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Effect of Different Dietary Protein Levels on Growth and Proximate Composition of the Eggs and Broodstock of Giant Murrel, *Channa marulius* (Forsskal)

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Authors' Contribution
MHR conceived and designed the study and wrote the manuscript. FA managed the brooders. GA formulated the feed and AR did chemical analysis. AH, SA and MA edited the manuscript. KJI analysed the data.

Key words
Body composition, Variable protein, Broodfish, Body growth, *Channa marulius*.

ABSTRACT

This research was performed to study the effect of different dietary protein levels on the growth and chemical conformation of broodfish and its eggs. There were three treatments (T_1, T_2 and T_3) and a control with three replicates in each group. Control group was exclusively fed on natural food composed of tilapia fry while fish in T_1 was fed on 30%, T_2 on 35% and T_3 on 40% protein diet, respectively. A total of 240 *Channa marulius* (945 g) were arbitrarily stocked in 12 earthen ponds (90 ft × 70 ft × 4 ft) @ 20 fish per pond. Fish were fed at 5% of their wet body weight two times per day. The net weight gained of broodstock was 103.65 g, 401.25 g, 484.85 g and 565.24 g in control, T_1, T_2, and T_3, respectively. Fish in T_3 yielded the highest weight, which was significantly higher than T_1, T_2 and control. Specific growth rates were not significant among treatments. Egg composition showed slight and statistically non-discernable variations. Same trend was observed when broodstock was subjected to analytical tests. Though there was slight increase in lipid and protein composition but statistically remained non-significant. Our studies suggest that *C. marulius* did respond to higher protein levels in growth and egg quality though with marginal improvements.

INTRODUCTION

Fish nutrition plays a pivotal role in the maintenance of brood stock quality and post-spawning performance which consequently affects breeding and rearing phenomena in fish ponds. Proper maintenance of brood stock is comparatively an expensive preposition, except few fish species, and is normally ignored (Brooks *et al.*, 1997; Izquierdo *et al.*, 2001). Fish hatcheries and commercial fish producers mainly depend on wild brood stock and/or their seed for stopgap arrangements, which is challenging for expansion of fish culture and mass seed production. However, some fish producers accepted such challenges and attempted to explore the role of protein, lipids, minerals, vitamins and ration size of different feeds to enhance reproductive potential and health of brood stock in certain fish species like sea bream, sea bass, common carp, rainbow trout, salmon and tilapia (Mourente and Odriozola 1990; Washburn *et al.*, 1990; Watanabe and Kiron, 1995; Fernandez-Palacios *et al.*, 1995, 1997; Zakeri *et al.*, 2009; Cerda *et al.*, 1994; Manissery *et al.*, 2001; Choubert and Blanc, 1993; Blom and Dabrowski, 1995; Choubert *et al.*, 1998; Pereira *et al.*, 1998; Hardy *et al.*, 1984; Eskelinen, 1989; Berglund, 1995; Christiansen and Torrissen, 1997; de Silva and Anderson, 1995; Cumaratunga and Mallika, 1991; Santiago and Reyes, 1993; Gunasekera *et al.*, 1995, 1996a, b, 1997; Siddiqui *et al.*, 1998).

Protein and lipid are considered as chief constituents of egg yolk which play a significant role in biosynthetic activities during early life stages of embryogenesis in

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specific, and post embryonic stages in general (Metcoff, 1986). Unlike aforementioned fish species, brood stock nutrition of snakeheads, in particular *Channa marulius* has not been given much attention it deserves. It is very hardy and can be successfully cultured in the areas which totally unfit for agriculture or for culture of other fish species. It exhibits reasonable growth rate and it is popular for its excellent flesh quality and also for its high consumption rate. Although, it is a carnivorous fish, its aquaculture strongly demands well balanced diet as continuous provision of live food will be impractical and uneconomical. Some researchers have devoted their efforts to this important issue but their investigative work was limited to *C. striata* and *C. punctata* (Hanifa and Sridhar, 2002; Marimuthu and Haniffa, 2007; Hafeez-ur-Rehman *et al.,* 2017). Nothing has so far been documented about development of feeding strategies including spawning of the murrels broodstock in Pakistan. Therefore, this research was planned to examine the effects of different levels of dietary protein on growth performance, biochemical composition of broodstock (*C. marulius*) and level of incorporation in these nutrients of eggs for proper development. Main objective of this study was to know its nutrient requirements both in somatic and reproductive tissue.

MATERIALS AND METHODS

Study site and duration

The experiment was conducted in earthen ponds (90 ft ×70 ft × 4 ft each) located in Fisheries and Aquaculture Department at Ravi Campus, University of Veterinary and Animal Sciences, Lahore-Pakistan. The trial was conducted for one year from June 2011 to May 2012. Experimental set up was arranged by following statistically Complete Randomized Design (Kothari, 2004). In this design, three dietary treatments with a control were studied. Each group including control had three replicates with 20 fish in each. The initial average body weight of *C. marulius* brood stock was 945.20±3.30 g, 952.83±2.884 g, 943.35±2.672 g and 947.88±2.551 g, in control, T_1, T_2 and T_3, respectively. Fishes in control group totally received natural food in the form of alive juvenile tilapia (78% water contents) at the rate of 40 % of wet body weight of *C. marulius* broodstock while treatments T_1, T_2, and T_3 received 30%, 35% and 40% protein diet, respectively.

Experimental diet

Each diet was formulated independently from well ground fish meal, soybean meal, maize gluten meal, rice polish, molasses, vitamin and mineral premixesin fixed and definite proportions to attain the desired protein level in each experimental group (Table I). Dry formulation was moistened, well kneaded, dried, then crumbled and sifted through 4 mm sieve to proportionate the particle size to fish mouth gape (3.5-4.0 mm). The feed was dispensed manually in each pond at 5% of the wet fish biomass twice a day at 09:00 and 16:30 hours (Javed and Sial, 1991).

Table I.- Ingredient composition of formulated feeds.

Ingredients	T_1 (30% CP)		T_2 (35% CP)		T_3 (40% CP) (Control)	
	%	CP	%	CP	%	CP
Fish meal	15	7.5	15	7.5	25	12.5
Soybean meal	20	8.4	20	8.4	20	8.4
Maize gluten	20	12	25	15	30	18
Rice polish	40	4.8	35	4.4	20	2.4
Molasses	4	-	4	-	4	-
Vitamins	1	-	1	-	1	-
Total	100	32.7	100	35.3	100	41.3

Proximate analysis

Experimental feeds, fish flesh and fish eggs were examined for biochemical constituents. The feed ingredients and the experimental diet were evaluated at the outset of studies (Tables II and III) while fishflesh and its eggs were analyzed at the end of the study (AOAC, 2005).

Growth indices

Fish were sampled from each pond fortnightly. Ten fish were arbitrarily chosen from each attempt. The sampled fish were weighed, measured, and then released back into the respective pond. In order to assess meat quality, samples of fish flesh ware taken from each replicate pond for chemical analysis. Growth performance and nutrient utilization were calculated according to the following formulae:

Net weight gain =
final average weight (g) - initial average weight (g)

Percent gain in weight =
net weight gain/initial weight x 100

Specific growth rate (SGR) was determined by the formula given by Hopkins (1992).

SGR (%) =ln (final wet body weight) – ln (initial wet body weight) x 100/number of days

Water quality

Water quality of the experimental ponds was determined by physical and chemical variables recorded fortnightly at 9.0 to 10.0 A.M. Physical and chemical parameters of pond water were determined by using YSI multi parameter analysis device and salinity was recorded by conductivity meter (Condi 330i WTW 82362 Weilheim, Germany).

Table II.- Chemical composition of feed constituents (% dry weight basis) used for *Channa marulius* brooder diet.

Percent (%) composition	Feed ingredients			
	Fishmeal	Soybean meal	Maize gluten	Rice polish
Dry matter	90.38±0.65	89.41±0.99	91.09±1.00	90.02±0.85
Crude protein	42.94±0.90	45.92±1.02	39.54±1.05	13.9±0.90
Crude lipid	12.38±0.86	0.72±1.03	1.03±1.02	10.9±0.95
Ash	31.7±0.75	8.96±1.23	7.27±1.05	15.69±0.80
Crude fiber	2.59±0.80	10.93±1.30	13.16±0.98	39.24±0.85
Nitrogen free extract	0.7±1.00	22.88±1.40	30.09±0.85	10.29±1.05
Gross energy (MJg^{-1})	17.26±1.02	18.99±1.20	19.12±0.75	18.6±1.06

Table III.- Chemical composition of formulated diet for brood fish(% ondry basis).

Percent (%) composition	T_1	T_2	T_3
Crude protein	30.5±0.52	35.21±0.43	40.34±0.64
Crude fibers	3.20±0.35	7.35±0.55	3.89±0.35
Crude lipid	3.75±0.40	4.02±0.60	5.52±0.56
Dry matter	86.30±0.52	89.55±0.42	92.07±0.50
Moisture	14.10±0.72	13.70±0.60	13.20±0.65
Ash	18.02±1.02	16.90±0.70	14.08±0.70
Nitrogen free extract (NFE)	45.30±0.95	36.52±0.85	26.24±0.85
Gross energy (MJg^{-1})	15.20±0.99	17.97±0.97	19.33±0.90

Statistical analysis

Fish length and weight data were statistically analyzed by using SAS 9.1 (SAS Institute, 2002) statistical package applying ANOVA. Differences among treatments were considered significant at $P<0.05$ and were compared by Duncan's Multiple Range Test to determine their significance across treatments.

RESULTS

Chemical composition of feed ingredients and feeds

Nutritional composition of feed ingredients and formulated feeds is given in Tables II and III. Crude protein content of feed ingredients ranged from 13.9% to 45.9% with gross energy contents of 17.26 to19.12 MJ g^{-1}. Similarly, three isolipidic diets contained protein 30.5% - 40.3% with dietary energy ranging from 15.2 MJg^{-1} to 19.3 MJg^{-1}.

Body weight gain

The initial average body weight of *C. marulius* brood stock was 945.20±3.30 g, 952.83±2.88 g, 943.35±2.67 g and 947.88±2.55 g, in control, T_1, T_2 and T_3, respectively. The final average body weight of *C. marulius* was 1048.85±4.20 g, 1354.08±3.15 g, 1428.20±2.89 g, and 1513.12±1.98 g, and net weight gained was 103.65±0.12 g, 401.25±0.14 g, 484.850.12 g and 565.24±0.141g in control, T_1, T_2 and T_3, respectively, which were significantly different ($P<0.05$) between fortnights and treatments (Tables IV and V). These results showed that T_3 yielded the highest weight followed by T_2, T_1, and control has the lowest. Differences were significant ($P<0.05$) in net weight gain and percent weight gain but values leveled off when we moved to specific growth rates.

Proximate composition of eggs in C. marulius

Crude protein, crude lipid, moisture and total ash remained uniform among all dietary treatments ($P>0.05$) (Table VI). There was slight increase in crude protein and moisture contents with a rise in protein levels in feed from 30% to 40% CP. Crude lipids were 8.02±0.85% in control while 10.06±0.10%, 9.85±0.12% and 10.27±0.42% in T_1, T_2 and T_3, respectively. Ash contents were found to be 0.80±0.03%, 0.62±0.02%, 0.35±0.04% and 0.24±0.01% in control, T_1, T_2 and T_3, respectively which decreased with increase in dietary protein levels. Crud fiber was not detected in any of the treatment and in control (Table VI).

Proximate composition of whole body

Crude protein (18.71%) and crude lipid (2.68) were observed in *C. marulius* fed on 40% protein and were significantly different from control, T_1 and T_2. Moisture contents were significantly higher in control than treatments, while there were significant differences in ash contents between control and treatments. Carbohydrates were significantly different among control and in all treatments except control. Carbohydrates were significantly different in control and treatments (Table VII).

Table IV.- Fortnightly average body weight gain(g) and total weight gain of *Channa marulius* brood stock in control and treatment ponds (June 2011-May 2012).

No. of Nettings	Fortnight	Control		T_1		T_2		T_3	
		Total body weight(g)	Fish weight gain(g)	Total body weight(g)	Fish weight gain(g)	Total body weight(g)	Fish weight gain(g)	Total body weight(g)	Fish weight gain(g)
Initial	01-06-11	945.20±3.30	-	952.83±5.88	-	943.35±5.39	-	947.88±6.56	-
1	15-06-11	949.30±5.15	4.10±1.10	972.08±6.10	19.25±2.32	965.40±7.19	22.05±2.99	973.18±6.29	25.30±3.29
2	30-06-11	953.35±4.12	4.05±1.15	992.88±8.17	20.80±3.37	988.45±5.39	23.05±2.88	1001.80±5.67	28.62±2.77
3	15-07-11	959.05±5.12	5.70±1.00	1014.10±7.14	21.23±3.45	1013.13±6.23	24.68±2.98	1032.15±7.34	30.35±3.68
4	30-07-11	964.3±5.55	5.25±1.25	1037.37±5.29	23.27±3.22	1040.15±8.34	27.03±3.99	1063.65±8.12	31.50±3.04
5	15-08-11	970.4±3.45	6.10±1.50	1061.30±7.95	23.93±3.56	1069.15±8.37	29.00±4.10	1096.40±9.24	32.05±3.49
6	30-08-11	976.6±3.45	6.20±2.00	1084.73±8.96	23.43±3.55	1098.85±8.93	29.70±3.77	1128.25±7.88	32.30±4.12
7	15-09-11	982.70±4.00	6.10±1.85	1106.02±8.24	21.29±4.22	1128.90±8.95	30.05±4.66	1160.07±9.29	31.83±4.23
8	30-09-11	987.95±4.30	5.25±1.50	1124.27±7.34	18.25±4.77	1155.60±9.65	26.70±5.22	1187.74±10.11	27.67±3.52
9	15-10-11	993.10±4.44	5.15±1.25	1139.49±9.81	15.23±3.22	1177.43±11.24	21.83±4.96	1211.80±9.88	24.06±5.22
10	30-10-11	996.45±3.55	3.35±1.00	1152.42±9.93	12.93±3.24	1194.78±8.66	17.35±2.99	1231.06±8.89	19.26±2.46
11	15-11-11	999.45±5.50	3.00±1.20	1162.14±7.68	9.73±2.89	1208.28±6.98	13.50±2.88	1248.39±9.66	17.33±2.97
12	30-11-11	1002.4±3.40	2.95±0.95	1171.39±13.34	9.25±4.12	1218.28±9.33	10.00±2.44	1261.64±11.90	13.25±4.11
13	15-12-11	1004.9±4.34	2.50±1.00	1180.62±11.33	9.23±3.59	1227.47±10.23	9.20±3.10	1273.24±8.96	11.60±3.22
14	30-12-11	1007.2±5.25	2.30±1.10	1189.54±15.44	8.93±3.22	1236.17±13.99	8.70±4.22	1283.96±12.66	10.72±4.11
15	15-01-12	1009.3±3.45	2.10±1.10	1198.74±9.66	9.20±3.11	1244.97±15.22	8.30±3.11	1294.96±14.61	11.00±3.22
16	30-01-12	1011.4±4.60	2.10±1.35	1209.04±12.95	10.30±4.13	1256.70±9.99	11.73±3.31	1308.28±12.33	13.33±2.99
17	15-02-12	1014.25±4.44	2.85±1.60	1220.39±13.45	11.35±4.11	1271.55±11.26	14.85±3.24	1324.63±9.88	16.35±4.33
18	28-02-12	1017.75±4.40	3.50±1.80	1233.80±14.55	13.41±3.91	1289.63±12.34	18.08±4.55	1347.39±10.66	22.76±4.43
19	15-03-12	1021.85±4.30	4.10±2.00	1250.16±9.88	16.36±4.76	1310.57±12.16	20.94±3.39	1372.49±15.11	25.10±5.11
20	30-03-12	1026.65±4.45	4.80±2.10	1269.66±14.33	19.50±4.19	1333.19±12.46	22.63±3.98	1399.98±9.66	27.49±5.23
21	15-04-12	1031.7±3.55	5.05±2.50	1289.91±15.25	20.25±5.23	1356.79±14.15	23.60±5.12	1428.61±14.99	28.63±6.14
22	30-04-12	1037.2±3.45	5.50±2.25	1311.41±14.99	21.50±5.33	1381.10±15.26	24.31±6.27	1458.01±15.11	29.40±4.99
23	15-05-12	1042.9±4.00	5.70±2.45	1333.23±12.68	21.83±4.55	1405.90±13.98	24.80±3.88	1486.67±14.33	28.67±6.11
24	30-05-12	1048.85±4.20	5.95±2.50	1354.08±13.22	20.85±5.55	1428.20±15.44	22.30±5.22	1513.12±11.99	26.45±5.99

Dissimilar superscripts horizontally show significant difference among each other at probability level of P≤0.05.

Table V.- Weight gain, SGR, and % survival of *Channa marulius* at the end of trial.

Parameters	Control	T_1	T_2	T_3
Initial weight (g)	945.20±3.30	952.83±2.88	943.35±2.67	947.88±2.55
Final weight (g)	1048.85±4.20	1354.08±3.16	1428.20±2.89	1513.12±1.99
Net weight gain (g)	103.65±0.12^{d}	401.25±0.14^{c}	484.85±0.13^{b}	565.24±0.14^{a}
Percent weight gain (%)	10.96±1.10^{d}	42.11±1.67^{c}	51.39±1.55^{b}	59.63±1.47^{a}
Specific growth rate (% wt. gain/day)	0.03±0.003^{b}	0.04±0.01^{a}	0.05±0.005^{a}	0.06±0.002^{a}
Survival (%)	70±4.20	100±2.88	100±2.76	100±2.45

Dissimilar superscripts horizontally show significant difference among each other at probability level of P≤0.05.

Table VI.- Chemical composition of the eggs of brooder *Channa marulius* supplied with diets having different protein levels.

Parameters	Control	T_1	T_2	T_3
Egg weight (g)	10.20±0.90^{d}	15.61±1.29^{c}	18.02±0.53^{b}	19.81±1.31^{a}
Crude protein (%)	63.20±1.03^{c}	69.49±0.93^{b}	71.25±1.38^{a}	72.56±1.75^{a}
Crude lipids (%)	8.02±0.85^{b}	10.06±0.10^{a}	9.85±0.12^{a}	10.27±0.42^{a}
Moisture (%)	5.85±0.03^{b}	6.02±0.02^{b}	6.08±0.02^{b}	6.50±0.21^{a}
Total ash (%)	0.80±0.03^{a}	0.62±0.02^{b}	0.35±0.04^{c}	0.24±0.01^{d}
Crude fiber (%)	ND	ND	ND	ND

ND, not detectable.

Table VII.- Body composition of whole *Channa marulius* brood stock fed on different dietary protein levels.

Parameters	Control	T_1	T_2	T_3
Crude protein (%)	16.50±0.12[bc]	17.80±0.02[b]	17.92±0.02[b]	18.71±0.20[a]
Crude lipids (%)	1.98±0.01[b]	2.03±0.01[b]	2.56±0.01[b]	2.68±0.07[a]
Moisture (%)	75.10±0.17[a]	74.38±0.18[a]	73.86±0.05[b]	73.69±0.04[b]
Total ash (%)	2.10±0.10[b]	2.11±0.07[a]	2.27±0.17[a]	2.25±0.13[a]
Carbohydrates (%)	4.25±0.03[a]	3.86±0.05[b]	3.38±0.13[c]	2.46±0.24[d]
Crude fiber (%)	ND	ND	ND	ND

ND, not detectable.

Table VIII.- Physico-chemical parameters of the experimental pond water.

Parameters	Control	T_1	T_2	T_3
Water temperature (°C)	29.50±1.30	29.32±1.30	31.10±2.61	30.20±1.72
Dissolved oxygen (DO) mg/l	5.80±0.95	5.40±0.95	5.32±0.65	5.20±1.12
pH	7.40±0.14	7.50±0.14	7.63±0.12	7.20±0.21
Salinity (mg/l)	0.82±0.08	0.83±0.08	0.82±0.06	0.82±0.05
Total dissolved solid (TDS)	1622.05±75.30	1624.05±75.30	1610.23±70.20	1540.10±65.71
Electrical conductivity (EC)	2.15±0.22	2.12±0.22	2.20±0.54	2.10±0.12

Water quality

Water quality parameters of control and treated ponds are given in Table VIII. The water temperature was maintained at 30.2±0.2°C. Dissolved oxygen w 5.5±0.3 mg/l. Ph was kept constant at 7.5 and salinity was 0.8±0.03%. Total dissolved solids (TDS) and electrical conductivity were 1620±1.5 mg/l and 2.2±0.1 mmho/cm, respectively.

DISCUSSION

In the present study, the dietary protein level of 40% with 19.33 MJg^{-1} energy was adequate to optimize growth, feed conversion and egg quality alongwith body composition of *C. marulius* broodstock. This level is in agreement with the reported values for variety of fish species (Harper, 1988; El-Sayed *et al*., 2003; Chong *et al*., 2004). They stated that better protein levels in broodstock feeds correlates with level of gonad development and quality of spawn. Moreover, El-Sayed and Kawanna (2008) described that *Tilapia nilotica* brood stock showed significant growth increments with rise in energy and dietary protein levels. These differences were more prominent when protein level was increased from 30% to 40% and the energy level from 15.20±0.99 MJ/Kg to 19.33±0.90 MJ/Kg at each level. Some other researchers who performed similar type of work on different fish species like *Dicentrarchus labrex* (Cerda *et al*., 1994) *Oreochromis niloticus* (El-Sayed *et al*., 2003) *Labeo rohita* (Afzal *et al*., 2005) and *Ictalurus punctatus* (Sink and Lochmann, 2008) documented similar findings which further support our observations on *C. marulius*. Our studies also suggest that 30% protein level is not enough for optimal growth and performance of other bodily functions in *C. marulius*. Energy is another important nutritional parameter, which retards growth if provided in insufficient amounts (NRC, 1993). Chow and Watanabe (1988) recommended that adult fishes need more energy for maintenance of their body functions, development of gonads, fecundity and better performance of hatchlings.

In this study, eggs in T_3 displayed maximum percentage of crude protein, crude lipid, moisture and total ash contents. These values corroborates with those of Sotolu and Kigbu (2011) who observed increased body protein and much better gonad development in catfish up to 40% protein inclusion in formulated feeds. Evidence to support this is available in studies of Gunasekara *et al*. (1996), Muchlisin *et al*. (2006) and Sotolu and Kigbu (2011). They suggested that 40% protein level in formulated feeds is advisable for proper growth and reproductive performance of this and other catfishes. The chemical analysis of fish meat indicated that protein, lipid, ash, carbohydrates and moisture contents were higher in T_3 as compared to T_2, T_1 and Control though statistically these differences were insignificant (P>0.05). The values of protein contents, moisture and lipid were higher in T_3 than those of other groups, which may be due to higher percentage of protein inclusion levels. Viola *et al*. (1982) while working on similar type of studies reported that nutrient levels present in feed are depicted in body composition. High concentration of protein and lipid results in significant increase in weight of the carps. In this study, it was observed that if lipid contents are found in low concentration, the protein contents will be lower and the growth will be lower as well. These findings corroborate with the observation of Jirasek *et al*. (1984). They mentioned that high feeding rate or daily ration level increases fish weight. In addition, it enhances the feed conversion as protein and lipid contents are increased in carps. Zeitler *et al*. (1984) with similar findings described a significant relationship between protein and fat contents. In the current research, the highest protein level was 18.22±0.22 % in T_3, 17.92±0.02 % in T_2 and 17.80±0.02 % in T_1 while the lowest was 16.50±0.12 % in Control. Moisture in control pond (30.0% CP) were high in which lower growth was found as compared with all other treatments. This might have been due to low protein deposition and accumulation of surplus water. Our findings corroborate with those of Riaz (2008) who reported that higher moisture contents in fish body related to low protein levels in the diet, experienced during predator-prey relationship studies in which *C. marulius* was deprived of sufficient live feed (tilapia). The present findings are also very close to those of Love (1980) who examined that fish consumed the fat from the liver during starvation and then started to draw muscle protein with depletion of available energy sources. Water contents replaced the exhausted protein reserves during this scenario.

CONCLUSION

The present study showed maximum feed utilization, growth, and the best egg quality and body composition in 40% CP containing feed group, followed by 35% CP, 30% CP and control. These studies suggest that 40% protein diet is required for better growth, health and egg development in *C. marulius*.

Statement of conflict of interest

Authors have declared no conflict of interest.

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