# **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 ecofriendly 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 (Dernekbası et al., 2017). SBM contains anti-nutritional factors such as lipase, protease, and αamylase 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 antinutrient 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, oilseed contains this 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; Dernekbası et al., 2017); 20-50% (Fagbenro et al., 2010a; Jimoh and Aroyehun 2011; Jimoh et al., 2014) and above 50% (Envidi 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 gariepinus parameters Clarias of (Fagbenro et al., 2010b; Lawal et al., 2016). This nutritional trial was the growth evaluate designed to 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<sup>-1</sup> crude protein) and isolipidic (80 g kg<sup>-1</sup> crude lipid) experimental diets were formulated, using WUFFFDA 2.0software, 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 °C (mean and 24.79 of 23.48±1.2°C) during the experimental period. The average dissolved oxygen and pH values were  $6.8\pm0.4$  mg l<sup>-1</sup> and 7.37±0.2, respectively. The photoperiod was based on natural environmental fluctuations (12-hour light and 12 hour throughout the experiment. dark) 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 anaesthetic2-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<sup>-1</sup>, 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 anesthetic (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 to analyze the biochemical tank 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 (AM B50, 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 40-60°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   | W  | was |  |
|---------------|-----------------|----|-----|--|
| measured      | by              | t  | the |  |
| cyanomethahen | noglobin method | at | a   |  |

of 540 nm using wavelength а 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 corpuscular (MCH) and mean hemoglobin concentration (MCHC) were calculated according to the following formula (Ellis and Campbell, 2007):

MCV= (Hct/ RBC)×1

MCH=Hem/RBC

MCHC g dL-1= (Hb/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 (IPF), ratio 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 (NRE), nitrogen 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:

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WG (g)= (Wt-W0) (Iqbal et al., 2022)

DGR= (Wt-W0)/t (Zaikov et al., 2008)

CF (gcm-3)=100×W<sub>1</sub>/L s<sup>3</sup> (Wang et al., 2015)

VSI(%)= 100 ×W<sub>y</sub>/W<sub>t</sub> (Wang et al., 2015)

IPF (%) =100 × W<sub>p</sub>f/W<sub>t</sub> (Yaghoubi et al., 2016)

HSI(%) =100 ×W<sub>1</sub>/W<sub>t</sub> (Wang et al., 2015)

SR (%)=100×Nt/N0 (Slawski et al., 2011; Iqbal et al., 2022)

FI (% day-1)=100×I/[(W0+Wt)/2×t] (Wang et al., 2015)

FCR=I/(Wt-W0) (Wang et al., 2015)

PER=(Wt-W0)/(I×CNf) (Yaghoubi et al., 2016)

NRE (%)=100×(Wt×CNt-W0×CN0)/(I×CNf) (Wang et al., 2015)

PRE(%)=100×(Wt×CPt-W0×CP0)/(I×CPf) (Wang et al., 2015)

NW [g N (kg fish gain)-1]=1000×(I×CNf)×(1-NRE)/[(Wt-W0)×6.25] (Wang et al., 2015)

PW [g P (kg fish gain)-1]=1000×(I×CPf)×(1-PRE)/(Wt-W0) (Wang et al., 2015)
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ECR =FCR×Cost of feed (Piedecausa *et al.*, 2007) RC=100-(100×(ECR diet/ECR Control diet)) (Hernández *et al.*, 2014) PI=Cf (kg)/CN (Hernández *et al.*, 2014)

Where I (g) is the total amount of the eaten experimental diets on a dry matter basis ; WO(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±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/=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 <i>C. carpio</i> for 90-day |      |       |       |       |        |  |
|---|------|-------|-------|-------|--------|--|
|   | SSC0 | SSC25 | SSC50 | SSC75 | SSC100 |  |
| Dietary ingredients (g. kg <sup>-1</sup> )  |      |       |       |       |        |  |
| Tuna by-product meal  | 70   | 70    | 70    | 70    | 70     |  |
| Poultry by-product  | 120  | 120   | 120   | 120   | 120    |  |
| meal  |      |       |       |       |        |  |
| 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 <sup>a</sup>   | 10   | 10    | 10    | 10    | 10     |  |
| Mineral Premix <sup>b</sup>   | 15   | 15    | 15    | 15    | 15     |  |
| Stay-C  | 5    | 5     | 5     | 5     | 5      |  |
| Betaine   | 5    | 5     | 5     | 5     | 5      |  |

#### **Dietary Proximate composition (% Dry-weight basis)**

| 2100019 2100111000 00111 |                        |                         |                         |                        |                     |
|--------------------------|------------------------|-------------------------|-------------------------|------------------------|---------------------|
| Dry matter               | 93.65±0.58             | 93.18±0.22              | $92.90 \pm 0.58$        | 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              | $7.38\pm0.26$          | 8.36±0.09               | 7.81±0.30               | $8.48 \pm 0.77$        | $7.86\pm0.57$       |
| Crude fiber              | $2.61 \pm 0.06^{e}$    | $3.12 \pm 0.04^{d}$     | 3.62±0.08°              | $4.13 \pm 0.10^{b}$    | $4.64 \pm 0.05^{a}$ |
| Ash                      | 7.57±0.17              | 7.34±0.16               | 7.72±0.05               | 7.73±0.04              | 7.79±0.01           |
| Phosphorus               | $1.50\pm0.07^{b}$      | $1.55 \pm 0.16^{b}$     | $1.65 \pm 0.09^{b}$     | 2.23±0.12 <sup>a</sup> | $2.50\pm0.09^{a}$   |
| Calcium                  | 1.92±0.05 <sup>b</sup> | 2.05±0.03 <sup>ab</sup> | 2.22±0.11 <sup>ab</sup> | 2.45±0.21ª             | $2.49 \pm 0.06^{a}$ |
|                          |                        |                         |                         |                        |                     |

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

SCC0 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          | $177.87 \pm 0.81$        | 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               | $87.68 \pm 2.78$         |  |  |
| DGR (g.day <sup>-1</sup> ) | $0.97 \pm 0.05$         | $1.07\pm0.07$        | $1.05\pm0.06$            | $1.01\pm0.04$            | $0.97 \pm 0.04$          |  |  |
| SL (cm)                    | 15.78±0.17              | 15.57±0.21           | 15.45±0.22               | 15.71±0.18               | $15.28 \pm 0.21$         |  |  |
| SR (%)                     | 95.56±4.44              | 97.78±2.22           | 97.33±3.85               | $100.00 \pm 0.00$        | 97.78±2.22               |  |  |
|                            |                         |                      |                          |                          |                          |  |  |
| feed utilization           |                         |                      |                          |                          |                          |  |  |
| FI (%.day <sup>-1</sup> )  | $1.26\pm0.01$           | $1.22\pm0.01$        | $1.22\pm0.00$            | $1.26\pm0.02$            | $1.24\pm0.02$            |  |  |
| FCR                        | $1.66 \pm 0.05$         | $1.50\pm0.05$        | $1.52\pm0.02$            | $1.64\pm0.04$            | $1.59 \pm 0.07$          |  |  |
| PER                        | $2.01\pm0.06$           | 2.23±0.07            | $2.19\pm0.02$            | 2.13±0.05                | $2.10\pm0.08$            |  |  |
| NRE (%)                    | $28.40 \pm 2.28^{b}$    | $36.10 \pm 1.44^{a}$ | $33.57 \pm 1.42^{ab}$    | $29.82 \pm 82^{ab}$      | 29.72±3.32 <sup>ab</sup> |  |  |
| PRE (%)                    | 16.55±1.75              | $20.0\pm2.68$        | 21.99±0.69               | $18.85 \pm 2.06$         | $19.55 \pm 0.42$         |  |  |
| NW (%)                     | 56.94±1.81 <sup>a</sup> | 45.92±1.03°          | 48.45±1.04 <sup>bc</sup> | 53.32±1.00 <sup>ab</sup> | $53.46 \pm 2.53^{ab}$    |  |  |
| PW (%)                     | 13.83±0.29 <sup>a</sup> | $11.92 \pm 0.40^{b}$ | $11.85 \pm 0.10^{b}$     | 12.46±0.34 <sup>ab</sup> | 12.75±0.07 <sup>ab</sup> |  |  |
| Dietary price              | 166805                  | 156680               | 146255                   | 135930                   | 125805                   |  |  |
| (Rial)                     |                         |                      |                          |                          |                          |  |  |
|                            |                         |                      |                          |                          |                          |  |  |
| Economic indexe            | s                       |                      |                          |                          |                          |  |  |
| 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       |                         |                      |                          |                          |                          |  |  |
|                            | $\mathbb{R}^2$          | B1                   | B2                       | Percentage of            | <b>Regression P-</b>     |  |  |
|                            |                         |                      |                          | replacement              | 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± SE, n=3).

| Diets                        | SSC0                    | SSC25                    | SSC50                    | SSC75                | SSC100                   |  |
|------------------------------|-------------------------|--------------------------|--------------------------|----------------------|--------------------------|--|
| Morphometric indices         |                         |                          |                          |                      |                          |  |
| CF(%)                        | $1.84\pm0.04$           | $1.81 \pm 0.05$          | 1.79±0.06                | $1.82 \pm 0.05$      | 1.75±0.05                |  |
| VSI                          | 19.76±1.28              | $17.44 \pm 1.67$         | $18.58 \pm 1.05$         | $15.07 \pm 2.25$     | $18.49 \pm 1.05$         |  |
| IPF                          | $0.64 \pm 0.21$         | $0.84\pm0.25$            | $0.92\pm0.32$            | 0.68±0.19            | $0.72 \pm 0.20$          |  |
| HSI                          | $1.04\pm0.32$           | $1.06\pm0.24$            | 1.43±0.21                | 1.54±0.36            | $1.32 \pm 0.32$          |  |
| Whole body proxi             | imate compositi         | on                       |                          |                      |                          |  |
| Moisture                     | $66.02 \pm 1.98$        | 63.01±1.08               | $62.20\pm0.88$           | 64.31±0.87           | 63.77±0.29               |  |
| *Crude Protein               | 45.89±0.86              | 45.14±0.34               | 42.42±0.56               | 42.50±0.50           | 42.76±1.55               |  |
| *Crude Lipid                 | 27.64±1.39              | $27.85 \pm 1.41$         | $29.03 \pm 1.68$         | 31.13±1.58           | 30.20±1.3                |  |
| *Ash                         | 15.72±0.21              | 15.55±0.26               | 14.73±0.16               | $15.22 \pm 0.55$     | 15.14±0.23               |  |
| Calcium                      | $1.73\pm0.21$           | $1.80\pm0.11$            | $1.80\pm0.32$            | $2.08\pm0.32$        | $1.57 \pm 0.14$          |  |
| Phosphorus                   | $2.02\pm0.05^{ab}$      | $1.89 \pm 0.09^{ab}$     | $1.97 \pm 0.04^{ab}$     | $2.06\pm0.06^{a}$    | $1.79 \pm 0.02^{b}$      |  |
| Fillet proximate composition |                         |                          |                          |                      |                          |  |
| Moisture                     | 67.40±0.95              | $65.94 \pm 2.45$         | 64.60±1.66               | 65.77±3.16           | 68.94±0.17               |  |
| *Crude Protein               | 53.96±0.83 <sup>b</sup> | 55.27±0.73 <sup>ab</sup> | 54.98±1.20 <sup>ab</sup> | $57.62 \pm 0.72^{a}$ | 54.96±0.75 <sup>ab</sup> |  |
| *Crude Lipid                 | 33.52±1.50              | 33.69±1.45               | 34.03±1.27               | 34.30±1.10           | 34.32±1.45               |  |
| *Ash                         | $3.25 \pm 0.25$         | 3.18±0.22                | 3.21±0.13                | 3.44±0.29            | 3.53±0.12                |  |
| Liver proximate composition  |                         |                          |                          |                      |                          |  |
| Moisture                     | 71.78±2.61ª             | $61.12 \pm 1.44^{ab}$    | $66.70 \pm 4.30^{ab}$    | $58.71 \pm 0.62^{b}$ | $66.17 \pm 0.86^{ab}$    |  |
| Crude Lipid                  | $43.67 \pm 1.05^{b}$    | $50.70\pm0.84^{a}$       | 46.07±0.39 <sup>b</sup>  | $51.73 \pm 1.07^{a}$ | 44.85±0.91 <sup>b</sup>  |  |

\* % 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±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  $(9.31\pm0.58 \text{ g. dL}^{-1})$  than in the (11.95±0.71 g.dL<sup>-1</sup>). SSC75 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 experimental between 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 + SE, n = 3).

| $(\text{incars} \pm 5E, n - 5).$ |                        |                        |                           |                         |                           |  |  |
|----------------------------------|------------------------|------------------------|---------------------------|-------------------------|---------------------------|--|--|
| Diets                            | SSC0                   | SSC25                  | SSC50                     | SSC75                   | SSC100                    |  |  |
| Hematological indices            |                        |                        |                           |                         |                           |  |  |
| PBC                              | $1884166.67 \pm$       | $1879166.67 \pm$       | $2015000.00 \pm$          | $1955000.00 \pm$        | 2033333.33±               |  |  |
| KDC                              | 98122.21               | 56858.51               | 77689.16                  | 69788.64                | 83014.54                  |  |  |
| Hct (%)                          | 35.92±1.04             | 39.0±0.88              | 37.75±0.82                | 36.17±1.00              | 39.83±1.51                |  |  |
| Hem <sup>*</sup>                 | 9.31±0.58 <sup>b</sup> | 9.53±0.28 <sup>b</sup> | 10.17±0.21 <sup>ab</sup>  | 11.95±0.71 <sup>a</sup> | 10.84±0.39 <sup>ab</sup>  |  |  |
| MCV                              | 195.93±10.65           | 210.12±9.30            | 190.11±7.54               | $186.02 \pm 4.42$       | 201.98±15.38              |  |  |
| MCH                              | 44.95±2.79             | 50.08±2.12             | 52.75±3.41                | 60.65±4.37              | 53.59±5.55                |  |  |
| $MCHC^*$                         | 26.55±2.55             | 26.63±1.43             | 27.37±0.99                | 32.50±2.79              | 26.82±1.05                |  |  |
|                                  |                        |                        |                           |                         |                           |  |  |
| Serum biocher                    | nical compositio       | n                      |                           |                         |                           |  |  |
| Albumin*                         | $1.11 \pm 0.18$        | 1.25±0.19              | $1.15 \pm 0.07$           | 1.187±0.25              | 1.24±0.19                 |  |  |
| Globulin <sup>*</sup>            | $1.86 \pm 0.47$        | 2.42±0.16              | $1.96 \pm 0.29$           | 2.22±0.43               | 1.90±0.13                 |  |  |
| Total protein <sup>*</sup>       | 2.96±0.29              | 3.67±0.08              | 3.11±0.22                 | 3.40±0.26               | 3.14±0.22                 |  |  |
| Glucose**                        | 83.67±9.17             | 82.00±12.50            | 78.33±8.33                | 79.33±10.90             | 93.00±9.29                |  |  |
| Cholesterol**                    | $115.00 \pm 4.36^{b}$  | $133.00 \pm 1.53^{a}$  | 125.00±5.77 <sup>ab</sup> | $126.00 \pm 3.06^{ab}$  | 124.33±2.91 <sup>ab</sup> |  |  |
| Triglyceride**                   | 229.00±11.27           | 230.67±19.24           | 210.00±6.66               | $218.00{\pm}12.86$      | 217.67±14.91              |  |  |
| Calcium**                        | 8.97±0.23              | 9.30±0.36              | 8.87±0.58                 | 9.63±0.27               | $8.80 \pm 0.47$           |  |  |
| phosphorus*                      | 4.97±0.61              | $7.40\pm0.78$          | 6.73±1.56                 | 6.47±0.34               | 5.97±0.18                 |  |  |

unit \*(g dL1) and \*\* (mg dl<sup>-1</sup>). 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, lectins and indigestible saponins, 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 growthlimiting 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 SBM and levels of 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 dietarv 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 short chain such as 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 increased with increasing content 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 globulin content of and 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<sup>-1</sup> 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 (Dicentrarchus seabass 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 reesterification 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, performance and growth 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 а 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 isoflavones. 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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