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Assessing the effects of partially replacing fishmeal with peanut meal on growth, body composition, digestibility and immunity in juvenile beluga (*Huso huso*)

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Abstract

In this feeding trial, the replacement of fishmeal (FM) with peanut meal (PM) was examined to assess its effects on growth performance, body composition, digestibility, and immunity in cultured juvenile beluga (*Huso huso*; initial weight: 132 ± 8.5 g). Four experimental diets were tested (3 replicates; 12 fish each), including a control diet (0% replacement) and diets with 10%, 20%, and 30% PM replacement levels, fed to fish over a 56-day period. The results showed that fish fed a diet with 10% PM replacement achieved the highest final weight, weight gain, and specific growth rate, as well as the lowest feed conversion ratio (FCR). As PM replacement levels increased, muscle protein content increased, while lipid content decreased ($p < 0.05$). Analysis of muscle fatty acids indicated that with higher replacement levels, saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFAs) increased ($p < 0.05$), while highly unsaturated fatty acids (HUFAs) decreased ($p < 0.05$). Additionally, the

10% replacement treatment exhibited the highest levels of essential amino acids in muscle tissue ($p < 0.05$), along with the greatest digestibility of dry matter and crude protein ($p < 0.05$). Immune parameters, specifically lysozyme and ACH50, showed significant increases compared to the control as PM replacement levels rose ($p < 0.05$). Based on the results, it can be concluded that partial replacement of FM with PM at a level of around 10% can enhance growth performance, nutrient digestibility, and immune response in Juvenile beluga.

KEYWORDS
chemical composition, growth performance, *huso huso*, immune response, peanut meal

1 | INTRODUCTION

The aquaculture industry has experienced rapid development over the last decade, achieving the fastest growth rate among global food production sectors (FAO, 2020). This expansion has driven increased demand for feed (Hodar et al., 2020). The nutritional value of aquafeeds largely depends on protein quality, a vital component in diet formulations. Fishmeal (FM) has traditionally been a prominent protein source, especially in diets for carnivorous fish (Hardy & Kaushik, 2022). While FM is widely recognized as a primary protein source because of its high nutritional value, its production presents environmental and economic challenges (Hodar et al., 2020; Wang et al., 2020). The reliance on FM is increasingly constrained because of diminishing global fish stocks (FAO, 2020), challenging the sustainability of aquaculture (Acar & Turker, 2018). As a result, considerable research has focused on identifying viable alternatives to FM in aquafeeds, particularly plant-based protein sources because of their availability and lower cost (Chakraborty et al., 2019).

Peanut meal (PM), a byproduct of peanut oil extraction, is emerging as a promising plant protein source in animal and aquaculture feeds because of its high protein content and palatability (Variath & Janila, 2017). The global average peanut production for 2016 to 2020 was 46.4 million metric tons (MMT) (Gelaye & Luo, 2024). PM is both accessible and economically viable. It is particularly rich in arginine, although it contains less lysine than soybean meal but more than cottonseed meal (Liu et al., 2012). However, the presence of anti-nutritional compounds like phytate in PM may adversely affect the digestion and absorption of proteins and can limit PM use at higher replacement levels by reducing growth performance in some aquatic species (Zhu et al., 2022). Investigating the effects of these compounds and determining the acceptable levels of PM substitution without compromising growth performance are key focuses of this study. In addition to growth effects, the impact of nutrition on the immune system of aquatic species is crucial (Wang et al., 2020; Xu et al., 2019). Because a robust immune system plays a vital role in disease resistance, evaluating the effects of PM on immune indicators, such as lysozyme activity and complement proteins, is among the significant objectives of this research. Studies on PM as a FM replacement have yielded mixed results across various aquatic species. For instance, Ye et al. (2020) found that up to 50% FM replacement with PM did not affect growth in juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂), while studies on Mozambique tilapia and rainbow trout identified optimal replacement levels at 20% and 10%, respectively, without adverse effects on growth parameters (Acar & Turker, 2018; Yildirim et al., 2014). Conversely, Qian et al. (2021) observed lower growth in red claw crayfish (*Cherax quadricarinatus*) fed a PM-based diet, indicating limited tolerance in this species. In a study on

Yellow River carp (*Cyprinus carpio*), the use of more than 50% mixed plant protein (A combination of rapeseed, cottonseed and PM) reduced lysozyme activity (Xu et al., 2019). The use of different levels of PM replacement in Pacific white shrimp (*Litopenaeus vannamei*) and juvenile hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂) did not significantly differ from the control treatment on lysozyme activity (Liu et al., 2012; Zhu et al., 2022).

Despite these studies, no research has yet explored PM as an alternative protein source in sturgeon diets. Sturgeons, especially beluga (*Huso huso*), are valuable in aquaculture for their rapid growth, disease resistance, adaptability to culture systems, high-quality meat, and prized caviar (Pelic et al., 2019). Thus, this study aimed to evaluate the effects of FM replacement with various levels of PM in the diet of *Huso huso*, focusing on growth performance, muscle composition, fatty acid and amino acid profiles, apparent digestibility, and immunity.

2 | MATERIALS AND METHODS

2.1 | Fish and experimental conditions

A total of 144 juvenile fish originated from broodstock in captivity with an initial weight of 132 ± 8.5 g were housed in 12 round fiberglass tanks (400 L, 12 fish per tank) at the Center for Restoration and Protection of Aquatic Genetic Resources, Shahid Rajaei (Sari, Iran). The fish were acclimated to the experimental conditions for 7 days and fed the control diet during this period. After acclimation, three tanks were designated per feed treatment, and the fish were fed to visual satiation three times daily (8:00, 12:00, and 16:00) over an 8-week period. Daily water temperature was maintained at 17 ± 0.5°C, with dissolved oxygen at 8.4 ± 0.35 mg/L, nitrite at 0.002 ± 0.05 mg/L, ammonia at 0.11 ± 0.005 mg/L, hardness at 70 ± 0.2 mg/L as $CaCO_3$, and pH at 8.15 ± 0.09. Water flow was continuous, and the photoperiod was set at 12 h light and 12 h dark.

2.2 | Diet preparation

Four isonitrogenous (50%) and isoenergetic (20 kJ/g) diets with 0%, 10%, 20%, and 30% replacement of FM with PM were formulated using Lindo software (Lindo System Inc., Chicago, IL, USA) (see Table 1). PM, sourced from a feed center in Mazandaran province, Iran, was milled to <250-μm particle size. Diets were prepared with soybean oil, soy lecithin, and water, then formed into strands using a 3-mm meat grinder die. These strands were dried at 60°C for 6 h and stored at −20°C (Esmaeili et al., 2017).

2.3 | Growth and nutrient efficiency indices

After 8 weeks, all juvenile fish were subjected to biometric analysis. Prior to sampling, fish were fasted for 24 h and then anesthetized with clove powder (300 ppm) (Falahatkar et al., 2018). Growth performance and nutritional efficiency indices such as survival rate (SR), feed conversion ratio (FCR), SGR, weight gain rate (WG), condition factor (CF), protein efficiency ratio (PER), visceral index (VSI), and hepatosomatic index (HSI) were calculated as follows (Wang et al., 2020; Ye et al., 2020):

$$\text{SR}\,(\%) = (\text{final fish number}/\text{initial fish number}) \times 100$$

$$\text{FCR} = \text{feed consumed}/\text{weight gain}$$

TABLE 1 The feed ingredients and chemical composition of the experimental diets of juvenile beluga (*Huso huso*) with 0% (control), 10%, 20%, and 30% substitution of FM with PM.

	Experimental diets containing different levels of FM substitution (%) with PM			
Ingredients (g kg^{-1})	**(control)**	**10**	**20**	**30**
FM[a]	536.88	483.20	428.64	375.82
Soybean meal[a]	250.00	250.00	250.00	250.00
Wheat flour[a]	135.42	135.42	135.42	135.42
PM[b]	0	50.98	102.74	152.87
Soybean oil[c]	5.00	7.70	10.50	13.19
Mineral mixture[d]	15.00	15.00	15.00	15.00
Vitamin mixture[e]	20.00	20.00	20.00	20.00
Antifungal[f]	2.50	2.50	2.50	2.50
Binder[g]	10.00	10.00	10.00	10.00
Organic acid[a]	10.00	10.00	10.00	10.00
Mono-calcium phosphate[a]	5.00	5.00	5.00	5.00
Antioxidant[a]	0.20	0.20	0.20	0.20
Lecithin[a]	5.00	5.00	5.00	5.00
Cr_2O_3[h]	5.00	5.00	5.00	5.00
Chemical composition (dry basis)				
Dry matter (%)	92.30	92.15	91.84	91.87
Crude protein (%)	50.10	50.12	50.11	50.14
Crude fat (%)	10.20	9.98	9.94	9.64
Carbohydrate[i] (%)	23.63	24.75	27.01	27.80
Ash (%)	16.07	15.15	12.94	12.42
Gross energy (Kj g^{-1})[j]	19.93	20.06	20.62	20.46

[a]Chapakrod Caspian Golden Yaghot Aquafeed Co, Mazandaran, Iran; Chemical composition of fish meal: 56% crude protein, 17% crude fat, 7.2% moisture, 2.3% carbohydrate, and 17.5% ash;
[b]Chemical composition of peanut meal: 59% crude protein, 6% crude fat, 6.2% moisture, 24.4% carbohydrate, and 4.4% ash;
[c]Behpak Co, Behshahr, Iran;
[d]Mineral mixture (mg kg^{-1}): Fe, 6000; Cu, 600; Mn, 2200; Zn, 3000; I, 600; Se, 20; Co, 60.
[e]Vitamin mixture (Unit kg^{-1}): A, 1,200,000 IU; D3, 400,000 IU; E, 50,000 IU; K3, 800 mg; C, 30,000 mg; B1, 2500 mg; B2, 4000 mg; B6, 25,000 mg; B9, 1000 mg; B12, 8 mg; Biotin, 150 mg; Niacin, 35,000 mg; Inositol, 50,000 mg.
[f]Domisha Co, Tehran.
[g]Fartak Co, Khorasan Razavi, Iran.
[h]Chromic oxide (Sigma-Aldrich®, USA).
[i]Carbohydrate = 100 – (crude protein + crude fat + ash + moisture).
[j]Gross energy was calculated based on the values of 23.6, 39.9, and 17.2 Kj/g^{-1} of protein, fat, and carbohydrate respectively (NRC, 1993).

$$\text{SGR}\left(\%\text{day}^{-1}\right) = [(\text{Ln final weight} - \text{Ln initial weight})/\text{duration}] \times 100$$

$$\text{WG}\,(\%) = [(\text{final weight} - \text{initial weight})/\text{initial weight}] \times 100$$

$$\text{CF}\,(\text{g/cm}^3) = \left(\text{fork length}^3/\text{body weight}\right) \times 100$$

$$\text{PER} = \text{weight gain}/\text{protein intake}$$

$$\text{VSI}\,(\%) = (\text{visceral weight}/\text{body weight}) \times 100$$

$$\text{HSI}\,(\%) = (\text{liver weight}/\text{body weight}) \times 100$$

From each tank, two fish were dissected to measure VSI and HSI and stored at −20°C for later chemical, fatty acid, and amino acid analyses.

2.4 | Apparent digestibility coefficients of experimental diets

Chromic oxide (0.5%) was added to each diet as a marker to measure apparent digestibility coefficients (ADCs) of dry matter, protein, and fat. In the final week, fish were fed diets with chromic oxide, and fecal samples were collected by gently stripping the abdominal area. Samples were stored at −20°C. Chromium content was measured using atomic absorption spectrophotometry (Williams et al., 1962), and ADC was calculated with the formula:

$$\text{ADC}\,(\%) = 100 - 100 \times [(\text{Cr in feed}/\text{Cr in feces}) \times (\text{nutrient in feces}/\text{nutrient in feed})]$$

2.5 | Chemical analysis

Moisture, protein, fat, and ash of feeds, feces, and muscle tissue were analyzed following AOAC (1995) methods. Moisture was determined by drying at 105°C for 12 h; ash was determined by heating at 550°C for 4 h in a Nabertherm furnace. Protein was measured by Kjeldahl (×6.25) (Kjeltec Analyzer 2300, Sweden), and fat by Soxhlet extraction with chloroform (Soxtec 2050, Switzerland).

2.6 | Fatty acid profile

Total lipids were extracted following Folch et al. (1957), with fatty acid esterification as per Metcalfe and Schmitz (1961). Fatty acids were analyzed using gas chromatography (GC, Varian CP 3800) with flame ionization and a polyethylene glycol column (PBX70, SGE; Australia). Chromatography Varian Software (version 6.41) was used to compare standards and calculate fatty acid composition, presented in Tables 2 and 6.

2.7 | Amino acid profile

Samples for amino acid analysis were hydrolyzed with 6 N HCl for 24 h at 110°C, then derivatized with o-phthaldialdehyde (OPA) (Antonine et al., 1999). High-performance liquid chromatography (HPLC, Knauer, Berlin) was used for amino acid quantification based on standards (Oujifard et al., 2012). Profiles are shown in Tables 3 and 7.

2.8 | Blood collection and sample preparation

Two fish per tank were sampled for blood. Fish were anesthetized with clove powder (300 ppm), and blood was drawn from the caudal vein into Eppendorf tubes (Montazeri Parchikolaei et al., 2020; Xu et al., 2019). Serum was separated by centrifugation (4000 *g*, 10 min) and stored at −80°C (Zhu et al., 2022).

TABLE 2 The fatty acid composition (% of total fatty acids) of the experimental diets of juvenile beluga (*Huso huso*) with 0% (control), 10%, 20%, and 30% substitution of FM with PM.

			Experimental diets containing different levels of FM substitution (%) with PM		
Fatty acids	**PM**	**Control**	**10**	**20**	**30**
C14	0.20	1.70	1.80	1.60	1.40
C16	10.30	17.00	18.30	18.30	15.10
C18	2.30	3.60	4.30	4.00	3.60
C20	n.d	0.10	0.20	0.40	0.60
C22	n.d	0.70	1.00	1.00	0.90
C16:1n-7	0.10	6.20	3.70	3.20	3.30
C18:1n-9	47.60	28.20	28.80	32.10	30.20
C18:2n-6	19.12	15.80	15.30	17.20	21.20
C18:3n-3	n.d	2.70	1.80	1.80	2.10
C20:1n-9	0.90	0.10	0.08	0.09	0.10
C20:2n-6	n.d	0.03	0.06	0.05	0.08
C20:3n-3	n.d	0.40	0.50	0.40	0.30
C20:4n-6	n.d	3.60	4.10	3.20	2.30
C22:1n-9	n.d	0.60	0.90	0.80	0.80
EPA[a]	n.d	0.50	0.70	0.50	0.60
DHA[b]	n.d	12.50	13.40	8.30	9.00
∑ SFA[c]	12.80	23.00	25.60	25.30	21.60
∑ MUFA[d]	48.60	35.10	33.50	36.20	34.40
∑ HUFA[e]	n.d	16.60	18.20	12.00	11.90
∑ PUFA[f]	19.12	35.10	35.30	31.00	35.20
∑ n-3	n.d	16.10	16.40	11.00	12.00
∑ n-6	19.12	19.43	19.46	20.45	23.58
n-3/n-6	n.d	0.83	0.84	0.54	0.51

Abbreviation: n.d, not determined.
[a]Eicosapentaenoic acid.
[b]Docosahexaenoic acid.
[c]Saturated fatty acids.
[d]Monounsaturated fatty acids.
[e]Highly unsaturated fatty acids.
[f]Polyunsaturated fatty acids.

2.8.1 | Lysozyme and alternative complement activity (ACH50)

Serum lysozyme activity was assessed by turbidimetry (Ellis, 1990). Lyophilized Micrococcus lysodeikticus (0.3 mg/mL) in phosphate buffer (pH 6.2) was used as a substrate. Absorbance was measured at 450 nm at 0.5 and 4.5 min intervals. ACH50 was measured using rabbit red blood cells (Amar et al., 2000), with absorbance measured at 414 nm.

2.9 | Statistical analysis

The research was conducted using a randomized design. Data analysis was performed with SPSS™ version 17 (Texas State University, San Marcos, TX, USA) for Windows. Normality of the data was assessed using the Kolmogorov–

TABLE 3 The amino acid composition (g 100 g^{-1}) of the experimental diets of juvenile beluga (*Huso huso*) with 0% (control), 10%, 20%, and 30% substitution of FM with PM.

			Experimental diets containing different levels of FM substitution (%) with PM		
	PM	Control	10	20	30
Essential amino acids (EAAs)					
Arginine	6.95	6.15	8.22	8.50	8.80
Isoleucine	5.65	7.47	7.83	7.20	7.71
Leucine	5.95	8.41	9.05	9.20	9.95
Lysine	1.94	1.20	0.80	0.73	0.61
Methionine	0.81	4.04	3.77	3.38	3.14
Phenylalanine	10.94	3.95	4.29	3.80	4.28
Threonine	1.29	6.25	5.00	5.09	3.63
Valine	12.69	8.50	8.56	7.55	8.39
∑EAA	46.22	45.97	47.45	45.52	46.51
Nonessential amino acids (NEAAs)					
Alanine	2.65	4.84	4.04	4.40	4.29
Aspartic	8.22	12.75	10.34	11.43	7.92
Glutamic	11.07	19.39	16.52	16.90	16.81
Glycine	2.83	3.50	3.45	3.15	4.63
Serine	4.54	2.78	2.75	3.20	3.55
Tyrosine	1.80	1.86	1.57	1.89	1.80
Taurine	n.d	6.69	7.31	7.86	8.81
∑NEAA	32.11	51.81	45.98	48.83	47.81

Abbreviations: ∑EAA, total essential amino acids; ∑NEAA, total nonessential amino acids; n.d, not determined.

Smirnov test, and homogeneity of variances was checked with Levene's test. Significant differences among the data were evaluated by one-way analysis of variance (ANOVA), followed by Duncan's test for multiple comparisons. A significance level of $p < 0.05$ was considered in this study.

3 | RESULTS

3.1 | Growth and nutritional parameters and digestibility

The results for growth and nutrient efficiency indices of juvenile fish fed diets with varying levels of PM replacing FM are shown in Table 4. The highest weight gain (229.7%) and PER, along with the lowest feed conversion ratio (FCR), were observed in the 10% replacement treatment ($p < 0.05$). The SGR was also highest in this treatment, recorded at 2.12% per day^{-1}. The survival rate (SR) showed an increasing trend in replacement treatments compared to the control, with the 10% and 20% treatments demonstrating the best performance at 100% and 96.3%, respectively ($p < 0.05$). No significant differences were observed for condition factor (CF), visceral somatic index (VSI), or HSI ($p > 0.05$).

The 10% replacement treatment, alongside the control, exhibited the highest apparent crude protein digestibility coefficient (ADCCP) at 90.17%. The treatments containing PM showed an increase in the apparent digestibility

TABLE 4 Means ± SD of indices growth performance, nutrient efficiency, and apparent digestibility coefficients of juvenile beluga (*Huso huso*) fed the experimental diets with 0% (control), 10%, 20%, and 30% substitution of FM with PM after 56 days.

		FM replacement levels with PM in diet (%)		
Growth parameters	**Control**	**10**	**20**	**30**
IW (g)	132.00 ± 8.50	132.00 ± 8.70	132.00 ± 8.40	132.00 ± 8.30
FW (g)	402.20 ± 9.80^{b}	435.20 ± 15.40^{c}	336.40 ± 20.30^{a}	296.60 ± 4.00^{a}
WG (%)	204.70 ± 7.40^{c}	229.70 ± 12.10^{d}	154.80 ± 13.60^{b}	124.70 ± 10.50^{a}
SGR (% day^{-1})	1.99 ± 0.18^{b}	2.12 ± 0.20^{c}	1.66 ± 0.12^{a}	1.44 ± 0.13^{a}
CF (g/cm^{3})	0.30 ± 0.02	0.30 ± 0.01	0.30 ± 0.02	0.20 ± 0.01
FCR	1.13 ± 0.06^{b}	0.87 ± 0.05^{a}	1.17 ± 0.11^{b}	1.48 ± 0.15^{c}
PER	1.80 ± 0.10^{c}	2.30 ± 0.10^{d}	1.70 ± 0.20^{b}	1.40 ± 0.20^{a}
HSI (%)	2.40 ± 0.10	2.10 ± 0.20	2.20 ± 0.20	2.40 ± 0.10
VSI (%)	9.60 ± 0.10	9.30 ± 0.10	10.40 ± 1.03	10.80 ± 1.00
SR (%)	86.70 ± 4.20^{a}	100.00^{b}	96.30 ± 0.50^{b}	89.10 ± 4.00^{a}
ADC_{DM} (%)	87.23 ± 0.16^{a}	89.37 ± 0.06^{b}	89.51 ± 0.08^{b}	89.62 ± 0.11^{b}
ADC_{CP} (%)	89.98 ± 0.01^{b}	90.17 ± 0.10^{b}	87.81 ± 0.08^{a}	87.59 ± 0.11^{a}
ADC_{CF} (%)	86.81 ± 0.08^{c}	85.80 ± 0.06^{b}	84.41 ± 0.06^{a}	84.46 ± 0.31^{a}

Note: Different letters indicate statistically significant differences between treatments ($p < 0.05$).
Abbreviations: ADC_{CF}, apparent digestibility coefficient of crude fat; ADC_{CP}, apparent digestibility coefficient of crude protein; ADC_{DM}, apparent digestibility coefficient of dry matter; CF, condition factor; FW, final weight; HIS, hepatosomatic index; IW, initial weight; PER, protein efficiency ratio; SGR, specific growth rate; SR, survival rate; VSI, visceral index; WGR, weight gain rate.

coefficient of dry matter (ADCDM) compared to the control ($p < 0.05$). Increasing PM replacement levels (10%–30%) led to a decrease in the apparent digestibility coefficient for crude fat (ADCCF) compared to the control (86.81) ($p < 0.05$) (Table 4).

3.2 | Muscle chemical composition

After 8 weeks of feeding on experimental diets, replacement levels did not significantly affect muscle moisture and ash content ($p > 0.05$). However, as PM replacement increased, the crude protein content in muscle rose, with the 20% and 30% treatments having the highest crude protein levels compared to the control ($p < 0.05$). The control treatment showed the highest crude muscle fat content (3.92%) ($p < 0.05$) (Table 5).

3.3 | Fatty acid profiles

Among saturated fatty acids (SFAs), palmitic acid (C16:00) was the most abundant, while myristic acid (C14:00) was the least. No significant differences were found in total monounsaturated fatty acids (MUFA) content between replacement treatments and the control ($p > 0.05$). However, polyunsaturated fatty acids (PUFAs) levels increased with higher PM replacement, relative to the control ($p < 0.05$). All replacement treatments had significantly lower ∑n-3 fatty acids than the control ($p < 0.05$). ∑n-6 fatty acids were significantly higher in replacement treatments than in the control (16.7) ($p < 0.05$). Conversely, docosahexaenoic acid (DHA) decreased as replacement levels

TABLE 5 Muscle chemical composition (Means ± SD) (wet weight basis; %) of juvenile beluga (*Huso huso*) fed the experimental diets with 0% (control), 10%, 20%, and 30% substitution of FM with PM after 56 days.

		FM replacement levels with PM in diet (%)		
Muscle composition	**Control**	**10**	**20**	**30**
Moisture	78.65 ± 0.31	78.21 ± 1.03	78.79 ± 1.83	78.09 ± 1.87
Crude protein	15.73 ± 0.44^{a}	16.91 ± 0.79^{ab}	17.40 ± 0.74^{b}	17.73 ± 0.93^{b}
Crude fat	3.92 ± 0.37^{b}	2.09 ± 0.20^{a}	2.17 ± 0.21^{a}	2.24 ± 0.18^{a}
Ash	0.96 ± 0.07	1.01 ± 0.04	1.05 ± 0.05	1.02 ± 0.03

Note: Different letters indicate statistically significant differences between treatments ($p < 0.05$).

increased ($p < 0.05$). The control (1.9) and 10% (1.8) treatments also exhibited the highest values of eicosapentaenoic acid (EPA) ($p < 0.05$) (Table 6).

3.4 | Amino acid profiles

The essential amino acids (EAAs) arginine and methionine remained unaffected by replacement levels, with no significant differences among treatments ($p > 0.05$). However, lysine content declined with higher substitution levels, and the control (2.2) showed a significant difference from other treatments except the 10% replacement (2.0) ($p < 0.05$). The 10% replacement treatment had the highest total essential amino acids ($\sum$EAA) at 54.1% and the lowest nonessential amino acids ($\sum$NEAA) at 41.8% ($p < 0.05$) (Table 7).

3.5 | Immune system parameters

As illustrated in Figure 1, increasing PM replacement levels improved immune indicators in juvenile beluga. Fish in the 30% replacement group exhibited the highest lysozyme activity (37.23) and ACH50 level (130.77). In comparison, the control treatment had significantly lower values for these immune parameters ($p < 0.05$).

4 | DISCUSSION

The substitution of FM with plant-based protein sources generally shows a negative impact on the growth performance of carnivorous fish species (Ye et al., 2020). In this study, the observed decrease in final weight (FW), weight gain rate (WG), and SGR, along with an increase in the feed conversion ratio (FCR) at higher replacement levels of PM, can be attributed to a deficiency of EAA like lysine and methionine in PM (see Table 3). The presence of antinutritional factors, such as phytate in PM, also contributes to reduced digestive enzyme activity, specifically proteases, thereby diminishing nutrient digestibility and absorption (Da Silva et al., 2017; Ye et al., 2020). The highest WG and SGR values and the lowest FCR values were found at a 10% PM replacement level. This may indicate an optimal level of digestion and nutrient absorption at this replacement level, possibly without significant changes in essential and nonessential amino acid (NEAA) profiles in the diet. In a related study, rainbow trout (*Oncorhynchus mykiss*) fed a diet with 10% PM substitution for FM exhibited similar weight gain to the control group, which had no PM (Acar & Turker, 2018). Studies on Mozambique tilapia (*Oreochromis mossambicus*) also suggested that PM substitution up to 20% is feasible without adverse effects on growth (Yildirim et al., 2014). Notably, PM is rich in arginine, an amino acid that competes with lysine for absorption sites in the intestine, potentially reducing lysine digestibility and

TABLE 6 The fatty acid composition (% of total fatty acids) muscle of juvenile beluga (*Huso huso*) fed the experimental diets with 0% (control), 10%, 20%, and 30% substitution of FM with PM after 56 days.

		Experimental diets containing different levels of FM substitution (%) with PM		
Fatty acids	**Control**	**10**	**20**	**30**
C14	0.62 ± 0.01	0.90 ± 0.05	0.63 ± 0.01	0.55 ± 0.04
C16	12.10 ± 0.20^{a}	15.50 ± 0.30^{b}	16.80 ± 0.60^{b}	14.90 ± 0.80^{b}
C18	1.90 ± 0.02^{a}	3.10 ± 0.20^{c}	2.80 ± 0.20bc	2.10 ± 0.03^{b}
C20	1.40 ± 0.10^{b}	0.90 ± 0.08ab	1.00 ± 0.09^{ab}	0.60 ± 0.04^{a}
C22	0.70 ± 0.05	0.50 ± 0.03	0.90 ± 0.08	0.80 ± 0.07
C16:1n-7	3.80 ± 0.30^{b}	2.90 ± 0.10^{ab}	2.10 ± 0.20^{a}	2.20 ± 0.10^{a}
C18:1n-9	30.70 ± 2.90	31.40 ± 2.90	30.40 ± 2.40	31.40 ± 2.50
C18:2n-6	14.20 ± 1.00^{a}	21.20 ± 1.10^{b}	24.50 ± 1.80^{c}	22.20 ± 0.90^{b}
C18:3n-3	1.30 ± 0.10	1.70 ± 0.08	1.60 ± 0.09	1.80 ± 0.10
C20:1n-9	0.80 ± 0.02^{ab}	0.90 ± 0.03^{b}	0.60 ± 0.05^{a}	0.80 ± 0.07^{ab}
C20:2n-6	0.32 ± 0.03	0.48 ± 0.05	0.55 ± 0.04	0.35 ± 0.03
C20:3n-3	0.75 ± 0.06	0.81 ± 0.07	0.92 ± 0.06	0.73 ± 0.05
C20:4n-6	2.20 ± 0.20^{d}	1.50 ± 0.10^{b}	0.70 ± 0.06^{a}	1.8 ± 0.10^{c}
C22:1n-9	1.00 ± 0.09^{a}	1.5 ± 0.10^{b}	2.60 ± 0.20^{c}	1.37 ± 0.10^{b}
EPA[1]	1.90 ± 0.10^{b}	1.80 ± 0.10^{b}	1.50 ± 0.10^{a}	1.50 ± 0.20^{a}
DHA[2]	13.60 ± 0.40^{b}	10.50 ± 0.50^{a}	10.20 ± 0.10^{a}	11.30 ± 0.10^{a}
∑ SFA[3]	16.70 ± 0.50^{a}	20.90 ± 0.50 ^{bc}	22.10 ± 1.10^{c}	18.80 ± 0.10^{b}
∑ MUFA[4]	36.30 ± 0.30	36.70 ± 0.70	35.70 ± 0.60	35.80 ± 0.70
∑ HUFA[5]	17.70 ± 1.20^{c}	13.80 ± 0.02^{b}	12.40 ± 0.90^{a}	14.60 ± 0.90^{b}
∑ PUFA[6]	33.20 ± 0.40^{a}	36.70 ± 0.09^{b}	38.50 ± 1.40^{c}	38.60 ± 0.90^{c}
∑ n-3	17.60 ± 0.50^{c}	14.80 ± 0.40^{a}	14.20 ± 0.80^{a}	15.30 ± 0.70^{b}
∑ n-6	16.70 ± 0.40^{a}	23.20 ± 0.90^{b}	25.80 ± 1.00^{b}	24.40 ± 0.70^{b}
n-3/n-6	1.05 ± 0.04^{b}	0.67 ± 0.02^{a}	0.56 ± 0.06^{a}	0.63 ± 0.01^{a}

Note: Different letters indicate statistically significant differences between treatments ($p < 0.05$).
[1]Eicosapentaenoic acid.
[2]Docosahexaenoic acid.
[3]Saturated fatty acids.
[4]Monounsaturated fatty acids.
[5]Highly unsaturated fatty acids.
[6]Polyunsaturated fatty acids.

absorption (Kim et al., 1992). In this study, the amino acid imbalance associated with PM did not adversely affect the PER. Increased PM levels in the diet may elevate fat deposition in the liver, potentially impacting the HSI (Zhu et al., 2022). However, HSI and other liver indicators showed no significant differences across treatments in the current study, indicating that PM inclusion did not negatively impact liver fat deposition in juvenile beluga. Similar findings were reported in hybrid grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*) (Ye et al., 2020), red claw crayfish (*Cherax quadricarinatus*) (Qian et al., 2021), and rainbow trout (Dernekbasi et al., 2021), where no significant changes in liver indices were observed with FM substitution, aligning with our findings.

Replacing FM with plant protein sources in carnivorous fish diets can disrupt digestion and nutrient absorption (Safari et al., 2016). In this study, increasing PM levels in the diet reduced the ADC of protein. This effect could be because of decreased bioavailability of EAAs like lysine and methionine, which are crucial for optimal growth

TABLE 7 The amino acid composition (g 100 g^{-1}) muscle of juvenile beluga (*Huso huso*) fed the experimental diets with 0% (control), 10%, 20%, and 30% substitution of FM with PM after 56 days.

		Experimental diets containing different levels of FM substitution (%) with PM		
	Control	10	20	30
Essential amino acids (EAAs)				
Arginine	7.40 ± 0.50	7.30 ± 0.60	7.00 ± 0.40	7.30 ± 0.50
Isoleucine	8.00 ± 0.90^{ab}	9.70 ± 0.70^{b}	7.60 ± 0.80^{ab}	6.60 ± 0.50^{a}
Leucine	10.10 ± 0.80^{b}	10.30 ± 0.40^{b}	8.60 ± 0.09^{a}	7.80 ± 0.20^{a}
Lysine	2.20 ± 0.10^{b}	2.00 ± 0.10^{b}	1.60 ± 0.10^{a}	1.90 ± 0.10^{a}
Methionine	4.10 ± 0.40	4.50 ± 0.30	4.00 ± 0.30	4.00 ± 0.40
Phenylalanine	4.00 ± 0.30	4.10 ± 0.20	3.60 ± 0.30	3.60 ± 0.40
Threonine	4.20 ± 0.40^{a}	7.00 ± 0.50^{b}	4.60 ± 0.80^{a}	3.80 ± 0.80^{a}
Valine	7.60 ± 0.40	9.20 ± 0.90	7.10 ± 0.60	7.80 ± 0.70
∑EAA	47.60 ± 2.70^{a}	54.10 ± 3.70^{b}	44.10 ± 3.50^{a}	42.80 ± 4.20^{a}
Non-essential amino acids (NEAAs)				
Alanine	8.00 ± 0.70^{b}	4.00 ± 0.40^{a}	7.80 ± 0.60^{b}	4.30 ± 0.10^{a}
Aspartic	8.40 ± 0.60^{b}	6.50 ± 0.40^{a}	9.60 ± 0.90^{b}	12.80 ± 1.10^{c}
Glutamic	16.10 ± 1.30	16.10 ± 0.90	15.60 ± 0.40	16.90 ± 1.40
Glycine	5.30 ± 0.50^{b}	1.70 ± 0.10^{a}	6.10 ± 0.40^{b}	1.90 ± 0.20^{a}
Serine	3.40 ± 0.30^{a}	3.10 ± 0.20^{a}	4.20 ± 0.40^{a}	7.30 ± 0.50^{b}
Tyrosine	3.00 ± 0.20^{c}	1.30 ± 0.10^{a}	2.60 ± 0.10^{b}	2.20 ± 0.20^{b}
Taurine	8.10 ± 0.70	9.10 ± 0.30	8.10 ± 0.30	8.00 ± 0.60
∑NEAA	52.00 ± 3.90^{b}	41.80 ± 1.10^{a}	54.00 ± 1.70^{b}	53.40 ± 0.30^{b}

Note: Different letters indicate statistically significant differences between treatments ($p < 0.05$).
Abbreviations: ∑EAA, total essential amino acids; ∑NEAA, total nonessential amino acids.

(Teodósio et al., 2022; Yildirim et al., 2014). Phytate, an anti-nutritional factor in PM, binds to proteins, inhibiting their digestibility (Zhu et al., 2022). Similar reductions in digestibility were observed in studies that replaced FM with soybean meal (Matani Bour et al., 2018; Wang et al., 2015) or PM (Liu et al., 2012; Yue et al., 2012) in other species, corroborating our results.

Diet composition significantly influences the body composition of fish (Xu et al., 2019). In this study, PM substitution enhanced the structural protein in muscle, which is consistent with observed increases in crude protein in rainbow trout muscle (Dernekbasi & Karayucel, 2017). However, while muscle crude fat levels in some species rise with increasing PM substitution, this trend was not observed in the present study. Dietary composition particularly affects muscle fatty acid profiles (Badiani et al., 1997). Here, higher PM replacement levels increased SFAs, likely because of palmitic acid in the diet. No significant difference in MUFA was observed across treatments, possibly because of biosynthetic processes that convert other dietary components into MUFA (Abi-Ayad et al., 2004). PUFA increased with higher PM substitution, likely because of desaturation and elongation of essential fatty acids (Sellner & Hazel, 1981). However, as PM levels rose, ∑n-3 fatty acids decreased while ∑n-6 fatty acids increased, reflecting PM's high linoleic acid content (47.6%) (Table 2). Plant proteins generally lack the important fatty acids EPA and DHA, which may explain the reduction of these fatty acids in both diet and muscle of the juvenile beluga in this study. Lysine and methionine are among the most limiting amino acids in fish diets, particularly when FM is replaced with plant proteins (Gatlin et al., 2007). Low lysine levels in replacement diets have been linked to reduced performance in Nile tilapia (Musita et al., 2015). The PM used in this study had low lysine and methionine levels, affecting their amounts in the experimental diets (Table 3). As PM substitution increased, muscle lysine levels

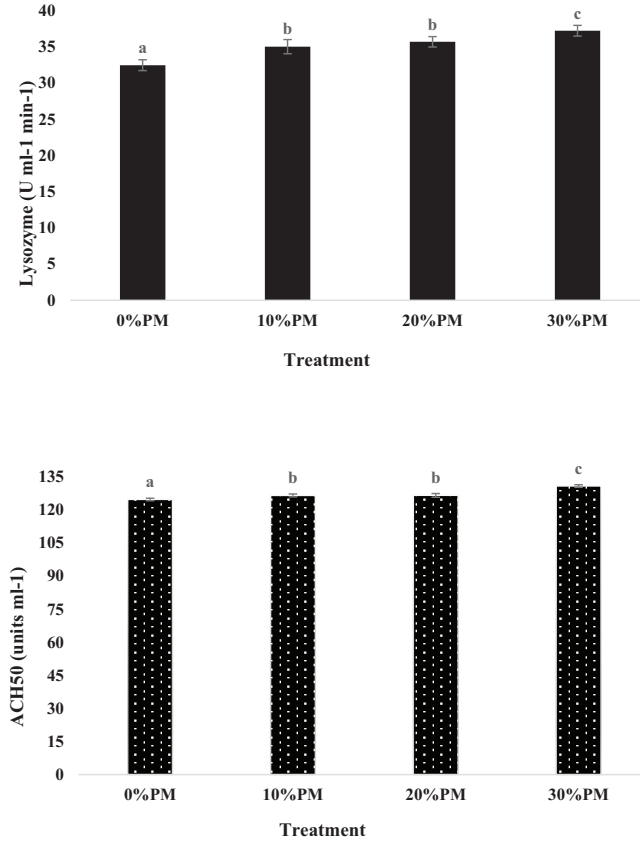

FIGURE 1 The immune response of juvenile beluga fed different dietary FM replacement levels with PM after 56 days. Values are the means ± standard deviation of the triplicate samples.

decreased, while methionine remained relatively stable across treatments, potentially because of methionine's preservation within whole-body protein (Wilson, 2003).

Aquatic animal nutrition is closely tied to immune system health (Zhu et al., 2022). Under stress, changes in immune parameters can reflect fish health status (Chesneau, 2018). Lysozyme, an enzyme with bacteriolytic activity, is a key immune marker that helps lyse Gram-positive bacteria and complements the destruction of Gram-negative bacteria (Zhu et al., 2022). In this study, lysozyme activity increased significantly at up to 30% PM replacement, suggesting a potential immune-boosting effect of PM for juvenile beluga. Increased lysozyme activity has also been observed with higher PM levels in hybrid grouper (Ye et al., 2020; Zhu et al., 2022) and Yellow River carp (Cyprinus carpio) (Xu et al., 2019). Complement proteins play a central role in the immune response, enhancing phagocytic abilities by identifying pathogens (Sunyer et al., 1995). High serum complement activity (ACH50) indicates robust fish health (Yano, 1992). In this study, ACH50 levels increased with higher PM replacement levels. The observed increases in lysozyme activity and ACH50 may be because of bioactive compounds in peanuts, such as flavonoids and resveratrol, as well as essential minerals like selenium, zinc, and copper, which are known to enhance immune function (Geulein, 2010; Yu et al., 2006).

5 | CONCLUSION

This study demonstrated that partial replacement of FM with PM in the diet of juvenile beluga (*Huso huso*) could be feasible up to certain levels without significant negative impacts on growth performance, hepatic indices, or immune

responses. Specifically, the substitution level of 10% PM showed growth results similar to the control group, suggesting that PM can be utilized at this rate without compromising essential growth parameters. Higher replacement levels, however, led to declines in growth metrics and protein digestibility, likely because of reduced bioavailability of key amino acids, such as lysine and methionine, and the presence of anti-nutritional factors in PM. Despite these limitations, PM substitution positively influenced immune parameters like lysozyme activity and complement protein (ACH50) levels, potentially because of bioactive compounds within PM that enhance immunity. Overall, PM may serve as a viable alternative protein source in aquaculture diets, especially when used in moderate amounts. Further research is recommended to optimize amino acid balance and mitigate anti-nutritional effects to maximize the benefits of PM in fish diets. This study contributes to sustainable aquaculture practices by highlighting the potential of PM as a partial FM replacement in beluga diets, helping to reduce reliance on traditional marine-derived protein sources.

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CONFLICT OF INTEREST STATEMENT

The authors stated that they have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

ANIMAL WELFARE AND FEED LEGISLATION

All procedures involving animals were done according to the Tarbiat Modares University protocols, which seek to optimize handling and minimize animal suffering (The guidelines, adopted from the Declaration of Helsinki (1975) and The Society for Neuroscience Animal Care and Use guidelines (1998) were approved for implementation by the Medical Ethics Committee, School of Medical Sciences of the Tarbiat Modares University on 28th Farvardin, 1385/17th April 2006).

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