Effects of varying dietary protein levels on growth, reproductive performance, body and egg composition of rohu, *Labeo rohita* (Hamilton)

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Abstract

A 360-day feeding trial was conducted to observe the influence of varying levels of dietary protein on growth, reproductive performance, body and egg composition of rohu, *Labeo rohita*. Twenty fish (40.4 ± 0.24 cm; 852 ± 4.9 g), stocked in outdoor concrete tanks (200 m^2), in duplicate, were fed diets with varying levels (200, 250, 300, 350 and 400 g kg^{-1}) of crude protein exchanged with carbohydrate to apparent satiation, twice daily, at 09:00 and 17:00 h. Higher ($P < 0.05$) weight increment was discernible in fish fed dietary protein ≥300 g kg^{-1}. Gonadosomatic index was comparable ($P > 0.05$) among fish of different dietary groups except those fed 200 g kg^{-1} protein diet which produced least values. Egg diameter remained unaffected ($P > 0.05$) by variations in levels of dietary protein. Relative fecundity was maximum ($P < 0.05$) in fish fed 250 and 300 g kg^{-1} protein diets. With the exception of fish fed 200 g kg^{-1} protein diet, fertilizability (%) remained unaffected ($P > 0.05$) by variations in dietary protein level. Hatchability (%) followed the trend of variations almost similar to that of fertilizability. Proximate composition of muscle and eggs varied significantly ($P < 0.05$) with dietary protein levels. For broodstock *L. rohita*, a dietary protein level of 250 g kg^{-1} was found optimum with regard to its reproductive performance, egg quality and composition.

KEY WORDS: broodstock, dietary protein, eggs, *Labeo rohita*, proximate composition, reproductive performance

Received 2 June 2003, accepted 5 July 2004

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Introduction

Despite intense research interest, broodstock nutrition remains one of the most poorly understood areas of finfish nutrition. Studies on broodstock nutrition are relatively expensive to conduct and limited to a few species (Brooks *et al*. 1997; Izquierdo *et al*. 2001). Numerous studies have demonstrated that reproductive performance and egg quality are influenced by nutrients like protein, lipid, minerals, vitamins and ration size in fish such as gilthead seabream, *Sparus aurata* (Mourente & Odriozola 1990; Fernández-Palacios *et al*. 1995, 1997), sea bass, *Dicentrarchus labrax* (Cerdá *et al*. 1994), red seabream, *Pagrus major* (Watanabe & Kiron 1995), rainbow trout, *Oncorhynchus mykiss* (Washburn *et al*. 1990; Choubert & Blanc 1993; Blom & Dabrowski 1995; Choubert *et al*. 1998; Pereira *et al*. 1998), Atlantic salmon, *Salmo salar* (Eskelinen 1989; Berglund 1995; Christiansen & Torrissen 1997), Coho salmon, *Oncorhynchus kisutch* (Hardy *et al*. 1984, 1989), tilapia, *Oreochromis niloticus* (De Silva & Radampola 1990; Cumaratunga & Mallika 1991; Santiago & Reyes 1993; Gunasekera *et al*. 1995, 1996a,b, 1997; Gunasekera & Lam 1997; Siddiqui *et al*. 1998) and common carp, *Cyprinus carpio* (Manissery *et al*. 2001).

Proteins and lipids, the main components of egg yolk, are considered to play pivotal role in reproduction. Further, proteins act as a source of amino acids and reservoir of materials used during biosynthetic activities essential for early stages of embryogenesis (Metcoff 1986). It has been pointed out that there is an optimal protein level for reproductive success and that this level is related to the growth of the concerned species (De Silva & Anderson 1995). However, very few studies have evaluated the effects of dietary protein level on the reproductive performance of fish (Dahlgren 1980; Watanabe *et al*. 1984a, 1985; De Silva & Radampola 1990;

Gunasekera *et al.* 1995, 1996a; Gunasekera & Lam 1997; Siddiqui *et al.* 1998; Manissery *et al.* 2001).

Several workers have maintained that diets for broodstock should be tailor-made to ensure good egg quality as different fish species have different dietary requirements (Brooks *et al.* 1997). Broodstock nutrition of carps, particularly Indian major carps (*Labeo rohita*, *Catla catla*, *Cirrhinus mrigala*), has not received much attention. Most nutritional studies on these carps remain confined to fry, fingerling and young fish. *Labeo rohita* is known to have a high fecundity, and has been successfully induced bred more than once in a year in some parts of India. However, nutrient requirements for broodstock of this fish have not been worked out.

The present study was undertaken to investigate the effects of varying dietary protein levels on growth, reproductive performance, body and egg composition of *L. rohita*.

Materials and methods

Experimental trial

Yearlings of female *L. rohita* (40.4 ± 0.24 cm; 852 ± 4.9 g) were stocked (20 fish per tank), in duplicate, in outdoor concrete tanks (200 m^2), supplied with groundwater. The experiment lasted 360 days. Natural photoperiod was maintained during the trial. Approximately, one-fourth of the total water volume in each tank was replenished daily. Diets were presented over an enamel tray kept about 30 cm below water level on two sides of the tank. Fish were fed to satiation, twice daily, at 09:00 and 17:00 h. Water temperature, pH and dissolved oxygen were monitored daily. Other water quality parameters were analysed on weekly basis. Average values for various parameters were: dissolved oxygen 4.0–8.6 mg L^{-1}; pH 7.81; total alkalinity 312 mg L^{-1} and total hardness (as $CaCO_3$) 380 mg L^{-1}.

At the end of the trial, 12 fish from each treatment (six fish per replicate) were induced bred, using Ovaprim (Syndel Laboratories Ltd, Vancouver, Canada), for assessment of reproductive performance of *L. rohita*. Intramuscular injection of ovaprim was given just below the lateral line in the region of the caudal peduncle at the rate of 0.4 and 0.2 mL kg^{-1} body weight of females and males, respectively. Thereafter, a set of fish (1 : 2, female : male) was released in a breeding hapa (2.4 × 1.2 × 1.0 m) fixed in outdoor pond with the help of bamboo poles (Chaudhuri & Singh 1984). All fish spawned 10–12 h after injection. Eggs were collected from the breeding hapa 2–3 h after spawning. Total number of eggs was estimated volumetrically to assess the relative fecundity and fertilizability. Eggs were incubated in indoor flow-up glass jar system. Hatching occurred 16–18 h after fertilization. To observe the fertilizability and hatchability (%), 50 samples of 5 mL were collected at random. Fertilized and unfertilized eggs were sorted out to calculate fertilizability (%) of total eggs. Hatchability (%) was assessed by counting the total number of hatchlings from the total number of fertilized eggs. On the day of induced spawning, six females from each treatment (three fish per replicate) were killed to assess the gonadosomatic index (GSI), egg diameter (using a compound microscope equipped with ocular micrometer), and also to collect muscle tissue and eggs for the analysis of proximate composition. Nearly 300 eggs from each female (approximately 100 eggs from three different portions of ovary) were examined for determining the egg diameter. Calculation of GSI and assessment of reproductive performance (relative fecundity, fertilizability % and hatchability %) were made following standard definitions (Hardy *et al.* 1989; Biswas 1993).

Experimental diets

Feed ingredients [steam cooked and solvent extracted fish meal (FM); solvent extracted soybean meal (SBM) and groundnut meal (GNM); rice bran; and corn flour] used for the trials were procured from local market (Pawan Feeds, Rohtak, India). Experimental diets were formulated to contain varying levels of crude protein (200, 250, 300, 350 and 400 g kg^{-1}) exchanged with carbohydrate. Ingredient and proximate composition of experimental diets are given in Table 1. Calculated amino acid composition (NRC 1993) of the diets is given in Table 2. All ingredients, and mineral premix, excepting vitamin and oil premixes, were mixed and cooked for 30 min to a dough form. After adding oil and vitamins, the ingredients were thoroughly mixed and mechanically extruded to get pellets of desired size. The pellets were dried in a convection oven at 25 °C to obtain a moisture level of approximately 100 g kg^{-1}.

Analytical methods

Proximate composition of feed ingredients, experimental diets, muscle and eggs was analysed using standard methods (AOAC 1995). Moisture was determined by drying the sample (105 °C for 24 h) to a constant weight. Crude protein (N × 6.25) was measured using the Kjeldahl method after acid digestion. Crude fat was estimated by Soxhlet exhaustive extraction technique using petroleum ether (40–60 °C, BP) as solvent. Ash was determined by incinerating the dried sample at 550 °C for 12 h. Crude fibre was estimated through 1.25%

Table 1 Ingredient and proximate composition of formulated diets containing varying levels of protein

Ingredients (g kg^{-1} diet)	Formulated diets (g kg^{-1} crude protein)				
	200	250	300	350	400
Tuna fish meal[1]	74.0	113.8	153.6	193.4	233.2
Soybean meal[1]	74.0	113.8	153.6	193.4	233.2
Groundnut meal[1]	74.0	113.8	153.6	193.4	233.2
Corn flour[1]	331.5	271.8	212.1	152.4	92.7
Rice bran[1]	331.5	271.8	212.1	152.4	92.7
Vitamin premix[2]	15.0	15.0	15.0	15.0	15.0
Mineral premix[3]	30.0	30.0	30.0	30.0	30.0
Oil premix (1 : 1 corn and cod liver oil)	70.0	70.0	70.0	70.0	70.0
Proximate composition[4]					
Crude protein	198.4	249.3	300.1	346.4	397.6
Crude fat	101.0	100.0	98.2	95.5	94.0
Ash	103.6	104.1	101.0	103.1	104.2
Crude fibre	93.0	86.8	81.0	78.4	73.6
Nitrogen-free extract	499.4	456.0	413.1	369.5	326.2
Metabolizable energy[5] (kJ g^{-1})	14.7	15.0	15.2	15.5	15.7

[1] Proximate composition as g kg^{-1} dry matter [FM (614 crude protein, 35 crude fat, 105 ash, 38 crude fibre, 139 NFE); SBM without hulls (522 crude protein, 10 crude fat, 68 ash, 65 crude fibre, 282 NFE); GNM (483 crude protein, 27 crude fat, 78 ash, 127 crude fibre, 225 NFE); corn flour (115 crude protein, 34 crude fat, 12 ash, 43 crude fibre, 728 NFE); rice bran (127 crude protein, 49 crude fat, 160 ash, 184 crude fibre, 415 NFE)].
[2] Halver (1989).
[3] Agrimin (Agrivet Farm Care, Glaxo India Limited, Mumbai, India) contains copper 312 mg, cobalt 45 mg, magnesium 2.114 g, iron 979 mg, zinc 2.13 g, iodine 156 mg, DL-methionine 1.92 g, L-lysine HCl 4.4 g, calcium 30% and phosphorus 8.25%, in 1 kg.
[4] Results are mean of triplicate estimations.
[5] Calculated using energy equivalents as proposed by Jauncey (1982).

acid and subsequent 1.25% alkali digestion, and incineration of the dried sample for 2 h at 550 °C. Nitrogen-free extract was calculated by difference. Water quality parameters were estimated using standard techniques (APHA 1985).

Statistical analysis

Data (mean ± SE values from each replicate with tank as unit) were analysed for comparison among different dietary treatments by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at $P = 0.05$ significance level (Duncan 1955; Gomez & Gomez 1984).

Results

Results on growth, GSI and egg diameter of *L. rohita* fed varying levels of dietary protein are given in Table 3. Higher

Table 2 Amino acid composition[1] of formulated diets

Amino acids (g kg^{-1} diet)	Formulated diets (g kg^{-1} crude protein)				
	200	250	300	350	400
Arginine	15.0	19.8	24.6	29.2	34.1
Histidine	5.6	6.9	8.4	9.7	11.5
Isoleucine	8.2	10.4	12.4	14.8	16.9
Leucine	16.9	19.8	23.2	26.2	29.3
Lysine	10.0	13.3	16.6	19.7	23.1
Methionine	3.5	4.3	5.2	6.2	7.0
Cystine	3.0	3.5	4.0	4.5	5.0
Methionine + cystine	6.5	7.8	9.2	10.7	12.0
Phenylalanine	9.4	11.9	14.3	16.5	18.9
Tyrosine	7.7	9.7	11.4	13.3	15.2
Threonine	7.5	9.6	11.5	13.6	15.6
Tryptophan	2.5	2.9	3.5	4.1	4.6
Valine	9.8	12.1	14.5	17.0	19.3

[1] Calculated values, based on tabular values of feed ingredients (NRC 1993).

($P < 0.05$) weight increment was discernible in fish fed 300, 350 and 400 g kg^{-1} protein diets than those receiving 200 g kg^{-1} protein diet. GSI was comparable ($P > 0.05$) among different dietary groups, except in fish fed 200 g kg^{-1} protein diet, which exhibited lower ($P < 0.05$) values than those receiving 300 and 350 g kg^{-1} protein diet. Egg diameter remained unaffected ($P > 0.05$) by variations in dietary protein level.

Results on reproductive performance of *L. rohita* fed varying levels of dietary protein are given in Table 4. Relative fecundity was maximum ($P < 0.05$) in fish fed 250 and 300 g kg^{-1} protein diets and minimum ($P < 0.05$) in fish receiving 200 g kg^{-1} protein diet. A significant ($P < 0.05$) decline in relative fecundity was also noticeable beyond 300 g kg^{-1} dietary protein intake. With the exception of fish fed 200 g kg^{-1} protein diet, fertilizability (%) was not affected ($P > 0.05$) by variations in levels of dietary protein. Hatchability (%) followed the trend of variations almost similar to that of fertilizability.

Proximate composition of muscle is given in Table 5. Muscle protein was higher ($P < 0.05$) in fish fed 300, 350 and 400 g kg^{-1} protein diets. Fat content was comparable ($P > 0.05$) among different dietary treatments, excepting in fish receiving 200 and 400 g kg^{-1} protein diets which exhibited significantly ($P < 0.05$) lower and higher values, respectively. Muscle ash content was comparable ($P > 0.05$) among fish of different dietary groups. Moisture content decreased with increasing dietary protein level.

Proximate composition of eggs is shown in Table 6. Protein content in the eggs was comparable ($P > 0.05$)

Table 3 Growth, gonadosomatic index and egg diameter of *Labeo rohita* fed formulated diets with varying protein levels

	Formulated diets (g kg^{-1} crude protein)				
	200	250	300	350	400
Initial body weight (g)	850 ± 25	840 ± 20	870 ± 30	850 ± 0.00	850 ± 20
Final body weight (g)	2000c ± 63	2325b ± 48	2500ab ± 50	2650a ± 80	2450ab ± 65
Weight increment (g)	1150c ± 88	1485b ± 68	1630ab ± 29	1800a ± 80	1600ab ± 85
Gonadosomatic index	23.5b ± 0.89	25.0ab ± 0.71	26.4a ± 0.64	27.0a ± 0.75	26.0ab ± 0.52
Egg diameter (mm)	1.30a ± 0.06	1.35a ± 0.04	1.41a ± 0.05	1.48a ± 0.04	1.40a ± 0.07

Mean ± SE values with different letters in each row are significantly ($P < 0.05$) different.

Table 4 Reproductive performance of *Labeo rohita* fed formulated diets with varying protein levels

	Formulated diets (g kg^{-1} crude protein)				
	200	250	300	350	400
Relative fecundity (eggs kg^{-1} body weight)	280 550c ± 1807	309 988a ± 1950	310 700a ± 300	300 675b ± 1238	300 012b ± 588
Fertilizability (%)	80.2b ± 1.1	86.7a ± 1.38	88.4a ± 1.33	90.4a ± 2.22	86.5a ± 1.86
Hatchability (%)	76.0b ± 1.19	79.3ab ± 1.07	83.6a ± 1.92	82.5a ± 1.60	82.2a ± 2.05

Mean ± SE values with different letters in each row are significantly ($P < 0.05$) different.

Table 5 Proximate composition (g kg^{-1}) of muscle at time of spawning of female *Labeo rohita* fed formulated diets with varying protein levels

	Formulated diets (g kg^{-1} crude protein)				
	200	250	300	350	400
Moisture	827.6a ± 9.2	821.0ab ± 8.0	807.9abc ± 9.3	796.6bc ± 3.8	784.4c ± 5.5
Crude protein[1]	775.0c ± 3.3	802.4b ± 4.4	833.1a ± 6.2	836.4a ± 3.5	827.9a ± 7.4
Crude fat[1]	48.4b ± 2.8	55.2ab ± 5.7	56.8ab ± 3.4	62.4ab ± 7.1	69.0a ± 6.0
Ash[1]	63.5a ± 6.9	54.8a ± 5.0	56.0a ± 2.2	55.5a ± 3.3	57.4a ± 1.7

[1] Dry-weight basis; mean ± SE values with different letters in each row are significantly ($P < 0.05$) different.

Table 6 Proximate composition (g kg^{-1}) of eggs of *Labeo rohita* fed formulated diets with varying protein levels

	Formulated diets (g kg^{-1} crude protein)				
	200	250	300	350	400
Moisture	628.7a ± 8.1	632.1a ± 10.8	606.9a ± 8.0	619.4a ± 5.2	618.5a ± 6.3
Crude protein[1]	694.8b ± 9.0	703.6ab ± 4.5	710.7ab ± 2.5	709.5ab ± 4.1	715.0a ± 4.7
Crude fat[1]	242.5a ± 3.2	250.1a ± 5.1	240.4a ± 4.4	241.5a ± 4.5	235.4a ± 8.3

[1] Dry-weight basis; mean ± SE values with different letters in each row are significantly ($P < 0.05$) different.

among fish of different dietary groups, excepting the groups fed 200 and 400 g kg^{-1} protein diets, which exhibited significantly ($P < 0.05$) lower and higher values, respectively. The eggs showed comparable ($P > 0.05$) values for moisture and fat.

Discussion

Growth in fish is affected by dietary protein level, however, only few reports are available on this aspect for broodfish. Gunasekera & Lam (1997) reported that broodstock of *O. niloticus* fed low (100 g kg^{-1}) protein diet attained less weight than those fed high (200 and 350 g kg^{-1}) protein diets. They found no difference in the growth of fish receiving 200 and 300 g kg^{-1} protein diets. In the present work on *L. rohita*, weight increment was discernible up to 300 g kg^{-1} dietary protein level beyond which fish growth remained unaffected. Singh & Dhawan (1996) observed that *C. carpio* showed higher weight gain when fed formulated diets containing 340 or 380 g kg^{-1} protein than those fed 310 g kg^{-1} protein diet.

Gonadosomatic index in *L. rohita* increased significantly at 250 g kg^{-1} dietary protein intake, thereafter, the values remained comparable. Female guppy, *Poecilia reticulata*, fed 470 g kg^{-1} protein diet produced higher GSI than those receiving 150 and 350 g kg^{-1} protein diets (Dahlgren 1980). Pathmasothy (1985) reported higher GSI in *Leptobarbus hoevenii* fed higher (320 and 400 g kg^{-1}) protein diets. However, in *O. niloticus*, there are reports of GSI not being affected by variations in dietary protein (Cumaratunga & Mallika 1991; Gunasekera & Lam 1997; Gunasekera *et al.* 1997). In *L. rohita*, lower GSI at 200 g kg^{-1} dietary protein could be the result of a change in protein quality, as protein contribution from FM, SBM and GNM was minimal (Tables 1 and 2). In *P. major*, diets containing cuttlefish meal reportedly produced higher GSI than those containing whitefish meal (Watanabe *et al.* 1984b).

Egg diameter is considered an important criterion for the assessment of reproductive performance in fish. Egg diameter in *L. rohita* was not influenced by levels of protein in diet. Similar findings have also been reported in *O. niloticus* (Gunasekera *et al.* 1996b, 1997), *P. reticulata* (Dahlgren 1980) and *P. major* (Watanabe *et al.* 1984a). Cerdá *et al.* (1994) reported that egg diameter was not affected in *D. labrax* fed different levels of protein and carbohydrate. However, Manissery *et al.* (2001) recorded larger egg diameter in *C. carpio* fed 350 g kg^{-1} protein diet in comparison with those fed higher (410 g kg^{-1}) protein diet. Variations in oocyte diameter, at different oocyte stages, have also been reported in *O. niloticus* fed varying levels of dietary protein (Cumaratunga & Mallika 1991).

Fecundity, another important parameter to assess reproductive performance of fish, is known to be affected by nutritional deficiencies in broodstock diet (Izquierdo *et al.* 2001). Diets containing 250 and 300 g kg^{-1} protein produced higher relative fecundity in *L. rohita*. Manissery *et al.* (2001) reported maximum fecundity in *C. carpio* at 350 g kg^{-1} protein diet. Higher dietary protein level (450 g kg^{-1}) reportedly enhanced the total number of eggs/female in Nile tilapia (Siddiqui *et al.* 1998). Santiago *et al.* (1991) noted higher number of eggs kg^{-1} body weight in bighead carp, *Aristichthys nobilis*, fed 400 g kg^{-1} than those receiving 200 g kg^{-1} protein diet. Similar enhanced fecundity at higher dietary protein levels (320 and 400 g kg^{-1}) has been reported for *L. hoevenii* (Pathmasothy 1985). In contrast, De Silva & Radampola (1990) noted that *O. niloticus* fed low (20 g kg^{-1}) protein diet produced higher relative fecundity than those fed high (250 and 300 g kg^{-1}) protein diets. There are reports of dietary protein level not affecting relative fecundity in *O. niloticus* (Gunasekera *et al.* 1996a), and mean number of eggs per spawning in *Sarotherodon niloticus* (Santiago *et al.* 1983). Similarly, Dahlgren (1980) found no effect of levels of dietary protein on fecundity in *P. reticulata*.

Nutrition influences the fertilizability and hatchability (%) of fish eggs. However, in *L. rohita* fertilizability and hatchability were not found affected by dietary protein levels, except at 200 g kg^{-1}, where lower values were noticeable for these parameters. Gunasekera *et al.* (1996b) found that at low (100 g kg^{-1}) level of dietary protein, *O. niloticus* continued to produce eggs but the eggs remained unfertilized. They also noted an improvement in fertilizability and hatchability of eggs with increase in level of dietary protein. In *C. carpio*, hatching increased with dietary protein, whereas maximum fertilization (%) occurred at 350 g kg^{-1} and a marked decline at 410 g kg^{-1} dietary protein level (Manissery *et al.* 2001). Watanabe *et al.* (1984a) reported low hatchability in the eggs of *P. major* at low protein diets. Similar results were reported for dwarf gourami, *Colisa lalia* by Shim *et al.* (1989), who suggested that high protein diets allowed female gouramis to produce more oocytes, and that these oocytes were more likely to hatch into larvae.

Proximate composition of muscle in *L. rohita* varied with levels of dietary protein. In *L. rohita*, an increase in muscle protein was evident up to a dietary protein level of 300 g kg^{-1}. Moisture content in muscle of *L. rohita* decreased with increasing levels of dietary protein. Low moisture and high protein values were similarly obtained in brood *O. niloticus* fed high protein diet (Gunasekera *et al.* 1997). There are, however, relatively few studies on the influence of dietary protein on body composition of broodfish.

In the eggs of *L. rohita*, moisture and fat contents were not affected with variations in dietary protein. However, egg protein was lower in fish fed 200 than those fed 400 g kg^{-1} protein diet. Similarly, Gunasekera *et al.* (1996a, 1997) reported higher protein content in the eggs of *O. niloticus* fed high protein diet. Gunasekera *et al.* (1995) did not find any effect of dietary protein content on chemical composition of eggs in *O. niloticus*. They noted comparable values for protein, lipid and moisture in the eggs of tilapia fed diets with different levels of protein. Similar observations were made in *P. major* (Watanabe *et al.* 1985), *O. mykiss* (Takeuchi *et al.* 1981; Washburn *et al.* 1990) and *D. labrax* (Cerdá *et al.* 1994).

It may thus be concluded that for *L. rohita* a dietary protein level of 250 g kg^{-1} is optimum with regard to its reproductive performance, egg quality and proximate composition. An increase in dietary protein level beyond 250 g kg^{-1} seems to generate more somatic growth with no further improvement in reproductive performance, egg quality or proximate composition of eggs.

Acknowledgements

Authors are thankful to the Director, Central Institute of Fisheries Education (Deemed University), Mumbai, India, and Chairman, Department of Zoology, Aligarh Muslim University, Aligarh, India for providing necessary facilities for the experiment. Financial assistance rendered by Indian Council of Agricultural Research, New Delhi, India (Sanction No. 4(13)/95-ASR-1) is gratefully acknowledged by the first author.

References

AOAC (1995) *Official Methods of Analysis of the Association of Official Analytical Chemists International*, 16th edn. Association of Official Analytical Chemists, Arlington, VA, USA.

APHA (1985) *Standard Methods for Examination of Water and Wastewater*, 16th edn. American Public Health Association, Washington, DC, USA.

Berglund, I. (1995) Effects of spring temperature and feeding regime on sexual maturation in Atlantic salmon (*Salmo salar* L.) male parr. In: *Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish* (Goetz, F.W. & Thomas, P. eds), pp. 170–172. Fish Symposium 95, Austin, TX, USA.

Biswas, S.P. (1993) *Manual of Methods in Fish Biology*. South Asian Publishers Pvt. Ltd, New Delhi, India.

Blom, J.H. & Dabrowski, K. (1995) Reproductive success of female rainbow trout (*Oncorhynchus mykiss*) in response to graded dietary ascorbyl monophosphate levels. *Biol. Reprod.*, **52,** 1073–1080.

Brooks, S., Tyler, C.R. & Sumpter, J.P. (1997) Egg quality in fish: what makes a good egg? *Rev. Fish Biol. Fish.*, **7,** 387–416.

Cerdá, J., Carrillo, M., Zanuy, S., Ramos, J. & de la Higuera, M. (1994) Influence of nutritional composition of diet on sea bass, *Dicentrarchus labrax* L., reproductive performance and egg and laval quality. *Aquaculture*, **128,** 345–361.

Chaudhuri, H. & Singh, S.B. (1984) *Induced Breeding of Carps*. Indian Council of Agricultural Research, New Delhi, India.

Choubert, G. & Blanc, J-M. (1993) Muscle pigmentation changes during and after spawning in male and female rainbow trout, *Oncorhynchus mykiss*, fed dietary carotenoids. *Aquat. Living Resour.*, **6,** 163–168.

Choubert, G., Blanc, J-M. & Poisson, H. (1998) Effects of dietary keto-carotenoids (canthaxanthin and astaxanthin) on the reproductive performance of female rainbow trout *Oncorhynchus mykiss* (Walbaum). *Aquacult. Nutr.*, **4,** 249–254.

Christiansen, R. & Torrissen, O.J. (1997) Effects of dietary astaxanthin supplementation on fertilization and egg survival in Atlantic salmon (*Salmo salar* L.). *Aquaculture*, **153,** 51–62.

Cumaratunga, P.R.T. & Mallika, K.L.G.P. (1991) Effects of different levels of dietary protein and a legume *Vigna catiang* on gonadal development in *Oreochromis niloticus* (L.). In: *Fish Nutrition Research in Asia* (De Silva, S.S. ed.), pp. 125–133. Proceedings of the Fourth Asian Fish Nutrition Workshop, Asian Fisheries Society Special Publication 5. Asian Fisheries Society, Manila, Philippines.

Dahlgren, B.T. (1980) The effects of three different dietary protein levels on the fecundity in the guppy, *Poecilia reticulata* (Peters). *J. Fish Biol.*, **16,** 83–97.

De Silva, S.S. & Anderson, T.A. (1995) *Fish Nutrition in Aquaculture*. Chapman & Hall Aquaculture Series 1, Chapman & Hall, London, UK.

De Silva, S.S. & Radampola, K. (1990) Effect of dietary protein level on the reproductive performance of *Oreochromis niloticus*. In: *The Second Asian Fisheries Forum* (Hirano, R. & Hanyu, I. eds), pp. 559–563. Asian Fisheries Society, Manila, Philippines.

Duncan, D.B. (1955) Multiple range and multiple F tests. *Biometrics*, **11,** 1–42.

Eskelinen, P. (1989) Effects of different diets on egg production and egg quality of Atlantic salmon (*Salmo salar* L.). *Aquaculture*, **79,** 275–281.

Fernández-Palacios, H., Izquierdo, M.S., Robaina, L., Valencia, A., Salhi, M. & Vergara, J. (1995) Effect of n-3 HUFA level in broodstock diets on egg quality of gilthead seabream (*Sparus aurata* L.). *Aquaculture*, **132,** 325–337.

Fernández-Palacios, H., Izquierdo, M.S., Robaina, L., Valencia, A., Salhi, M. & Montero, D. (1997) The effect of dietary protein and lipid from squid and fish meals on egg quality of broodstock for gilthead seabream (*Sparus aurata*). *Aquaculture*, **148,** 233–246.

Gomez, K.A. & Gomez, A.A. (1984) *Statistical Procedures for Agricultural Research*. John Wiley & Sons, Singapore.

Gunasekera, R.M. & Lam, T.J. (1997) Influence of dietary protein level on ovarian recrudescence in Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, **149,** 57–69.

Gunasekera, R.M., Shim, K.F. & Lam, T.J. (1995) Effect of dietary protein level on puberty, oocyte growth and egg chemical composition in the tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, **134,** 169–183.

Gunasekera, R.M., Shim, K.F. & Lam, T.J. (1996a) Effect of dietary protein level on spawning performance and amino acid composition of eggs of Nile tilapia, *Oreochromis niloticus*. *Aquaculture*, **146,** 121–134.

Gunasekera, R.M., Shim, K.F. & Lam, T.J. (1996b) Influence of protein content of broodstock diets on larval quality and performance in Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, **146,** 245–259

Gunasekera, R.M., Shim, K.F. & Lam, T.J. (1997) Influence of dietary protein content on the distribution of amino acids in oocytes, serum and muscle of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture*, **152,** 205–221.

Halver, J.E. (1989) The vitamins. In: *Fish Nutrition*, 2nd edn (Halver, J.E. ed.), pp. 31–109. Academic Press Inc., San Diego, CA, USA.

Hardy, R.W., Shearer, K.D. & King, I.B. (1984) Proximate and elemental composition of developing eggs and maternal soma of pen-reared coho salmon (*Oncorhynchus kisutch*) fed production and trace element fortified diets. *Aquaculture*, **43,** 147–165.

Hardy, R.W., Masumoto, T., Fairgrieve, W.T. & Stickney, R.R. (1989) The effects of dietary lipid source on muscle and egg fatty acid composition and reproductive performance of coho salmon (*Oncorhynchus kisutch*). In: *Proceedings of Third International Symposium on Feeding and Nutrition in Fish* (Takeda, M. & Watanabe, T. eds), pp. 347–355. Toba, Japan.

Izquierdo, M.S., Fernández-Palacios, H. & Tacon, A.G.J. (2001) Effect of broodstock nutrition on reproductive performance of fish. *Aquaculture*, **197,** 25–42.

Jauncey, K. (1982) The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapias (*Sarotherodon mossambicus*). *Aquaculture*, **27,** 43–54.

Manissery, J.K., Krishnamurthy, D., Gangadhara, B. & Nandeesha, M.C. (2001) Effect of varied levels of dietary protein on the breeding performance of common carp Cyprinus carpio. *Asian Fish. Sci.*, **14,** 317–322.

Metcoff, J. (1986) Intracellular amino acid levels as predictors of protein synthesis. *J. Am. Coll. Nutr.*, **5,** 107–120.

Mourente, G. & Odriozola, J.M. (1990) Effect of broodstock diets on lipid classes and their fatty acid composition in eggs of gilthead sea bream (*Sparus aurata* L.). *Fish Physiol. Biochem.*, **8,** 93–101.

NRC (1993) *Nutrient Requirements of Fish*. National Research Council, National Academy Press, Washington, DC, USA.

Pathmasothy, S. (1985) The effect of three diets with variable protein levels on ovary development and fecundity in *Leptobarbus hoevenii*. In: *Fish Nutrition Research in Asia* (Cho, C.Y. ed.), pp. 107–112. IDRC, Ottawa, Canada.

Pereira, J.O.B., Reis-Henriques, M.A., Sanchez, J.L. & Costa, J.M. (1998) Effect of protein source on the reproductive performance of female rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquacult. Res.*, **29,** 751–760.

Santiago, C.B. & Reyes, O.S. (1993) Effects of dietary lipid source on reproductive performance and tissue lipid levels of Nile tilapia *Oreochromis niloticus* (Linnaeus) broodstock. *J. Appl. Ichthyol.*, **9,** 33–40.

Santiago, C.B., Aldaba, M.B. & Laron, M.A. (1983) Effect of varying dietary crude protein levels on spawning frequency and growth of *Sarotherodon niloticus* breeders. *Fish. Res. J. Philippines*, **8,** 9–18.

Santiago, C.B., Camacho, A.S. & Laron, M.A. (1991) Growth and reproductive performance of bighead carp (*Aristichthys nobilis*) reared with or without feeding in floating cages. *Aquaculture*, **96,** 109–117.

Shim, K.F., Landesman, L. & Lam, T.J. (1989) Effect of dietary protein on growth, ovarian development and fecundity in the dwarf gourami, *Colisa lalia* (Hamilton). *J. Aquacult. Trop.*, **4,** 111–123.

Siddiqui, A.Q., Al-Hafedh, Y.S. & Ali, S.A. (1998) Effect of dietary protein level on the reproductive performance of Nile tilapia, *Oreochromis niloticus* (L.). *Aquacult. Res.*, **29,** 349–358.

Singh, R. & Dhawan, A. (1996) Effect of formulated diets on the growth and ovarian maturation in common carp *Cyprinus carpio communis* Linn. *Indian J. Fish.*, **43,** 349–353.

Takeuchi, T., Watanabe, T., Ogino, C., Saito, M., Nishimura, K. & Nose, T. (1981) Effects of low protein-high calory diets and deletion of trace elements from a fish meal diet on reproduction of rainbow trout. *Bull. Jpn. Soc. Sci. Fish.*, **47,** 645–654.

Washburn, B.S., Frye, D.J., Hung, S.S.O., Doroshov, S.I. & Conte, F.S. (1990) Dietary effects on tissue composition, oogenesis and the reproductive performance of female rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, **90,** 179–195.

Watanabe, T. & Kiron, V. (1995) Red sea bream (*Pagrus major*). In: *Broodstock Management and Egg and Larval Quality* (Bromage, N.R. & Roberts, R.J., eds), pp. 398–413. Blackwell Science, Oxford.

Watanabe, T., Itoh, A., Kitajima, C. & Fujita, S. (1984a) Effect of dietary protein levels on reproduction of red sea bream. *Bull. Jpn. Soc. Sci. Fish.*, **50,** 1015–1022.

Watanabe, T., Ohashi, S., Itoh, A, Kitajima, C. & Fujita, S. (1984b) Effect of nutritional composition of diets on chemical components of red sea bream broodstock and eggs produced. *Bull. Jpn. Soc. Sci. Fish.*, **50,** 503–515.

Watanabe, T., Itoh, A., Satoh, S., Kitajima, C. & Fujita, S. (1985) Effects of dietary protein levels and feeding period before spawning on chemical components of eggs produced by red sea bream broodstock. *Bull. Jpn. Soc. Sci. Fish.*, **51,** 1501–1509.