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Dietary Crude Protein, Citric Acid and Microbial Phytase and Their Interacts to Influence Growth Performance, Muscle Proximate Composition and Hematocrite of Common Carp, *Cyprinus carpio* L, Juveniles

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Abstract: A 2×2×2 factorial experiment was conducted to evaluate the effect of citric acid (CA), phytase (P) supplementation and crude protein (CP) on growth, muscle composition and hematocrite (Hct) of common carp, *Cyprinus carpio* L, Juveniles. Two isoenergetic diets were formulated using plant-based ingredients to contain either subnormal protein (25%) or normal protein (35%) containing two levels of CA (0 and 30 g kg^{-1}) and two levels of P (0 and 500 IU kg^{-1}) were fed to triplicate groups of fish for 8 weeks. The results revealed that adding CA increased ($P<0.05$) the weight gain (WG), specific growth rate (SGR) and decreased ($P<0.05$) food conversion rate (FCR) whereas dietary CP and P supplementation no affect ($P>0.05$) growth performance. In addition, CP as well as P supplementation and their interaction ($P>0.05$) don't affected muscle composition and Hct. No differences ($P>0.05$) were detected in moisture, protein of muscle sample among treatments, but lipid content was reduced ($P<0.05$) while ash content increased ($P=0.035$) by CA. Results of the present study indicate that CA can improve growth in common carp.

Key words: *Cyprinus carpio* · Crude protein · Phytase · Citric acid · Growth · Hematocrite

INTRODUCTION

Growth rate, one of the most important parameters determining the economic efficiency of commercial fish culture, is influenced by several factors [1]. Feed cost and feed efficiency are among the prime factors that control the farm economy. Due to the limited and unpredictable supply of fish meal, attention has been given to the possibility of increasing the inclusion of vegetable protein sources in diets for carnivorous fish. Soybean meal (SBM) is considered a promising alternative protein source because of its availability, high crude protein (CP) content and low P content relative to fish meal. However, the content of anti-nutritional factors such as phytic acid is a major impediment in efforts to increase the use of SBM in diets for carnivorous fish [2]. A major portion of P (60-70%) in vegetable protein ingredients is bound in phytic acid. Like other monogastric organisms, carnivorous fish lack the enzymes capable of hydrolysing P from phytic acid [2]. This might reduce the availability of P when dietary plant meal increases [3], as well as the availability of other minerals such as zinc, magnesium and calcium (Ca) [4, 5].

The addition of microbial phytase in the diet has been shown to increase the availability of phytate-P in fish. However, P availability has been reported to be no more than 60% [6, 7]. Jongbloed [8] had reported that lower intestinal pH increases the solubility of P and improves its absorption in the small intestine. Besides these, addition of organic acids in aquafeed may act as a chelating agent and bind with various cations along the intestine [9], resulting in increased intestinal absorption of minerals. Addition of CA to diets has been reported to be able to increase the release of P from phytate *in vitro* [10]. Reduced intestinal pH increases the solubility of P and phytate and improves P absorption in the small intestine [9-11]. In addition to the effect on intestinal pH, supplementary organic acid can bind various cations along the intestine and may act as chelating agent resulting in increased intestinal absorption of minerals [12]. There has been considerable research regarding the effect of dietary acidification on mineral utilization in terrestrial animals, yet studies on fish have been very limited [13]. A recent study has shown that the addition of CA to the feed improved P and Ca of content in Beluga fed SBM diets [14]. Experiments with pigs

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have shown that CA promotes growth performance (Sugiura *et al.* 2001). Citric acid supplementation to rainbow trout diet chelates Ca and P, which increases the solubility of Ca and P and improves mineral utilization [15]. Therefore, the present study aimed to investigate the effect of dietary CA and P supplementation and CP dietary on growth, muscle composition and Hct of common carp Juveniles.

MATERIALS AND METHODS

Diets and Experimental Design: Two isoenergetic diets (Table 1) were formulated using plant-based ingredients to contain either subnormal protein (25%) or normal protein (35%) containing two levels of CA (0 and 30 g kg^{-1}) and two levels of P (0 and 500 IU kg^{-1}) were fed to triplicate groups of fish for 8 weeks. Diets were supplemented with 0 and 30 gm kg^{-1} of CA (Table 1). The CP content of the diets was balanced by adjusting barley and wheat meal levels. Fish oil was added to keep lipid and gross energy constant in all treatment. Citric acid and oil were added to the dry ingredients and dough was prepared with the required amount of water. The required amount of microbial phytase was dissolved in 50 ml of distilled water and sprayed over one kg of the finished diet as reported by Jackson [16]. The basal diet was sprayed with distilled water to maintain an equal level of moisture. Pellets (2 mm) were prepared using a hand pelletiser, air-dried to about 100 gm kg^{-1} moisture and sealed in vacuum-packed bags and frozen at -18°C until use.

Fish Experimental Condition and Feeding: Fingerlings (average weight of 10.6±1.5 gm) were obtained from Sturgeon Propagation and Rearing Complex of Shahid Marjan (Gorgan, Iran). Each dietary treatment was replicated three times with 10 fish per replicate. Fish were randomly distributed into aquariums (70 × 40 × 30 cm) of 80 L capacity. Important water quality parameters such as temperature, pH and oxygen were monitored daily and adjust by aeration and heater. All the measurements were observed to be within the acceptable limit for culture. Average daily water temperature was 28.6±1.5°C. fish were fed to satiation and that occurred with an amount of somewhere between 3 and 4% of bi-weekly body weight measurements for four times a day by hand (06:00, 12:00, 18.00, 24:00) over 8 weeks. There was any feed refusal at the all treatments.

Table 1: Formulation of experimental diets (g kg^{-1} diets)

Ingredients	CP 250	CP 350
Kilka fish meal (645)X	110	110
Soybean meal (485)X	240	440
Corn meal	220	120
Wheat meal	340	240
Vitamin PermixY	10	10
Mineral PermixZ	10	10
Kilka fish oil	70	70
Proximate composition(g Kg^{-1})		
Dry matter	915	907
Crude protein	245	336
Crude lipid	94	91
Ash	8	7.7
Phosphorus	12.3	11.7
Calcium	14.2	13.7
Lysine	18.9	19.5
Methionine	7.1	7.94
Gross energy (KJ g^{-1}DM)	18.4	18.9

X Values in parentheses are the crude protein content (g kg^{-1}).

Y itamin mixture (mg or IU if mentioned/kg diet): 5000 IU retinylacetate,; 2000 IU cholecalciferol; 80 IU all-rac-a-tocopheryl acetate; 10 menadione sodium bisulfite; 10 thiamin; 5 riboflavin; 10 pyridoxine; 50 d-calcium pantothenate; 120 niacin; 500 choline; 1 biotin; 5 folic acid; 400 myoinositol; 50 vitamin C,; 0.05 vitamin B12.

Z Mineral mixture (mg/kg diet): 40 Fe; 150 Zn; 25 Mn; 3 Cu; 5 K; 0.09 Na.

Citric acid (0 and 30 g kg^{-1}) and Phytase (0 and 500 IU kg^{-1}) added to diets.

Samples Collection and Chemical Analyses: All fish were weighed at the start of the experiment and bi-weekly. Growth performance calculated according to Khajepour and Hosseini [17]:

WG = 100× (Final Weigh -Initial Weigh)/Initial Weigh

SGR = 100 × ln (Final Weigh /Initial Weigh)/days of experiment

FCR = Feed consumed (g, dry weight)/(weight gain, g)

Two fish from each tank (6 fish from each treatment) were sampled for chemical analyses. Fish were anaesthetized using clove solution (3000 mg l^{-1} for 40-60 s) and then killed by a sharp blow on the head. Muscles of fish were sampled, sealed in plastic bags and stored frozen at -18°C until analysis for muscle nutrient compositions. Crude protein, lipid, moisture and ash in the diets and muscle samples were determined following standard methods [18]. Crude protein (N×6.25) was determined by the Kjeldahl method after acid digestion (Gerhardt, Königswinter, Germany). Crude lipid analysis with or without acid hydrolysis was determined by the ether-extraction method using a Soxtec System (Gerhardt, Königswinter, Germany). Moisture was determined by oven drying at 105°C until a constant weight was achieved. Ash content was estimated by incinerating the

Table 2: Growth performance and Hematocrite of Common carp, Cyprinus carpio fed on experimental diets.

		0% CA		3% CA			Analysis of variance						
	MP[1]												
Parameters	(U kg_1)	25%CP	35% CP	25%CP	35%CP	SEM_2	CA	MP	CP	CA ×MP	MP ×CP	CA ×CP	CA ×MP × CP
WG	0	103.43[a]	104.83[a]	112.56[b]	111.9[b]	2.11	0.000	0.56	0.32	0.65	0.45	0.70	0.26
	500	10.3.70[a]	103.96[a]	112.23[b]	113.33[b]								
SGR	0	1.14[a]	1.13[a]	1.20[b]	1.23[b]	0.01	0.001	1.00	0.52	0.09	0.63	0.85	0.75
	500	1.14[a]	1.12[a]	1.21[b]	1.23[b]								
FCR	0	1.23[a]	1.22[a]	1.17[b]	1.17[b]	0.01	0.00	0.35	0.35	0.88	0.74	0.86	0.88
	050	1.22[a]	1.21[a]	1.17[b]	1.15[b]								
HCT	0	42[a]	40[a]	46[b]	45[b]	0.95	0.00	0.54	0.40	0.40	0.54	0.71	0.71
	500	41[a]	42[a]	45[b]	46[b]								

[a, b] Values in the same column different superscripts are significantly different ($p< 0.05$).

1 Natuphoss 5000G produced from Aspergillus niger (MERK , GERMANY).

2Standard Error of Mean

Table 3: Muscle composition of Common carp, Cyprinus carpio fed on experimental diets.

		0% CA		3% CA			Analysis of variance						
	MP[1]												
Parameters	(U kg_1)	25%CP	35% CP	25%CP	35%CP	SEM_2	CA	MP	CP	CA ×MP	MP ×CP	CA ×CP	CA ×MP × CP
Dry matter	0	24.26[a]	24.1[a]	24.36[a]	23.9[a]	0.34	0.06	0.50	0.06	0.84	0.46	0.08	0.42
	500	24.4[a]	24.2[a]	24.2[a]	24.2[a]								
Protein	0	15.9[a]	16.12[a]	16.20[a]	15.8[a]	0.23	0.81	0.09	0.36	0.76	0.68	0.06	0.30
	500	16.03[a]	16.48[a]	16.80[a]	15.9[a]								
Lipid	0	4.45[a]	4.44[a]	3.77[b]	3.67[b]	0.03	0.00	0.00	0.06	0.06	0.78	0.73	0.19
	500	4.26[a]	4.20[a]	3.70[b]	3.69[b]								
Ash	0	3.70[a]	3.62[a]	3.67[b]	3.73[b]	0.02	0.00	0.55	0.45	0.45	0.36	0.06	0.28
	500	3.67[a]	3.59[a]	3.72[b]	3.96[b]								

[a] b Values in the same column different superscripts are significantly different ($p< 0.05$).

1 Natuphoss 5000G produced from Aspergillus niger (MERK , GERMANY).

2Standard Error of Mean

samples in a muffle furnace at 600°C for 6 h. Lysine and methionine of diets were determined by HPLC as described by Ovissipour *et al.* [19]. Gross energy content of the diets was determined by bomb calorimetry (Digital Bomb Calorimeter, New Delhi, India). By caudal severance at end experiment, Hct was determined by the microhaematocrit method as described by Brown [20].

Statistical Analysis: All data were analyzed for homogeneity of variance using Levene´s test. All data were subjected to three-way ANOVA. When significant differences occurred, fisher least significance difference test was used for comparison of means. All statistical analyses were performed using SAS (Institute, Cary, NC).

RESULTS

Data for WG, SGR, FCR and Hct are reported in Table 2. The interaction term was not significant for all measurements. Crude protein and P had no effect on FW, WG and SGR. Weigh Gain and SGR was increased ($P<0.05$) in fish fed CA supplemented diets. No significant differences was observed for Hct among treatments ($P>0.05$) with the exception whereas CA supplementation increased Hct. The muscle composition data are presented in Table 3. No differences among treatments were detected for moisture and protein contents of muscle samples. However, fish fed diets added 30 gm kg^{-1} CA contained more ash ($P=0.035$) and less lipid ($P=0.002$) than fish fed the similar diets without added CA. There are no significant interaction between among treatments ($P>0.05$).

DISCUSSION

The present study is dealing with CP, P and CA. Citric acid supplementation significantly increases the growth performance and Hct which is in agreement with findings by Khajepour and Hosseini [17] on the Beluga and other species as *Labeo rohita* [21], red sea bream [13] and rainbow trout [15, 22]. Furthermore, in the present study addition of 30 gm kg^{-1} CA to diet that 500 IU kg^{-1} P supplemented improved growth performance. Supplementation P no affect on growth. This result

disagrees with finding by Baruha *et al*. [21]. Moreover, level CP dietary no affect on growth that this is agree with study by Baruha *et al*. [21] in *Labeo rohita*. Carp has high feed intake. Studies also revealed that, free hydrochloric acid levels in the stomach are reduced during periods of high feed intake; the animals are young or the feeds are high in CP, [23]. This reduction negatively impacts pepsin activation and pancreatic enzyme secretion and impairs digestion. Providing acidifiers in the feed tackles this problem and aid in feed digestion [23]. Positive effects of organic acids on protein hydrolysis have been demonstrated in pigs [24]. Likewise feed supplementation with organic acids has been shown to lead to lower duodenal pH, improved nitrogen retention and overall increased nutrient digestibility [8]. Erdman [25] reviewed literature and suggested that the phytate molecule binds minerals such as Ca. Perhaps CA, a strong chelator of Ca, removes Ca or decreases Ca binding to the phytate molecule. In addition, CA might enhance P activity because it can decrease PH of stomach. Data showed that dietary addition of CA (3%) significantly increased ($P<0.05$) Hct compare to other treatments, in agreement with the results of [27] and Baruha *et al.* [26] in Rohu, *L Rohita* Khajepour *et al.* [27] in Beluga. Higher muscle ash content was determined in fish fed CA supplemented diets. This suggests that CA enhanced the mineral availability from fish and plant meal and mitigated inhibitory effect of some dietary component (e.g. phytate) on mineral availability in plant meal. Similar results were observed by Khajepour and Hosseini [14] [28] feeding in beluga where, beluga showed increase in mineral status of scute and muscle as a result of CA addition to plant and animal protein based diets. Addition of CA decreased muscle lipid content. This finding is agreed by our recent study that it shown addition CA decreased content lipid of muscle at Beluga [17]. The exact mechanism which citrate acts in the present study is unknown. Results of the present study indicated that CA enhanced growth performance of common carp juveniles at sub-optimal protein level.

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