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Effect of Partial Replacement of Fish Meal with Fermented Soybean Meal on Growth, Feed Efficiency, Body Composition, Amount of Lactic Acid Bacteria in Diet and Intestine in Carp (*Cyprinus carpio* L. 1758)

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Research Article

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Abstract

This trial was conducted to determine the effects of feeding a diet containing fermented soybean meal with whey on growth performance, body composition, hepatosomatic index (HSI), and viscerosomatic index (VSI) of carp. Fermented soybean meal (FSM) was used to replace fish meal (FM) protein in diets for juvenile carp (*Cyprinus carpio*) at rates of 10% (FSM10), 20% (FSM20), 30% (FSM30), 40% (FSM40) and 50% (FSM50). The control diet contained no fermented soybean meal. All experimental diets were prepared as isonitrogenous and isocaloric. The feeding trial was conducted in 18 glass aquaria. At the beginning of the experiment, 30 fish (average weight 1.66±0.14 g) were randomly stocked into each aquarium with 3 replications per treatment. At the end of the experiment, the highest final weight, weight gain, and specific growth rate were obtained in carp fed with FSM20 group. However, growth parameters and feed conversion ratio (FCR) of fish fed with diets containing fermented soybean meal up to 50% of fish meal protein were similar to those of the fish fed the control diet. There were no significant differences between body compositions, HSI, and VSI of all groups. Fermentation with whey of soybean meal increased the number of lactic acid bacteria in diet. However, lactic acid bacteria levels in the intestine not changed with the use of fermented soybean meal in the diet. The results of this experiment were showed that fermented soybean meal with whey can be replaced by up to 50% of fish meal protein in diets for juvenile carp.

Keywords: whey, fermentation, soybean meal, growth, carp

Sazan balıklarında (*Cyprinus carpio* L. 1758) fermente soya küspesi ile balık ununun yer değiştirmesinin büyüme, yem etkinliği, vücut kompozisyonu, yemde ve bağırsakta laktik asit bakteri miktarı üzerine etkileri

Özet

Bu çalışma, peynir altı suyu ile fermente edilmiş soya küspesi içeren yemlerle sazan yavru balıklarını beslemenin büyüme performansı, vücut kompozisyonu, hepatosomatic indeks (HSİ) ve visserosomatik indeks (VSİ) üzerindeki etkilerini araştırmak için yapılmıştır. Fermente soya küspesi (FSM) sazan balıklarının (*Cyprinus carpio*) yemlerinde balık unu proteinini %10 (FSM10), %20 (FSM20), %30 (FSM30), %40 (FSM40) ve %50(FSM50)'si yerine kullanılmıştır. Kontrol yeminde fermente soya küspesi kullanılmamıştır. Yemler izonitrojenik ve izokalorik olarak hazırlanmıştır. Deneme de 18 adet akvaryum kullanılmıştır. Araştırmanın başında, her akvaryuma 30 balık (ortalam ağırlığı 1.66±0.14 g) konulmuş ve deneme 3 tekerrür yürütülmüştür. Denemenin sonunda, FSM20 grubu ile beslenen sazanlarda en yüksek ağırlık, ağırlık artışı ve spesifik büyüme oranı elde edilmiştir. Balık unu proteininin %50'si yerine fermente soya küspesi içeren yemlerle beslenen balıkların büyüme parametreleri ve yem dönüşüm oranları (FCR) kontrol yemiyle beslenen balıklarla benzer bulunmuştur. Tüm grupların vücut kompozisyonları, HIS ve VSI değerleri arasında istatiktiksel olarak önemli bir farklılık bulunmamıştır. Bununla birlikte, yemlerde fermente soya küspesi kullanımı ile bağırsaktaki laktik asit miktarı değişmemiştir. Bu deneme sonuçları, peynir altı suyu ile fermente edilmiş soya küspesinin, sazan yavru yemlerinde balık unu proteininin %50'sine kadar kullanılabileceğini göstermiştir.

Anahtar kelimeler: Peynir altı suyu, fermententasyon, soya küspesi, büyüme, sazan

INTRODUCTION

Fish meal has been a major ingredient in fish diets because of its good protein quality and palatability. However, fish meal is one of the most expensive ingredients in prepared fish diets. Nutritionists have tried to use less expensive plant protein sources to partially or totally replace fish meal. Among plant proteins, soybean meal is an important source of dietary protein in fish diet.

However, like other plant, soybeans contain numerous antinutritional factors such as phytic acid, tanen and trypsin inhibitors which decrease the nutritional value of food and digestibility of nutrients (Rumsey et al., 1994; Anderson and Wolf, 1995; Smits and Annison, 1996; Cheng and Hardy, 2003, Karakurt et al., 2019).

Fermentation is one of the processes known to reduce the level of antinutrients in food grains, increases protein digestibility and nutritive value (Sharma and Kapoor, 1996; Sripriya et al., 1997; Egounlety and Aworh, 2003; Saha and Ray, 2010). Mukhopadhyay and Ray (1999) reported that lactic acid bacteria fermentation has been shown to give a significant reduction in antinutrients such as tannin and phytic acid in sesame. In the same study, Rohu, *Labeo rohita*, fed diet containing sesame seed meal fermented with lactic acid bacteria gave better growth performance than feeding raw sesame seed meal. Egounlety and Aworh (2003) showed that phytic acid decreased during fermentation in soybean, cowpea, and groundbean.

Whey is a by-product of the manufacture of cheese. In the nutritional aspect, whey includes a high amount of lactose as prebiotic and some major minerals as vital nutrients (Reeves, 1982; Kilara, 1994). Also, whey is containing lactic acid bacteria (Mondragón-Parada et al., 2006; Shiphrah et al., 2013). There are few studies on fermentation with whey. Fermented soybean meal with whey could replace up to 40% of fish meal protein in juvenile tilapia diets (Yigit and Tulay, 2016). *Coturnix coturnix japonica* fed wheat fermented with whey showed significantly high weight gain and feed efficiency (Yasar and Gok, 2014).

This study was investigated to determine the effect of partial substitution of fish meal protein with fermented soybean meal on growth performance, feed utilization, body composition, and amount of lactic acid bacteria in the intestine of carp.

MATERIALS and METHODS

Fermentation of soybean meal

Soybean meal was fermented using whey according to a process reported by Yasar and Gok (2014) and Yasar et al., (2016). Soybean meal was grinded to approximately 3mm and transferred to bioreactor with 75 L capacity. Soybean meal was soaked with whey at a ratio of 1:2, to ensure the growth of lactic acid bacteria, the lemon pulp was added to the mixture to provide 4.5 pH at every two hours (2. 4. and 6. hours). and incubated at 30°C for 8 h. After, fermented soybean meal was spread onto a clean flat table in a room in a thickness of 5 cm layer and left for drying at room temperature for 24 h. The mixture was frequently turned upside down by a hand scraper. When the moisture content of mixture reached 10%, fermented soybean meal ground to pass 300-500µ sieve before their inclusion in the experimental diets. Proximate analyses of fermented soybean meal, soybean meal, and fish meal were presented in table 1.

Table 1. Nutrient analyses of soybean meal, fermented soybean meal and fish meal (%)

	Dry matter	Moisture	Crude protein	Crude fat	Crude fiber	Crude ash
Bean meal	91.8	8.2	44.53	1.28	4.66	5.86
Fermented soybean meal	92.39	7.61	47.33	1.28	4.51	6.17
Fish meal	92.34	7.66	67.54	8.25	-	17.33

Experiment diets and analysis

Experimental diets were prepared as isonitrogenous and isocaloric. Six experimental diets were prepared by replacing 10%, 20%, 30%, 40%, and 50% of the fish meal protein in the control diet by fermented soybean meal. Ingredients and chemical composition of the experimental diets are given in table 2. All experimental diets were prepared as isonitrogenous (36% crude protein) and isocaloric (16.7 MJ/kg). In preparing the diets, the ingredients were first ground to a small particle size in a mill. The ingredients were thoroughly mixed in a mixer, and then oil and water were added. The diets were pelleted into 1.5 mm-diameter sizes using a mincing machine. The pellets were dried at room

temperature for 24 h. After drying, the diets were broken up into appropriate pellet sizes, sieved and stored at –20 °C.

The moisture, crude protein, crude fiber, ash contents of experimental diets, and body composition were determined according to standard AOAC methods (AOAC, 2002). The total lipids of all samples were determined by the chloroform-methanol extraction method (Bligh and Dyer, 1959).

Lactic acid bacteria count

Lactic acid bacteria count (LAB) in diet and the intestine of fish were determined the end of the experiment. LAB in diet and the intestine of fish were determined according to Baumgart (1993)

Table 2. Ingredients and chemical composition of the experimental diets

Ingredients	Groups					
	Control	**FSM10**	**FSM20**	**FSM30**	**FSM40**	**FSM50**
Fish meal	48.48	43.64	38.79	33.94	29.09	24.24
Fermented soybean meal	0.00	7.11	14.22	21.33	28.44	35.56
Wheat flour	32.20	32.20	32.20	32.20	32.20	32.20
Corn starch	17.26	13.81	10.35	6.89	3.44	0.00
Fish oil	0.46	1.60	2.75	3.91	5.05	6.19
Vitamin[1]	1.50	1.50	1.50	1.50	1.50	1.50
Mineral[2]	0.60	0.60	0.60	0.60	0.60	0.60
Methionine	0.00	0.04	0.09	0.13	0.17	0.22
Chemical composition						
Dry matter	91.52	91.49	91.45	91.42	91.39	91.35
Crude protein	36.62	36.68	36.74	36.79	36.85	36.90
Crude fat	5.43	6.21	7.00	7.79	8.57	9.34
Crude fiber	1.62	1.90	2.17	2.44	2.71	2.99
Gross energy (MJ/g)	16.7	16.7	16.7	16.7	16.7	16.7

[1]Vitamin premix contained the following per kilogram; 4 000 000 IU vitamin A, vitamin D3 480 000 IU, 2400 mg vitamin E, 2400 mg vitamin K3, 4000 mg vitamin B1, 6000 mg vitamin B2, 4000 mg Niacin, 10 000 mg Cal. D. Pantothenate, 4000 vitamin B6, 10 mg vitamin B12, 100 mg D-Biotin, 1200 mg folic acid, 40 000 mg vitamin C, 60 000 mg inositol. [2] Mineral premix contained the following per kilogram; 23 750 mg manganese, 75 000 mg zinc, copper 5000 mg, cobalt 2000 mg, iodine 2750 mg, selenium 100 mg, magnesium 200 000 mg.

Fish and experimental conditions

Carp (*Cyprinus carpio,* L. 1758) were obtained from Mediterranean Fisheries Research, Production and Training Institute, Antalya. Turkey. This experiment was carried out in 18 glass aquariums (70 x 30 x 40 cm). At the beginning of the experiment, 30 fish (average weight 1.66±0.14 g) were randomly stocked into each aquarium with 3 replications per treatment. All of the fish were fed ad libitum for 60 days. At the end of the trial, five fish from each aquarium were sacrificed by a lethal dose of anesthesia (150 mg/L MS-222), homogenized in a blender, and stored at -20°C for subsequent protein, ash, and moisture analysis. During the experimental period, the dissolved oxygen level, temperature, and pH were maintained at 6.4±0.06 mg/L, 24±0.21°C and 7.08±0.45 respectively.

Statistical analysis

One-way ANOVA was used to compare growth parameters, FCR, body composition, and somatic indices among the treatments. All data were analyzed using the SPSS computer program (SPSS 2000).

Duncan's multiple range tests were used to determine the mean differences among the treatments. Differences were considered significant at $p<0.05$.

RESULTS

The growth performance and feed efficiency of carp fed fermented soybean meal is presented in table 3. At the end of the experiment, the highest final weight (5.38 g), weight gain (3.71 g), and specific growth rate (1.96) were obtained in carp fed with FSM20 group. Final weights, weight gain, and specific growth rates of carp were ranged 4.78-5.38 g, 3.09-3.71g, 1.73-1.96 in all groups. Growth parameters and FCR of fish fed with diets containing fermented soybean meal up to 50% of fish meal protein were similar to those of the fish fed the control diet ($P>0.05$).

Table 3. Growth performance and feed efficiency of juvenile carp fed with fermented soybean meal

	CONTROL	FSM10	FSM20	FSM30	FSM40	FSM50
Growth Parameters						
Initial weight (g)	1.67±0.03	1.69±0.05	1.67±0.04	1.65±0.04	1.67±0.02	1.64±0.05
Final weight (g)	4.98±0.24	4.78±0.10	5.38±0.10	5.09±0.64	5.03±0.22	4.93±0.14
WG (g)	3.31±0.22	3.09±0.06	3.71±0.08	3.44±0.61	3.36±0.21	3.30±0.10
SGR (% day^{-1})	1.82±0.06	1.73±0.03	1.96±0.03	1.86±0.18	1.84±0.07	1.84±0.03
Feed Efficiency						
FCR	2.18±0.25	1.91±0.09	1.91±0.08	1.87±0.09	2.08±0.26	2.18±0.23
PEO	0.86±0.08	0.71±0.02	0.87±0.04	0.82±0.18	0.85±0.04	0.82±0.02
Feed intake	2.41±0.16	2.56±0.01	2.35±0.10	2.55±0.41	2.48±0.05	2.34±0.07

Weight gain (WG) = (final body weight - initial body weight)
Specific Growth Rate (SGR) (% day^{-1}) = [(ln final body weight - ln initial body weight)/days] x 100
Feed conversion ratio (FCR) = feed consumed (g)/weight gain (g)
Protein efficiency ratio (PER) = weight gain (g)/protein intake (g)
Feed intake (FI) = total feed intake (g)/fish number/days of the experiment

There were no significant differences between body composition, HSI, and VSI off all groups ($P>0.05$) (Table 4). The number of lactic acid bacteria in the diet increased with an increase of fermented soybean meal level ($P<0.05$). Lactic acid bacteria levels in the intestine not changed with the use of fermented soybean meal in the diet ($P>0.05$) (Table 4).

Table 4. Somatic indices, body composition and amount of lactic acid bacteria in diet and intestine of carp fed diets containing different levels of fermented soybean meal

	Control	FSM10	FSM20	FSM30	FSM40	FSM50
Somatic Indices						
HSI	1.55±0.06	2.04±0.11	2.09±0.19	1.88±0.25	1.82±0.15	2.02±0.19
VSI	15.32±1.01	14.31±0.67	15.05±0.58	16.31±0.87	16.21±0.81	16.48±0.41
Body Composition (%)						
Moisture	73.33±2.19	71.00±1.73	71.00±1.53	74.33±2.73	71.67±2.19	70.00±3.21
Crude protein	18.85±0.14	18.85±0.49	18.61±0.18	18.00±0.05	17.82±0.03	17.66±0.17
Crude ash	1.89±0.11	1.89±0.39	1.72±0.19	1.94±0.16	1.23±0.05	1.37±0.31
Amount of LAB (log CFU/g)						
LAB in diet	$4.33±0.19^{c}$	$4.67±0.07^{bc}$	$4.80±0.12^{ab}$	$4.97±0.12^{ab}$	$5.00±0.10^{ab}$	$5.17±0.09^{a}$
LAB in intestine	3.64±0.44	3.80±0.24	3.94±0.35	3.88±0.43	4.58±0.12	4.60±0.29

$^{a-c*}$ Values in the line having the same superscript are not significantly different ($P>0.05$)
Hepatosomatic index (HSI) = 100 x liver weight (g)/body weight (g)
Viscerosomatic index (VSI) = 100 x viscera weight (g)/ body weight (g)
LAB: Lactic acid bacteria amount

DISCUSSION

At the end of the experiment, growth parameters and feed efficiency of fish fed with diets containing fermented soybean meal up to 50% of fish meal protein were similar to those of the fish fed the control diet (P>0.05). Therefore, the result of this experiment was showed that fermented soybean meal with whey can be replaced by up to 50% of fish meal protein in diets for juvenile carp. In the studies on the use of fermented soybean meal in a fish diet, Yigit and Tulay (2016) showed that fermented soybean meal with whey could replace up to 40% of fish meal protein in juvenile tilapia diets. Zhou et al., (2011) was reported that fermented soybean meal by *Candida utilis* could be replaced by up to 20% of fish meal protein in the diet of black sea bream (*Acanthopagrus schlegelii*). Azarm and Lee (2012) were showed that fermented soybean meals using *Bacillus subtilis* could be replaced by up to 40% of fish meal in juvenile black sea bream diets. Barnes et al., (2012) was reported that PepSoyGen (commercial fermented soybean meal) could be replaced by up to 30% of fish meal protein in juvenile rainbow trout (*Oncorhynchus mykiss)*.

In the current study, fermented soybean meal with whey does not affect palatability and acceptability of the diets and feed intake was not influenced by dietary fermented soybean meal levels. Similar results were reported in juvenile rainbow trout (*O. mykiss)* by Barnes et al., (2012) and in juveniles black sea bream (*Acanthopagrus schlegelii*) by Sun et al., (2015). Also, feed conversion ratio and protein efficiency ratio were not influenced by dietary fermented soybean meal levels in the present study. Azarm and Lee (2012) and Zhou et al., (2011) informed that the protein efficiency ratio showed a decreasing tendency with increasing dietary fermented soybean meal level. This discrepancy could be due to the fermentation process and fish species.

In the present study, body composition was unaffected by the dietary fermented soybean meal level. Also, fish fed diets containing different levels of fermented soybean meal did not result in noticeable differences in HSI and VSI indices in the carp diet. The results were also consistent with previous studies (Refstie et al., 2005; Azarm and Lee, 2012; Lin et al., 2012, Yigit and Tulay, 2016).

The number of lactic acid bacteria in the diet increased with an increase of fermented soybean meal level (P<0.05). Lactic acid bacteria levels in the intestine not changed with the use of fermented soybean meal in the diet (P>0.05) (Table 4). Similar results have also been obtained in studies with tilapia by Yigit and Tulay (2016).

The result of this experiment showed that soybean meal fermented by whey could be replaced by up to 50% of dietary fish meal protein without any significant negative impact on growth performance, feed efficiency, body composition, HSI, and VSI in juvenile carp diet.

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