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INVESTIGATION OF CHANGES IN WATER QUALITY PARAMETERS BY INCREASING THE LEVEL OF DIETARY PROTEIN IN INTENSIVE POLYCULTURE OF CARPS

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خلاصہ

Carps مچھلیوں (Catla catla، Labea rolita اور Cirhinus migala) کے ایک Intensive polyculture میں مختلف پروٹین ماخذ اجزاء کے استعمال سے پانی کے خواص پر اثرانداز ہونے والی تبدیلیوں کا ایک سال تک مطالعہ کیا گیا۔ اس تحقیق کا مقصد یہ معلوم کرنا تھا کہ آیا کہ کسی مخصوص پروٹین ماخذ جز میں 45% سے 25% کمی کا اثر پانی کے خواص اور پیداوار پر ہوتا ہے یا نہیں۔ اگرچہ ان پروٹین ماخذ اجزاء کی مقدار میں اضافہ امونیا پر نمایاں طور پر اثرانداز نہ ہو سکا لیکن دیگر عوامل گہرے متاثر ہوئے۔ نتائج سے یہ بات اخذ کی گئی کہ اجزا کی خاصیت اور مقدار ایک آبی ماحول کے ماحولیاتی عوامل پر گہرا اثر رکھتی ہیں۔

Abstract

Changes in water quality parameters of an intensive polyculture of carps (*Catla catla*, *Labeo rohita* and *Cirhinus mrigala)* by different dietary protein ingredients were assessed for a period of one year. The aim of this research was to evaluate whether decrease in the level of protein from 45% to 25% of a certain ingredient could alter various water quality parameters and hence production. Although ammonia was not significantly affected by increasing the level of these protein based ingredients but all other parameters were greatly influenced on ecology in raceways treated with different diets. The results attributed that amount and nature of ingredients; certainly have huge impact directly or indirectly on environmental condition of a pond ecosystem.

Introduction

Intensive aquaculture requires an exchange of large amount of water to lower down nitrogen input and toxic metabolites which are associated to the amount of formulated feed and its level of protein (Brune and Drapcho, 1991; Westerman et al, 1993; Hopkins et al, 1995). Nitrogenous waste, which is produced by the metabolic breakdown of protein to liberate energy, is necessary to remove from water to avoid ecosystem degradation (Sedgwick, 1979). However, excess amount of dietary protein can easily poor down water quality of ponds (Kureshy and Davis 2002); but on the other hand it is the most essential ingredient from the standpoint of growth, among all ingredients of formulated feed (Lim, 1997).

Pellet quality is greatly influenced by the inclusion level of its ingredients. Pellet durability is enhanced by increasing the concentration of protein, whereas the result is vice versa in case of oil content. Quantity of oil above 7.5% reduces pellet durability drastically (Briggs et al. 1999).

By increasing the level of protein of feed, the cost also increases (Lee and Wickins 1992). Majority of formulated animal diets are prepared on a least-cost basis to decrease costs of the production. Feed manufacturer can minimize cost of the feed by utilizing a variety of locally available cheap ingredients to fulfill the nutrient requirements of the animal (Briggs et al., 1999).

The protein level of carp feed has been investigated for a variety of carps. In most of the cases inexpensive alternative sources of protein such as by-product meals, grains and soluble may reduce pellet quality to a considerable level (Koch, 2007; Buchanan and Moritz, 2009), while, addition of expensive feed constituents, such as soy protein isolate, cellulose, and soybean meal improve pellet quality to a high extent (Buchanan and Moritz, 2009; Briggs et al., 1999).

The aim of this research was to pursue a study to evaluate the effects of various animal and plant based protein ingredient on water quality parameters and whether decrease in the level of protein from 45% to 25% could alter various water quality parameters and production. These findings are critical for the reason that feed nutritionists formulate diets exclusively on the basis of least-cost which may unintentionally reduce pellet quality.

Materials and Methods

2.1. Experimental design

Twelve experimental diets were formulated by using animal and plant based protein ingredients i.e. fish meal (FM), all parts chicken meal (APCM), corn gluten (CGM) and soybean meal (SBM). Each ingredient was added in three different inclusion levels (25%, 35% and 45%) with various other additives and inclusions to formulate a balanced fish diet. By increasing the level of protein some other constituents were altered among the formulated diets. (Table: 1). Powdered ingredients were mixed thoroughly with canola oil. The dough after passing through a pellet feed maker, dried out at room temperature.

Table 1: Formulation of diets and percentages of ingredients per 100 g with proximate values.

	FM			APCM			CGM			SBM		
	25%	35%	45%	25%	35%	45%	25%	35%	45%	25%	35%	45%
Ingredients (%)												
Fish meal	25	35	45	-	-	-	9.13	22.40	35.67	8.69	21.79	34.87
All parts chicken meal	-	-	-	25	35	45	-	-	-	-	-	-
Corn gluten meal	1.16	16.69	30.98	1.74	16.90	32.06	25	35	45	-	-	-
Rice polish	63.83	38.30	14.00	63.24	38.08	12.93	55.85	32.59	9.31	56.29	33.20	10.12
Soybean meal	-	-	-	-	-	-	-	-	-	25	35	45
Starch	5	5	5	5	5	5	5	5	5	5	5	5
Canola oil	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Vitamins and mineral mixture	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Proximate composition (%)												
Crude protein	24.99	35.2	44.98	24.98	34.98	44.99	24.98	34.98	44.98	24.98	34.98	44.98
Crude fat	15.19	12.74	10.42	16.85	15.12	13.39	13.51	11.48	9.43	14.26	12.52	10.79
Crude fiber	2.56	2.48	2.38	2.95	3.02	3.07	3.29	3.05	2.81	3.74	3.69	3.64
Ash	13.7	13.42	13.25	12.02	11.13	10.24	9.83	10.4	10.98	10.61	11.49	12.37
Nitrogen – free extract	42.99	35.6	28.39	42.63	35.17	27.74	47.82	39.54	31.23	45.82	36.77	27.64
DE (K cal/Kg)	3109.9	3186.4	3263.4	3231.2	3356.8	3482.6	3018.5	3114.5	3209.9	3037.6	3141.2	3244.9
GE (K cal/Kg)	4520.4	4554.3	4587.6	4655.9	4824.1	4832.7	4565.3	4590.2	4614.3	4552.3	4572.0	4591.3

FM = Fish meal, APCM = All parts chicken meal, CGM = Corn gluten meal, SBM = Soybean meal

Fingerlings of *Catla catla, Labeo rohita* and *Cirhinus mrigala* were stocked in rectangular concrete raceways (22′×50′) with the ratio of 33:33:34 respectively (Wahab et al. 2002).

2.2. Estimation of Physico - chemical parameters

Physico-chemical parameters estimate the water quality by influencing the biological productivity of the pond. Water temperature, pH, dissolved oxygen, total suspended solids, total dissolved solids, total alkalinity, total hardness and ammonia were the main parameters which were estimated daily and the average was noted on monthly basis. Tables show mean annual values of these factors. The temperature and dissolved oxygen of the water was recorded with the help of Dissolved Oxygen Meter (HI-9146) by fixing the temperature factor at 0˚C unit. To measure the pH (hydrogen ion concentration) the microprocessor pH meter (HANNA-HI-8520) was used after setting its range at pH point.

Total dissolved solid were measured by TDS meter (HANNA-HI-98302) and was used after setting its range at “TDS” point.

Table 2: Mean, maximum and minimum annual values of various Physico-chemical parameters of water received various protein levels of different animal and plant based ingredients in intensive polyculture system.

		Temp (˚C)	pH	DO (mg/l)	TS (mg/l)	TDS (mg/l)	T. Alk (mg/l)	T. Hd (mg/l)	Ammonia (mg/l)
FM 25%	Mean	21.6	8.2	6.5	1398.4	1302.9	393.6	168.1	0.06
	±SE	±2.30a	±0.04bcd	±0.35ab	±20.7a	±15.7a	±9.87d	±5.15de	±0.01bc
	Max	30.3	8.4	8.4	1483.2	1395	440.5	195	0.16
	Min	10.2	8.0	5.2	1280.5	1240	353	140	0.01
FM 35%	Mean	21.2	8.1	6.8	1372.7	1295.6	426.7	183.9	0.05
	±SE	±2.37a	±0.04de	±0.27a	±20.2ab	±17.4a	±15.9ab	±5.64bcde	±0.01bc
	Max	30.3	8.4	8.3	1478.2	1365	462.5	215	0.16
	Min	10.3	7.9	5.6	1270	1190	365.5	162	0.01
FM 45%	Mean	21.1	8.1	6.7	1417.3	1269.1	438.5	187.3	0.05
	±SE	±2.34a	±0.04d	±0.29a	±21.4a	±16.2a	±9.65abc	±4.26bcd	±0.00bc
	Max	30.5	8.4	8.4	1518.5	1350	490	210	0.09
	Min	10.3	8.0	5.1	1244.2	1180	370.5	160	0.01
APCM 25%	Mean	21.1	8.1	6.7	1396.2	1287.5	419.5	167.3	0.05
	±SE	±2.37a	±0.05de	±0.27a	±22.6a	±19.0a	±14.8bcd	±4.26e	±0.01bc
	Max	30.1	8.4	8	1550.6	1380	531	190	0.14
	Min	10.1	7.8	5.2	1284.3	1130	349.5	140	0.02
APCM 35%	Mean	20.9	8.2	6.7	1400.8	1291.6	455.7	180.5	0.05
	±SE	±2.31a	±0.05cd	±0.25a	±16.8a	±14.8a	±11.1a	±4.11cde	±0.00bc
	Max	30.3	8.4	8.1	1486.7	1390	510.5	210	0.09
	Min	10.5	7.8	5.3	1308.5	1230	381.5	163	0.01
APCM 45%	Mean	20.9	8.1	6.7	1390.1	1294.1	447.2	189.3	0.05
	±SE	±2.35a	±0.05de	±0.22a	±20.5a	±12.2a	±6.07ab	±3.94bc	±0.01bc
	Max	30.5	8.4	8	1494.6	1350	470.5	215	0.14
	Min	10.2	7.8	5.5	1306.2	1250	395.5	170	0.01
CGM 25%	Mean	21.4	7.9	6.0	1100.2	981.4	402.5	166.1	0.06
	±SE	±2.12a	±0.14e	±0.54abc	±23.1d	±27.2d	±9.70d	±6.56e	±0.02bc
	Max	30.1	8.8	9.1	1225	1135	440	195	0.30
	Min	12	7.3	3.5	946	833	350	116	0.00
CGM 35%	Mean	21.5	8.1	5.7	1208.7	1075.8	402.5	183.3	0.05
	±SE	±2.14a	±0.07de	±0.54bcd	±28.0c	±31.2c	±9.4d	±7.28bcde	±0.01c
	Max	30.6	8.5	10.7	1352	1242	440	222	0.16
	Min	11.8	7.4	3.8	1025	878	320	138	0.01
CGM 45%	Mean	21.6	8.1	5.0	1316.3	1169.5	411.6	201.5	0.06
	±SE	±1.99	±0.10cd	±0.41d	±46.9b	±44.9b	±10.9cd	±9.14b	±0.01bc
	Max	30.9	8.6	7.9	1561	1364	450	270	0.13
	Min	12.4	7.2	3.8	1104	923	330	152	0.01
SBM 25%	Mean	21.7	8.4	5.5	1147.5	1122.7	407.5	256.2	0.08
	±SE	±2.01a	±0.088abc	±0.20cd	±32.1cd	±27.1bc	±7.08d	±9.85a	±0.00abc
	Max	30.9	8.8	6.8	1325	1280	460	310	0.13
	Min	12.7	7.7	4.6	960	935	380	200	0.03
SBM 35%	Mean	21.8	8.4	5.9	1175.0	1078.4	404.1	260.2	0.10
	±SE	±2.00	±0.11ab	±0.20abcd	±38.9cd	±30.1c	±5.83d	±8.93a	±0.01ab
	Max	30.4	8.9	6.9	1350	1230	440	305	0.15
	Min	12.9	7.5	4.6	950	840	370	215	0.06
SBM 45%	Mean	22.2	8.4	6	1155.8	1132	400.8	247.1	0.11
	±SE	±2.10a	±0.09a	±0.16abc	±34.1cd	±27.2bc	±6.57d	±11.3a	±0.02a
	Max	30.8	8.8	6.8	1291	1280	440	310	0.30
	Min	12.5	7.6	4.9	945	950	360	182	0.04

Temp=Water Temperature (°C), DO=Dissolved oxygen (mg/l), TS=Total solids (mg/l), TDS=Total dissolved solids (mg/l), T.Alk=Total alkalinity (mg/l), T.Hd=Total hardness (mg/l).
FM = Fish meal, APCM = All parts chicken meal, CGM = Corn gluten meal, SBM = Soybean meal
Values are means ± SE of one year.
Means in a column followed by different letter were significantly different from each other at $P = 0.05$ by the Fisher's least-significant-difference (LSD) test.

The total alkalinity and total hardness were determined by MERCK chemical test kits for testing water and waste waters. In present research work concentrations of ammonia in raceways were determined by following the method of John and Craig (2004).

2.3. Statistical analysis of experimental data

The obtained data was subjected to statistical analysis by using MINTAB Release 16. The comparison of mean values for various parameters was carried out by using two-way analysis of variance. Pearson′s coefficient was also performed to find out relationships among growth variables.

Results

Table 2 shows mean annual values of water temperature, pH, dissolved oxygen, total suspended solids, total dissolved solids, total alkalinity, total hardness and ammonia. Fish being a cold blooded animal is directly or indirectly affected by the temperature of its surrounding water in terms of growth rate, feed consumption and other body function. In the present result trial the mean range of temperature was between 20.9 to 22.2℃ throughout the whole duration of the study for all formulated diets. In water, traces of some substances may convert the neutral pH of water into acidic or alkaline in reactions. Mean values of pH were within 7.9 to 8.4. Mean values of other parameters such as; dissolved oxygen, total solids, total dissolved solids, total alkalinity, total hardness and ammonia were within the range of 5.0 - 6.8 mg/l, 1100.2 - 1417.3 mg/l, 981.4 - 1302.9 mg/l, 393.6 - 455.7 mg/l, 166.1 - 260.2 mg/l and 0.05 - 0.11 mg/l respectively. In all diets except temperature, all parameter were significantly different from each other at $P = 0.05$ by the Fisher's least-significant-difference (LSD) test (Table 2).

It was revealed by one-way analysis of variance that except SBM, all diets showed significant relationships among water quality parameters and levels of protein (Table 3). Total alkalinity and total hardness were significantly affected by levels of protein in the raceways receiving fish meal based diets, while total hardness was greatly altered by varying level of APCM. Great influence of protein ingredient was also observed by CGM based diets in which total solids, total dissolved solids and total hardness were significantly associated with increasing level of protein. Ammonia was not significantly affected by increasing the level of these protein based ingredients, as might be expected from the digestibility of protein in high amount.

Table 3: One-way analysis of variance for various Physico-chemical parameters of water enriched with different formulated diets.

Variables	FM		APCM		CGM		SBM	
	R-Sq (%)	P-Value	R-Sq (%)	P-Value	R-Sq (%)	P-Value	R-Sq (%)	P-Value
Temperature (°C)	0.09	0.985	0.00	0.999	0.01	0.998	0.14	0.977
pH	4.02	0.508	1.95	0.722	7.54	0.274	1.09	0.835
Dissolved oxygen (mg/l)	1.35	0.799	0.21	0.967	6.36	0.338	9.64	0.188
Total solids (mg/l)	6.58	0.325	0.43	0.931	37.65	0.000**	0.97	0.852
Total dissolved solids (mg/l)	6.60	0.324	0.28	0.954	30.15	0.003**	5.89	0.367
Total alkalinity (mg/l)	27.36	0.006**	14.65	0.073	1.65	0.760	1.56	0.771
Total hardness (mg/l)	19.91	0.026**	30.59	0.002**	24.03	0.011**	2.61	0.646
Ammonia (mg/l)	1.77	0.745	0.54	0.914	0.11	0.722	5.10	0.421

** = Significant;
FM = Fish meal, APCM = All parts chicken meal, CGM = Corn gluten meal, SBM = Soybean meal

All physico-chemical features of water were interrelated with each other in fish ponds and raceways. Strong correlations were observed among variables (Table 4). Dissolved oxygen was highly affected by temperature, ammonia, total solids and total dissolved solids, while total hardness and total alkalinity were altered by amount of total solids and total dissolved solids. A significant correlation was also observed between pH and ammonia.

Table 4: Pearson correlation of various Physico-chemical parameters of water enriched with different formulated diets.

	Temp (˚C)	pH	DO (mg/l)	TS (mg/l)	TDS (mg/l)	T. Alk (mg/l)	T. Hd (mg/l)
pH	-0.160 (0.055)						
DO (mg/l)	-0.193 (0.020)**	-0.053 (0.527)					
TS (mg/l)	-0.015 (0.857)	0.104 (0.214)	0.454 (0.000)**				
TDS (mg/l)	0.038 (0.652)	0.027 (0.214)	0.474 (0.000)**	0.823 (0.000)**			
T. Alk (mg/l)	-0.006 (0.944)	-0.135 (0.108)	0.008 (0.920)	0.260 (0.002)**	0.213 (0.011)**		
T. Hd (mg/l)	0.111 (0.186)	0.146 (0.081)	-0.088 (0.292)	-0.249 (0.003)**	-0.170 (0.042)**	0.034 (0.689)	
Ammonia (mg/l)	0.477 (0.000)**	0.666 (0.000)**	-0.103 (0.221)	-0.126 (0.132)	0.035 (0.673)	-0.029 (0.123)	0.157 (0.060)

*Pearson correlation
(P-Value)
** = Significant;
*Temp=Water Temperature (°C), DO=Dissolved oxygen (mg/l), TS=Total solids (mg/l), TDS=Total dissolved solids (mg/l), T.Alk=Total alkalinity (mg/l), T.Hd=Total hardness (mg/l).

Discussion

Inclusion level of feed ingredients and feeding regime play a vital role in the dynamics of associated parameters of pond water ecosystem such as temperature, pH, oxygen, ammonia, nitrates, nitrites and chlorine (Bociec et al. 2011). The variation intensity generally depends upon the quantity of input i.e. fertilizers or formulated diets.

In the present research trial, all parameter were significantly dissimilar from each other at P = 0.05 by the Fisher's least-significant-difference (LSD) test except temperature. Although ammonia was not significantly affected by increasing the level of these protein based ingredients but all other parameters were greatly influenced on ecology in raceways treated with different diets. These parameters may also be altered more or less by the excretion of fecal matter as a result of digestion on other side.

Likewise the tested levels of protein in present feeding trial, the influence of three different levels of dietary protein i.e. 25%, 35% and 45% from soybean meal, blood meal and bone meal with yellow maize on water quality of pond and feed utilization were also evaluated by Bechara et al. (2005). Higher protein levels gave higher values of alkalinity, electrical conductivity and nitrate while lower levels gave better results in term of growth, feed intake and carcass composition.

Dulic *et al.,* (2010) worked on the effects of different formulated feeds from unprocessed cereals and other inclusions on water quality of intensive polyculture of carps. The work of Winowiski (1988) and Stevens (1987) also supported the strong influence of protein in diet. Increase in the quantity of wheat from 0 to 60%, resulted in an augment in pellet durability indices (PDI) from 32 to 73 respectively (Winowiski, 1988). In the study by Stevens (1987), feed with 72.4% wheat improved PDI an average of seven points as compared to 72.4% corn. These finding clearly depict the significant role of protein in pellet quality. Influence of ingredients on pellet quality was also assessed by Wood (1987), who produced considerably stronger pellets by the inclusion of raw protein than by that of denatured protein apart from conditioning scheme (with or without steam).

Excess protein may reduce water quality by increasing the amount of nitrogen in wastes (Thompson et al. 2004; Tomasso, 1994). In the present research trial, no significant differences were evident in the concentration of ammonia by increasing the level of protein, as might be expected from the high protein digestibility. This result coincides with the findings of McIntosh et al., (2001). They accounted no significant differences in water quality and total ammonia by diets with 21% and 31% protein, when fed to *Litopenaeus vannamei.* In contrast, a significant increase in ammonia with 45% protein diet as compare to 20% protein diet was reported by Samocha et al. (1998a, b) when fed to *Penaeus setiferus*.

The result of this finding attributed that quantity and nature of ingredients, certainly have huge impact on environmental condition of a pond ecosystem but excess protein may be regulated by proper removal of organic matter. In intensive culturing, it is necessary to regulate water quality by selecting a suitable protein ingredient in optimum level to sustain ecosystem healthy.

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