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Effects of Protamine Sulfate on Growth Performance, Body Composition and Blood Constituents in Catfish (*Clarias lazera*)

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Abstract: This study was carried out to investigate the effects of protamine (Merck), when used as dietary additive, on growth performance, body composition and blood constituents of catfish *Clarias lazera*. The initial mean body weight 200g, the dose concentration used in this experiment was 0, 200, 400, 600, 800 and 1000 mg protamine sulfate/kg diet. However, catfish *Clarias lazera* fed protamine supplemented diet showed significant increase in growth rate, body consumption (crude protein, lipid and moisture.) and blood parameter (RBCs, Hb, MCV, MCH, MCHC, blood glucose, total protein and total lipid). It was concluded that the protamine sulfate supplemented diets improve the growth performance, body composition and biochemical constituents of catfish *Clarias lazera*.

Key words: Protamine sulfate % Growth performance % Body composition % Blood constituents

INTRODUCTION

Protamines are basic proteins which have been isolated from sperm and ova of fish, characterized by a high content of amino acids; arginine, lysine, cysteine, serine and alanine. These 4 amino acids represented 80-84% of the total number of amino acid residues formed protamine [1]. Scylliorhinine Zl is one of the four protamines of mature sperm of dogfish [2]. Also, it was isolated from sperm of the bivalve *Spisula solidissima*, with very large size protamine residues (297 amino acids) and a high molecular weight [3]. The mature sperm of the flounder contains a high molecular weight basic proteins ranging from 80,000 to 200,000[4]. Two types of protamines were detected including protamine of the dog-fish *(Scylliorhinus caniculus)* named Scylliorhinines which has a high MW and greater amino acid diversity[5]. The other type is Teleost protamines which are generally represented by a family of arginine-rich polypeptides [6]. Elizabeth and Graham [7] isolated protamine from unfractionated testis and ova of rainbow trout (*Salmo gairdnerii*) [7].

Growth and feed efficiency were the responses used to establish essential amino acid requirements[8]. Sulfur amino acid nutrition in fish has become increasingly important as dietary formulations incorporate lower levels of fish meal and higher levels of plant feedstuffs, which are often low in methionine. Also methionine is the starting point of an important catabolic pathway in vertebrates that includes cyst(e)ine, betaine, choline and phosphatidylcholine, all important nutrients in fishes[9]. Protamines, was found to inhibit the proteolytic activity of arginine-specific cysteine protease (RC-protease) from *Porphyromonas gingivalis*[10]. Replacing fish meal by a plant protein did not significantly affected feed intake, feed conversion ratio, protein efficiency ratio, crude protein and ether extract [11]. Yeast protein *Saccharomyces cerevisiae* with biogenic L-carintine (methionine and lysine mixture) can totally replace fish meal in tilapia diet without any adverse effect on growth performance[12].

The objective of the present work was to investigate the effects of protamines (rich in arginine, cystein, lysine,..) on the growth performance, feed efficiency and body composition of catfish.

MATERIALS AND METHODS

Protamine sulfate powder was obtained from Merck chemical company.

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Table 1: Formulation and chemical composition of experimental diets

Ingredients	Diet 1 (Control)	Diet 2 Group2	Diet 3 Group 3	Diet 4 Group 4	Diet 5 Group 5	Diet 6 Group 6
Dextrin(gm)	456.75	456.75	456.75	456.75	456.75	456.75
Gelatin(gm)	90.18	90.18	90.18	90.18	90.18	90.18
Casein(gm)	188.85	188.85	188.85	188.85	188.85	188.85
Soya been oil (gm)	114.22	114.22	114.22	114.22	114.22	114.22
Protamine(mg)	0.00	200	400	600	800	1000
C.M.C(gm)	30.0	30.0	30.0	30.0	30.0	30.0
Cellulose(gm)	30.0	30.0	30.0	30.0	30.0	30.0
Fish oil(gm)	30.0	30.0	30.0	30.0	30.0	30.0
Mineral(gm)	30.0	30.0	30.0	30.0	30.0	30.0
Vit. mix. (gm)	30.0	30.0	30.0	30.0	30.0	30.0

Vitamin & Mineral mixture was the same as described by Lim and Lovell [13].

Preparation of Experimental Diets: All diets were prepared as shown in Table 1. The diet was pelleted trough fodder machine. The pellets were dried in a drying oven for 48 hrs at 45°C, then cooled and saved in plastic bags and stored in refrigerator at 4°C.

Experimental Design: A total number of 180 Cat fish *(Calaries Lazera)* with average body weight of 200g /fish was divided into six groups, fed on diets containing protamine sulfate with different levels as food additives (Table 1). The control group1 fed on diet free from protamine. The experiment period extended for three months. The fish were fed with the experimental diets (each treatment was represented by three aquaria as replicates) at the rate of 3% of body weight.

Blood Samples Collection: At the end of experiment, heparinzed blood samples were collected, from the caudal vein of fish after three months. Samples were divided into 2 parts, a part for examination of blood picture and the other part was centrifuged at 3000 r.p.m for 15 minutes and blood plaa samples were separated and kept at –20°C until biochemical analysis.

Growth Measurements: Specific growth rate (S.G.R.), Feed conversion ratio (F.C.R.), Protein efficiency ration (P.E.R), Protein productive value (P.P.V%), Energy utilization (E.U) and, Survival rate were calculated according to Jobling [14].

Analyses:

Fish Body Composition: Moisture content was determined by oven drying at 105°C for 10 hrs (till obtaining constant weight). Crude protein was indirectly measured by analysis of total nitrogen (CP = N X 6.25) using the Kjeldahl method [15]. Crude lipid was determined by using Soxhlet apparatus and ash was detected by weighting samples in a porcelain crucible placed in a furnace at 550°C for 4hrs. Crude fiber was estimated according to Goering and Van Soest [16].

Whole blood was used for assaying erythrocyte count [17], hemoglobin content and hematocrit value [18]. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formulae mentioned by Dacie and Lewis [17].

Total plasma protein was determined by the Biuret method described by Wootton [19]. Total lipids were determined colorimetrically using a kit supplied by El Nasr Pharmaceutical Chemical Co., according to Knight *et al*.[20]. Glucose concentration was measured according to Trinder [21].

Statistical Analysis: Data were analyzed by analysis of variance using the SAS program [22]. Duncan's multiple-range test [23] was used to verify significance of the mean differences among treatments.

RESULTS

The changes in growth performances of catfish after three month of feeding on protamine supplemented diet were summarized in Table 2. The final body weight, weight gain and SGR of catfish fed on diet with protamine supplemented diets showed significant increase as compared to control. FCR decreased ($P<0.01$) in catfish groups fed on protamine supplemented diet, compared to the control group. But, PER increased with different treatment groups fed on protamine. No significant differences were detected in the hepatosomatic index between treated groups and the control.

Table 2: The growth performance of Catfish *(Clarias lazera)* fed on protamine supplemented diet (Mean + SE).

Parameters	Control group	Group2	Group 3	Group 4	Group 5	Group 6
Initial weight	200.0 ±0.50 [a]	200.0±0.0[a]	202.0±1.0[a]	200.0±2.0[a]	201.0±1.0[a]	200.0±0.0[a]
Final weight	291.1±2.25c	304.0±1.00b	305.1±0.76b	303.1±0.76b	333.8±0.763a	304.8±1.04b
Body gain	91.10±0.51d	104.0±0.78c	103.1±0.88c	103.1±0.78b	132.8±1.0a	104.8±0.66c
Specific growth rate%	0.56±0.022d	0.64±0.032b	0.66±0.020b	0.68±0.029b	0.80±0.031a	0.61±0.01c
Feed consumption	437.76±3.45e	440.94±2.05d	442.92±3.41c	462.59±4.43b	476.06±3.13a	436.7±2.51e
feed conversion ratio %	4.8±0.05a	4.2±0.05b	4.3±0.04b	4.4±00.04b	3.6±0.03c	4.1±0.06b
Protein efficiency ratio%	0.643±0.03 c	0.672±0.02 b	0.684±0.03 b	0.691±0.042b	0.840±0.045a	0.639±0.02c
productive value (PPV) %	27.66±3.7d	42.42±2.7c	41.76±1.5c	42.15±2.3c	61.92±4.2a	47.62±3.0b
energy utilization(EU)%	17.54±1.0e	35.38±3.3c	36.33±5.9c	41.34±5.1b	61.39±2.1a	31.32±1.0 d
HIS	1.77±0.09a	1.66±0.01a	1.68±0.01a	1.67±0.01a	1.69±0.03a	1.64±0.19a
Survival rate(%)	96.97±1.92c	100.0±0.0a	98.67±1.93b	100.0±0.00a	100.0±0.0a	99.0±0.20b

Means with the different letters for each parameter is significantly different at P < 0.01

Table 3: Body tissues composition of Catfish *(Clarias lazera)* fed on protamine supplemented diet (Mean + SE)

Body composition	Moisture	T. Protein	T. Lipid	Ash	Gross energy
Treatments					
Group1 (Control)	74.20±0.97b	57.47±0.72c	10.80±0.90c	31.73±0.51a	426.08±4.11e
Group2	±74.231.03b	62.63±0.64b	13.50±0.80b	23.87±0.98b	480.67±1.95c
Group 3	75.601.00±a	61.87±0.90b	14.10±0.40b	24.03±0.33b	482.05±3.76c
Group 4	75.80±0.80a	61.83±0.43b	15.70±1.60b	22.47±0.29b	498.78±1.99b
Group 5	76.60±1.04a	67.43±0.43a	17.50±0.30a	15.07±0.45d	545.51±3.38a
Group 6	72.44±1.00c	66.55±0.5a	10.33±0.76c	23.12±0.20b	472.86±2.24d
Initial body composition	74.18±0.91b	62.34±1.51b	17.58±1.22a	20.18±0.49c	517.55±2.09 a

Means with the different letters for each parameter is significantly different at P < 0.01

Table 4: Blood constituents of Catfish *(Clarias lazera)* fed on protamine supplemented diet (Mean + SE)

Blood constituents	Control group	Group2	Group 3	Group 4	Group 5	Group 6
Erythrocyte count (c/mm3)	1.67±0.06c	1.62±0.05c	1.72±0.07b	1.87±0.04b	1.96±0.04a	1.92±0.04a
Hemoglobin (g/100ml)	6.32±0.22c	6.142±0.23c	7.43±0.25b	6.784±0.21c	8.072±0.13a	8.165±0.20a
Hematocrit values (%)	14.74±0.58b	14.48±0.74b	14.64±0.74b	16.0±0.83a	15.0±0.44a	16.2±0.86a
MCV(ìm3)	83.40±1.56 c	71.58±2.21f	90.00± 1.16a	75.74±2.05e	77.0±1.62d	85.00±1.62b
MCH(pg)	43.73±1.80bc	43.69±1.62c	46.45±1.72a	44.46±1.65ab	45.67±1.42a	45.67±1.42a
MCHC(%)	36.11± 1.50	41.46±0.80b	36.45± 1.26a	34.45±1.35a	32.67±1.26a	32.67±1.26a
Total Protein(g/dl)	3.40±0.10c	4.76±0.55a	4.52±0.10b	4.72±0.13a	4.74±0.12a	4.54±0.12b
Total Lipids(mg/dl)	7.99±0.16a	7.05±0.20b	7.19±0.19b	7.18±006b	7.00±0.10c	6.84±0.19d
Glucose(mg/dl)	113.51±1.18 a	106.49±1.58c	109.51±1.18b	100.51±1.18d	101.51±1.18d	105.01±0.73c

Means with the different letters for each parameter is significantly different at P < 0.01

Protein content in the body of catfish was higher (P<0.01) in the groups fed on protamine supplemented diet than control group (Table. 3). Total lipids content in catfish body increased (P<0.01) in all catfish groups fed on protamine supplemented diet as compared to control. Ash content decreased (P<0.01) in catfish fed on protamine as compared to control. Moisture content in catfish body was significantly different with protamone treated groups as compared to the control.

RBCs counts, hemoglobin content and hematocrit percentage were significantly high (P<0.01) in *clarias lazera* fed on diets containing different levels of protamine, except in the fish group fed on 200mg protamine (Table 4). MCH and MCHC increased (P<0.01) in *clarias lazera* fed on diets containing high levels of protamine.

Plasma protein increased(P<0.01) in catfish fed on higher levels protamine diet as compared to control

(Table 4). The mean value of total plasma lipid decreased ($P<0.01$) in catfish fed on diets containing 1000mg protamine / kg diet. Plasma glucose decreased in catfish fed on protamine supplemented diets compared to control (Table 4).

DISCUSSION

This experiment investigated the effects of different levels of protamine sulfate on growth performance in Catfish, the final weight gain and SGR were ssignificantly increased in all groups fed on protamine supplemented diets which may be due to increase of essential amino acids of diets in form of protamines. These results were consistent with observation of Griffin *et al*. [8] who showed that arginine increase growth performance, also it was reported that methionine is the starting point of an important meatabolic pathway in fishes [9]. Yeast protein supplemented with biogenic L-carintine (methionine plus lysine mixture), can totally replace fish meal in tilapia diet without any adverse effect on growth performance and body composition [12]. Methionine and lysine in diet improved growth performance and feed utilization,and lowest mortality ratio in tilapia [24]. Effects of dietary methionine were confirmed in pigs [25] and effects of dietary arginine was clear inn catfish [26]. On the other hand, Shouqi and Xiqin [27] reported that growth rate and feed utilization. feeding rate and the contents of dry matter, fat and energy of fish body were not affected after replacement of fishmeal by soybean in Chinese catfish.

S. G. R. Feed intake increased, but F. C. R. decreased with increasing protamine levels in the diets. PER are used as quality indicators for fish diet and amino acid balance. So, these parameters are used to assess protein utilization and turnover. These results are in agreement with Khattab *et al*. [28] who found that the dietary of Biogen® increased feed intake, FCR, PER and body composition (crude protein, ether extract, ash and moisture) in fish.

Body composition of Catfish, crud protein increased significantly with diets containing different levels of protamine sulfate while total lipid content decreased significantly with the same levels of protamine. These results agree with that obtained by Abd-Elhamid *et al*. [29], Khattab *et al*., [28] and Sardar *et. al,* [30] who found that dietary lysine and methionine increase carcass compositions in major carp, *Labeo rohita* fingerlings.

Protamine supplemented diets induced significant increases in all blood parameters (erythrocyte count, hemoglobin content and hematocrit value) in treated fish, which agrees with Sardar *et. al*. [30] who found that dietary lysine and methionine increase, haematological status in major carp. The addition of protamine to fish diets increased erythrocytes number, hemoglobin content, hematocrit value. Protamine may have some constituents that play a role in the stimulation of the immune system and in the function of organs related to blood cell formation such as thymus, spleen and bone marrow. Blood indices (MCV, MCH and MCHC) are particularly important for the diagnosis of anemia in most animals [31]. Significant increase in MCH and MCHC of catfish fed on the highest level protamine may be attributed to a improvement of fish health, Lysine and methionine showed significantly increase in haematological status than fish fed control diet [30].

Plasma protein is a biochemical system, reflecting the condition of the organism and the changes happening to it under influence of internal and external factors. Total plasma protein was significantly increased in catfish fed on protamine supplemented diet, due to improve liver and other organs functions which synthesized plasma protein. These results are in agreement with those obtained by Khattab *et al*. [28, 29] who found that the dietary of Biogen® improve composition of fish.

Reduction of total plasma lipid of *Clarias lazera* fed on diets containing protamine in different levels are in agreement with the study of James *et al.* [32] who found that semipurified diets with arginine, cystine, methionine or tryptophan, fed to channel catfish (*Ictalurus punctatus*) fingerlings significanly enhanced of growth preformance. Also, methionine improved weight gain, energy efficiency of catfish fed plant protein diets [33]. The sulfur containing amino acid present in protamine structure may increase the oxidation of plasma and cell lipids by improving fish health and enhancement of protein anabolism

Plasma glucose concentration was significantly decreased in fish fed on diets containing different levels of protamine supplemented diets. This condition was attributed to improving of the metabolism system and improving of the antioxidant system in â cell of pancreas to produce insulin. These results agree with those of Kiss and Zamfirova [34] who showed that protamine is an effective inhibitor of the various activated forms of adenylate cyclase of liver plasma membranes. Other studied amino acids (arginine, leucine, phenyl alanine and methionine) when given orally stimulate insulin release [35]. Methionine and lysine was effective in improving the nutritive value for juvenile kuruma shrimp[36], blue caffish *Ictalurus furcatus* or channel caffish *I. punctatus*

[37]. Tryptophan, methionine and arginine improved larval quality and growth performance of *Diplodus sargus* [38]. In conclusion, protamine supplemented diets improve growth performance, body composition and biochemical constituents of catfish *Clarias lazera*.

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