9
Ile	3.3	3.0	2.8	2.8	5.1	5.0	5.1	5.1	7.6	6.9	5.2	4.8
Leu	3.9	3.4	3.0	3.0	6.2	6.2	6.2	6.2	9.3	8.5	6.4	5.8
Tyr	2.4	2.4	1.2	1.2	2.4	1.7	1.2	1.2	2.5	2.2	1.2	1.1
Phe	4.9	5.0	5.1	3.2	4.7	4.3	3.7	3.7	4.5	4.2	3.8	3.4
His	5.2	4.9	3.5	2.7	5.0	3.0	2.8	2.8	4.9	2.9	2.8	2.6
Lys	1.5	1.4	1.4	1.2	1.4	1.9	1.1	1.3	1.5	2.0	1.1	0.98
Arg	4.9	4.9	4.7	3.6	4.9	4.4	4.3	4.3	4.7	4.5	4.4	4.0
Trp	2.5	2.0	1.0	1.1	2.4	1.8	1.7	1.7	1.9	1.8	1.7	1.7

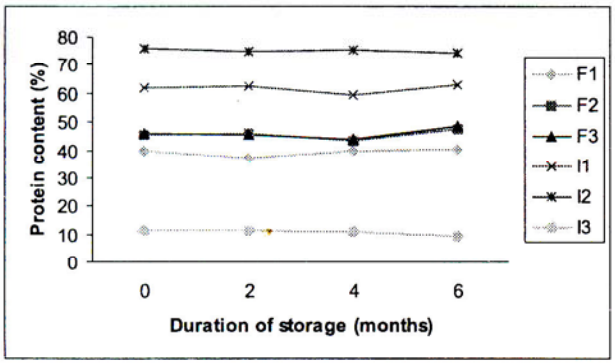

Fig. 2. Changes in the crude protein content of the feeds and ingredients during storage

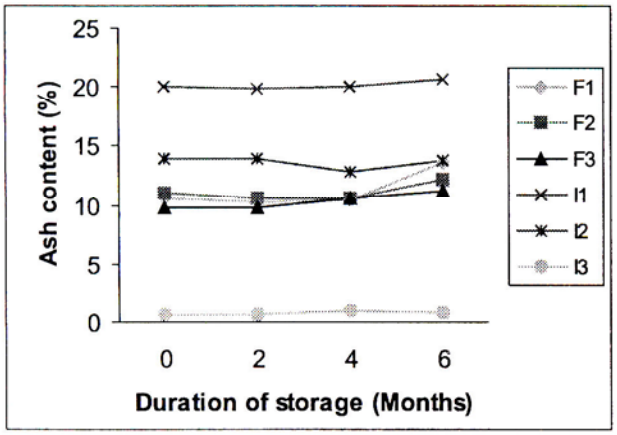

Fig. 3. Ash content of the feeds and ingredients during storage

Lipids are important source of energy and they serve as a source of the essential fatty acids for normal growth and survival of the fishes (Rath, 1993). Besides this they are also carriers of fat-soluble nutrients like vitamin A, D, E and K. A serious problem of lipids during storage is their easy susceptibility to oxidation. In the present study, no significant variations were observed in the fat content of the feed sample during the period of storage. But the ingredients showed a different pattern. In all the ingredients a significant reduction in the fat content was reported at the end of the storage period (Fig 4). Reduction in the fat content was observed in feeds with higher moisture content during storage (Bartov *et al.*, 1982). Oxidation of the fat and binding of the oxidation products to the other constituents might be the reason for the reduction noticed in the fat content. There were no significant changes observed in the

Table 3. Changes in the fatty acid profile of the ingredients during storage

	Percentage of fatty acid								
	Indian fish meal			Fish meal Danish			Wheat flour		
	Initial	After 3 months	After 6 months	Initial	After 3 months	After 6 months	Initial	After 3 months	After 6 months
Fatty acid									
C14:0	9.64	8.71	7.60	5.01	4.99	4.62	-	-	-
C15:0	0.99	0.68	-	0.39	0.29	-	-	-	-
C16:0	31.5	34.0	35.6	17.4	17.6	18.0	18.6	17.8	14.1
C16:1	12.9	14.0	13.9	5.93	6.57	6.57	-	-	-
C17:0	3.05	1.78	1.35	1.06	1.06	1.06	-	-	-
C18:0	7.72	8.58	9.37	1.68	1.74	1.73	2.5	2.5	3.0
C18:1	13.8	17.4	17.4	18.1	19.1	19.8	16.6	12.3	12.1
C18:2	2.86	2.50	2.62	2.3	2.14	2.28	57.9	44.4	40.5
C18:3	0.5	0.48	-	1.19	1.16	1.04	2.7	2.7	2.0
C18:4	-	-	-	8.26	8.28	8.42	-	-	-
C20:1	1.61	1.12	0.87	-	-	-	0.7	-	-
C20:4	1.13	0.88	0.79	0.86	0.88	0.67	0.9	0.9	0.9
C20:5	4.88	4.07	1.90	17.5	16.0	15.8	1.3	1.1	0.9
C24:1	0.31	-	-	0.25	0.17	-	0.5	-	-
C22:6	5.89	4.63	3.46	18.7	17.9	17.3	-	-	-

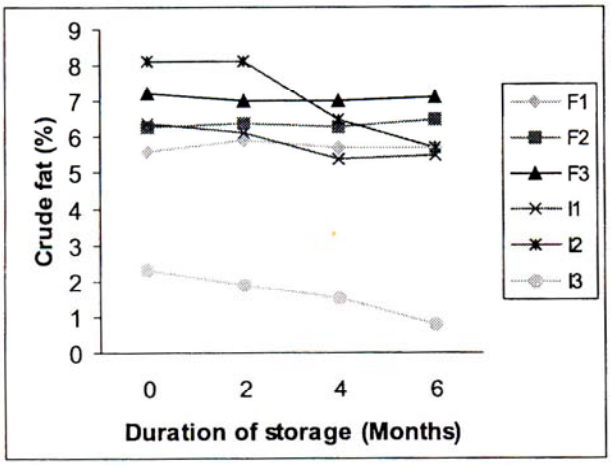

Fig. 4. Changes in the crude fat content of feeds and ingredients during storage

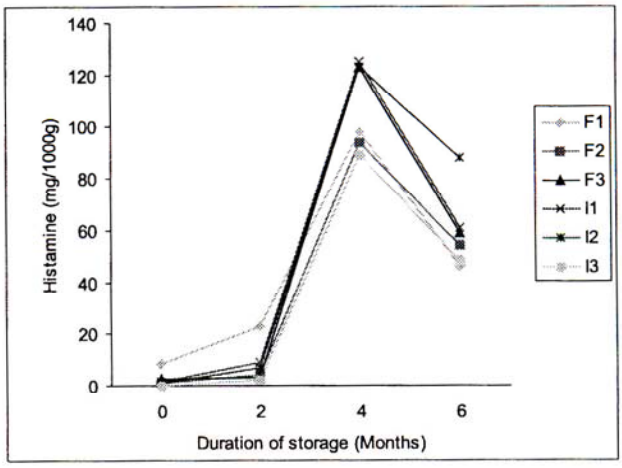

Fig. 5. Changes in the histamine content of feeds and ingredients during storage

ash content of the feeds and ingredients during storage (Fig. 3).

Formation of volatile nitrogenous bases including ammonia and toxic amines like histamine is a major problem encountered during storage. This was evident from the notable increase in the histamine content observed in the samples during storage (Fig. 5), which is in line with an earlier report (Kim *et al.*, 2001). Increase in histamine content is an index of the growth of microorganisms in the stored feeds (Belitz and Grosch, 1999). But towards the end of the storage period, histamine content was found to be decreasing, which is in accordance with the report by Edmunds and Eitenmiller (1975).

Table 4. Changes in the fatty acid profile of the feeds during storage

	Percentage of fatty acid								
	1000 Grower 15+			3000 Grower			6000 Grower A		
	Initial	After 3 months	After 6 months	Initial	After 3 months	After 6 months	Initial	After 3 months	After 6 months
Fatty acid									
C14:0	3.8	3.9	3.9	3.85	4.03	3.7	3.7	3.3	3.2
C15:0	0.18	-	-	0.17	-	-	-	-	-
C16:0	19.3	19.51	19.63	17.99	17.72	16.9	20.72	19.95	19.4
C16:1	6.01	6.9	6.9	5.5	5.7	5.6	5.5	5.4	5.3
C17:0	0.86	0.84	-	0.7	0.7	-	0.83	0.86	0.85
C18:0	3.6	3.4	3.4	-	-	-	3.38	3.00	3.1
C18:1	17.8	17.7	17.4	20.47	19.11	18.33	18.8	18.7	18.5
C18:2	23.4	23.05	22.85	21.49	21.33	21.17	23.13	22.65	22.43
C18:3	2.2	2.2	1.9	-	-	-	2.27	2.25	1.97
C18:4	0.3	-	-	0.34	-	-	-	-	-
C20:1	3.7	3.5	2.9	4.77	4.49	4.23	4.97	4.8	4.87
C20:4	0.8	0.8	-	0.76	0.65	-	0.63	0.60	0.56
C20:5	8.5	8.5	8.4	11.2	11.02	10.99	10.48	10.27	10.2
C24:1	0.4	-	-	0.5	0.4	-	-	-	-
C22:6	5.9	5.6	5.3	7.2	7.2	7.1	3.38	2.99	2.7

The amino acid composition of feeds and ingredients during storage showed significant changes in some amino acids (Tables 1 & 2). It is well established that fish requires ten essential amino acids viz. methionine, arginine, tyrosine, tryptophan, valine, isoleucine, leucine, phenylalanine, histidine and lysine for normal growth (Reddy and Rao, 1999) and the quality of the protein is determined by the presence of these amino acids in a balanced proportion. Significant alterations in the levels of various amino acids were observed in both feeds and ingredients during the period of storage. Mavromichalis & Baker (2000) reported that the bioavailability of amino acids was affected upon storage. The amino acids might be acted upon by microbes to form toxic amines. This might be the reason for the observed reduction in the amino acid levels. Interaction of the amino acids with the oxidation products of the lipids also could be another factor.

Lipids are liable to oxidation or hydrolysis during storage and it affects the flavour characteristics of the aged feeds (Dhaliwal *et al.*, 1990). The physical, chemical and functional characteristics of the feed may get altered producing desirable or undesirable changes (Deka *et al.*, 2000). Study conducted by Shin and Godber (1996) showed that there was a significant increase in the amount of saturated fatty acids and the fatty acid C18:1 during a storage period of 52 weeks in rice. But all other unsaturated fatty acids showed gradual decrease in their quantity. In the present study, analysis of the fatty acid profiles of the ingredients showed that in the case of Indian fish meal, there was a significant reduction in the proportion of C14:0 among saturated fatty acids during the storage (Table 3). But even though it was not significant, C16:0 and C18:0 showed an increasing tendency during the period as reported by Shin and Godber (1996). Among the mono unsaturated fatty acids, C20:1 and C24:1 levels showed a significant decrease. All polyunsaturated fatty acids showed a significant reduction in their levels during storage, which is in line with earlier reported studies (Shin and Godber, 1996; Deka *et al.*, 2000). But in the case of fish feeds (Table 4), there were no significant changes noticed in the levels of both saturated and unsaturated fatty acids. The difference observed in the fatty acid profiles between the feeds and ingredients during storage could be attributed to the antioxidants present in the feed.

In conclusion the most important adverse effects, which affect the nutritive value of the feed during storage, include loss of amino acid, formation of histamine, degradation of PUFA and the possible deleterious effects of the products of lipid oxidation.

References

A.O.A.C. (1975) *Official Methods of Analysis*. Association of Analytical Chemists, 12th Edn., Washington, D.C

Agrawal, V.P. (1999) Nutrition of cultured fishes. In: *Recent Trends in Aquaculture*, pp 87-114, Society of Biosciences, Muzaffar nagar, India

Al Hafedh, Y.S. (1999) Effect of dietary protein on growth and body composition of Nile tilapia, *Orechromis niloticus* L. *Aqua. Res.* **30**, pp 385-393

Ammu, K., Stephen, J. and Devadasan, K. (1994) Retention of amino acids in the carcass of fish protein fed rats. *Fish. Technol.*, **31**, pp 29-35

Bartov, I., Paster, N. and Lisker, N. (1982) The nutritional value of moldy grains fo broiler chicks. *Poult. Sci.* **61**. pp 2247-54

Belitz, H.D. and Grosch, W. (1999) Meat. In: *Food Chemistry*, 2nd Edn., Springer-Verlag Berlin Heidelberg (pub) pp 527-578.

Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, pp 911-917.

Branen, A.L. (1993) Introduction to use of antimicrobials. In: *Antimicrobials in Foods*, pp 1-10, 2nd Edn., Michael Davidson P and Alfred Larry Branen (eds.), Marcel Dekker Inc. New York

Chow, C.K. (2000) Biological effects of oxidized fatty acids. In: *Fatty acids in Foods and Their Health Implications*. 2nd Edn. (Cow C.K ed.) Marcel Dekker Inc. New York

Deka, S.C., Sood, D.R. and Gupta, S.K. (2000) Effect of storage on fatty acid profiles of Basmati Rice (*Oryza sativa* L.) Genotypes. *J. Food Sci. Technol.* **37**, pp 217-221

Dhaliwal, Y.S., Sekhon, K.S. and Negi, N.P.S. (1990) Effect of drying and storage on the fatty acid composition of rice. *J. Food Sci. Technol.* **27**, pp 107-108

Edmunds, W.J. and Eitenmiller, R.R. (1975) Effect of storage time and temperature on histamine content and histidine decarboxylase activity of aquatic species. *J. Food Sci.* **40** pp 516-519

El-Sayed, A.M. and Teshima, S. (1991) Tilapia nutrition in aquaculture. *Rev. Aqua. Sci.* **5**, pp 247-265

Gadient, M., Fenster, R. and Latscha, T. (1992) Vitamin stability in aquaculture feeds. Fish Farmer Jan/Feb pp 27-28

Garcia-Ortega, A., Verreth, J., Van Hoornyck, A. and Segner, H. (2000) Heat treatment affect protein quality and protease activity in decapsulated cysts of Artemia when used as starter feed for larvae of African catfish (*Clarias gariepinus* (Burchell). *Aquacult. Nutr.* **6**, pp 25-31

Gomez, R., Vioque, M., Sanchez, E. and Fernandez-Salguero, J. (1997) Changes in water activity and some microbial groups during storage of pet feed. *Acta Microbiol. Immunol. Hung.* **44,** pp 155-164

Hardy, R. and Smith, J.G.M. (1976) The storage of mackerel (Scomber scombrus): development of histamine and rancidity. *J. Sci. Food Agri.* **27**, pp 595-599

Jain, K.K. (1998) Storage of aqua feeds. In: *Modern Approach to Aqua Feed Formulation and on Farm Feed Management*. (Takur, N.K., Jain, K.K. & Sahu, N.P. eds.), pp 135-137

Kim, S.H., Field, K.G., Chang, D.S., Wei, C.I. and An, H. (2001) Identification of bacteria crucial to histamine accumulation in pacific mackerel during storage. *J. Food Prot.* **64**, pp 1556-1564

Mavromichalis, I. and Baker, D.H. (2000) Effects of pelleting and storage of a complex nursery pig diet on lysine bioavailability. *J. Anim. Sci.* **78**, pp 341-347

Metcalfe, L.D., Schimitz, A.A. and Petta, J.R. (1966) Rapid preparation of fatty acid esters from lipids for chromatographic analysis. *Anal. Chem.* **38**, pp 514-515

Rath, R.K. (1993) Fish feed. In: *Fresh Water Aquaculture*. Scientific Publishers, pp 234-280, Jodhpur, India

Reddy, M.S. and Rao, K.R.S.S. (1999) Nutrition and feeds. In: A Text Book of Aquaculture (Rao, D. ed.), pp 135-209, Discovery Publishing House, New Delhi

Sastry, C.S.P. and Tummuru, M.K. (1985) Spectrophotometric determination of tryptophan in proteins. *J. Food Sci. Tech.* **22**, pp 146-147

Shin, T.S. and Godber, J.S. (1996) Changes of endogenous anti oxidants and fatty acid composition in irradiated rice bran during storage. *J. Agric. Food Chem.* **44**, pp 567-573.