

# Effects of replacing fish meal with sunflower meal on growth performance, body composition, hematological and biochemical indices of common carp (*Cyprinus carpio*) fingerlings

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Abstract. The aim of the present study was to evaluate the effects of replacing fish meal with sunflower meal had on common carp, Cyprinus carpio L. fingerlings. A total of 455 fish with an average weight of  $3.03 \pm 0.36$  g were distributed in 15 fiberglass tanks and fed five isonitrogenous and isoenergetic diets with replacement levels of 0 (control), 25, 50, 75, and 100% of fish meal with sunflower meal for 10 weeks. Based on the results, the highest and lowest final weights were observed at 25 and 100%, respectively, although the differences at 50 and 75% were not significant compared to the control. Significant differences were observed in body composition excluding ash content. Differences in all hematological indices among treatments were not significant, but in plasma biochemical indices, there was a significant decline in triglyceride levels at 100%, and cholesterol was significantly higher in the control. The results of the current study demonstrate that replacing fish meal with sunflower meal is possible up to 75% for common carp fingerlings without negative impacts on growth, body composition, or hematological and plasma biochemical indices.

**Keywords**: substitution, plant ingredients, protein utilization, blood indices, cyprinids

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# Introduction

Fish meal (FM) is the most important feed ingredient in aquaculture because of its nutritional features such as highly digestible protein, palatability, and low antinutritional factors, but there are limitations in ready access to FM because of its rising cost and global production decline, so the necessity of finding alternative sources for FM is inevitable (Ljubojević et al. 2015). Plant products cost less and their supply is more sustainable (Hardy 2010), which makes them suitable as replacements for FM.

Sunflower meal (SFM) is a by-product of sunflower (*Helianthus annuus*) seeds oil extraction. SFM has less antinutritional factors except tannin and phytic acid compared to other oilseed meals (Francis et al. 2001). It is rich in sulfur amino acids content (Olvera-Novoa et al. 2002) but low in lysine (Tacon et al. 1984). Some previous studies have used SFM in fish nutrition (Tacon et al. 1984, Olvera-Novoa et al. 2002, Gill et al. 2006, Sánchez Lozano et al. 2007, Nogales Mérida et al. 2011).

Cyprinids are one of most widespread families of farmed aquatic animals. Common carp, *Cyprinus* 

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*carpio* L. is the most well-known member of this family and is ranked third among farmed aquatic animals (FAO 2014). Most common carp products come from ponds and semi-intensive culture systems (Ljubojević et al. 2014).

Formulated diets increase production output, but supplying it elevates costs, and low cost plant ingredients can reduce expenses (Ljubojević et al. 2015). Many experiments have studied the effects of feeding plant ingredients on the hematological indices of fish (Kaushik et al. 1995, Bransden et al. 2001, Dias et al. 2005, Sitjà-Bobadilla et al. 2005, Kumar et al. 2010, Jahanbakhshi et al. 2013), but no study has yet examined the effects of feeding by SFM on fish blood indices, so the present study was conducted to determine the effects of replacing FM with SFM on growth performance, proximate body composition, hematological and plasma biochemical indices of common carp fingerlings.

# Materials and methods

#### Fish and experimental design

This study was conducted at the fish rearing facilities of the Faculty of Natural Resources at the University of Guilan (Sowmeh Sara, Guilan, Iran) for 10 weeks. The fish were obtained from a local farm and were adapted with a diet free of SFM in a culture system for 2 weeks before starting the experimental period. After the acclimation period, four hundred and fifty fish with an average weight of  $3.03 \pm 0.36$  g were distributed in 15 circular fiberglass tanks with 500 L capacity (30 fish per tank) which were filled with water up to 300 L. The water flow rate was  $5.23 \pm 0.27$  L min<sup>-1</sup>. Water quality parameters were monitored during the experimental period, and mean values of temperature of  $16 \pm 1^{\circ}$ C, dissolved oxygen of  $6.51 \pm$  $0.27 \text{ mg L}^{-1}$ , and pH of 7.61 ± 0.52 were recorded. The photoperiod using artificial light was 12 hours light and 12 hours dark.

#### **Experimental diets**

Five isonitrogenous and isoenergetic diets with SFM replacement levels of 0 (control), 25, 50, 75 and 100% of FM were formulated. The fish were fed these diets four times daily at 08:00, 12:00, 16:00, and 20:00 to satiation. The ingredients and proximate composition of the diets are presented in Table 1.

#### Growth performance

Biometric measurements were conducted bi-weekly, and feeding was stopped 24 hours before any handling. The fish were anesthetized with 100 ppm clove powder extract, and then they were weighed and their total length was measured. At the end of the experiment, 3 fish per tank were sampled and their liver and viscera were weighed and the other growth indices mentioned in Table 2 were calculated.

## Body composition analysis

The proximate composition of the whole body and the diets was determined using standard methods described in AOAC (2005). At the end of the trial, 6 fish per tank were collected randomly and sacrificed by applying blunt trauma to the head. The samples were homogenized and dried at 105°C in an oven (Laboven, Tehran, Iran) to a constant weight. Ash content was determined by combusting dried samples in an electric furnace (Atbin, Tehran, Iran) at 550°C for 8 hours. Crude protein was determined with the Kjeldahl method, and crude fat was extracted with a Soxhlet set (Bakhshi, Tehran, Iran) and n-Hexane solvent.

# Hematological and plasma biochemical indices

At the end of the experiment, blood was drawn radonly from 12 fish per tank. Blood samples were drawn from the caudal vein with 2 mL heparinized syringes. Half of each of sample was used to

#### Table 1

	Replacement levels (%)					
	0 (control)	25	50	75	100	
Ingredients (%)						
Fish meal	28	21	14	7	-	
Sunflower meal	-	7	14	21	28	
Soybean meal	19	23	26	29	34	
Meat meal	12	16	19	23	25	
Corn meal	18	15	12	8	3	
Wheat flour	14.5	9.5	6.5	3.5	1.5	
Fish oil	1.5	1.5	1.5	1.5	1.5	
Canola oil	1.5	1.5	1.5	1.5	1.5	
Vitamin premix <sup>1</sup>	1.5	1.5	1.5	1.5	1.5	
Mineral premix <sup>2</sup>	1.5	1.5	1.5	1.5	1.5	
Lysine	1	1	1	1	1	
Methionine	0.5	0.5	0.5	0.5	0.5	
Dicalcium phosphate	1	1	1	1	1	
Proximate composition (% of wet weight)						
Moisture	12.25	11.97	13.30	12.93	13.31	
Crude protein	35.54	35.98	35.65	35.78	35.75	
Crude fat	9.97	10.24	10.40	10.64	10.63	
Ash	8.87	9.53	8.62	9.57	10.21	
Nitrogen free extract <sup>3</sup>	33.37	32.28	32.03	31.08	30.10	
Gross energy (kcal kg <sup>-1</sup> ) <sup>4</sup>	4230.20	4234.20	4220.30	4210.40	4176.60	

Ingredients and proximate composition of experimental diets used to feed common carp (*C. carpio*) fingerlings with different levels of fish meal replaced with sunflower meal

<sup>1</sup>Science Laboratories Co. (Qazvin, Iran), including (g kg<sup>-1</sup>): A (1600000 IU), D<sub>3</sub> (400000 IU), E (40), K<sub>3</sub> (2), B<sub>1</sub> (6), B<sub>2</sub> (8), B<sub>3</sub> (12), B<sub>5</sub> (40), B<sub>6</sub> (4), B<sub>9</sub> (2), B<sub>12</sub> (0.008), H<sub>2</sub> (0.24), C (60), Inositol (20), Biotin (0.2)

<sup>2</sup>Science Laboratories Co. (Qazvin, Iran), including (g kg<sup>-1</sup>): Iron (6), Zinc (10), Selenium (0.02), Cobalt (0.1), Cupper (6), Manganese (5), Iodine (0.6), Choline chloride (6)

<sup>3</sup>Nitrogen free extract = 100 - (moisture + crude protein + crude fat + ash)

<sup>4</sup>Calculated by: crude protein (55 kcal kg<sup>-1</sup>), crude fat (91 kcal kg<sup>-1</sup>), NFE (41 kcal kg<sup>-1</sup>)

determine hematological indices and the other half was used to assess plasma biochemical parameters. The hematological parameters including the number of red blood cells (RBC) and white blood cells (WBC), differential leukocyte count, hemoglobin (Hb), and hematocrit (Hct) were determined with methods described in Blaxhall and Daisley (1973) and Řehulka (2000). RBC and WBC were counted in a Neubauer hemacytometer. Hb was determined with the cyanomethemoglobin method. Hct determinations was done by centrifuging (Nüve, Ankara, Turkey) blood samples at 14000 rpm for 5 minutes and packed cell volume was measured with a micro-hematocrit reader. Differential leukocyte counts

#### Table 2

Growth performance and nutritional efficiency of common carp (*C. carpio*) fingerlings after 10 weeks of being fed diets with different levels of fish meal replaced by sunflower meal. Mean  $\pm$  SE, n = 3 fish per tank for HSI and VSI

	Replacement levels (%)						
	0 (control)	25	50	75	100		
Wi(g) <sup>1</sup>	$3.00 \pm 0.01$	$3.05 \pm 0.03$	$2.99 \pm 0.04$	$3.08 \pm 0.02$	$3.01 \pm 0.06$		
$_{\rm Wf}$ (g) <sup>2</sup>	$12.56 \pm 0.30^{\rm b}$	$15.35 \pm 0.41^{a}$	$13.07 \pm 0.34^{\rm b}$	$12.19 \pm 0.22^{b}$	$10.19 \pm 0.17^{\rm c}$		
WG $(g)^3$	$9.56 \pm 0.29^{b}$	$12.30 \pm 0.38^{a}$	$10.07 \pm 0.32^{\rm b}$	$9.11 \pm 0.19^{b}$	$7.18 \pm 0.12^{c}$		
SGR (% day <sup>-1</sup> ) <sup>4</sup>	$2.04 \pm 0.03^{\rm bc}$	$2.31 \pm 0.02^{a}$	$2.10 \pm 0.03^{b}$	$1.97 \pm 0.01^{\rm c}$	$1.74 \pm 0.01^{d}$		
$\mathrm{CF}^5$	$1.87 \pm 0.07$	$1.98 \pm 0.02$	$1.95 \pm 0.01$	$1.92 \pm 0.02$	$1.83 \pm 0.01$		
HSI (%) <sup>6</sup>	$2.25 \pm 0.11$	$2.15 \pm 0.08$	$2.14 \pm 0.05$	$2.20 \pm 0.05$	$2.31 \pm 0.07$		
VSI (%) <sup>7</sup>	$15.85 \pm 0.86$	$15.68 \pm 0.53$	$16.32 \pm 0.64$	$15.37 \pm 0.30$	$14.18 \pm 0.11$		
$SR(\%)^8$	$97.78 \pm 1.11$	$93.33 \pm 1.93$	$95.56 \pm 1.11$	$94.44 \pm 1.11$	$92.22 \pm 1.11$		
FCR <sup>9</sup>	$1.61 \pm 0.04^{\rm b}$	$1.62 \pm 0.04^{b}$	$1.66 \pm 0.02^{b}$	$1.74 \pm 0.11^{b}$	$2.05 \pm 0.04^{a}$		
PER <sup>10</sup>	$1.75 \pm 0.04^{a}$	$1.71 \pm 0.04^{a}$	$1.69 \pm 0.02^{a}$	$1.62 \pm 0.09^{a}$	$1.36 \pm 0.03^{\rm b}$		
LER <sup>11</sup>	$6.31 \pm 0.22^{a}$	$6.02 \pm 0.15^{a}$	$5.78 \pm 0.07^{a}$	$5.45 \pm 0.30^{ab}$	$4.59 \pm 0.09^{b}$		
$PPV^{12}$	$0.23 \pm 0.00^{\rm b}$	$0.26 \pm 0.01^{ab}$	$0.28 \pm 0.01^{a}$	$0.26 \pm 0.01^{ab}$	$0.19 \pm 0.00^{\rm c}$		
LPV <sup>13</sup>	$0.80 \pm 0.02^{a}$	$0.65 \pm 0.01^{\rm b}$	$0.62 \pm 0.01^{\rm b}$	$0.60 \pm 0.06^{\rm b}$	$0.43 \pm 0.01^{\circ}$		

<sup>\*</sup>different superscript letters in the same row indicate significant differences (P < 0.05).<sup>1</sup>W<sub>i</sub>: initial weight, <sup>2</sup>W<sub>f</sub>: final weight, <sup>3</sup>WG: weight gain, <sup>4</sup>SGR: specific growth rate, <sup>5</sup>CF: condition factor, <sup>6</sup>HSI: hepatosomatic index, <sup>7</sup>VSI: viscerosomatic index, <sup>8</sup>SR: survival rate, <sup>9</sup>FCR: feed conversion ratio, <sup>10</sup>PER: protein efficiency ratio, <sup>11</sup>LER: lipid efficiency ratio, <sup>12</sup>PPV: protein productive value, <sup>13</sup>LPV: lipid productive value

were determined with Giemsa staining. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated with the following formulas:

- MCV (fL) =  $10 \times [Hct (\%) / RBC (\times 10^6 \text{ mm}^{-3})];$
- MCH (pg cell<sup>-1</sup>) =  $10 \times [Hb (g dL^{-1}) / RBC (\times 10^6 mm^{-3})];$
- MCHC (g dL<sup>-1</sup>) =  $100 \times [\text{Hb} (\text{g dL}^{-1}) / \text{Hct} (\%)].$

Plasma was obtained by centrifuging (Hettich, Kirchlengern, Germany) blood samples at 3000 rpm for 10 minutes. Separated plasma samples were transferred to 1.5 mL micro tubes and stored at -80°C until analysis. Total protein was measured with the refractometery method (Campbell 2015), while the other biochemical indices including triglyceride, cholesterol, and glucose were determined using commercial diagnostic kits (Pars Azmoon, Karaj, Iran) and a spectrophotometer (Unico, New Jersey, USA) at 546 nm (Burtis et al. 2012).

#### Statistical analysis

Data analysis was performed by One-Way ANOVA in SPSS 19 (IBM, Armonk, NY, USA). The Kolmogorov-Smirnov test was applied to verify the normality of values. Homogeneity of variance was verified with Levene's test, and Tukey's test was used to verify the presence of significant differences among treatments at a level of 5% (P  $\leq$  0.05). The results are presented as mean  $\pm$  standard error (SE).

# Results

The growth parameters are shown in Table 2. Based on the results, the highest final weight and SGR were obtained at 25% SFM (P < 0.05), while the lowest values were at 100% SFM (P < 0.05). The proximate body composition is presented in Table 3. According to the results, the protein values were significantly higher at 50% and 75% SFM, while the highest and lowest fat content values were in the control group and at 100% SFM, respectively (P < 0.05). The hematological indices are presented in Table 4. The results indicated that none of the indices differed significantly among the treatments (P > 0.05). The results of plasma biochemical indices are presented in Table 5. According to the results, triglyceride was

#### Table 3

Proximate body composition of common carp (*C. carpio*) fingerlings after 10 weeks feedings with different levels of fish meal replaced by sunflower meal. Mean  $\pm$  SE, n = 6 fish per tank

	Replacement levels (%)						
	0 (control)	25	50	75	100		
Moisture (%)	$73.57 \pm 0.43^{ab}$	$72.92 \pm 0.39^{ab}$	$71.42 \pm 0.47^{b}$	$72.79 \pm 0.53^{b}$	$75.34 \pm 0.77^{a}$		
Protein (%)	$12.97 \pm 0.20^{\mathrm{b}}$	$14.22 \pm 0.21^{ab}$	$15.64 \pm 0.30^{a}$	$14.84 \pm 0.57^{a}$	$13.05 \pm 0.14^{\rm b}$		
Fat (%)	$11.43 \pm 0.08^{a}$	$10.19 \pm 0.26^{\mathrm{b}}$	$9.97 \pm 0.05^{\rm b}$	$10.06 \pm 0.32^{\rm b}$	$8.89 \pm 0.06^{\circ}$		
<u>Ash (%)</u>	$1.98 \pm 0.15$	$2.03 \pm 0.15$	2.15 ± 0.10	$2.11 \pm 0.08$	2.38 ± 0.23		

\*different superscript letters in the same row show significant difference (P < 0.05)

#### Table 4

Hematological indices of common carp (*C. carpio*) fingerlings after 10 weeks feeding with different levels of fish meal replaced by sunflower meal. Mean  $\pm$  SE, n = 6 fish per tank

	Replacement levels (%)						
	0 (control)	25	50	75	100		
Hct (%) <sup>1</sup>	$35.50 \pm 0.58$	$36.00 \pm 1.76$	$39.17 \pm 0.44$	$41.33 \pm 1.67$	$36.67 \pm 1.86$		
Hb $(g dL-1)^2$	$6.38 \pm 0.10$	$6.43 \pm 0.26$	$6.97 \pm 0.12$	$7.30 \pm 0.22$	$6.57 \pm 0.24$		
RBC $(\times 106 \text{ mm-3})^3$	$0.77 \pm 0.01$	$0.79\pm0.03$	$0.85 \pm 0.01$	$0.89 \pm 0.03$	$0.80 \pm 0.04$		
WBC $(\times 103 \text{ mm-3})^4$	$4.32 \pm 0.29$	$4.38 \pm 0.16$	$3.95 \pm 0.78$	$3.80 \pm 0.21$	$3.33 \pm 0.24$		
MCV $(fL)^5$	$460.17 \pm 2.35$	$455.33 \pm 3.12$	$458.33 \pm 1.33$	$461.17 \pm 3.09$	$458.00 \pm 0.58$		
MCH (pg cell-1) <sup>6</sup>	$82.83 \pm 0.44$	$81.33 \pm 0.17$	$81.33 \pm 0.44$	$81.17\pm0.60$	$80.67 \pm 0.33$		
MCHC (g dL-1) <sup>7</sup>	$17.67 \pm 0.17$	$17.83 \pm 0.17$	$17.50 \pm 0.29$	$17.50 \pm 0.29$	$17.33 \pm 0.33$		
Lymphocytes (%)	$72.33 \pm 1.76$	$72.50 \pm 1.73$	$74.00 \pm 3.22$	$75.33 \pm 1.42$	$78.67 \pm 1.20$		
Neutrophils (%)	$22.00 \pm 1.04$	$22.83 \pm 1.76$	$21.33 \pm 2.46$	$19.17 \pm 1.17$	$17.33 \pm 0.67$		
Monocytes (%)	$4.67 \pm 0.44$	$4.33 \pm 0.44$	$4.17 \pm 0.44$	$4.67 \pm 0.33$	$3.00 \pm 0.58$		
Eosinophils (%)	$1.00 \pm 0.29$	$0.34 \pm 0.17$	$0.50 \pm 0.50$	$0.83 \pm 0.44$	$1.00 \pm 0.00$		

<sup>1</sup>Hct: hematocrit, <sup>2</sup>Hb: hemoglobin, <sup>3</sup>RBC: red blood cells, <sup>4</sup>WBC: white blood cells, <sup>5</sup>MCV: mean corpuscular volume, <sup>6</sup>MCH: mean corpuscular hemoglobin, <sup>7</sup>MCHC: mean corpuscular hemoglobin concentration

#### Table 5

Plasma biochemical indices of common	carp (C. carp	io) fingerlings	after	10 weeks	feeding with	n different	levels	of fish	1 meal
replaced by sunflower meal. Mean ± SE,	n = 6 fish pe	r tank							

	Replacement levels (%)						
	0 (control)	25	50	75	100		
Triglyceride (mg dL <sup>-1</sup> )	$254.93 \pm 17.03^{a}$	$239.58 \pm 6.16^{a}$	$244.46 \pm 9.00^{a}$	$246.38 \pm 13.85^{a}$	$171.49 \pm 10.64^{\mathrm{b}}$		
Cholesterol (mg dL <sup>-1</sup> )	$124.80 \pm 5.00^{a}$	$86.36 \pm 6.82^{b}$	$89.10 \pm 8.73^{b}$	$65.32 \pm 9.42^{b}$	$66.57 \pm 7.11^{\mathrm{b}}$		
Glucose (mg dL <sup>-1</sup> )	$57.37 \pm 4.76$	$63.44 \pm 8.07$	$79.48 \pm 3.87$	$61.85 \pm 7.00$	$64.02 \pm 3.33$		
Total protein (g dL <sup>-1</sup> )	$5.06 \pm 0.18$	$4.86 \pm 0.09$	$4.97 \pm 0.22$	$4.73 \pm 0.48$	$4.63 \pm 0.50$		

\*different superscript letters in the same row show significant difference (P < 0.05)

significantly lower at 100% SFM (P < 0.05). The highest cholesterol value was in the control group (P < 0.05). No significant differences were noted in glucose or total protein among treatments (P > 0.05).

## Discussion

In the present study, the final weight was significantly higher at 25% SFM and was the lowest at 100% SFM. Better growth performance at 25% SFM compared to the control could have resulted from the synergic effect of both the animal and vegetal protein sources in the diets. Significant declines in growth performance at 100% SFM could also have been the consequence of proteolytic enzyme suppression in the digestive tract by antinutrient factors present in plant protein sources such as SFM (Lin et al. 2010). Antinutrients like tannin present in plant ingredients (Francis et al. 2001, Hardy 2010) can inhibit the bioavailability of amino acids and vitamins and lead to growth reduction, which has been reported by other authors (Tacon et al. 1984, Olvera-Novoa et al. 2002) when FM is replaced by SFM. Olvera-Novoa et al. (2002) observed decreased growth in tilapia, Tilapia rendalli (Boulenger) fed diets including SFM at replacement levels exceeding 20%, which differs from the results of the present study. Gill et al. (2006) also failed to observe significant changes in growth rates with increasing levels of SFM in the diets of Atlantic salmon, Salmo salar L. Moreover, Nogales Mérida et al. (2011) report no significant difference among sharp snout sea bream, Diplodus puntazzo (Walbaum) fed different levels of SFM compared to the control. The FCR was significantly higher at 100% SFM, which indicated the lower nutritional efficiency of SFM compared to FM. The PER and LER decreased as SFM levels increased, and this trend was significant at 100% SFM. Higher values of PER and LER with increasingly higher FM levels resulted from the amino acid balance and better fatty acid profiles (Zhou et al. 2005) of FM compared to plant protein sources. These results are consistent with the findings of Olvera-Novoa et al. (2002), Ye et al. (2011), and Wang et al. (2015) with plant protein substitution in fish diets.

No significant differences in the HSI and VSI indexes were noted among the treatments. The lack of significant shifts in HSI among groups indicated the suitability of SFM for lipid metabolism. The liver is the main organ of lipid metabolism and food deficiencies can be harmful and cause disorders in liver function that manifest as liver fattening and increasing HSI values (Rocha et al. 1994).

Proximate body composition, excluding ash content, was significantly different among treatments in the current study, and the differences in body protein deposition among treatments was significant. Increases in carcass protein at the lowest SFM replacement percentage was also reported by Nogales Mérida et al. (2011), but higher SFM replacement percentages led to decreased protein retention. In contrast, Olvera-Novoa et al. (2002) observed that increasing the percentages of FM replaced by SFM led to reductions in body protein. Increases in body protein content in the treatments with SFM replacement compared to the control in the present study were explained by the synergic effect caused by the presence of both animal and vegetal protein sources in the diets. The reduction in body protein deposition at the highest SFM replacement percentages indicated a reduction in feed digestibility stemming from high levels of indigestible carbohydrates such as dietary fiber that lead to intensified intestinal evacuation rates (Hardy 2010), which caused a considerable part of dietary nutrients, such as proteins, to be excreted before absorption.

Body fat contents decreased with increasing levels of FM replacement with SFM and were significantly higher in the control and were the lowest at 100%. In contrast, Gill et al. (2006) observed similar body fat contents in different treatments of FM replacement with SFM. Sánchez Lozano et al. (2007) reported no significant changes in body fat levels stemming from the replacement of FM with SFM in the diets of gilthead sea bream, Sparus aurata L.. Nogales Mérida et al. (2011) also failed to find any significant differences in body fat among treatments fed different levels of SFM in comparison with the control. Olvera-Novoa et al. (2002) first observed reduced carcass fat content with SFM replacement up to 20%, but higher levels of SFM led to increased body fat. No significant differences in ash content were noted among treatments, which was similar to the results of Gill et al. (2006), Sánchez Lozano et al. (2007), and Nogales Mérida et al. (2011).

None of the hematological indices were significantly different among treatments. Kumar et al. (2010) observed a similar trend in WBC, but the status of RBC differed when FM was replaced by *Jatropha curcas* kernel meal in common carp diets. The differential leukocyte count was not significantly different among treatments. Bransden et al. (2001) did not observe any significant difference in the neutrophils of Atlantic salmon fed plant proteins compared to those fed FM, which is in agreement with our results. Likewise, Jahanbakhshi et al. (2013) reported no significant alterations in differential leukocyte counts in great sturgeon. *Huso huso* (L.) fed plant protein as a substitution for FM, which corresponds with the results of the current study.

No significant differences were noted in hemoglobin or hematocrit levels among the treatments. Other authors, such as Kumar et al. (2010), report similar results for common carp. This indicated that replacing FM with SFM in the current study had no negative effect on blood parameters, because the effect of plant ingredients lowering hematocrit values is considered to be a risk for farmed fish health (Hardy 2010), and this was not observed in the current study. According to the results, hematological indices, including MCV, MCH, and MCHC, did not differ significantly among treatments. Increases in these parameters could be signs of disorders in hematopoietic tissues like the liver and the spleen, blood toxicity, or anemia (Munker et al. 2007). Therefore, the similar values of these parameters in all treatments of the present study indicated that the hematopoietic tissues were functioning normally.

Of the plasma biochemical indices, triglycerides were significantly lower at the full substitution of SFM. Dias et al. (2005) reports a reduction in plasma triglyceride levels caused by plant-based diets in European seabass, Dicentrarchus labrax (L.). Significant increases in plasma triglyceride levels could result from essential amino acids being unavailable, which leads to liver dysfunction, increasing lipogenesis, and reduced fatty acid beta oxidation (Matter et al. 2004). Therefore, no significant differences at 25, 50 and 75% SFM substitution with the control indicated that liver function was normal in these groups. Significantly reduced triglycerides at 100% SFM along with significant decreases in body fat indicated lipid mobilization to provide energy that led to decreased growth.

The plasma cholesterol level was the highest in the control, whereas it was lower in all the other treatments. Cholesterol reduction in fish fed plant diets has also been reported in other studies (Kaushik et al. 1995, Sitjŕ-Bobadilla et al. 2005, Kumar et al. 2010). Sources of animal protein, such as FM, cause elevated cholesterol levels, while, conversely, plant ingredients have an anti-cholesterol effect (Wester 2000). Protein catabolic metabolites are the main materials of cholesterol in the liver (Baghchi et al. 1963), so diets containing highly digestible protein sources like FM lead to the production of more protein metabolites and facilitate more cholesterol synthesis. This explains the high level of plasma cholesterol in the control of the present study.

Rising levels of glucose were observed with increasing percentages of SFM replacement in the diets up to 50%, which was a consequence of the high percentage of plant ingredients used in the diets. This observation is similar to the results of other studies (Bransden et al. 2001, Kumar et al. 2010). No significant differences in plasma total protein were noted among the treatments. These results were similar to observations reported by other authors (Bransden et al. 2001, Zhou et al. 2005, Kumar et al. 2010). Using different alternative protein sources can affect the activity of enzymes that are involved in protein metabolism (Jahanbakhshi et al. 2013). No significant changes in plasma total protein in the current study showed that replacing FM with SFM did not affect the plasma protein regulation mechanisms.

# Conclusions

In conclusion, the results of current study showed that the partial replacement of FM with SFM of up to 75% has no negative effects on growth performance, feed efficiency, or blood indices. Therefore, SFM is a potencial feed ingredient in commercial common carp diets.

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experiment, analyzed the data, and drafted the manuscript, A.B.L .analyzed the data.

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