

Research Article



Replacement effects of soybean meal with sesame seed cake on growth, biochemical body composition, and economic efficiency of *Cyprinus carpio* formulated diet

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Abstract

A 90-day feeding trial was run to evaluate the effect of replacement of soybean meal (SBM) with sesame seed cake (SSC) at 0, 25, 50, 75, and 100% in the isonitrogenous (30% crude protein) and iso-lipidic (8% crude lipid) experimental diets on growth, nutrient and economic efficiency, and biochemical body composition of juvenile *Cyprinus carpio*. Common carp with an average initial weight of 83.09±0.06 g with a density of 30 numbers were randomly fed experimental diets in 15 tanks (300 L) with the flow-through system. The results showed that survival and growth rate, visceral indices, and proximate biochemical composition of the whole body and the fillet except protein did not significant between treatments. The feed intake, feed conversion ratio and protein efficiency ratio, calcium and phosphorus, nitrogen retention efficiency, nitrogen wastage except for SSC25, and phosphorus wastage except for SSC25 and SSC50 were not significantly affected by the dietary treatments. Economic conversion ratio of diets decreased, whereas profit index increased with increasing levels of dietary SSC. Reduced cost of SSC100 was about 35.10%. The number of white blood cells was significantly higher in SSC0 than in other experimental treatments. Hemoglobin was higher in SSC75 than in SSC0. Among serum parameters, cholesterol was significantly lower in SSC0 treatment than in SSC25 treatment. Comparable growth performance, nutrient wastage, economic efficiency, and some blood factors indicated that SBM could be replaced by 75 to 100% SSC in the formulated diet of *C. carpio* juveniles.

Keywords: Replacement, Sesame seed cake, Soybean meal, Body composition, Economic efficiency, *Cyprinus carpio*

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Introduction

Common carp (*Cyprinus carpio*) is widely cultured in Iran due to its nutritional value, market demand, and economic value (Modaberi *et al.*, 2022). The protein requirement in the diet of omnivorous fish is often met by soybean meal (SBM) (Roy *et al.*, 2014; Lawal *et al.*, 2016; Olude *et al.*, 2016). Among the reasons for the widespread use of SBM in aquafeed are its protein content, high digestibility and almost balanced amino acid profile (Abdel-Warith *et al.*, 2019; Biswas *et al.*, 2019; Arriaga-Hernández *et al.*, 2021). Due to the increasing demand and dependence of the food industry on SBM, its use and accessibility in aquafeed are limited. Consequently, increased demand has also led to higher prices (Guo *et al.*, 2011; Lawal *et al.*, 2016; Dernekbaşı *et al.*, 2017). As a result, the cost of farmed fish increases with the increase in the price of plant protein input in the diet. Therefore, it is necessary to find eco-friendly and economically feasible plant protein sources to replace SBM (De Boer *et al.*, 2014). Among plant protein sources, oilseeds are more important than legumes in fish nutrition. Sesame seed (SS) (*Sesamum indicum* L.) has comparable nutritional value to other protein sources of oilseeds, including SBM and other legumes (Wei *et al.*, 2022). Sesame seed cake (SSC) is obtained by mechanical pressing of SS (Onsaard *et al.*, 2010). This cake consists of about 35-45% protein. The amino acid composition of SSC, except for the lower lysine content and the higher methionine content, is similar to that of

SBM (Dernekbaşı *et al.*, 2017). SBM contains anti-nutritional factors such as protease, lipase, and α -amylase inhibitors, lectins, tannin, phytic acid, saponin, antivitamins, beta-conglycinin, estrogenic isoflavones, phytohemagglutinin, and low sulfur amino acid such as methionine and cysteine (Hekmatpour and Mozanzadeh, 2021) that may have negative impacts on hematological indices, growth performance and feed utilization (Francis *et al.*, 2001; Gatlin III *et al.*, 2007). The SSC almost lacks anti-nutrient factors, except oxalate and phytate (Wei *et al.*, 2022). Sesame oil seed also contains zinc, iron, copper, and vitamin B6 for the production and function of red blood cells. In addition, this oilseed contains antioxidants, including vitamin E and ligands, and is a good source of lecithin, selenium, magnesium, calcium, and phosphorus (Sauvant *et al.*, 2002; Nang Thu *et al.*, 2011). This oil seed is cultivated in 16 provinces of Iran. As a locally produced crop, it is easily available at a lower cost compared to SBM. The price of imported soybean meal: sesame seed cake is 3:1.

It was reported that low levels (25-50%) of SBM replacement with sesame seed meal (SSM) did not decrease growth and nutrient efficiency of African Catfish (*Clarias gariepinus*; Jimoh and Aroyehun 2011; Lawal *et al.*, 2016). Information on the overall performance of omnivorous fish in response to dietary replacement of SBM with SSC is limited. Based on growth, feeding efficiency, nutrient apparent

digestibility coefficient, and body composition of omnivorous species, 25% (Hasan *et al.*, 1997; Ray 1999; Olude *et al.*, 2016) to 50% (El-Saidy *et al.*, 2009; Roy *et al.*, 2014) was reported as the optimum replacement level of fish meal with SS meal. In carnivorous fish, replacement of plant protein sources with SS meal at levels less than 20% (Fagbenro *et al.*, 2013; Dernekbaşı *et al.*, 2017); 20-50% (Fagbenro *et al.*, 2010a; Jimoh and Aroyehun 2011; Jimoh *et al.*, 2014) and above 50% (Enyidi *et al.*, 2014; Lawal *et al.*, 2016) did not show negative effects on fish performance. Replacing SBM with SS meal did not adversely affect hematological parameters of *Clarias gariepinus* (Fagbenro *et al.*, 2010b; Lawal *et al.*, 2016). This nutritional trial was designed to evaluate the growth performance, nutrient and economic efficiency, body composition, blood parameters responses of *C. carpio* to dietary SBM replacement by SSC.

Materials and methods

Formulated diets preparation

Five isonitrogenous (300 g kg⁻¹ crude protein) and isolipidic (80 g kg⁻¹ crude lipid) experimental diets were formulated, using WUFFFDA 2.0 software, in which 0 (SSC0 or the control diet, where poultry by-product meal (PBM), tuna by-product meal (TBM) and SBM were the main protein sources), 25 (SSC25), 50 (SSC50), 75 (SSC75), 100% (SSC100) of SBM were replaced with SSC. The proximate analyses of the diets are given in Table 1. Fish oil, tuna by-product meal, PBM

and the other feed ingredients were provided by the Beyza Feed mill company. Starch and gelatin were cooked separately and blended with the ingredients to produce a homogeneous mixture in a Hobart-type mixer. Oils and water were then added and thoroughly mixed. Pellets (3.0 mm diameter) were produced using a pelletizing machine (CPM, model CL series; USA), air-dried (Iran khodsaz oven) at 40 °C for 48 h to about 10% moisture, sealed in plastic bags and stored frozen (-20°C) before use in the feeding trial (Table 1).

Feeding trial

This study was carried out in the South Iranian Aquaculture Research Center, Ahvaz, Iran (SIARC). Four hundred and fifty healthy juveniles of common carp (mean body weight 83.27±0.1 g, mean±SD), produced at the hatchery of SIARC, were randomly distributed into fifteen 300 L cylindrical polyethylene tanks with a flow-through system (1 L min⁻¹). Fish were acclimated to the experimental condition for 2 weeks before the onset of the feeding trial. Water temperature ranged between 22.17 and 24.79 °C (mean of 23.48±1.2°C) during the experimental period. The average dissolved oxygen and pH values were 6.8±0.4 mg l⁻¹ and 7.37±0.2, respectively. The photoperiod was based on natural environmental fluctuations (12-hour light and 12 hour dark) throughout the experiment. Triplicate groups of fish were hand-fed one of the experimental diets to visual satiation thrice daily (08:00 h, 13:00 h and 17:00 h) for 90 days.

Sampling methods

Before starting the feeding test, the weights of 20 fish were measured to the nearest 0.1 g and whole body was kept at -80°C until the biochemical composition analysis. At the termination of the feeding trial, 24 h after the last feeding, fish in each tank were anesthetized with anaesthetic 2-phenoxyethanol (0.5 mL L^{-1} ; (Zahl *et al.*, 2012; Utne-Palm and Smith 2020) and individually weighed to the nearest 0.01 g (Wang *et al.*, 2015; Yaghoubi *et al.*, 2016).

Blood samples were taken from the caudal vein of four fish (per replicate) using a 2.5 mL heparin syringe (Heparinsodium 5000 IU mL^{-1} , Alborz Daru, Iran). The extracted blood sample was aliquoted into two parts (one for measuring blood factors, and the other for separating serum) in the microtubes. Separating serum of the blood sample was done by centrifuging at 3000g for 10 min at 4°C and stored in Eppendorf tubes at -80°C until further serum biochemical analysis (Carobene *et al.*, 2016). Three fish from each tank were sacrificed with the high levels of anaesthetic (4 mL L^{-1}). The visceral contents of the fish were isolated on dry ice, and the total visceral contents were weighed to the nearest 0.01 g and then weighed separately for liver and visceral fat. The fillet was removed from the rest of the carcasses. In addition, three fish were isolated from each experimental tank to analyze the biochemical composition of the whole body. Fillets and whole body were transferred in labeled bags to a freezer at -80°C until further analysis (Qin *et al.*, 2016).

Biochemical composition of diets and body

Proximate biochemical composition of ingredients, diets, whole body, fillet, and liver was measured based on AOAC, 2005 methods. Dry matter was determined using a moisture analyzer (AMB5 0, ADAM, UK) and gravimetric calculation. Using the Kjeldahl method and device (BÜCHI, Auto-KjeldahlK-370, Switzerland), after acidic digestion of the sample, distillation, and titration with 4% boric acid, the amount of nitrogen obtained was multiplied by 6.25 for protein content calculation. Total lipid was measured by Soxhlet (Barnstead/ Electrothermal, UK) using petroleum ether with a boiling point of $40\text{-}60^{\circ}\text{C}$ as a solvent. Raw dietary fiber after acidic and alkaline digestion of the sample by the raw fiber extractor (VELP® Scientifica, Italy) and placing the dry sample in the furnace at 550°C for four hours and calculating the amount of sample lost in the furnace (Finetech, Shin Saeng Scientific, South Korea) was calculated. Ash of ingredient, diet, whole body, and fillets were measured using a furnace at 550°C for 8 hours. Calcium and phosphorus content were measured on the ash obtained from items and then measured by titration and spectrophotometer (at a wavelength of 700 nm), respectively (AOAC, 2005).

Blood parameters measurement methods

Hemoglobin concentration was measured by the cyanomethahemoglobin method at a

wavelength of 540 nm using a commercial kit (Zistshemi, Iran; (Houston 1990). The red blood cells (RBC) were determined optically with a Neubauer chamber using the Natt and Herrick (1952) solution as diluent. Hematocrit (Hct) was estimated by the micro-hematocrit method (Brown, 1988). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the following formula (Ellis and Campbell, 2007):

$$\text{MCV} = (\text{Hct} / \text{RBC}) \times 1$$

$$\text{MCH} = \text{Hem} / \text{RBC}$$

$$\text{MCHC} \text{ g dL}^{-1} = (\text{Hb} / \text{Hct}) \times 100$$

Serum biochemical parameters were analyzed using an auto-analyzer (Mindray BS-200, China) with commercial clinical investigation kits (Pars Azmoon Kit, Tehran, Iran). Biochemical measurements were carried out for glucose, total protein (TP;

(Morris *et al.*, 1996), albumin, triglyceride and total cholesterol (CHO; (McGowan *et al.*, 1983). Globulin was calculated by subtracting the albumin values from the total plasma protein (McClatchey, 2002).

Standard formulae were used to assess growth performance and morphometric indices: weight gain (WG;), daily growth rate (DGR); viscera somatic index (VSI), intraperitoneal fat ratio (IPF), hepatosomatic index (HSI), condition factor (CF), survival rate (SR); feed utilization: feed intake (FI), feed conversion ratio (FCR), the protein efficiency ratio (PER), retention efficiency of nitrogen (NRE), phosphorus (PRE), waste output of total nitrogen (NW) and total phosphorus (PW); economic indices: economic coefficient ratio (ECR), reduced cost (RC), profit index (PI) were calculated as follows:

$$\text{WG (g)} = (\text{Wt} - \text{W0}) \text{ (Iqbal } et al., 2022)$$

$$\text{DGR} = (\text{Wt} - \text{W0}) / t \text{ (Zaikov } et al., 2008)$$

$$\text{CF (gcm}^{-3}) = 100 \times \text{W}_t / \text{L}_s^3 \text{ (Wang } et al., 2015)$$

$$\text{VSI (\%)} = 100 \times \text{W}_v / \text{W}_t \text{ (Wang } et al., 2015)$$

$$\text{IPF (\%)} = 100 \times \text{W}_{pf} / \text{W}_t \text{ (Yaghoubi } et al., 2016)$$

$$\text{HSI (\%)} = 100 \times \text{W}_l / \text{W}_t \text{ (Wang } et al., 2015)$$

$$\text{SR (\%)} = 100 \times \text{Nt} / \text{N0} \text{ (Slawski } et al., 2011; \text{Iqbal } et al., 2022)$$

$$\text{FI (\% day}^{-1}) = 100 \times I / [(\text{W0} + \text{Wt}) / 2 \times t] \text{ (Wang } et al., 2015)$$

$$\text{FCR} = I / (\text{Wt} - \text{W0}) \text{ (Wang } et al., 2015)$$

$$\text{PER} = (\text{Wt} - \text{W0}) / (I \times \text{CNf}) \text{ (Yaghoubi } et al., 2016)$$

$$\text{NRE (\%)} = 100 \times (\text{Wt} \times \text{CNt} - \text{W0} \times \text{CN0}) / (I \times \text{CNf}) \text{ (Wang } et al., 2015)$$

$$\text{PRE (\%)} = 100 \times (\text{Wt} \times \text{CPt} - \text{W0} \times \text{CP0}) / (I \times \text{CPf}) \text{ (Wang } et al., 2015)$$

$$\text{NW [g N (kg fish gain)}^{-1}] = 1000 \times (I \times \text{CNf}) \times (1 - \text{NRE}) / [(\text{Wt} - \text{W0}) \times 6.25] \text{ (Wang } et al., 2015)$$

$$\text{PW [g P (kg fish gain)}^{-1}] = 1000 \times (I \times \text{CPf}) \times (1 - \text{PRE}) / (\text{Wt} - \text{W0}) \text{ (Wang } et al., 2015)$$

$ECR = FCR \times \text{Cost of feed}$ (Piedecausa *et al.*, 2007)

$RC = 100 - (100 \times (ECR \text{ diet} / ECR \text{ Control diet}))$ (Hernández *et al.*, 2014)

$PI = Cf \text{ (kg)} / CN$ (Hernández *et al.*, 2014)

Where I (g) is the total amount of the eaten experimental diets on a dry matter basis ; W0(g) is the total initial body weight and Wt (g) is the total final body weight; Ls (cm), Wv (g), Wpf and Wl(g) are the final body length, viscera weight, intraperitoneal fat weight and liver weight; t (day) is the duration of the feeding trial; Nt is the number of fish at the end of the feeding trial and N0 at the start; CNt, CPt, and CN0, CP0, are the final and initial levels (%) of crude protein, phosphorus; CNf, CPf, (%) are the crude protein, phosphorus contents of the test diets. Cf is the value of fish; CN is the cost of feed. Cost of feeds were presented in Table 1

Data analysis

The data are presented as means \pm standard error of the mean calculated from three replicates. The data of each parameter were tested for normality and homoscedasticity by applying Brown-Forsythe and Welch tests respectively (Iqbal *et al.*, 2021). A one way analysis of variance (ANOVA) was performed with diet as the independent variable. A Tukey's HSD test was used for post hoc after a significant ANOVA ($p < 0.05$). Data were analyzed using SPSS ver.25.0 (Chicago, Illinois, USA) and figures were prepared using Microsoft Excel 2010. Quadratic Regression analysis was done to model relationship between the measured

parameters and the SBM replacement level. The result is a regression equation that can be used to predict the maximum and minimum SBM replacement levels.

The equation has the form:

$$y = ax^2 + bx + c$$

Where $a \neq 0$, $x = -b/2a$

Results

Experimental diets

The sesame seed cake used in this experiment was higher in crude lipid (8.6%), crude fiber, phosphorus, and calcium content than SBM. The crude lipid, crude fiber, phosphorus, and calcium contents of SSC were 7.4, 1.75, 1.5, and 2.7 times higher than in SBM, respectively. With increasing level of replacement of SBM with SSC, the dietary crude fiber was significantly increased ($p < 0.05$, Table 1). Dietary phosphorus content was significantly higher in SSC75 and 100 than in other experimental diets ($p < 0.05$). Dietary calcium content was significantly higher in SS75 and 100 than in SSC0 ($p < 0.05$).

Survival, production, and nutrient utilization

The juvenile common carp remained in good health throughout the feeding trial and neither deformity nor disease was observed. Percentage SR without significant differences was 95 in SSC0 to 100% in SSC75 ($p > 0.05$). FBW, WG, DGR and SL did not show significant differences between treatments

($p>0.05$). In SSC treatments, the FI, FCR and PER did not show a significant difference with SBM-based treatment (SSC0; $p>0.05$; Table 2). The value of FCR in treatments containing SSC was lower than in the control treatment. The value of PER was higher in SSC

treatments than in SSC0. Significantly higher NRE was observed in SSC25 in comparison with SSC0 ($p<0.05$). The NW in SSC treatments was lower than in the control treatment (SSC0).

Table 1: Formulation of the experimental diets were fed by juvenile *C. carpio* for 90-day

	SSC0	SSC25	SSC50	SSC75	SSC100
Dietary ingredients (g. kg⁻¹)					
Tuna by-product meal	70	70	70	70	70
Poultry by-product meal	120	120	120	120	120
Soybean meal	350	262.5	175	87.5	0
Sesame seed cake	0	87.5	175	262.5	350
Wheat middlings	200	200	200	200	200
Corn meal	90	90	90	90	90
Barely meal	85	85	85	85	85
Fish Oil	25	25	25	25	25
Sunflower oil	25	20	12	5	0
Corn Starch	0	5	13	20	25
Vitamin Premix ^a	10	10	10	10	10
Mineral Premix ^b	15	15	15	15	15
Stay-C	5	5	5	5	5
Betaine	5	5	5	5	5
Dietary Proximate composition (% Dry-weight basis)					
Dry matter	93.65±0.58	93.18±0.22	92.90±0.58	92.62±0.55	92.34±0.63
Crude protein	29.60±0.14	29.97±0.17	29.34±0.28	29.70±0.26	29.47±0.37
Crude lipid	7.38±0.26	8.36±0.09	7.81±0.30	8.48±0.77	7.86±0.57
Crude fiber	2.61±0.06 ^e	3.12±0.04 ^d	3.62±0.08 ^c	4.13±0.10 ^b	4.64±0.05 ^a
Ash	7.57±0.17	7.34±0.16	7.72±0.05	7.73±0.04	7.79±0.01
Phosphorus	1.50±0.07 ^b	1.55±0.16 ^b	1.65±0.09 ^b	2.23±0.12 ^a	2.50±0.09 ^a
Calcium	1.92±0.05 ^b	2.05±0.03 ^{ab}	2.22±0.11 ^{ab}	2.45±0.21 ^a	2.49±0.06 ^a

Composition of ingredients (% Dry-weight basis):

Tuna by product meal: 60.52% crude protein, 9.42% crude lipid, 13.09% ash, 1.52% calcium, 3.95% phosphorus

Poultry by-product meal: 55.62% crude protein, 23.38% crude lipid, 4.11% ash, 3.9% calcium, 6.62% phosphorus

Soybean meal: 42.12% crude protein, 1.16% crude lipid, 7.04% ash, 0.79% calcium, 0.73% phosphorus

Sesame seed cake: 40.32% crude protein, 8.6% crude lipid, 7.04% ash, 2.12% calcium, 1.13% phosphorus

Tuna by-product meal, poultry by-product meal, soy bean meal and the other feed ingredients were provided by the Beyza Feed mill company. a) Vitamin premix: Vit. A, 2000 IU/Kg; Vit. D3, 800 IU/Kg; Vit E, 88 IU/Kg; Vit K, 3 mg/kg; Vit C, 200 mg/kg; Vit B1, 12 mg/kg; Vit B2, 14 mg/kg; Vit B5, 70 mg/kg; Vit B3, 50 mg/kg; Vit B6, 12 mg/kg; Vit B9, 3 mg/kg; Vit B12, 0.016 mg/kg; Vit H2, 0.14 mg/kg. Damloran Pharmaceutical Company, Broujerd, Iran. b) Mineral Premix: Selenium, 0.168 mg/kg; Iron sulfate, 20 mg/kg; Copper sulfate, 2 mg/kg; Calcium iodate, 2 mg/kg; Zinc oxide, 33.2 mg/kg; Cobalt, 0.336 mg/kg; Manganese oxide, 16.8 mg/kg. c) SSC.CP: The portion of sesame seed cake protein in crude protein; d) SSC. TDP: The portion of sesame seed cake protein in total dietary protein.

A significantly lower NW was observed in SSC25 and SSC50 treatments

($p<0.05$). Significant differences were not detected in PRE between fish fed

SSC0 and other dietary treatments ($p>0.05$). A significantly lower PW was measured in SSC25 and SSC50 than in SSC0 ($p<0.05$). According to the quadratic regression, 47.81–56.34% replacement of SBM by SSC could result in the highest FBW (Table 2; Fig. 1), PER (Fig. 2) and the lowest values of PW (Fig. 3).

The price of experimental diets presented in Table 1. The value of ECR decreased with increasing levels of SSC. RC of SSC100 was about 35.10% in comparison with the control diet. PI increased with an increased replacement level of dietary SBM.

Table 2: Growth performance and feed utilization of juvenile *C. carpio* and economic indexes of experimental diets and quadratic regression between experimental diets and FBW and PER and PW

Indices	SSC0	SSC25	SSC50	SSC75	SSC100
Growth performance					
IBW (g)	83.09±1.21	83.29±1.13	83.44±1.12	83.00±1.12	83.28±1.17
FBW (g)	169.99±2.70	179.27±3.00	177.87±0.81	173.95±3.63	170.96±2.27
WG (g)	86.90±1.56	95.99±3.98	94.43±2.87	90.95±1.10	87.68±2.78
DGR (g.day ⁻¹)	0.97±0.05	1.07±0.07	1.05±0.06	1.01±0.04	0.97±0.04
SL (cm)	15.78±0.17	15.57±0.21	15.45±0.22	15.71±0.18	15.28±0.21
SR (%)	95.56±4.44	97.78±2.22	97.33±3.85	100.00±0.00	97.78±2.22
feed utilization					
FI (%.day ⁻¹)	1.26±0.01	1.22±0.01	1.22±0.00	1.26±0.02	1.24±0.02
FCR	1.66±0.05	1.50±0.05	1.52±0.02	1.64±0.04	1.59±0.07
PER	2.01±0.06	2.23±0.07	2.19±0.02	2.13±0.05	2.10±0.08
NRE (%)	28.40±2.28 ^b	36.10±1.44 ^a	33.57±1.42 ^{ab}	29.82±82 ^{ab}	29.72±3.32 ^{ab}
PRE (%)	16.55±1.75	20.0±2.68	21.99±0.69	18.85±2.06	19.55±0.42
NW (%)	56.94±1.81 ^a	45.92±1.03 ^c	48.45±1.04 ^{bc}	53.32±1.00 ^{ab}	53.46±2.53 ^{ab}
PW (%)	13.83±0.29 ^a	11.92±0.40 ^b	11.85±0.10 ^b	12.46±0.34 ^{ab}	12.75±0.07 ^{ab}
Dietary price (Rial)	166805	156680	146255	135930	125805
Economic indexes					
ECR	19389.63	16002	14630.76	14092.52	12053
RC	0	8.76	17.59	26.43	35.10
PI	2.75	2.81	3.12	3.49	3.96
Quadratic regression					
	R ²	B1	B2	Percentage of replacement	Regression P-value
FBW	0.795	-30.926	29.574	Xmax=47.81	0.002
PER	0.784	-0.789	0.889	Xmax=56.34	0.002
PW	0.8185	5.806	-6.454	Xmin=56	0.002

A different superscript in the same row denotes statistically significant differences ($p<0.05$).

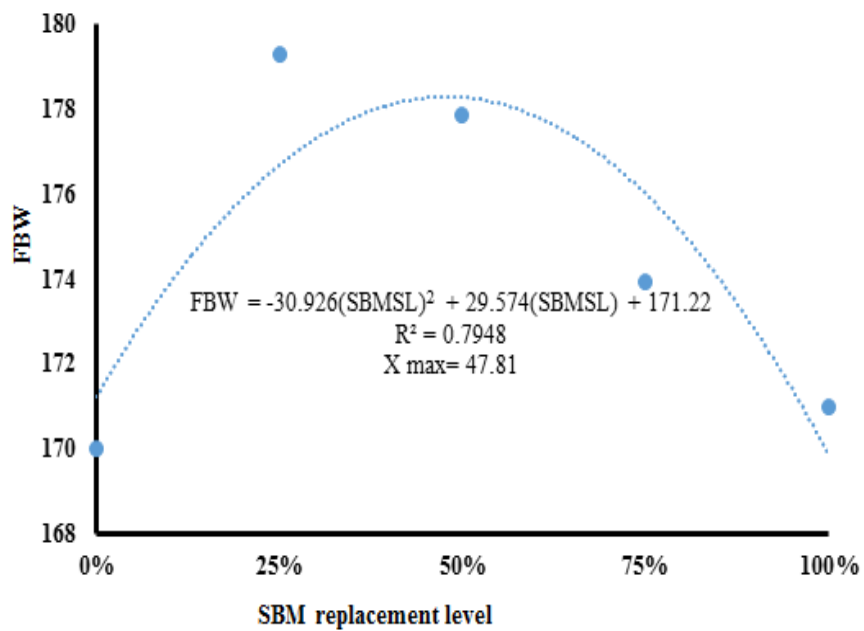


Figure 1: The relationship between final body weight (FBW) and dietary SBM replacement level (SBMSL) with SSC in juvenile common carp diet.

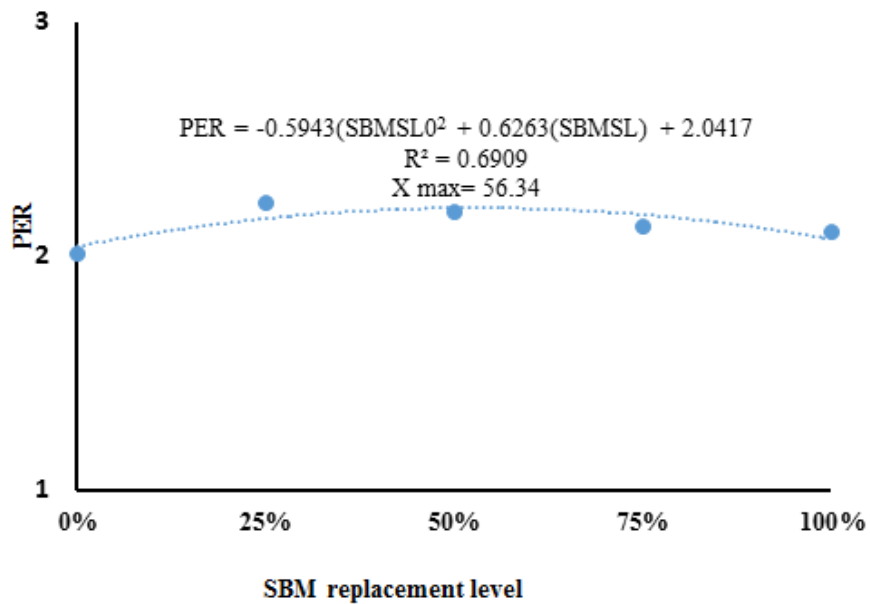


Figure 2: The relationship between protein efficiency ratio (PER) and dietary SBM replacement level (SBMSL) with SSC in juvenile common carp diets.

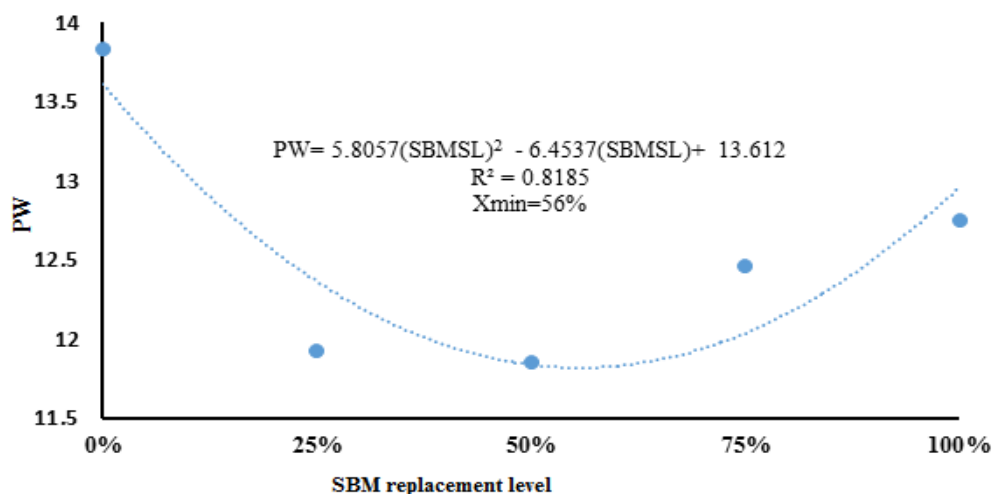


Figure 3: The relationship between phosphorous wastage (PW) and dietary SBM replacement level (SBMSL) replacement with SSC in juvenile common carp diets.

Body composition

The value of VSI, IPF, HSI, and CF did not differ significantly between experimental treatments. IPF and HIS were higher in SSC treatments than in the control treatment. HIS varied from 1.04 in SSC0 to 1.54 in SSC75. IPF ranged from 0.64 to 0.92. The whole body proximate biochemical composition such as moisture, protein,

lipid, ash, and calcium, did not show significant differences between treatments ($p > 0.05$; Table 3). Common carp's total protein, lipid, and ash content ranged from 42.42% to 45.89%, 27.65 to 31.31, and 14.73 to 15.72, respectively. Phosphorus content was significantly higher in SSC75 than in SSC100 ($p < 0.05$).

Table 3: Morphometric indices, whole body, fillet and liver proximate composition of common carp fed the experimental diets (means \pm SE, n=3).

Diets	SSC0	SSC25	SSC50	SSC75	SSC100
Morphometric indices					
CF(%)	1.84 \pm 0.04	1.81 \pm 0.05	1.79 \pm 0.06	1.82 \pm 0.05	1.75 \pm 0.05
VSI	19.76 \pm 1.28	17.44 \pm 1.67	18.58 \pm 1.05	15.07 \pm 2.25	18.49 \pm 1.05
IPF	0.64 \pm 0.21	0.84 \pm 0.25	0.92 \pm 0.32	0.68 \pm 0.19	0.72 \pm 0.20
HSI	1.04 \pm 0.32	1.06 \pm 0.24	1.43 \pm 0.21	1.54 \pm 0.36	1.32 \pm 0.32
Whole body proximate composition					
Moisture	66.02 \pm 1.98	63.01 \pm 1.08	62.20 \pm 0.88	64.31 \pm 0.87	63.77 \pm 0.29
*Crude Protein	45.89 \pm 0.86	45.14 \pm 0.34	42.42 \pm 0.56	42.50 \pm 0.50	42.76 \pm 1.55
*Crude Lipid	27.64 \pm 1.39	27.85 \pm 1.41	29.03 \pm 1.68	31.13 \pm 1.58	30.20 \pm 1.3
*Ash	15.72 \pm 0.21	15.55 \pm 0.26	14.73 \pm 0.16	15.22 \pm 0.55	15.14 \pm 0.23
Calcium	1.73 \pm 0.21	1.80 \pm 0.11	1.80 \pm 0.32	2.08 \pm 0.32	1.57 \pm 0.14
Phosphorus	2.02 \pm 0.05 ^{ab}	1.89 \pm 0.09 ^{ab}	1.97 \pm 0.04 ^{ab}	2.06 \pm 0.06 ^a	1.79 \pm 0.02 ^b
Fillet proximate composition					
Moisture	67.40 \pm 0.95	65.94 \pm 2.45	64.60 \pm 1.66	65.77 \pm 3.16	68.94 \pm 0.17
*Crude Protein	53.96 \pm 0.83 ^b	55.27 \pm 0.73 ^{ab}	54.98 \pm 1.20 ^{ab}	57.62 \pm 0.72 ^a	54.96 \pm 0.75 ^{ab}
*Crude Lipid	33.52 \pm 1.50	33.69 \pm 1.45	34.03 \pm 1.27	34.30 \pm 1.10	34.32 \pm 1.45
*Ash	3.25 \pm 0.25	3.18 \pm 0.22	3.21 \pm 0.13	3.44 \pm 0.29	3.53 \pm 0.12
Liver proximate composition					
Moisture	71.78 \pm 2.61 ^a	61.12 \pm 1.44 ^{ab}	66.70 \pm 4.30 ^{ab}	58.71 \pm 0.62 ^b	66.17 \pm 0.86 ^{ab}
Crude Lipid	43.67 \pm 1.05 ^b	50.70 \pm 0.84 ^a	46.07 \pm 0.39 ^b	51.73 \pm 1.07 ^a	44.85 \pm 0.91 ^b

* % of dry weight. A different superscript in the same row denotes statistically significant differences ($P < 0.05$).

Moisture, lipid, and ash of fish fillets fed different levels of SSC did not show significant difference with fish-fed control diet ($p>0.05$). Compared to the control treatment, significantly higher fish fillet protein was observed in SSC75 ($57.62\pm 0.72\%$; $p<0.05$). The Lipid content of the liver was significantly higher in SSC25 and SSC75 than in other treatments ($p<0.05$). The liver moisture was significantly higher in the control treatment than in SSC75 ($p<0.05$).

Blood parameters

There was no significant difference between the treatments in RBC, Hct, MCV, MCH, and MCHC ($p>0.05$; Table 4). The RBC was higher in SSC50, 75, and SSC100 than in the control

treatment. The highest percentage of hematocrit was recorded in SSC100 ($p>0.05$). The amount of hemoglobin was significantly lower in the control treatment (9.31 ± 0.58 g. dL⁻¹) than in the SSC75 (11.95 ± 0.71 g.dL⁻¹). The hemoglobin and hematocrit percentage in treatments containing SSC were higher than in the control treatment. Serum albumin, globulin, total protein, glucose, triglyceride (TG), calcium, and phosphorus, did not significantly differ between experimental treatments ($p>0.05$; Table 4). Serum CHO was significantly lower in SSC0 than in SS25 ($p<0.05$). The serum calcium and phosphorus did not significantly differ between treatments ($p>0.05$). The serum phosphorus was lower in SSC0 than in other treatments.

Table 4: Blood and serum biochemical parameters of juvenile *C. carpio* fed the experimental diets (means \pm SE, n = 3).

Diets	SSC0	SSC25	SSC50	SSC75	SSC100
Hematological indices					
RBC	1884166.67 \pm 98122.21	1879166.67 \pm 56858.51	2015000.00 \pm 77689.16	1955000.00 \pm 69788.64	2033333.33 \pm 83014.54
Hct (%)	35.92 \pm 1.04	39.0 \pm 0.88	37.75 \pm 0.82	36.17 \pm 1.00	39.83 \pm 1.51
Hem*	9.31 \pm 0.58 ^b	9.53 \pm 0.28 ^b	10.17 \pm 0.21 ^{ab}	11.95 \pm 0.71 ^a	10.84 \pm 0.39 ^{ab}
MCV	195.93 \pm 10.65	210.12 \pm 9.30	190.11 \pm 7.54	186.02 \pm 4.42	201.98 \pm 15.38
MCH	44.95 \pm 2.79	50.08 \pm 2.12	52.75 \pm 3.41	60.65 \pm 4.37	53.59 \pm 5.55
MCHC*	26.55 \pm 2.55	26.63 \pm 1.43	27.37 \pm 0.99	32.50 \pm 2.79	26.82 \pm 1.05
Serum biochemical composition					
Albumin*	1.11 \pm 0.18	1.25 \pm 0.19	1.15 \pm 0.07	1.187 \pm 0.25	1.24 \pm 0.19
Globulin*	1.86 \pm 0.47	2.42 \pm 0.16	1.96 \pm 0.29	2.22 \pm 0.43	1.90 \pm 0.13
Total protein*	2.96 \pm 0.29	3.67 \pm 0.08	3.11 \pm 0.22	3.40 \pm 0.26	3.14 \pm 0.22
Glucose**	83.67 \pm 9.17	82.00 \pm 12.50	78.33 \pm 8.33	79.33 \pm 10.90	93.00 \pm 9.29
Cholesterol**	115.00 \pm 4.36 ^b	133.00 \pm 1.53 ^a	125.00 \pm 5.77 ^{ab}	126.00 \pm 3.06 ^{ab}	124.33 \pm 2.91 ^{ab}
Triglyceride**	229.00 \pm 11.27	230.67 \pm 19.24	210.00 \pm 6.66	218.00 \pm 12.86	217.67 \pm 14.91
Calcium**	8.97 \pm 0.23	9.30 \pm 0.36	8.87 \pm 0.58	9.63 \pm 0.27	8.80 \pm 0.47
phosphorus*	4.97 \pm 0.61	7.40 \pm 0.78	6.73 \pm 1.56	6.47 \pm 0.34	5.97 \pm 0.18

unit *(g dL⁻¹) and ** (mg dl⁻¹). RBC: Red blood cell, Hct: Hematocrit, Hem: Hemoglobin, MCV: Mean corpuscular volume, MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration, A different superscript in the same row denotes statistically significant differences ($p<0.05$).

Discussion

In the present study, based on feed intake, it could be confirmed that the use of SSC did not have a negative effect on

feed attractiveness. Also, the bioavailability of nutrients was not affected by the attractiveness of the experimental diets. In our study, changes

in PER showed an analogous trend to GP. The existence of a positive relationship between PER and GP was attributed to the opportunity for nutrition to the satiation, which ensures that the fish receives adequate food (Zhou *et al.*, 2010). In our trial, treatment with lower NRE was consistent with treatment with lower GP. Quadratic regression analysis of growth and feed performance against soybean meal replacement level revealed that around 50-60% (47.81-56.34%) replacement level resulted in maximized feed utilization and growth performance. Increasing protein absorption and decreasing its excretion leads to higher GP (Green and Hardy 2002). Kumar *et al.* (2020) and Arriaga-Hernández *et al.* (2021) suggested that the reduction in GP and NE of fish fed high levels of SBM could be attributed to high level of anti-nutritional factors such as beta-conglycinin, phytate, saponins, lectins and indigestible carbohydrates, trypsin inhibitor, lower protein digestion and lower amino acid absorption. The results of other study, showed the effect of trypsin inhibitor on reducing NRE, and increasing metabolic NW (Kader *et al.*, 2012). Kumar *et al.* (2020) observed higher crude protein content and NRE in fish fed dietary SBM contains lower trypsin inhibitors, lectin, oligosaccharides, and carbohydrate. The researchers reported that at low levels of plant protein sources, physiological mechanisms in fish could offset the negative effects of anti-nutritional substances (Jimoh and Aroyehun, 2011). In our trial, the phosphorus content of dietary SSC was 1.5 times that of SBM.

Treatments containing SSC showed higher phosphorus, PRE, GP and lower PW than SBM-based treatment.

The dietary SSC helps reduce phosphorus pollution of the aquatic environment by improving the phosphorus bio-availability, increasing PRE, and reducing the demand for phosphorus supplementation in aquafeed (Yoo *et al.*, 2005). The researchers have cited that amino acid deficiency in SBM is not a growth-limiting factor, but a lack of phosphorus bio-availability reduces the growth rate (Brown *et al.*, 1997; El-Sayed and Tacon 1997). In several studies, the use of high levels of SBM and its products significantly reduced the dietary phosphorous digestibility and PRE (Yoo *et al.*, 2005; Biswas *et al.*, 2019). A decrease in phosphorus efficiency, NRE, and PRE were observed in fish fed high levels plant protein levels, which may due to high levels of phytic acid (Plaipetch and Yakupitiyage, 2014). Guo *et al.* (2011) reported that the phytic acid content of SSC was less than half that of SBM. Removal of phytin with microbial phytase from soy protein increased PRE and NRE (Plaipetch and Yakupitiyage, 2014; Biswas *et al.*, 2019).

Morphometric indices of *C. carpio* did not significantly differ between dietary treatments. In other studies, lower levels of SBM replacement (50-60%) with plant protein sources did not affect the visceral indices of Nile tilapia (Guo *et al.*, 2011) and hybrid tilapia, *O. niloticus* × *Oreochromis aureus* (Yue and Zhou, 2008). In the present study,

the liver moisture was lower, but liver lipid, whole body lipid, fillet lipid content, and HIS was higher in SSC treatments than in SBM-based treatment. Dernekbaşı *et al.*, (2017a), cited that the increase in body lipid at high levels of SS meal could be due to the higher lipid content of SS meal than SBM. The crude fiber content of SSC was almost twice that of SBM. The treatments with higher crude fiber had higher HSI, liver, whole body, and fillet lipid. The main part of the polysaccharides extracted from sesame seed includes arabinose, glucose, xylose, galactose, and mannose with trace amounts of rhamnose. The occurrence of xylose, glucose and fucose suggested the possibility of xyloglucans (Ghosh *et al.*, 2005). Improved growth performance and feed utilization in kutum (*Rutilus frisii* kutum) fed dietary galactooligosaccharide and xylooligosaccharide as fermentable fiber were reported by Kumar *et al.* (2008) and Hoseinifar *et al.* (2013). The fermentable fraction of the dietary fiber enhanced the production of metabolites such as short chain fatty acids (Yarahmadi *et al.*, 2014; Mirghaed *et al.*, 2018). Fillet protein was significantly higher in fish fed SSC75 diet than in fish fed SBM-based diet. Dernekbaşı *et al.* (2017), reported that the body protein content increased with increasing sesame seed meal level in replacement with soybean meal. The researchers attributed the decrease in body protein to a decrease in PER and NRE (Kader *et al.*, 2012; Dernekbaşı *et al.*, 2017) and lack of complete absorption of dietary

protein in the body (Egerton *et al.*, 2020).

The RBC, Hem, Hct, MCH, and MCHC value were higher in the SSC treatments than in the SBM-based treatment. Similar to our observations, higher blood indices were reported in fish fed diets containing lower levels of SBM (Zheng *et al.*, 2012; Lawal *et al.*, 2016; Viana *et al.*, 2019). Higher Hem and RBC are indicators of the health (Ye *et al.*, 2019) and higher oxygen-carrying capacity in fish fed this level of nutrients (Nazir *et al.*, 2021). It could also be stated that the total SBM replacement with SSC did not result in symptoms of diet-induced anemia (Demir *et al.*, 2014; Grant 2015; Nazir *et al.*, 2021). Decreased blood factors in fish fed SBM diet were attributed to the effects of ANs such as phytic acid (Hassaan *et al.*, 2018), which may lead to iron mal-absorption in fish intestines (Barros *et al.*, 2002; Kasiga 2018; Ye *et al.*, 2019). SBM contains lectin, phyto-hemagglutinin, and saponin (NRC 1993; Francis *et al.*, 2001), which have a high capacity for clotting and hydrolysis of red blood cells (Lim and Lee 2009; Lim *et al.*, 2011). Sesame seed is almost devoid of the above ANs (Wei *et al.*, 2022). The serum protein and NRE were higher in SSC treatments than in the control treatment. Similar to the present study, high levels of dietary soy products reduced serum protein level (Xu *et al.*, 2012; Shamna *et al.*, 2017; Nazir *et al.*, 2021). No significant difference in serum albumin and globulin content of dietary treatments could indicate that the alternative plant protein source did not

affect the content of some components of the intrinsic defense mechanism such as total protein, albumin, and globulin (Magnadóttir 2006; Shahsavani *et al.*, 2010; Soltanzadeh *et al.*, 2016). The absence of significant differences in serum glucose content, showed that fish were not affected by replacing two plant protein sources and are in good and stress-free conditions (Svoboda *et al.*, 2001; Wagner and Congleton 2004). It also indicates that the sesame seed cake did not affect energy metabolism (Zhou *et al.*, 2005; Soltanzadeh *et al.*, 2016). The serum phosphorus in fish-fed SSC diets was higher than in the fish-fed SBM-based diet. Similarly, it was reported that plasma phosphorus decreased due to the reduced availability of phosphorus in a diet containing high soy content (Lim *et al.*, 2011; Yaghoubi *et al.*, 2016). Most phosphorus in soy is associated with phytic acid and is unavailable (Liener 1994; Yaghoubi *et al.*, 2016). The results showed no significant differences in serum calcium between treatments. Half of all plasma calcium is combined with plasma protein (Andreasen, 1985); it seems that the absence of significant differences in plasma protein levels may lead to a stable plasma calcium concentration between experimental treatments. In the present feeding trial, compared to the SBM-based treatment (350 g.kg⁻¹ SBM), the treatments containing SSC, had higher serum cholesterol, HSI, and IPF. Similar to our observations, it was reported that soy products reduced the activity of fat-degrading enzymes and interrupted the absorption of bile acids

and total cholesterol in fish diet (Makino *et al.*, 1988), consequently reducing serum total cholesterol levels in European seabass (*Dicentrarchus labrax*; Dias *et al.*, 2005). This is mainly due to the fat-reducing effect and high levels of estrogenic iso-flavones, phytates, saponins, phytosterols in the soy products which may interfere with various stages of fat digestion, including emulsion, hydrolysis, binding of fatty acids to micelles, reabsorption and re-esterification in intestinal cells (Setchell and Cassidy, 1999; Biswas *et al.*, 2019).

In conclusion, juvenile *Cyprinus carpio* fed SSC diets showed higher blood indices, serum phosphorus, phosphorous retention efficiency, growth performance and lower phosphorous wastage than fish fed SBM-based diet as a consequence of lower ANs such as phytic acid in the diets. Also SSC treatments had higher crude fiber, as well as higher lipid of liver, whole body, and fillet, serum cholesterol, HSI, and IPF as a consequence of the higher fermentable fraction of the dietary fiber and due to the fat-enhancing effect and lower levels of some ANs such as estrogenic iso-flavones, phytates, saponins, phytosterols in the SSC. Comparable growth performance and measured physiological responses indicated that SBM could be replaced by 75 to 100% SSC in the formulated diet of *C. carpio* juveniles.

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