Effects of fish meal replacement with fish biosilage on some haematological and biochemical parameters in common carp Cyprinus carpio fingerlings

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Abstract

This study was carried out to investigate the effects of replacing fish meal with locally produced fish biosilage on some haematological and biochemical parameters in common carp *C. Carpio* fingerlings. Biosilage was prepared by fermenting marine by-catch fish with date fruit residues, domestic vinegar and citric acid. The produced biosilage was incorporated in feeds to replace 0, 25, 50 or 75% of fish meal protein. Fish were fed for 14 weeks and haematological analysis showed improvements in all studied parameters (RBC, WBC, Hb and Hct) of feed groups in comparison with initial fish. Plasma proteins showed similar trend of improvement. Plasma lipid profile indicated significant improvements where total cholesterol decreased by 30% and triglycerides by 40% while HDL increased by 49%. The study concluded that fish silage could be a good replacer for fish meal without adverse effects on fish blood characteristics.

Key words: biosilage, *C. carpio*, hematology, plasma biochemistry.

Ϯ:-This paper is a part of doctoral thesis for the first author

1. Introduction

Protein is a very important macronutrient in fish feeding. Fish meal is the widest protein source which used in aquaculture feeds because of its high protein content, good protein quality and balanced amino acid profile[1]. However, fish meal supplies witnessed significant fluctuations in supplies and thus in prices during the last decade. This encouraged the search for fish meal alternatives from plant and animal sources. Plant materials suffer from low digestibility, high fibre content and antinutritional factors which limit their use effectively in aquaculture feeds[2,3]. Animal protein concentrates like blood meal and meat and bone meal were banned due to the outbreaks of BSE disease[4]. All these reasons make fish silage a viable alternative for fish meal in aquaculture. It is characterized by similar or even better proximate composition in comparison with fish meal. The high quality content of fish oil rich in PUFA fatty acids makes fish silage an excellent source of essential fatty acids. It could be made easily even at farm level from different raw materials like by-catch fish or fish wastes without need for advanced technology[5,6].

Haematological examination became a basic part of fish health monitoring programs. Fish blood haematological and biochemical parameters could be influenced by several factors such as species, sex, stress, environmental conditions and physiological nutritional status (7,8). This study was carried out to investigate the general haematological parameters and biochemical blood plasma indices in common carp fingerlings in response to different partial replacements of fish meal by fish biosilage produced from local raw materials.

2. Materials and methods

Fish biosilage was produced using marine by-catch fish obtained from marine shrimp fisheries at Al-Fao city southern Basrah. Ensiling fermentation process was carried out by adding date fruit residues (10%) as carbohydrate substrate, domestic vinegar (20%) as an acidulant and inoculant and citric acid as starting acidifying agent. Ensiling mixture was incubated at 35^{o}C for 10 days. Fish meal was produced by the standard method from the same fish sample for comparison purposes.

Fingerlings of the common carp (5.81± 0.29 gm) were obtained from outdoor ponds of fish farm at Marine Science Center, University of Basrah. Upon arrival to the Fish Hatchery at the Department of Fisheries and Marine Resources, a sample of fish was taken for proximate analysis and the rest of fish were distributed in culture system of 12 glass aquariums (60 x 30 x 42 cm) each containing about 57 liters of dechlorinated tab water. Fish were acclimatized to the laboratory conditions for 3 days and to the experimental diets for 2 other days. Before the

Table 1. Feed formulation and proximate composition using fish silage as a partial replacement for fish meal

Feedstuff, %	Feed formulation			
	A	B	C	D
Fishmeal	34	25.5	17	8.5
Fish silage	0	10.1	20.2	30.3
Soybean meal	15	15	15	15
Corn meal	15	15	15	15
Barley flour	18	18	18	18
Wheat bran	11	11	11	11.2
Corn oil	5	3.4	1.8	0
Premix*	2	2	2	2
Proximate composition, %				
Moisture	6.50±0.52	6.88±0.64	6.91±0.49	7.15±0.81
Protein	36.63±1.17	35.41±1.79	35.24±1.58	34.42±0.92
Lipid	10.90±0.91	11.28±0.85	11.15±1.04	12.09±1.11
NFE	35.76±2.69	34.84±1.71	35.58±2.15	34.41±2.17
Ash	11.21±0.83	11.59±1.21	11.12±1.50	11.93±1.43
Energy, Kcal/kg	4473 ± 33	4402 ± 45	4411 ± 38	4404 ± 39
P/E Ratio, mg/Kcal	81.90±1.54	80.43±1.71	79.88±1.66	78.15±1.67

*Vapcomix, VAPCO Veterinary and agricultural product manufacturing Co., Amman, Jordan.

Table 2. Water quality parameters of rearing water for common carp fingerlings during the experiment

Parameter	Feed group			
	A	B	C	D
Temperature, °C	27.7 ± 0.743	27.9± 0.889	27.7± 0.676	27.8± 0.512
pH	7.54± 0.131	7.66± 0.114	7.81± 0.101	7.99± 0.136
Oxygen, mg/l	8.99± 0.203	9.02± 0.219	9.01± 0.234	8.85± 0.242
Salinity, ‰	1.81± 0.215	1.80± 0.217	1.83± 0.221	1.79± 0.206
Nitrate, mg/l	0.94± 0.101	1.09± 0.121	1.34± 0.137	1.41± 0.099
Ammonia, mg/l	0.038± 0.005	0.039± 0.005	0.055± 0.009	0.057± 0.011

beginning of the experiment, weak and abnormal fish were excluded and the remaining fish redistributed on aquariums at 15 fish/ aquarium. The experiment included 4 treatments with 3 replicated aquariums for each. Feeds A, B, C and D were designed according to NRC [9] criteria to be isonitrogenous (35% crude protein) and isocaloric (4400 Kcal/kg) replacing 0, 25, 50 and 75% of fish meal protein content by fish biosilage (table 1). Each aquarium was equipped with air flow and thermostat controlled underwater heater fixed at 28± 1°C. Fish were fed 5% of body weight daily twice daily (8 am and 2 pm) sex days a week. About 30% of aquarium water was changed daily before morning feeding. Fish were weighed biweekly and feed ration was adjusted accordingly. The experiment lasted for 14 weeks from 6 October 2013 to 12 January 2014. Water quality parameters (temperature, oxygen, salinity, pH and nitrate NO_3 concentrations) were monitored on a daily basis using YSI Professional Plus (YSI Incorporated, USA) except for ammonia concentrations which determined twice weekly during the last three weeks on different replicates by Nessler method (Morrison, 1971). Proximate composition of feeds and ingredients was analyzed according to AOAC [11] methods.

Haematological study was carried out on fish before and after feeding on the four experimental feeds. Fish were killed by pithing with a fine needle immediately before severing the caudal peduncle to collect the freely flowing blood. Red and white blood cells were counted manually after diluting blood with Dacie and Lewis formal citrate solution and Natt-Herrigs solution, respectively, using an improved Neubauer's chamber haemocytometer[12]. Hemoglobin was measured by Drabkin's method [13]. Haematocrit was determined on centrifuged microhematocrit capillaries [14]. Total plasma protein and albumin concentrations were determined using Biolabo kits (Biolabo SA, Maizy, France). Plasma globulin concentration was calculated according to [15]. Total plasma cholesterol, triglycerides, HDL-Cholesterol were measured using Cromatest® kits (Linear Chemicals, S.L., Barcelona, Spain) while LDL and VLDL-cholesterol concentrations were calculated according to [16,17], respectively.

The data were analyzed by one-way analysis of variance (ANOVA, F test) using IBM® SPSS® version 19. The differences between means were tested by least significant difference LSD test on SPSS with significance level of $p \leq 0.05$. All means with standard deviations are produced from

Table 3. General haematology of common carp fingerlings before and after feeding on experimental feeds

Parameter	Initial	Feed group			
		A	B	C	D
RBC x 10^6	2.251± 0.32^a	3.808± 0.19^b	3.791± 0.18^b	3.799± 0.19^b	3.812± 0.16^b
WBC x 10^4	1.49± 0.47^a	1.66± 0.41^b	1.63± 0.44^b	1.59± 0.40^b	1.65± 0.39^b
Hb gm/dl	7.81± 0.91^a	11.07± 0.65^b	10.99± 0.62^b	11.05± 0.71^b	11.18± 0.69^b
Hct, %	19.88± 2.79^a	27.41± 2.28^b	26.89± 2.31^b	26.97± 3.05^b	27.55± 2.99^b

Values in the same raw which carry different superscript letters are significantly ($p \leq 0.05$) different.

Table 4. Blood plasma biochemistry (mg/dl) of common carp fingerlings before and after feeding on experimental feeds

Parameter	Initial	Feed group			
		A	B	C	D
Total protein	3.35± 0.49^a	4.26± 0.38^b	4.20± 0.31^b	4.27± 0.50^b	4.36± 0.39^b
Albumin	1.68± 0.47^a	2.88± 0.54^b	2.83± 0.48^b	2.88± 0.51^b	2.91± 0.46^b
Globulin	1.67± 0.25^a	1.38± 0.23^b	1.37± 0.18^b	1.39± 0.25^b	1.45± 0.27^b
Alb/Glob	1.01± 0.31^a	2.09± 0.37^b	2.07± 0.36^b	2.07± 0.39^b	2.01± 0.29^b
Total cholesterol	255.3± 33.2^a	212.1± 29.3^b	201.6± 23.7^{bc}	191.9± 25.4^{bc}	178.2± 27.7^c
Triglycerids,	121.6± 25.1^a	78.11± 23.1^b	75.74± 22.6^b	73.54± 25.2^b	71.14± 20.9^b
HDL	118.2± 17.9^a	101.7± 15.2^b	93.31± 15.7^{bc}	86.91± 17.1^{bc}	79.15± 14.3^c
LDL	112.8± 11.5^a	94.78± 10.4^b	93.14± 9.7^{bc}	90.28± 9.9^{bc}	84.82± 8.8^c
VLDL	24.32± 5.22^a	15.62± 4.17^b	15.15± 4.26^b	14.71± 5.06^b	14.23± 4.89^b

Values in the same raw which carry different superscript letters are significantly ($p \leq 0.05$) different.

at least three replicates.

3. Results and discussion

Water quality parameters during this study are shown in table 2. They were adequately stable (temperature 27.7-27.9°C, pH 7.54-7.99, oxygen 8.85-9.02 mg/l, salinity 1.79-1.81, nitrate 0.94-1.41 mg/l and ammonia 0.038-0.057 mg/l. These values are within suitable ranges for culture of this species [18,19].

General hematological indices of common carp fingerlings before and after feeding on experimental feeds which contain different inclusion ratios of fish biosilage are presented in table 3. RBC counts in initial fish averaged 2.251 $x10^6$ /mm^3 and increased by 69.3% to a maximum of 3.812 $x10^6$ /mm^3 in feed D group. WBC counts showed similar, though lower, trend of increasing by only 11.3% from initial 1.49 $x10^4$/mm^3 to 1.66 $x10^4$ /mm^3 in feed A group. Hb increased from initial 7.81 to 11.18 gm/dl in feed D group (43.1% increase). Similar increases were observed in Hct which increased from initial 19.88 to 27.55% in feed D group (38.6% increase). Initial values of all investigated general hematological indices were significantly different ($p < 0.05$) from the four feed groups which in turn did not differ significantly ($p > 0.05$) from each other. There were significant positive correlations between RBC counts and each of Hb ($r = 0.999$, $p < 0.05$) and Hct ($r = 0.997$, $p < 0.05$). Hb and Hct was significantly and positively correlated also ($r = 0.999$, $p < 0.05$).

Abdelghany[20] showed that replacement of fish meal partially or totally by *Gambusia* meal in diets for common carp fingerlings did not affect significantly RBC counts (3.08-3.17 x 10^6/mm^3) but it improved significantly Hct (27.53-35.07%). His figures are very close to the findings of the current study in feed groups. Moradi*et al.* [7]evaluated the hematological and biochemical changes induced by replacing fish meal with plant protein in the common carp *Cyprinus carpio* fed for 8 weeks and indicated significant differences in Hb and Hct while no significant differences were found in RBC and WBC. They concluded that the maximum levels of fish meal replacement by corn gluten and sesame oil cake in diets of *Cyprinus carpio* could be 68 % of total protein without significant alterations in fish hematological indices. Their values reported for fish fed on fish meal diet were very close to the findings of the current study for fish feed groups. Nasir and Al-Sraji[8] investigated the effects of different dietary protein and fats on some blood parameters in common carp fingerlings reared in floating cages for 180 days and demonstrated that fish fed on high fish meal diet containing 23.68% dietary protein had Hb, Hct, RBC and WBC counts higher than those fed on lower protein level of

13.82%. They concluded that in order to prevent any adverse effects on fish hematology, diets containing lower than 23% dietary protein should be avoided. This supports the findings of this study that replacing fish meal by fish biosilage on protein content basis in isonitrogenous feeds (35% CP) did not affected significantly all fish feed groups.

Table 4 presents some blood plasma biochemistry indices of common carp fingerlings before and after feeding on experimental feeds. Total plasma protein showed significant ($p < 0.05$) increases from initial value 3.35 to 4.36 gm/dl in feed D group (30.1% increase). Plasma albumen showed similar trend, increasing significantly ($p < 0.05$) by 73.2% (initial 1.68 to 2.91 gm/dl in feed D group) and represents the main contributor in rising levels of total plasma proteins. In contrast, plasma globulin levels decreased significantly ($p < 0.05$) by 18% from an initial value of 1.67 to 1.37 gm/dl in feed D group. Ratio of Alb/Glob increased significantly ($p < 0.05$) by 164% (initial 1.01 to 2.09 in feed A group).

Plasma lipid indices showed rather similar trends in comparison with plasma proteins (table 4). Total plasma cholesterol decreased significantly ($p < 0.05$) from initial 255.3 to 178.2 mg/dl in feedD group (30.2% decrease). Plasma triglycerides decreased further by 41.5% from initial 121.6 to 71.14 mg/dl in feedD group. HDL cholesterol showed an opposite trend and increased significantly ($p < 0.05$) from initial 79.15 to 118.2 mg/dl in feedD group (49.3% increase). LDL cholesterol levels decreased significantly ($p < 0.05$) by 69.9% from initial 151.8 to 45.77 mg/dl in feedD group. As VLDL cholesterol is the result of dividing total cholesterol by factor 5, its levels followed the same trend of decrease in feed treatments (table 4).

Abdelghany[20] reported values of total plasma protein between 2.71 to 4.37 gm/dl in common carp fingerlings fed diets with different levels of *Gambusia* meal as partial or total replacements of fish meal. These values resemble well those reported in the current study. The study of Moradi*et al*. [7] on the hematological and biochemical changes induced by replacing fish meal with plant protein in the common carp *Cyprinus carpio* which was fed for 8 weeks, indicated that experimental diets showed significant differences in total protein levels while no significant differences were found in cholesterol, albumin and triglycerides. They determined the maximum levels of fish meal replacement by corn gluten and sesame oil cake in diets of *Cyprinus carpio* at 68 % of total protein sources of diet without significant alterations in major biochemical indices in fish. Their values reported for fish meal feed group were comparable to the findings of the current study for fish feed groups. Significant differences in plasma total protein, total cholesterol and triglycerides were found in the study of Nasir and Al-Sraji[8]who indicated that fish fed on high fish meal diet containing 23.68% dietary protein had better blood biochemical profile than those fed on lower protein level of 13.82%. Based on these observations, they concluded that 23% dietary protein is more adequate for growth and health of this fish species under rearing conditions in floating cages. This agree well with the results of this study that using isonitrogenous feeds (35% CP) by replacing fish meal with different levels of fish biosilage did not affect significantly the blood profile of fish feed groups.

In addition to the proved adequacy of fish biosilage as a fish meal replacer in common carp fingerling feeds, it is important to note that low values of hematological and biochemical parameters in initial fish were lower than the normal levels reported for this species indicating some kind of anemia [21]. This phenomenon was attributed to many factors like stress, disease and starvation [9, 22] which is the most likely reason. The significant improvements in all the studied blood profile indices of fish which supplemented with different experimental feeds in the current study could support this explanation since all feeds contained suitable quantity and quality of the major nutrients. Previous studies have shown that nutrition had a significant influence on blood profile of the common carp and other cultured species [8, 23, 24].

4. Conclusions

Fish biosilage which was produced from marine fish by-catch, date fruit residue, domestic vinegar and citric acid could be used effectively as a fish meal alternative in common carp feeds. Locally produced fish biosilage could replace up to 75% of fish meal protein in feeds for common carp fingerlings without any adverse influence on general hematology or blood plasma biochemistry of fish which are important indices of fish health..

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