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EDITED BY
Cláudia Aragão,
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REVIEWED BY
Marce Herrera,
IFAPA Centro Agua del Pino, Spain
Khaled Mohammed Geba,
Menoufia University, Egypt

*CORRESPONDENCE
Valiollah Jafari
v.jafari.sh110@gmail.com

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The effects of dietary *Agaricus bisporus* powder on growth performance, haematological indices, and serum immune response in beluga (*Huso huso*) juvenile

Afshin Aminzadeh, Valiollah Jafari* and Seyed Hossein Hoseinifar

Department of Fisheries, Faculty of Fisheries and Environmental Sciences, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran

Introduction: The present study investigates the effects of mushroom powder (MP) on growth parameters, haematological indices, innate immune response, and serum biochemical indices in beluga (*Huso huso*) juveniles.

Methods: A total of 120 fish (45 ± 0.5 g) were stocked with 10 fish in each 300-L tank. Experimental diets were prepared by inclusion of 0, 0.5, 1.0, and 2.0% MP. At the end of the feeding trial haemato-immunological parameters as well growth performance were measured.

Results: The growth parameters results revealed that body weight, specific growth rate (SGR), and body length increased significantly in the fish fed diets containing 1% and 2% MP ($P<0.05$). Haematological indices results indicated that none of the experimental diets showed significant effects on red blood cell count (RBC), hemoglobin, mean corpuscular volume (MCV), mean cell hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) ($P>0.05$). However, the haematocrit increased significantly in the fish fed diets containing 1% MP compared to the control ($P<0.05$). White blood cell count (WBC) in the fish fed 2% MP was significantly higher than that in the fish fed 0% and 0.5% MP ($P<0.05$). Furthermore, MP treatments caused no significant change in the activity of complement C_3 ($P>0.05$), while C_4 activity increased significantly in the fish fed 2% MP ($P<0.05$). Total immunoglobulin in 1% MP had no significant difference when compared with control ($P>0.05$). Albumin level was significantly higher in fish fed 2% MP compared to control ($P<0.05$).

Discussion: In conclusion, MP (2%) can be considered to improve growth parameters and immune indices in beluga juveniles.

KEYWORDS

humoral immunity, mushroom, beluga, growth, prebiotic

1 Introduction

Sturgeon (Acipenseridae) are known as ancient species dating back to 80 million years ago (Kasumyan, 1994). The Caspian Sea hosts approximately 90% of the total stocks of these invaluable fish worldwide. They are included in the list of the Convention on International Trade in Endangered Species (Bronzi et al., 1999), which has led to more attention paid to the culture of the species rather than uncontrolled catch in Iran and other countries.

Aquaculture has gained a considerably higher share in worldwide food production practices over the past few decades. It encompasses more than half of the overall fish and mollusk consumption by humans (FAO, 2023). Developments in culture techniques and the introduction of new species have accelerated aquaculture industry growth. It should follow the principles of sustainable development considering the global importance of the food sector. Economic losses in aquaculture are predominantly rooted in infectious diseases, especially in the primary steps of production. Treatment methods to fight against these infections mainly include the use of antibiotics as well as chemical treatments, which are neither very efficient nor environmentally friendly and adaptable. Existing concerns regarding the adverse effects of using antibiotics, such as the appearance of resistant bacteria, accumulation of antibiotic residues in edible tissues, and attenuation of the immune system, have resulted in the enforcement of strict laws and regulations in the application of antibiotics in many countries (Reverter et al., 2014). Food and Agriculture Organization of the United Nations has encouraged scientific communities to take preventive practices. These include proper feeding practices to improve the health status of aquatic organisms, the use of innate and adaptive immune systems of the host organism to control diseases, the incorporation of growth and adaptive immune promoters to reduce susceptibility to diseases, and the reduction of using chemicals and medicines in aquaculture (Subasinghe, 1997). It seems necessary to consider feeding approaches to reduce disease in aquatic organisms. In the past two decades, there have been efforts to achieve a better understanding of the relationship among feeding, immune response, and resistance to diseases. Feeding imbalance, especially during larval and juvenile periods, greatly undermines growth, disease resistance, and survival (Lieke et al., 2020). Modern practices in the aquaculture industry are focused on the methods to improve the health status of aquatic organisms and to reach environment compatibility.

During the past years, there have been progressive attempts to discover naturally occurring prebiotics. According to the literature, mushrooms seem to be a perfect candidate to be considered natural prebiotics due to their contents of carbohydrates like chitin, hemicellulose, β- and α-glucans, mannans, xylans, and galactans. Mushrooms contain several polysaccharides such as linear and branched glucans attached to different types of glycosides such as (1→3)-α-glucan and (1→6)-β-glucan. Although mushrooms contain a variety of polysaccharides, the polysaccharides are mostly limited to β-glucans (Van Loo and Gibson, 2006). The indigestibility of mushroom carbohydrates has put them at the center of attention as a source of naturally occurring prebiotics. However, this needs extensive research to be proved because not all carbohydrates in the diet are regarded as prebiotics (Gibson, 2004). Some studies have considered the use of mushrooms as a feed supplement in aquaculture. For example, Khodadadian Zou et al. (2016) reported that using white button mushroom powder in the common carp diet led to significant elevations of mucus and serum immune parameters, immune-related gene expressions, and growth promotion at the end of the trial. Furthermore, Uluköy et al. (2016) stated that the incorporation of *Pleurotus ostreatus* in rainbow trout diet improved some immune parameters such as lysozyme activity, but had no significant effect on serum immunoglobulin. Moreover, Van Doan et al. (2016) found that the singular or combined use of *Pleurotus eryngii* and *Lactobacillus plantarum* in the diet of *Pangasius bocourti* enhanced serum innate immune parameters and growth indicators such as feed conversion ratio (FCR). Although studies have shown that the use of different species of mushroom as a feed supplement in animal diets, especially in aquatic organism diet, improves immune and growth parameters, there are only limited findings on the effects of different mushroom species in fish nutrition. In addition, to the best of our knowledge, no report has been made on the use of different mushroom species in sturgeon, especially beluga, diet and its influence on immune and growth parameters.

Therefore, the present study was performed to investigate the effects of dietary mushroom powder on growth performance, haematological indices, and serum immune response of beluga (*Huso huso*) juveniles.

2 Materials and methods

2.1 Ethics

All experiments were performed following the protocol approved by the committee of ethics of the faculty of sciences of the University of Tehran (357; 8 November 2000).

2.2 Preparation of experimental diets

White button mushroom (*Agaricus bisporus*) was provided by a reputable producer, cut into sheets, and kept in an oven at 45°C for 48 h (Şevik et al., 2013). Then, *A. bisporus* was powdered using a grinder. A commercial fish feed (Coppens, Germany, Table 1) was milled followed by blending with different levels of mushroom powder (0, 5, 10, or 20 g kg^{-1}) (Hoseinifar et al., 2019). Afterwards,

TABLE 1 Proximate composition of the basal diet (%).

Proximate composition dry matter basis	(%)
Crude protein	47
Crude lipid	9.0
Moisture	7.5
Fiber	1.2
Ash	9.9

the diet was sprayed with 3% gelatin to obtain a paste-like tissue and then, the resulting paste was tuned into 2-mm strips using a mincer (Khanzadeh et al., 2024). The dried paste strips were placed in zipper storage bags and kept in the refrigerator.

2.3 Feeding and rearing conditions

The present study was carried out at Shahid Rajaee Aquaculture Center of Sari, Iran, for 8 weeks. 120 juvenile belugas (45 ± 0.5 g) were stocked in four (300 L) tanks and acclimated to experimental conditions for two weeks. Thereafter, the fish were randomly stocked in twelve 300-L tanks, each containing 10 fish. During the 8-week trial, the fish were fed manually, three times a day at 3% of biomass (Hoseinifar et al., 2011). Thereafter, the fish were randomly stocked in twelve 300-L tanks each containing 10 fish. Tank water was aerated using air stones connected to the central air compressor and 50% of water was replaced daily. The photoperiod consisted of 12 hours of light and 12 hours of darkness. Water temperature was maintained at 20.2 ± 1°C, dissolved oxygen levels were kept at 7.5 ± 0.2 mg/L, and pH was regulated at 7.3 ± 0.2 on a daily basis.

2.4 Growth parameters

To analyze the growth rate, the fish were subjected to biometric measurements at the end of the trial. Growth performance in beluga was measured using the following formulas:

Body weight Increase (BWI) = Final weight (Wt_2) – Initial weight (Wt_1) (Tacon, 1990)

Percentage of BWI (PBWI) = [(final weight (g) – initial weight (g)]/(initial weight) × 100 (Bekcan et al., 2006)

Specific growth rate (SGR) = 100(Ln final weight – Ln initial weight)/day (Hevrøy et al., 2005)

Feed Conversion Ratio (FCR) = feed intake (g)/weight gain (g) (Hevrøy et al., 2005)

2.5 Blood serum preparation

At the end of the experiment, blood samples were obtained from caudal fins. The samples were divided into two parts and were aliquoted into either heparinized microtubes for the analysis of blood parameters or non-heparinized microtubes for the investigation of serum immune parameters. The blood samples in heparinized microtubes were placed in containers filled with ice and transferred to the laboratory. For analyzing serum immune indices, the samples were kept in a refrigerator for 4 h for coagulation and then, they were centrifuged at 5000 g for 5 min using a clinical centrifuge (Hettich Micro 200R, Germany) and serum was separated. The separated sera samples were transferred into vials and kept at -80 °C until analysis.

2.6 Haematological parameters

The blood samples were poured into blood-diluting pipettes by suction and were diluted (Natt and Herrick, 1952) for red blood cell (RBC) and white blood cell (WBC) count. The pipettes were shaken for 3 min and then, one drop of each was spread on a Neubauer hemocytometer slide for RBC and WBC using a microscope (Hoseinifar et al., 2011). White blood cell differential count (Diff), Hemoglobin (Hb), Haematocrit (HCt) were measured as described in detail in our previous paper (Hoseinifar et al., 2011).

2.7 Immunological parameters

2.7.1 Total immunoglobulin

Total immunoglobulin was measured by the method proposed by Siwicki and Anderson (1993). The protein content of the sample was measured and then, polyethylene glycol (PEG) 12% was added. Total immunoglobulin was acquired by considering the difference between the protein concentrations of the sample before and after the addition PEG.

2.7.2 Complement system activity

Complements C3 and C4 were measured using special kits (Nanjing, China) and a turbidimetry following the manufacturer protocol. The assay is based on the reaction between antigen and antibody. This reaction forms an insoluble complex producing a turbidity, which is measured spectrophotometrically. The amount of complex formed is directly proportional to the amount of C3 in the sample. The results were expressed as $\mu g.ml^{-1}$.

2.7.3 Total protein

Total protein was measured through the biuret method (Gornall et al., 1994) using a biuret kit (Pars Azmun, Iran) by reading through a spectrophotometer and expressed as $mg.ml^{-1}$ (Yusefi et al., 2022).

2.7.4 Albumin

Albumin level was measured by using a serum albumin kit (Pars Azmun, Iran) through photometry and expressed as $g.dL^{-1}$ following the protocol suggested by the company (Yusefi et al., 2022).

2.8 Statistical analysis

The current study was performed in a completely randomized design. The normal distribution of data was checked and confirmed with Kolmogorov-Smirnov test. One-way ANOVA and Duncan's test were used to compare means at 95% confidence interval by SPSS version 20 software ($P< 0.05$) (Zar, 1994). Values are presented as the mean ± S.E.

3 Results

3.1 Growth parameters

The effects of different levels of mushroom powder (MP) on the growth parameters of the fish after 8 weeks are shown in Table 2. The incorporation of 1% and 2% MP to the beluga diet significantly increased growth compared with control (P<0.05), while the addition of 0.5% MP to the diet resulted in a negligible growth promotion with no significant difference with control (P >0.05). Furthermore, no significant difference was observed in terms of length increase among control and fish fed 0.5% and 1% MP (P >0.05). However, the length of the fish fed 2% MP was significantly higher than that in other groups (P<0.05).

The results also showed that the incorporation of MP had no significant effect on FCR (P >0.05). The fish fed 1% and 2% MP had significantly higher SGR compared to the control (P<0.05), but SGR in the fish fed 0.5% MP showed no significant difference with other groups (P >0.05). The same trend was observed for the condition factor (K).

3.2 Blood indices

The results of the analysis of blood indices are summarized in Tables 3, 4. No significant difference was detected in RBC, hemoglobin, MCV, MCH, and MCHC between experimental and control groups (P >0.05). Nevertheless, the hematocrit percentage increased significantly in the fish fed level of MP 1% compared with the control (P<0.05). Although the other treatment groups have similar values to the MP 1%, they aren't significantly different from the control group (P >0.05). Significant differences were noticed in WBC between the fish fed 2% MP and those fed 0% and 0.5% MP (P<0.05), but no significant difference was found in this regard between the fish fed 2% and 1% MP (P >0.05). The results of the differential count also revealed that there was no significant difference among the samples in terms of the number of eosinophils, neutrophils, and lymphocytes (P >0.05) (Table 4).

3.3 Complement system

The effects of different levels of MP on complement system activity are shown in Figures 1, 2. According to the results, the use of MP in the beluga diet at any tested levels showed no significant change in the complement C3 activity (P >0.05). The C4 activity in the MP 0.5% group showed a significant decrease compared to the control group (P<0.05), whereas the MP 2% group exhibited a significant increase in C4 levels relative to the control group (P<0.05).

3.4 Total immunoglobulin

The effect of MP incorporation in beluga diet on serum total immunoglobulin is presented in Figure 3. Total immunoglobulin significantly decreased in the fish fed 0.5% and 2% MP compared with control (P<0.05), whereas no significant difference was noticed in terms of this parameter between the fish fed 1% MP and control (P >0.05).

3.5 Total protein

Figure 4 presents the effects of different levels of MP in the beluga diet on total protein. The fish fed 1% and 2% MP had significantly higher total protein content than those fed 0.5% MP (P<0.05), while no other significant difference was observed among the groups tested (P >0.05).

3.6 Albumin

The influence of adding MP into the beluga diet at different levels is shown in Figure 5. Total albumin in the fish fed 2% MP was significantly higher than that in control (P<0.05); however, no significant difference was observed between the fish fed 0.5% and 1% MP and control in terms of albumin content (P >0.05).

TABLE 2 The growth parameters of Beluga fed with different levels of mushroom powder (%) after 8 weeks.

Parameters	Control (0)	MP 0.5%	MP 1%	MP 2%
IW (g)	45.10 ± 1.31	45.21 ± 1.01	45.93 ± 1.29	45.60 ± 1.62
FW (g)	236.11 ± 24.86^{b}	255.21 ± 8.90^{ab}	269.85 ± 7.85^{ab}	279.55 ± 8.64^{a}
FL (cm)	36.76 ± 1.47^{b}	37.94 ± 0.90^{ab}	38.11 ± 0.71^{ab}	38.97 ± 1.0^{a}
CF	0.44 ± 0.013^{b}	0.46 ± 0.012^{ab}	0.46 ± 0.016^{a}	0.44 ± 0.00^{a}
WG (g)	190.01 ± 24.37^{b}	209.99 ± 9.85^{ab}	224.04 ± 9.69^{a}	233.95 ± 9.20^{a}
SGR (% d^{-1})	5.32 ± 0.10^{b}	5.47 ± 0.03^{ab}	5.53 ± 0.01^{a}	5.56 ± 0.03^{c}
FCR	1.40 ± 0.05 ^{a}	1.45 ± 0.03 ^{a}	1.45 ± 0.02 ^{a}	1.48 ± 0.05 ^{a}

IW, initial weight; FW, final weight; WG, weight gain; SGR, specific growth rate; FCR, Feed conversion ratio; FL, Final length; CF, Condition factor; MP, mushroom powder. Values are presented as the mean ± SD. Different letters (a–d) in the same row indicate significant differences (P< 0.05).

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TABLE 3 The effects of dietary mushroom powder (%) on haematological indices of beluga juveniles.

Groups	Erythrocyte count ($\times 10^4$ mm^{-3})	Haemoglobin (g dl^{-1})	Haematocrit (%)	MCH (pg)	MCHC (g dl^{-1})	MCV (fl) ($\times 10^2$)
Control	81.71 ± 14.40 ^{a}	3.77 ± 0.40 ^{a}	16.33 ± 1.52 ^{b}	47.13 ± 9.25 ^{a}	23.10 ± 1.57 ^{a}	2.02 ± 0.26 ^{a}
MP 0.5 %	82.70 ± 21.00 ^{a}	3.99 ± 0.49 ^{a}	19.33 ± 1.57 ^{ab}	51.20 ± 19.61 ^{a}	20.56 ± 1.88 ^{a}	2.45 ± 0.71 ^{a}
MP 1 %	82.30 ± 19.30 ^{a}	4.37 ± 0.48 ^{a}	19.66 ± 1.57 ^{a}	51.83 ± 11.60 ^{a}	21.10 ± 3.12 ^{a}	2.50 ± 0.72 ^{a}
MP 2 %	82.63 ± 5.10 ^{a}	4.62 ± 0.44 ^{a}	21.33 ± 1.50 ^{ab}	39.96 ± 13.60 ^{a}	21.66 ± 1.83 ^{a}	1.86 ± 0.65 ^{a}

MP, mushroom powder; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume. Values are presented as the mean ± SD. Different letters in the same column indicate significant differences ($P< 0.05$).

TABLE 4 The effects of dietary mushroom powder (%) on total and differential leukocyte counts of beluga juveniles. .

Groups	Leukocyte count ($\times 10^3$ mm^3)	Lymphocyte (%)	Neutrophil (%)	Eosinophil (%)
Control	16.18 ± 0.99^{b}	66.00 ± 4.00 ^{a}	7.66 ± 2.08 ^{a}	26.33 ± 2.88 ^{a}
MP 0.5 %	17.10 ± 0.76^{b}	58.33 ± 7.68 ^{a}	7.00 ± 2.64 ^{a}	34.66 ± 8.08 ^{a}
MP 1 %	19.31 ± 0.76 ^{a}	62.66 ± 17.47^{a}	6.30 ± 2.78 ^{a}	37.00 ± 2.07 ^{a}
MP 2 %	19.33 ± 0.64^{a}	62.66 ± 3.51 ^{a}	3.87 ± 0.08 ^{a}	30.66 ± 1.03 ^{a}

MP, mushroom powder. Values are presented as the mean ± SD. Different letters in the same column indicate significant differences ($P< 0.05$).

4 Discussion

In recent years, mushrooms have emerged as a significant source of functional foods and medicinal products, gaining recognition for their health benefits and nutritional value (Bhushan and Kulshreshtha, 2018). Mushrooms are highly regarded for their rich content of various bioactive compounds. These include B and D2 vitamins, β-glucans, essential minerals, terpenes phenolic compounds, and sterols (Sławińska et al., 2022). Agaricus is among the largest genera of macrofungi, encompassing numerous edible species known for their medicinal properties and high nutritional content (Zhang et al., 2017). *A. bisporus*, belonging to the Agaricaceae family, is the most widely cultivated mushroom and is renowned for its edibility. It holds significant importance due to its culinary applications and medicinal properties, making it one of the foremost mushrooms in terms of both nutritional and health benefits (Usman et al., 2021). Consequently, mushrooms are classified as functional foods and are increasingly utilized to enhance the nutritional and health benefits of a wide range of food products.

The results obtained from the current study indicated that the 1% and 2% MP in the beluga diet improved growth parameters. Previous studies reported that the addition of *Phellinus linteus* mushroom and *Inonotus obliquus* mushroom powder into the grouper diet (Harikrishnan et al., 2011), and β-glucan into the common carp diet (Kühlwein et al., 2014) enhanced growth parameters. Furthermore, Van Doan et al. (2016) concluded that the enrichment of the catfish (*Pangasius bocourti*) diet with the mushroom *Pleurotus eryngii* increased SGR and decreased FCR. It was also reported that the replacement of fishmeal with the

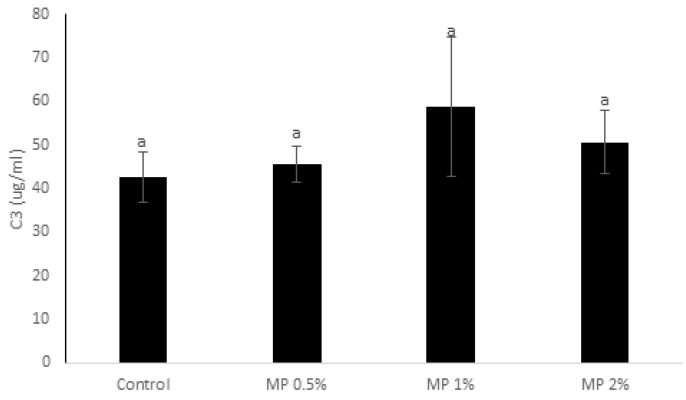

FIGURE 1
The serum C3 activity of Beluga fed with different levels of mushroom powder (%) after 8 weeks. Bars assigned with same letter indicate no significant differences ($P>0.05$). Values are presented as the mean ± SD.

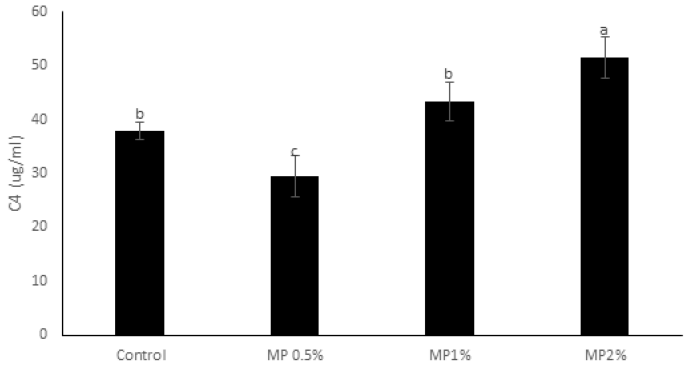

FIGURE 2
The serum C4 activity of Beluga fed with different levels of mushroom powder (%) after 8 weeks. Different letters (a-c) in the columns indicate significant differences ($P<0.05$). Values are presented as the mean ± SD.

mushroom *Pleurotus sajorcaju* powder (at 0%, 33%, 67%, and 100%) in the tilapia diet did not affect the fish growth.

Owing to their content of indigestible carbohydrates, mushrooms are known as a naturally occurring prebiotic, which provides nutrients for gut microbiota and increases the level of useful bacteria such as lactic acid bacteria (Yin et al., 2020). These bacteria reduce intestine pH, which leads to limited growth of harmful bacteria in the intestine and better health status (Ringø et al., 2018). Although it remains to be found which compounds in mushrooms account for the positive effects in aquatic organisms, it can be claimed that glucan, whose positive roles in the growth and immunity of aquatic animals have been substantiated, is the most abundant polysaccharide in mushrooms (Saha et al., 2023). Nonetheless, the influence of other polysaccharides and other compounds of mushrooms should not be overlooked. The synergistic effects of different compounds in mushrooms are likely responsible for the role of the compounds in fish growth (Van Doan et al., 2019).

Blood indices in fish can be influenced by various components such as species, size, age, physiological status, environmental conditions, and diet (Brunt and Austin, 2005). To the best of our knowledge, no comprehensive report has been presented regarding the accurate mechanism of feed supplement (e.g. prebiotics) effectiveness on fish blood indices. However, there is a unanimous ground that indicates blood indices vary in different species and they are highly dependent on environmental conditions, feeding, age, etc (Ross et al., 2008). The results acquired from the present study pointed to the ineffectiveness of different levels of MP applied to blood indices such as Hb, RBC, MCV, MCH, and MCHC. Yet, Hct significantly increased in the fish fed 1% MP, and WBC was significantly higher in the fish fed 2% MP, while no significant difference was seen in blood differential counts among the groups analyzed ($p>0.05$). Harikrishnan et al. (2012) reported that enrichment of the diet of *Epinephelus bruneus* with the mushroom *Inonotus obliquus* resulted in improved blood indices in the fish fed different levels of mushroom. Other investigations also highlighted the positive effects of fish diet enrichment with medicinal plants or mushrooms on blood parameters (Harikrishnan et al., 2011; Kim et al., 2012). In addition, Hisano et al. (2007) stated that the incorporation of 2% dehydrated yeast (as the main source of mannan oligosaccharides) in the tilapia diet had no influence on hematological parameters, which is consistent with the findings of the present study. According to our research, Safari and Sarkheil (2018) investigated how *Pleurotus eryngii* mushroom powder affects the hematological parameters in *Cyprinus carpio koi*. Their findings indicated that this mushroom powder had a notable

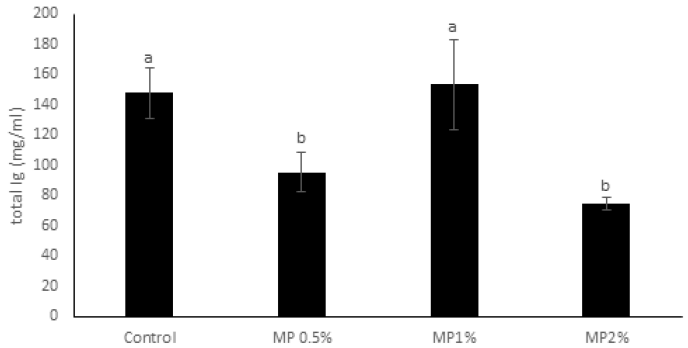

FIGURE 3
The serum total Ig level of Beluga fed with different levels of mushroom powder (%) after 8 weeks. Different letters in the columns indicate significant differences ($P<0.05$). Values are presented as the mean ± SD.

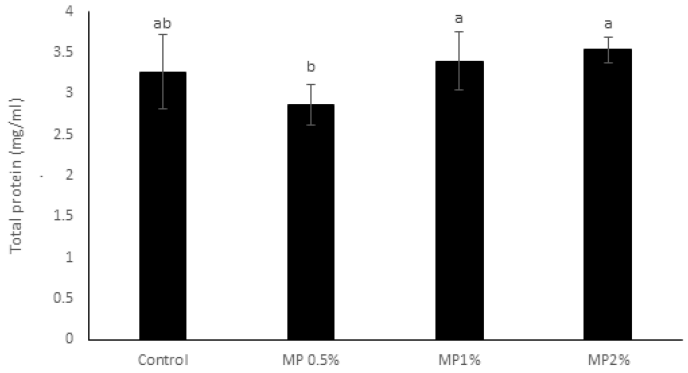

FIGURE 4
The serum total protein level of Beluga fed with different levels of mushroom powder (%) after 8 weeks. Different letters in the columns indicate significant differences (P<0.05). Values are presented as the mean ± SD.

impact on hematological parameters including WBCs, lymphocytes and monocytes (Safari and Sarkheil, 2018).

The complement system is one component of the immune system of osteichthyes and it is composed of several proteins, which are activated, like evolved vertebrates, through three pathways, i.e. classic, alternative, and lectin. These three pathways lead to opsonization or the decomposition of microorganisms (Whyte, 2007). The determination of complement system activity at the end of the trial in the present study showed that the administration of appropriate levels of MP caused no change in complement C3 activity. However, complement C4 in the fish fed 2% MP significantly increased compared to control, but there was no significant difference in complement C4 activity between the fish fed 1% MP and control. Chang et al. (2013) stated that the incorporation of β-glucan extracted from the mushroom in the *Epinephelus coioides* diet improved complement system activity. Furthermore, Sirimanapong et al. (2015) evaluated the effects of incorporating 0.05%, 0.1%, and 0.2% β-glucan in the diet on the immune system of *Pangasianodon hypophthalmus*. They witnessed a significant elevation of complement system activity in the serum of the fish fed 0.2% β-glucan at the end of the first week, while the highest level of complement system activity at the end of the second week belonged to the fish fed 0.1% β-glucan. At 0.5%, the concentration of β-glucans may not be high enough to effectively stimulate immune cells. This could result in a diminished response, leading to lower C4 levels as the immune system is not adequately activated. In contrast, the significant increase in C4 levels in the MP 2% group indicates a stimulatory effect at this higher concentration. The increased availability of β-glucans likely enhances the activation of immune cells, promoting a more robust immune response and leading to elevated C4 levels.

Immunoglobulins are among natural antibodies, which are formed in a regulated manner in the absence of external antigenic stimulators and they provide immediate and extensive protection against pathogens. This characteristic has made them a vital part of the innate immune system (Magnadóttir, 2006). Total immunoglobulin of the fish fed 0.5% and 2% MP in the current study was significantly lower than that in control, whereas no significant difference existed in terms of this factor between the fish fed 1% MP and control. Uluköy et al. (2016) administered 1% and 2% water-alcohol extract of oyster mushrooms in rainbow trout diet for 6 weeks and examined fish survival rate at the end of the trial in exposure to *Lactococcus garvieae*. They reported that phagocytic, lysozyme, and myeloperoxidase activities of serum had no significant difference from those in

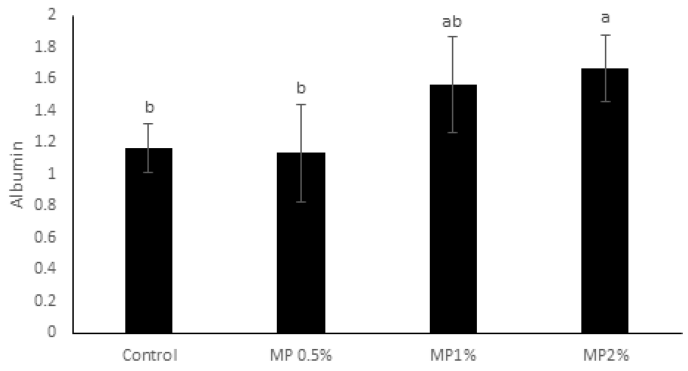

FIGURE 5
The serum albumin level of Beluga fed with different levels of mushroom powder (%) after 8 weeks. Different letters in the columns indicate significant differences (P<0.05). Values are presented as the mean ± SD.

control. In addition, they announced that the administration of mushroom extract in the diet reduced fatality caused by *L. garvieae* infection compared with control, which is in agreement with the results of the present study regarding total immunoglobulin. In contrast with our contradictory findings about Ig levels following MP administration, the application of the mushroom *Lentinula edodes* extract in the rainbow trout diet (Baba et al., 2015) and β-glucan in the *Sparus aurata* diet (Guzmán-Villanueva et al., 2014) increased the level of immunoglobulin. In accordance with our findings, Safari and Sarkheil examined the impact of *P. eryngii* mushroom powder on the immune parameters of *C. carpio koi*. They reported that a 2% inclusion of this mushroom powder significantly influenced immune parameters, including total immunoglobulin and lysozyme levels (Safari and Sarkheil, 2018).

The results of this study also showed that the administration of 1% and 2% MP in the beluga diet increased total protein compared with the fish fed 0.5% MP. Furthermore, albumin, which is crucial to preserve osmotic pressure for proper distribution of body fluids and act as an innate ligand and plasma carrier (Holeton and Randall, 1967), increased in the fish fed 1% and 2% MP. Elevation of total protein and albumin in the fish fed MP could reflect the innate immune strength in the fish (Awad and Austin, 2010; Jha et al., 2007). In line with the results of the present study, previous studies reported increased total protein in common carp fed β-glucan (Siwicki, 1989) and increased total protein and albumin in *Epinephelus bruneus* fed the mushroom *P. linteus* (Harikrishnan et al., 2012).

Several studies have emphasized the relationship between the performance of the digestive system and the immune system. Part of the innate immune system in fish and other vertebrates is gut-associated lymphoid tissue (GALT), which plays a pivotal role in the occurrence and regulation of food-related immune responses (Lazado and Caipang, 2014). Digestive system flora may have regulatory and evolutionary roles in this system. Changes in innate immune responses are explained by the compounds found in mushrooms such as β-glucans and other polysaccharides. Moreover, mushrooms could be regarded as a naturally occurring prebiotic. Prebiotics improve the innate immune system by changing digestive system flora, increasing lactic acid bacteria and bacillus bacteria, and having a direct effect on the GALT system (Hoseinifar et al., 2015). In mushrooms, in addition to β-glucan that stimulates macrophages, other compounds such as lentinan and schizophyllan trigger the proliferation of T lymphocytes and humoral immune parameters. Overall, although the results obtained from the present study are indicative of improved growth parameters and innate immune factors in beluga, further research is required on other immune parameters to make a fine judgment in this regard.

5 Conclusion

The current study demonstrates that incorporating mushroom powder (MP) into the diet of beluga (*Huso huso*) juveniles significantly influences growth parameters and immune indices. The results indicate that a 2% MP inclusion is particularly beneficial, as it led to notable increases in body weight, specific growth rate, and serum albumin levels, alongside enhanced white blood cell counts and complement C4 activity. This suggests that a higher concentration of MP not only promotes better growth but also strengthens the immune response in these fish. It is also recommended that future research explore various dosages and examine other commercially farmed fish species. Additionally, to gain a deeper understanding of the effects of *A. bisporus*, studies should focus on the mechanisms involved, particularly the role of specific bioactive compounds like β-glucans.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

All experiments were performed following the protocol approved by the ethics committee of the faculty of sciences of the University of Tehran (357; 8 November 2000). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

AA: Data curation, Formal analysis, Methodology, Resources, Writing – original draft. VJ: Conceptualization, Formal analysis, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. SH: Conceptualization, Data curation, Formal analysis, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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