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Research Article

Effect of dietary *Ganoderma lucidum* extract on growth performance, blood biochemical parameters, and antioxidant status in juvenile beluga (*Huso huso*)

Omidzahir S.[1*]; Fallah F.[1]

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Abstract

In the present study, the dietary effect of *Ganoderma lucidum* extract (GLE) on growth performance, antioxidant status, and some blood biochemical parameters was investigated in *Huso huso*. The fish were divided into four groups and fed with different concentrations of 0, 0.5, 1, and 2 g/kg GLE for 6 weeks. The results showed that the growth indices including weight gain and specific growth rate were increased significantly compared to the control group. However, the food conversion rate was significantly decreased in 1 and 2 g/kg GLE-supplemented groups. An increasing trend of serum total protein and IgM was observed in the groups fed with GLE, while the fish fed with 1 and 2 g/kg GLE showed significant differences compared to the control group (p<0.05). The serum glucose level decreased in the groups fed with GLE compared to the control group, however, it was not significant. The triglycerides and cholesterol levels were significantly reduced in 1 and 2 g/kg GLE added groups compared to 0.5 g/kg GLE and control group (p<0.05). An increase in the serum total antioxidant capacity was observed in GLE-supplemented groups, which significantly raised in 1 and 2 g/kg GLE (p<0.05). Serum malondialdehyde decreased in the groups fed with GLE, which significantly reduced in 2 g/kg GLE compared to the other groups (p<0.05). In conclusion, dietary GLE showed a proper effect on growth performance, antioxidant capacity, hypolipidemia, and immunity in *H. huso*.

Keywords: Lingzhi mushroom, Sturgeon, Growth, Blood biochemistry, Antioxidant enzymes

1-Department of Marine Biology, Faculty of Marine and Environmental Sciences, University of Mazandaran, Babolsar, Iran.
* Corresponding author's Email: sh.omidzahir@umz.ac.ir

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Introduction

The increasing demand for seafood has caused a remarkable development in the aquaculture industry and therefore, aquatic health management is critical for farming fish species. In the meantime, different stressors, pathogens, and environmental pollutants lead to disease outbreaks in fish farms. Immune and antioxidant systems play an essential role in combating different stresses and diseases in fish (Galina *et al*., 2009). Using bioactive compounds as nutritional supplements in the fish diet seems to boost the immune and antioxidant systems against stressors (Amar *et al*., 2004; Martinez-Álvarez *et al*., 2005; Hoseinifard *et al*., 2018).

Ganoderma lucidum commonly known as 'Reishi' or 'Lingzhi' is a medicinal fungus with several interesting compositions such as polysaccharides, terpenoids, nucleotides, steroids, fatty acids, proteins, flavonoids, alkaloids, antioxidants, glycopeptides, vitamins, and minerals, which triterpenoids and polysaccharides are the major components (Zjawiony, 2004; Paterson, 2006; Liu *et al*., 2016). *G. lucidum* has been widely used as an oriental mushroom for centuries to improve some disorders like hypertension, bronchitis, immunological disease, anorexia, hepatitis, and cancer (Boh *et al*., 2007; Zhao *et al*., 2016).

Because of the great properties and multifunctional ingredients of *G. lucidum*, it has a high potential to treat several diseases and use in the nutraceutical and pharmaceutical industries (Li *et al*., 2013; Stojković *et al*., 2014).

A few researches have been reported on the effect of *G. lucidum* as a nutritional supplement in aquatic animals. Dietary *G. lucidum* polysaccharides increased immune inflammatory response and antioxidant enzyme activity in (*Cyprinus carpio*) against CCl4, which caused hepatocyte lesions (Liu *et al*., 2015). *G. lucidum* polysaccharides revealed the desirable effect on the survival and growth performance of *Ctenopharyngodon idella* (Chithra *et al*., 2016). Yin *et al*. (2009) reported *G. lucidum* extract can increase the immune system status of common carp (*Cyprinus carpio*) against *Aeromonas hydrophila*. In other research, *G. lucidum* extract enhanced the survival rate, growth performance, digestive enzymes, and antioxidant activities in giant freshwater prawn (*Macrobrachium rosenbergii*) (Mohan *et al*., 2016). *G. lucidum* extract showed beneficial effects on growth and health status as well as antioxidant enzymes stimulation in the red hybrid Tilapia (*Oreochromis* sp.) (Wan *et al*., 2021). *G. lucidum* polysaccharides increased both specific and non-specific immunity as effective immunostimulants against *Vibrio harveyi* in pearl gentian grouper (*Epinephelus* sp.) (Zhang *et al*., 2022).

The effect of *G. lucidum* extract as a dietary supplement on sturgeons has not been reported so far. Sturgeons are valuable fish species belonging to the Acipenseridae family. Unfortunately, conservation threats have endangered sturgeons critically (Carmona *et al*.,

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2009). The culture of these highly endangered species, especially the great sturgeon (*Huso huso*), can relieve the pressure on the sturgeon populations in the Caspian Sea (Hoseinifar *et al*., 2011). The great sturgeons or beluga are suitable fish species for aquaculture because of their valuable caviar and meat (Mohseni *et al*., 2008, Yadolahi et al., 2022).

In aquatic animals, stress happens in the conditions of water physicochemical changes, nutritional deficiencies, water pollution, xenobiotics, and diseases (Hwang and Lin, 2002; Yeganeh Kari *et al.,* 2022). Therefore, using the proper dietary supplements which can improve growth performance, and increase the immune and antioxidant systems may enhance the survival of fish in culture (Trichet, 2010; Taleghani *et al*., 2019). The present study aimed to evaluate the dietary effect of *G. lucidum* extract (GLE) on growth performance, some serum biochemical parameters, and antioxidant status in beluga (*H. huso*) juvenile.

Materials and methods

Experimental setup

120 healthy juvenile beluga (*H. huso*) with an average weight of 34.63±4.77 g were collected from the Culture and Breeding Center of Shahid Rajaei (Sari, Iran). After two weeks adaptation period, ten fish were randomly distributed into separate tanks as four groups with three replications. Each tank with 500 cm^3 size and 250 L volume of water was supplied with an inlet water flow rate of 2.47 L min^{-1}. Based on the previous studies (Chithra *et al.,* 2016; Mohan *et al.,* 2016), the groups received different concentrations of 0, 0.5, 1, and 2 g/kg GLE in the diet. Ingredients of the diet were mixed well with GLE and then made into pellets. The composition of experimental diets is shown in Table 1.

Fish were fed 4% of body weight four times a day (Adel *et al*., 2016) for 6 weeks. Water physicochemical parameters of fish tanks were checked daily during the experimental period including temperature 25±1°C, dissolved oxygen 6.5 mg/L and 7.2−7.4 pH.

Table 1: Dietary ingredients and proximate composition.

Ingredients (g/kg)	Ganoderma lucidum extract (g/kg)			
	0	**0.5**	**1**	**2**
Fish meal	460	460	460	460
Soybean oil	58	58	58	58
Wheat flour	150	150	150	150
Soybean meal	100	100	100	100
Meat meal	90	90	90	90
Cellulose	2	1.5	1	0
Vitamin mixture[a]	35	35	35	35
Mineral mixture[b]	25	25	25	25
Fish oil	60	60	60	60
Binder	20	20	20	20
Ganoderma lucidum extract	0	0.5	1	2
Proximate composition (%)				
Dry matter	91.3	91.5	91.2	91.5
Crude protein	39.6	39.5	39.7	39.7

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Table 1 continued:				
Crude lipid	16.9	16.9	16.8	16.7
Ash	10.0	9.8	10.1	10.2
Crude fiber	2.5	2.6	2.5	2.3
Moisture	8.0	7.9	8.0	8.2
NFE[c]	22.3	22.7	22.1	22.6
Gross energy (kcal g^{-1})	3.6	3.6	3.6	3.6

a Unit/kg of mixture: vitamin A, 1,600,000 IU; D3, 400,000 IU; E, 40 IU; K3, 2000 mg; H2, 240 mg; B1, 6000 mg; B2, 8000 mg; B3, 12,000 mg; B5, 40,000 mg; B6, 4000 mg; B9, 2000 mg; B12, 8000 mg; vitamin C, 60000 mg; inositol, 20,000 mg; BHT, 20,000 mg. [b]Unit/kg of mixture: mineral: Fe, 26,000 mg; Zn, 12,500 mg; Se, 2000 mg; Co, 480 mg; Cu, 4200 mg; Mn, 15,800 mg; I, 1000 mg; choline chloride, 12,000 mg. [C] Nitrogen-free extracts (NFE) = dry matter - (crude protein + crude lipid + ash + fiber).

Ganoderma lucidum extract

The fruiting bodies of *G. lucidum* were provided from Iran Ganoderama (Karaj, Iran). The *G. lucidum* specimens were cut into small pieces and mixed to obtain powdered samples for extraction. The GLE was performed according to the procedure described by Taofiq *et al*. (2017). Briefly, the powder of *G. lucidum* was extracted in a Soxhlet apparatus using ethanol. Eventually, the dried ethanolic extracts were obtained by rotary evaporator under reduced pressure (Stuart RE 300, UK).

Growth parameters

The initial and final body weight and length of each fish in different groups were measured at the beginning and the end of the experiment. The weight gain (WG), length gain (LG), specific growth rate (SGR), condition factor (CF), and feed conversion ratio (FCR) were calculated as follows:

WG (g) = final weight (W_2, g) - initial weight (W_1, g)
LG (cm) = final length (L_2, cm) - initial length (L_1, cm)
SGR (%) =100 (ln final weight− ln initial weight) / number of days
CF (%) = 100× final weight (g) / final length $(cm)^3$
FCR = feed intake (g)/weight gain (g)

Serum biochemical parameters

At the end of the experiment, six fish from each group were randomly sampled. Blood samples were collected from the caudal vein and transferred to the non-heparinized microtube for serological examination. The serum samples were separated by 1006 ×g centrifuging for 10 min. The serum biochemical parameters including total protein, albumin, immunoglobulin G (IgM), glucose, triglyceride, cholesterol, aspartate aminotransferase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) were analyzed by commercial kits (Pars azmoon, Iran) using an auto-analyzer (Cobas Mira plus, Germany).

Antioxidant and oxidative stress analysis

Serum total antioxidant capacity (TAC) was measured using commercial kit (Teb Pazhouhan Razi (TPR), Iran) according to the manufacturer protocol.

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Serum malondialdehyde (MDA) was evaluated based on a thiobarbituric acid reaction by commercial kit (Teb Pazhouhan Razi (TPR), Iran).

Statistical analysis

Statistical analysis was performed using SPSS version 22. Data were analyzed using a One-way test of variance (ANOVA) to compare means. Duncan's test was performed to analysis of significant differences among groups. The statistical significance level was $p<0.05$. Data are presented as mean± standard error (SE).

Results

Growth and feed utilization indices

In this study, the growth indices including WG, LG, and SGR were increased significantly in the groups supplemented with GLE compared to the control group ($p<0.05$). The highest increase was observed in 2 g/kg GLE. However, it was not significantly different from the group fed with 1 g/kg GLE ($p>0.05$). FCR decreased in GLE supplemented-group with significant differences in 1 and 2 g/kg GLE compared to the other groups ($p<0.05$). There was no significant difference in CF of all groups ($p>0.05$; Table 2).

Table 2: Growth performance of *Huso huso* fed diets supplemented with *Ganoderma lucidum* extract for 6 weeks.

Growth parameters	*G. lucidum* extract (g/kg)			
	0	0.5	1	2
W_1 (g)	34.21 ± 4.9	34.87 ±4.7	34.9 ±4.9	34.57 ±4.6
L_1 (cm)	19.67 ±1.2	19.80 ±1.1	19.29 ±1.09	19.62 ± 1.0
W_2 (g)	89.27 ± 3.7^a	96.67 ± 3.5^a	108.51 ± 3.8^b	110.93 ± 4.4^b
L_2 (cm)	27.34 ± 0.3^a	28.45 ± 0.4^a	29.64 ± 0.4^b	30.22 ± 0.3^b
WG (g)	55.06 ± 0.9^a	61.79 ± 1.2^b	73.69 ± 1.5^c	76.36 ± 1.0^c
LG (cm)	7.84 ± 0.16^a	8.64 ± 0.13^b	10.35 ± 0.34^c	10.60 ± 0.27^c
SGR (%)	$1.03^a \pm 0.00$	1.10 ± 0.02^b	1.22 ± 0.02^c	1.26 ± 0.01^c
CF	0.43 ± 0.01	0.41± 0.00	0.40 ±0.01	0.41± 0.00
FCR	1.70 ± 0.03^a	1.62 ± 0.03^a	1.50 ± 0.03^b	1.47 ± 0.01^b

Data are presented as mean ± SE. Different letters above the values indicate significant difference among groups ($p<0.05$). W_1, initial weight; L_1, initial length; W_2, final weight; L_2, final length; WG, weight gain; LG, length gain; SGR, specific growth rate; CF, condition factor; FCR, Feed conversion ratio.

Serum biochemical parameters

The results of this study showed an increasing trend of serum total protein in the groups supplemented with GLE, which significantly raised in 1 and 2 g/kg GLE compared to control group ($p<0.05$).

Also, the IgM level increased in GLE-supplemented groups. The lowest and highest IgM levels were observed in control group and 2 g/kg GLE-supplemented group, respectively. Meanwhile, the amount of albumin in different groups was not significantly different ($p>0.05$; Fig.1).

The amount of glucose in the groups fed with GLE showed a decreasing trend. However, there was no significant difference among the groups ($p>0.05$). The triglyceride level was reduced in GLE-supplemented groups and showed a significant difference in 1 and 2 g/kg GLE groups compared to 0.5 g/kg GLE and control group ($p<0.05$). The lowest

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cholesterol level was observed in 2 g/kg GLE, which was significantly different compared to 0.5 g/kg GLE and the control group (p<0.05), however, it was not significantly different from 1 g/kg GLE (p>0.05; Fig. 2).

There were no significant differences in the amount of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in all groups (p>0.05; Table 3).

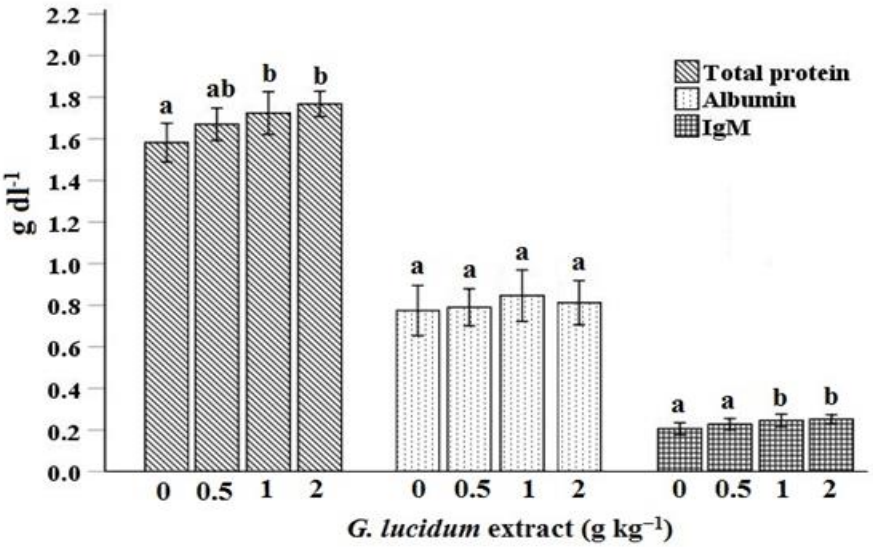


Figure 1: Serum biochemical parameters including total protein, immunoglobulin M (IgM), and albumin in *Huso huso* fed diet supplemented with *Ganoderma lucidum* extract for 6 weeks. Data are presented as mean±SE. Different letters above the bars indicate significant difference among groups (p<0.05).

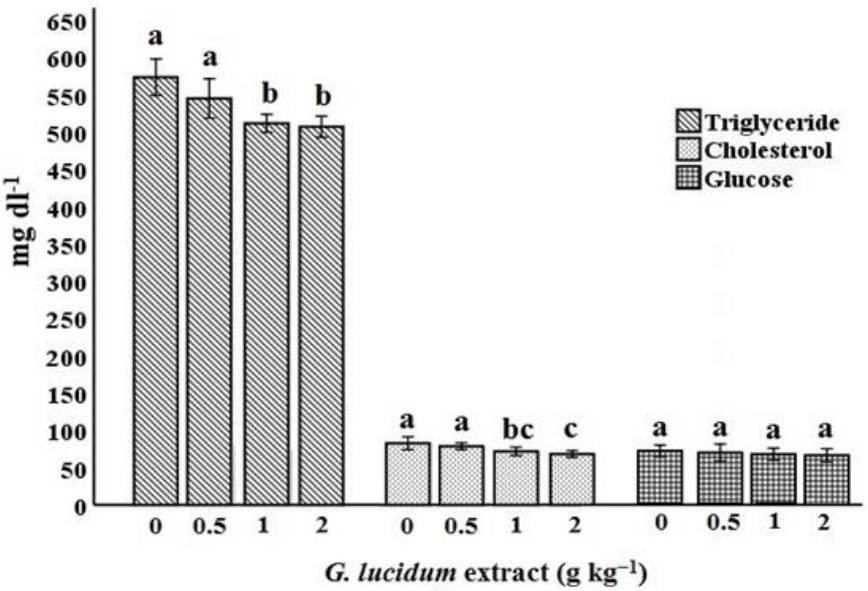


Figure 2: Serum biochemical parameters including glucose, triglycerides, and cholesterol in *Huso huso* fed diet supplemented with *Ganoderma lucidum* extract for 6 weeks. Data are presented as mean ± SE. Different letters above the bars indicate significant differences among groups (p<0.05).

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Antioxidant and oxidative stress analysis

In the present study, serum TAC was affected by GLE. The highest TAC level was observed in 2 g/kg GLE, which was significantly different from 0.5 g/kg GLE and control group ($p< 0.05$). In contrast, serum MDA decreased in the groups with dietary supplementation, which was significantly lower in fish fed with 1 and 2 g/kg GLE compared to the other groups ($p< 0.05$; Table 3).

Table 3: Serum metabolic enzymes and antioxidant status in *Huso huso* fed diets supplemented with *Ganoderma lucidum* extract for 6 weeks.

Parameters	*G. lucidum* extract (g/kg)			
	0	0.5	1	2
AST (UL^{-1})	426.6±22.95	443.6±37.79	471.0± 32.48	431.6±23.91
ALT (UL^{-1})	12.6±1.07	11.2±1.24	11.6±1.02	12.0±0.70
ALP (UL^{-1})	265.4±16.86	250.4±8.68	255.0±14.36	245.0±0.05
TAC ($\mu M\ l^{-1}$)	116.70± 1.49^{a}	121.28± 1.27^{a}	138.66±1.51^{b}	143.57± 2.84^{b}
MDA ($\mu M\ l^{-1}$)	9.72±0.37^{a}	8.96±0.45^{a}	6.24±0.39^{b}	5.01±0.29^{c}

Data are presented as mean ± SE. Different letters above the values indicate significant difference among groups ($p<0.05$). AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; TAC, total antioxidant capacity; MDA, malondialdehyde.

Discussion

Ganoderma lucidum contains beneficial biological compounds such as polysaccharides and triterpenoids. Recently, more attention has been paid to the therapeutic effects of *G. lucidum* (Wachtel-Galor *et al*., 2004; Paterson, 2006). This study showed an increase in the WG, LG, and SGR values as well as a decrease in the FCR in the groups fed diets supplemented with GLE. These results indicate the appropriate effect of GLE on the growth performance of fish during the experimental period. The ability to enhance the growth performance of GLE is due to the effect of immune stimulation caused by *G. lucidum*, which regulates immunity and prevents disease. In the study of Chithra *et al*. (2016) *G. lucidum* polysaccharides caused a significant increase of body weight and SGR in grass carp (*Ctenopharyngodon idella*) (Mohan *et al*., 2016).

Also, Mohan *et al*. (2016) reported an increase in the digestive enzymes including protease, amylase, and lipase in giant freshwater prawn (*M. rosenbergii*) fed with *G. lucidum* polysaccharides. The dietary supplementation with *G. lucidum* polysaccharides increased the secretion of digestive enzymes and enhanced the absorption of nutrients from the gastrointestinal tract and finally, improved the growth performance of *M. rosenbergi* (Mohan *et al*., 2016).

In the present study, the amounts of total protein and IgM increased with the increment of GLE in the diet. Total protein includes albumin and globulins. Globulins are the main constituents of serum protein that makeup immunoglobulins which are essential in the immune response. Serum

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immunoglobulin level is a substantial indicator of immune status. B lymphocytes that originate from the anterior part of the kidney, spleen, and anterior part of the heart become the cells that secrete plasma cell antibodies and produce immunoglobulins (Yu *et al*., 2008; Yildiz *et al*., 2009). The increase of immunoglobulin in this study indicates an improving effect of GLE on the immune system.

This study showed hypoglycemia in the groups that supplemented with GLE, however, this reduction was not significantly different among the groups. *G. lucidum* facilitates the inflow of calcium to pancreatic cells by releasing insulin which leads to hypoglycemia (Zhang and Lin, 2004). In the present study, triglycerides and cholesterol were reduced in the GLE-supplemented groups, which significantly decreased in 1 and 2 g/kg GLE compared to 0.5 g/kg GLE and control group (p<0.05).

Triglycerides and cholesterol are important lipid metabolism biomarkers (Chen *et al*., 2014). Serum cholesterol reduction could be due to plant sterols (Fremont *et al*., 2000, Avci *et al.*, 2006), which are being considerably supplemented to the diet for preventing hypercholesterolemia and hyperlipidemia (Rubel *et al.*, 2011). The results of this study confirm former researches that reported hypolipidemia caused by *G. lucidum* (Kabir *et al*., 1988; Berger *et al*., 2004).

In this study, an increase in TAC was observed with a significantly different in 1 and 2 g/kg GLE compared to 0.5 g/kg GLE and control group. TAC protects biological molecules against oxidation (Yousefi *et al*., 2019). Antioxidants reduce oxidative stress by scavenging reactive oxygen (Lee *et al*., 2009). TAC indicates total antioxidant capacity, which illustrates antioxidant ability to resist oxidants (Taheri Mirghaed *et al*., 2018; Yousefi *et al*., 2019).

The result of this study indicated that GLE reduces the concentration of serum lipid peroxidation which showed its effect by decreasing serum MDA. Lipid peroxidation is an adverse event that leads to oxidation of unsaturated fatty acid, because of antioxidant system failure (Yilmaz, 2019). MDA which is produced by lipid peroxidation cause oxidative stress. Therefore, MDA is a biomarker used to evaluate lipid peroxidation and oxidative stress (Hwang *et al*., 2013;Taheri Mirghaed *et al*., 2018; Yousefi *et al*., 2019).

It can be concluded from this study that dietary GLE improved growth performance and antioxidant capacity in *H. huso*. Moreover, results revealed that GLE possesses hypoglycemic, hypolipidemic, and immunostimulatory properties.

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