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Research Article

Improvement of Growth Performance, Hepatic and Erythrocyte Antioxidant Capacity, Innate Immunity, and Biochemical Parameters of Persian Sturgeon, *Acipenser persicus*, by Sulfur Amino Acids' Supplementation

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The present study was aimed at determining the effects of taurine and/or methionine addition to a plant-based diet on growth performance and immunological and antioxidant responses of Persian sturgeon, *Acipenser persicus*. Four plant-based diets were formulated, including control (C), control+1 g/kg taurine (T), control+2 g/kg methionine (M), and control+1 g/kg taurine and 2 g/kg methionine (T+M). Fish were fed each diet in triplicate for 70 days, and growth performance, hematological, plasma biochemical, and immunological parameters, and hepatic and erythrocyte antioxidant parameters were determined after that. Dietary taurine or methionine supplementation significantly increased growth performance, hematological parameters, antioxidant enzymes' activities, plasma lysozyme, alternative complement, and total immunoglobulin but decreased plasma alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase activities, and malondialdehyde. Interaction additive effects of dietary taurine and methionine were observed in erythrocyte count, hematocrit, alternative complement, total immunoglobulin, hepatic malondialdehyde, superoxide dismutase, and glutathione peroxidase. In conclusion, dietary taurine (1 g/kg) and methionine (2 g/kg) supplementation is necessary to support higher growth rate, immunological responses, and antioxidant capacity in Persian sturgeon, when fed a plant-based diet. According to the present results, the highest performance is obtained when both amino acids are simultaneously added to the fish diet.

1. Introduction

Sustainable aquaculture depends on finding feedstuffs to replace fishmeal in the fish diet, as there has been a decline in global fishmeal supply and upsurge in its demand [1]. Plant protein sources have been the first choice as alternative to fishmeal in aquafeeds [2]. Despite the benefits such as availability, ease to store, and pellet quality, plant protein sources have some limitations that affect fish growth performance and welfare [2]. Among these limitations, amino acid shortage is of great concern, as it strongly affects growth rate, physiological performance, and welfare of fish [3].

Sulfur amino acid shortage may be the most important limitation of plant protein sources [4]. Methionine is considered an essential amino acid in fish, and methionine shortage has been found to greatly depress the fish growth, when fed on a plant-based diet [4]. Methionine is one of the most important immunostimulant amino acids; thus, methionine shortage causes disease susceptibility in fish [5]. This amino acid plays roles in cell proliferation, thus affecting humoral cellular immune responses [6]. Studies on different fish have demonstrated that adequate dietary methionine levels support higher leukocyte count, humoral innate immunity, and disease resistance [6–9]. A body of evidence suggests that methionine modulates the antioxidant system in fish; thus, methionine shortage affects fish welfare. Studies on different fish species have shown that methionine supplementation decreases antioxidant enzymes' activity as well as lipid peroxidation and protein carbonylation [7, 10, 11]. Therefore, methionine supplementation is crucial, when a plant-based diet is used for fish culture. Commonly used plant proteins such as soybean meal and glutens contain as low as half of fishmeal methionine level [12], which gives emphasis on the necessity of methionine supplementation in the plant-based diets.

Beside methionine, taurine shortage is another limitation of plant protein sources, as these sources almost lack taurine [4]. Fish have a huge quantity of taurine in their tissues, which show the importance of this sulfur amino acid. Although taurine does not contribute to protein structure, it has several important roles in fish, and recent evidences have demonstrated that taurine deficiency leads to growth retardation, hepatic damage, hemolysis, oxidative stress, and disease susceptibility [4, 13–15]. Requirement for dietary taurine depends on the species ability to synthetize it from its precursor, methionine [4]. Generally, marine/carnivorous fish have lower ability to synthetize taurine, but freshwater/herbivorous ones have the highest ability [16]. Therefore, taurine and methionine may have interaction effects on fish.

Due to the relationships between taurine and methionine, several studies have investigated interaction effects of simultaneous supplementation of taurine and methionine in plant-based fish diets. Regarding this, variable results have been obtained in different studies. In Asian seabass, *Lates calcarifer*, and largemouth bass, *Micropterus salmoides*, taurine has no beneficial effects on the fish performance, but methionine supplementation improves the fish growth rate [17, 18]. On the other hand, in meagre, *Argyrosomus regius*,

and California yellowtail, *Seriola dorsalis*, taurine supplementation has supported higher growth rate, but no positive effects of methionine supplementation have been observed [19, 20]. Some species such as Atlantic salmon, *Salmo salar*, and Nile tilapia, *Oreochromis niloticus*, have shown the best growth performance, when both taurine and methionine have been added to their diet [21, 22]. Therefore, there is a need for further studies on other fish species to better understand the interaction between these sulfur amino acids.

Persian sturgeon, *Acipenser persicus*, is a native fish in Iran with great aquaculture potentials, which is reared for caviar and meat production [23]. Data about nutritional requirement of this species is scarce, and there is a need for further studies on this topic. Regarding this, there has been a desire to lower fishmeal in the diet of Persian sturgeon [24], which is a step toward sustainability. It is believed that with further progress of aquaculture of this species, fishmeal proportion will further decrease in its diet. So, it is necessary to investigate the roles of dietary sulfur amino acids in this species. A previous study has demonstrated that Persian sturgeon requirement for dietary taurine may be lower than other studied species, as 2.5 g/kg taurine supplementation to a low fishmeal diet (19%) has produced no benefits in the fish and higher levels (5-16 g/kg) have negatively affected the fish performance [25]. So, in the present study, lower dietary taurine supplementation level (1 g/kg) was applied along with lower dietary fishmeal percentage (15%) and methionine supplementation (2 g/kg) to further illustrate the roles of sulfur amino acids in sturgeons. For this, the fish growth performance and immunological and antioxidant responses have been monitored.

2. Materials and Methods

2.1. Experimental Diets. Four plant-based diets were formulated including control (C): no taurine/methionine supplementation; taurine-supplemented (T): supplemented with 1 g/kg taurine; methionine-supplemented (M): supplemented with 2 g/kg methionine; and taurine+methionine-supplemented: supplemented with 1 g/kg taurine and 2 g/kg methionine (Tables 1 and 2).

Feedstuffs were sieved (500 μm) and mixed together using a mixture. 1 kg of the mixture was moisturized by adding 350 mL water to create paste. The paste was passed through a die (3 mm), and the resultant sticks were dried against a fan blow. The dried sticks were crushed in appropriate size and kept at -20°C until use.

Proximate composition of the diets was determined based on AOAC [26]. The amino acid profile of the diets was determined by HPLC and fluorescent detector (Agilent 1090 system, Palo Alto, CA). Briefly, the samples were digested in 6 N HCl for 22 h. Then, the digested samples were centrifuged, and the supernatants were used for derivatization by *O*-phthalaldehyde before injection into the HPLC system [27]. For the taurine assay, the samples were extracted by 0.1 N HCl for 15 min. Derivatization was performed by using dansyl chloride, before the HPLC assay [28].

TABLE 1: Feedstuffs and chemical composition of the experimental diets.

Ingredients (g/kg)	Experimental diets			
	C	T	M	T+M
Soybean meal	230	230	230	230
Fish meal	150	150	150	150
Wheat gluten	240	240	240	240
Wheat meal	192.3	192.3	192.3	192.3
Plant oil[1]	130	130	130	130
Vitamin mix[2]	1.5	1.5	1.5	1.5
Mineral mix[3]	42	42	42	42
Phytase	0.2	0.2	0.2	0.2
Cellulose[4]	7	6	5	4
Lysine[5]	7	7	7	7
DL-methionine[6]	0	0	2	2
Taurine[7]	0	1	0	1
Moisture	65	64	65	66
Crude protein	421	422	421	422
Crude lipid	147	148	149	148
Crude ash	84	82	83	82
Gross energy (kJ/g)	19.66	19.62	19.58	19.60

[1]Mixture of soybean oil (60 g), canola oil (50 g), and linseed oil (20 g). [2]The premix provided following amounts per kg of feed: A: 1000 IU; D_3: 5000 IU; E: 20 mg; B_5: 100 mg; B_2: 20 mg; B_6: 20 mg; B_1: 20 mg; H: 1 mg; B_9: 6 mg; B_{12}: 1 mg; B_4: 600 mg; and C: 50 mg. [3]The premix provided following amounts per kg of diet: Se: 0.15 mg; iodate: 0.05 mg; Mg: 700 mg; Fe: 25 mg; Co: 4 mg; Cu: 5 mg; Zn: 80 mg; NaCl: 10 g; and dicalcium phosphate: 26 g. [4]Sigma, St. Louise, MO, USA. [5]Faravar Lysine Pars Co., Tehran, Iran. [6]Evonik Co., Essen, Germany, 99% purity. [7]Xi'an Biosunny Biological Technology Co., Xi'an, China; 99% purity.

TABLE 2: Amino acid profiles of the experimental diets (g per kg diet).

	Experimental diets			
	C	T	M	T+M
Indispensable amino acids				
Arginine	19.1	19.3	19.5	19.4
Histidine	8.00	8.30	8.10	7.60
Isoleucine	14.6	14.9	14.9	14.8
Leucine	28.1	27.2	26.8	26.0
Lysine	23.4	22.5	23.1	23.0
Phenylalanine	17.8	17.6	17.6	16.7
Tryptophan	4.00	4.10	4.30	4.70
Valine	2.32	23.5	24.0	23.3
Threonine	14.4	14.9	14.5	15.2
Methionine	6.97	7.00	9.20	9.20
Dispensable amino acids				
Taurine	0.41	1.50	0.40	1.60
Aspartic acid	46.3	47.5	46.2	46.0
Glutamic acid	140.0	137.7	136.2	138.5
Serine	19.0	19.2	19.5	19.3
Glycine	16.3	16.0	16.5	17.0
Alanine	15.7	15.8	16.1	15.2
Tyrosine	12.8	12.2	13.1	12.4

After 70 d rearing, weight gain, food conversion ratio (FCR), and specific growth rate (SGR) were calculated according to the following formulas:

$$SGR(\%/d) = 100 \times \frac{Ln\,(final\,weight) - Ln\,(initial\,weight)}{70},$$

$$FCR = \frac{Consumed\,feed\,(g)}{Gained\,biomass\,(g)},$$

$$Weight\,gain(\%) = 100 \times \frac{Final\,weight\,(g) - Initial\,weight\,(g)}{initial\,weight\,(g)}.$$

(1)

2.2. Experimental Conditions. This study was conducted in accordance with the National Ethical Framework for Animal Research in Iran. Persian sturgeon juveniles were purchased from a private sector, transported to the laboratory, treated with 1.5% NaCl solution (10 min) as a prophylactic procedure, and distributed in 12 fiberglass tanks (filled with 200 L water). The tanks were continuously aerated and received water flow rate of 0.5 L/min. The fish were allowed to acclimatize with the experimental conditions for two weeks. Then, the tanks were assigned to four treatments; each received one of the aforementioned diets for 70 days. The fish weight at this point was near 26 g, and they were offered each diet based on 20 g/kg of biomass per day (divided into two meals at 08:00 and 16:00). The tanks' biomass was recorded biweekly to adjust the daily feed amount. The water used in this experiment was dechlorinated tape water, which was renewed in the tanks based on 50% per day. The water quality during the experiment was monitored, with dissolved oxygen 6.87 ± 1.18 mg/L, temperature $20.9 \pm 0.98°C$, pH 8.11 ± 0.11, total hardness 294 ± 33.4 mg/L, total alkalinity 273 ± 24.7 mg/L, N-nitrite 0.02 ± 0.005 mg/L, and N-ammonia 0.01 ± 0.004 mg/L. The water parameters were determined using a digital probe (Hach Co., HQ40d, Loveland, Colorado, USA) and a customized photometer (Palintest Photometer model 7100, Gateshead, UK).

2.3. Sampling and Preservation. At the end of the rearing period, six fish were sampled per treatment for hematological and biochemical assays. The fish were gently caught from the experimental tanks and anesthetized in clove extract (2 g/L). Then, the fish blood samples were collected using heparinized syringes. The fish were then killed by decapitation, and hepatic samples were collected and kept at -70°C for antioxidant assays.

The blood samples were divided into two aliquots: one used for hematological assays and the next for plasma and erythrocyte separation. The later aliquot was centrifuged for 10 min ($2500 \times g$), and the supernatant plasma was collected in new tubes and frozen at -70°C until analysis. The packed erythrocytes were collected in other tubes and frozen at -70°C for antioxidant assays.

2.4. Hematological Assays. Red blood cell (RBC) and white blood cell (WBC) were counted using a Neubauer chamber, after blood dilution in Dacie's solution [29]. Capillary tubes and centrifugation were used to determine the blood hematocrit percentage (Hct). A commercial kit (Zist Chem Co., Tehran, Iran) was used to measure the blood hemoglobin (Hb). A blood smear was prepared from each blood sample, fixed with methanol, and stained with Giemsa to determine lymphocyte, neutrophil, and monocyte percentages.

2.5. Plasma Biochemical Assays. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were measured kinetically, using commercial kits provided by Pars Azmun Co. (Tehran, Iran). One unit of ALT was equal to the amount of enzyme that converts one-molecule alanine to pyruvate and lactate. One unit of AST was equal to the amount of enzyme that converts one-molecule aspartate to oxoglutarate and malate. One unit of ALP was defined as the enzyme amount that breaks down one-molecule P-nitrophenylphosphate.

Plasma lysozyme activity was measured based on the turbidimetric method [30]. A suspension of *Micrococcus luteus* was prepared in phosphate buffer (50 mM; pH 6.2). To 1 mL of this suspension was added 30 μL of the plasma sample, and decrease in optical density was recorded for 6 min at 450 nm. One unit of lysozyme activity was equal to 0.001 unit decrease in the optical density per min.

Plasma alternative complement activity (ACH50) was determined based on hemolytic activity of the sample against sheep erythrocyte. A suspension of sheep erythrocyte was prepared in veronal buffer containing EGTA, magnesium, and gelatin. The plasma samples were serially diluted in the same buffer. To 50 μL of the sheep erythrocyte suspension was added 25 μL of each sample dilution. The mixture remained at room temperature for 60 min and was then centrifuged (10 min; 2500 × g). The optical density of the supernatant was recorded at 412 nm, and ACH50 was calculated at the dilution-induced 50% hemolysis, as described by Yano [31].

Plasma total immunoglobulin (Ig) was measured after precipitation of Ig by polyethylene glycol. 100 μL of polyethylene glycol (12%) was added to 100 μL of plasma sample and shaken at room temperature for 120 min. Then, the mixture was centrifuged (10 min; 2500 × g) to precipitate Ig. Total protein of the supernatant and the original sample were determined using a commercial kit (Zist Chem Co., Tehran, Iran). Difference in the total protein values was equal to the sample total Ig [32].

2.6. Antioxidant Assays. Plasma malondialdehyde (MDA) was measured based on the reaction of the sample aldehydes with thiobarbituric acid at 95°C, using the ZellBio Co. (Germany) kit.

The frozen fish erythrocytes were defrosted and mixed with equal volume of phosphate buffer (50 mM; pH 7.0). The mixture was vigorously vortexed before centrifugation (4°C; 10 min; 2500 × g). The supernatant was used for erythrocyte antioxidant enzyme assays. Superoxide dismutase (SOD) activity was measured based on the reduction of the

cytochrome C, using a commercial kit provided by ZellBio Co. (Germany). Catalase (CAT) activity was measured based on the decomposition of hydrogen peroxide per min, using the ZellBio Co. (Germany) kit. Glutathione peroxidase (GPx) activity was measured based on the conversion of the reduced glutathione to oxidized form, using the ZellBio Co. (Germany) kit. The supernatant Hb levels were determined, and the enzyme activities were expressed per mg Hb. Diluting was performed by the same phosphate buffer, when necessary.

The liver samples were defrosted and homogenized in 100 mM phosphate buffer (pH 7.0). The mixture was centrifuged for 15 min at 4°C (6000 × g), and the supernatant was used to measure SOD, CAT, GPx, and MDA. These parameters were determined using the aforementioned methods but expressed based on the sample protein content. The sample protein content was determined based on the Bradford method [33].

2.7. Statistical Analysis. The data were checked for normal distribution (Shapiro-Wilk's test) and homoscedasticity (Levene's test). After confirmation of normal distribution and homoscedasticity, the data were analyzed by two-way ANOVA (taurine×methionine). When there was an interaction effect of taurine×methionine, multiple comparison was done by Duncan's test. The analyses were conducted in SPSS v.22, and the results were expressed as mean ± SE.

3. Results

3.1. Growth Performance. The fish growth performance, feed efficiency, and survival are presented in Table 3. Dietary supplementation with either taurine or methionine significantly ($P < 0.05$) improved the fish final weight, weight gain, and SGR; however, FCR was improved only in the T treatment. There was no significant difference ($P > 0.05$) in the fish survival among the treatments. No significant interaction effects of dietary taurine and methionine supplementation have been observed on growth performance and feed efficiency ($P > 0.05$).

3.2. Hematological Parameters. Dietary taurine, methionine, and taurine+methionine supplementation significantly ($P < 0.05$) affected the fish blood RBC, Hct, and Hb levels (Table 4). Taurine and methionine alone significantly ($P < 0.05$) increased the fish blood RBC and Hct levels; nevertheless, combination of taurine and methionine resulted in further increases in these parameters. The fish received the diets T (1.35-fold), M (1.33-fold), and T+M (1.46-fold) exhibited similar blood Hb levels, which was significantly ($P < 0.05$) higher than the value in the C treatment. Dietary supplementation with either taurine or methionine significantly ($P < 0.05$) increases the fish blood WBC count. There was no significant difference in the fish blood MCV, MCH, MCHC, lymphocyte, neutrophil, and monocyte among the treatments ($P > 0.05$).

3.3. Plasma Biochemical and Immunological Parameters. Plasma lysozyme, ACH50, total Ig, ALT, and AST levels were significantly ($P < 0.05$) affected by dietary taurine,

TABLE 3: Growth performance, feed efficiency, and survival of Persian sturgeon during 70 d feeding with taurine- and/or methionine-supplemented diets (mean ± SE; $n = 3$).

| | Dietary treatments | | | | P value | | |
	C	T	M	T+M	T	M	T×M
Initial weight (g)	26.0 ± 0.99	26.0 ± 0.69	26.5 ± 0.53	26.6 ± 1.09	0.971	0.528	0.970
Final weight (g)	57.4 ± 1.12	67.1 ± 1.68	63.8 ± 0.64	70.4 ± 1.91	<0.001; C < T	0.009; C < M	0.305
Specific growth rate (%/d)	1.13 ± 0.03	1.35 ± 0.01	1.25 ± 0.02	1.39 ± 0.02	<0.001; C < T	0.005; C < M	0.079
Weight gain (%)	121 ± 4.35	158 ± 2.21	141 ± 2.66	165 ± 4.46	<0.001; C < T	0.006; C < M	0.114
Feed conversion ratio	1.33 ± 0.04	1.20 ± 0.03	1.25 ± 0.03	1.16 ± 0.02	0.005; C>T	0.072	0.534
Survival (%)	93.3±2.37	100	100	100	0.081	0.081	0.081

C: control treatment without taurine and methionine supplementation; T: supplemented with 1 g/kg taurine; M: supplemented with 2 g/kg methionine; T+M: supplemented with 1 g/kg taurine and 2 g/kg methionine.

TABLE 4: Hematological parameters of Persian sturgeon during 70 d feeding with taurine- and/or methionine-supplemented diets (mean ± SE; $n = 6$). Different letters within a row show significant differences among the treatments based on multiple comparison (Duncan's test).

| | Dietary treatments | | | | P value | | |
	C	T	M	T+M	T	M	T×M
RBC (10^6 cell/μL)	0.98 ± 0.03a	1.32 ± 0.03b	1.24 ± 0.03b	1.44 ± 0.04c	<0.001; C < T	<0.001; C < M	0.045
Hct (%)	23.8 ± 0.98a	31.7 ± 0.71b	31.8 ± 0.48b	34.8 ± 1.08c	<0.001; C < T	<0.001; C < M	0.010
Hb (g/dL)	7.58 ± 0.27a	10.3 ± 0.32b	10.1 ± 0.48b	11.1 ± 0.33b	<0.001; C < T	<0.001; C < M	0.023
MCV (fL)	243 ± 6.72	240 ± 5.59	256 ± 4.98	242 ± 5.74	0.175	0.187	0.337
MCH (pg)	77.2 ± 1.70	78.2 ± 2.25	81.3 ± 1.97	77.2 ± 0.65	0.387	0.377	0.168
MCHC (mg/dL)	31.9 ± 0.76	32.5 ± 0.48	31.8 ± 1.26	31.9 ± 0.96	0.703	0.706	0.786
WBC (10^3 cell/μL)	3.89 ± 0.18	6.03 ± 0.31	7.05 ± 0.24	8.08 ± 0.33	<0.001; C < T	<0.001; C < M	0.053
Lymphocyte (%)	84.5 ± 0.43	84.0 ± 0.73	84.0 ± 0.73	84.3 ± 0.66	0.909	0.913	0.545
Neutrophil (%)	12.2 ± 0.40	12.0 ± 0.45	11.8 ± 0.48	12.0 ± 0.26	0.685	1.00	0.685
Monocyte (%)	3.33 ± 0.21	4.00 ± 0.37	4.17 ± 0.31	3.67 ± 0.42	0.465	0.806	0.097

C: control treatment without taurine and methionine supplementation; T: supplemented with 1 g/kg taurine; M: supplemented with 2 g/kg methionine; T+M: supplemented with 1 g/kg taurine and 2 g/kg methionine.

methionine, and taurine+methionine supplementation; however, the plasma ALP activity was only affected ($P < 0.05$) by dietary taurine or methionine (Table 5). There were significant ($P < 0.05$) elevations in plasma lysozyme, ACH50, and total Ig in T, M, and T+M treatments, compared to C. The highest lysozyme activity was observed in M and T+M treatments, whereas the highest total Ig was observed in T and T+M treatments. Dietary taurine and methionine showed significant ($P < 0.05$) additive effects on plasma ACH50 activity. The plasma ALT and AST levels in M and T+M treatments were similar and significantly ($P < 0.05$) lower than those in C treatment. Dietary taurine or methionine significantly ($P < 0.05$) decreased the plasma ALP activity.

3.4. Erythrocyte and Hepatic Antioxidant Parameters. Dietary taurine or methionine significantly ($P < 0.05$) increased the erythrocyte SOD, CAT, and GPx activity, but there were no significant ($P > 0.05$) interaction effects of taurine and methionine on these parameters. On the other hand, taurine or methionine significantly ($P < 0.05$) decreased the plasma MDA levels and created a significant ($P < 0.05$) interaction

effect on this parameter, characterized by the lowest plasma MDA in T+M treatment (Figure 1).

Dietary taurine or methionine significantly ($P < 0.05$) increased the hepatic SOD and GPx activity; there were significant ($P < 0.05$) interaction effects of dietary taurine and methionine on these parameters as the highest activities were observed in T+M treatment. Dietary taurine or methionine significantly ($P < 0.05$) increased the hepatic CAT activity, but there were no significant ($P > 0.05$) interaction effects of taurine and methionine on the enzyme's activity. Dietary taurine or methionine significantly ($P < 0.05$) decreased the hepatic MDA level; there were significant ($P < 0.05$) interaction effects of dietary taurine and methionine on the hepatic MDA levels as the lowest level was observed in T+M treatment (Figure 2).

4. Discussion

Plant protein sources such as soybean meal and wheat gluten (used in the present study) have approximately no taurine and near half methionine levels, compared to fishmeal [19, 34]. Therefore, inclusion of high dietary plant protein leads

TABLE 5: Plasma immunological and enzymatic parameters of Persian sturgeon during 70 d feeding with taurine- and/or methionine-supplemented diets (mean ± SE; $n = 6$). Different letters within a row show significant differences among the treatments based on multiple comparison (Duncan's test).

| | Dietary treatments | | | | P value | | |
	C	T	M	T+M	T	M	T×M
Lysozyme (U/mL)	41.8 ± 1.76a	61.2 ± 2.70b	70.1 ± 1.67c	78.6 ± 2.76c	<0.001; C < T	<0.001; C < M	0.001
ACH50 (U/mL)	84.0 ± 2.42a	114 ± 2.56b	115 ± 2.50b	134 ± 4.70c	<0.001; C < T	<0.001; C < M	0.008
Total Ig (g/L)	12.8 ± 0.48a	17.9 ± 0.36c	16.4 ± 0.27b	17.9 ± 0.31c	<0.001; C < T	<0.001; C < M	<0.001
ALT (U/L)	13.9 ± 0.80b	9.16 ± 0.44a	9.50 ± 0.50a	8.42 ± 0.52a	<0.001; C > T	<0.001; C > M	0.001
AST (U/L)	243 ± 6.97b	188 ± 5.82a	193 ± 4.60a	184 ± 6.21a	<0.001; C > T	<0.001; C > M	<0.001
ALP (U/L)	405 ± 35.1	210 ± 10.6	288 ± 19.9	180 ± 18.3	<0.001; C > T	0.004; C > M	0.072

C: control treatment without taurine and methionine supplementation; T: supplemented with 1 g/kg taurine; M: supplemented with 2 g/kg methionine; T+M: supplemented with 1 g/kg taurine and 2 g/kg methionine.

FIGURE 1: Blood antioxidant parameters of Persian sturgeon during 70 d feeding with taurine- and/or methionine-supplemented diets (mean ± SE; $n = 6$). Different letters above the bars show significant differences among the treatments based on multiple comparison (Duncan's test). C: control treatment without taurine and methionine supplementation; T: supplemented with 1 g/kg taurine; M: supplemented with 2 g/kg methionine; T+M: supplemented with 1 g/kg taurine and 2 g/kg methionine.
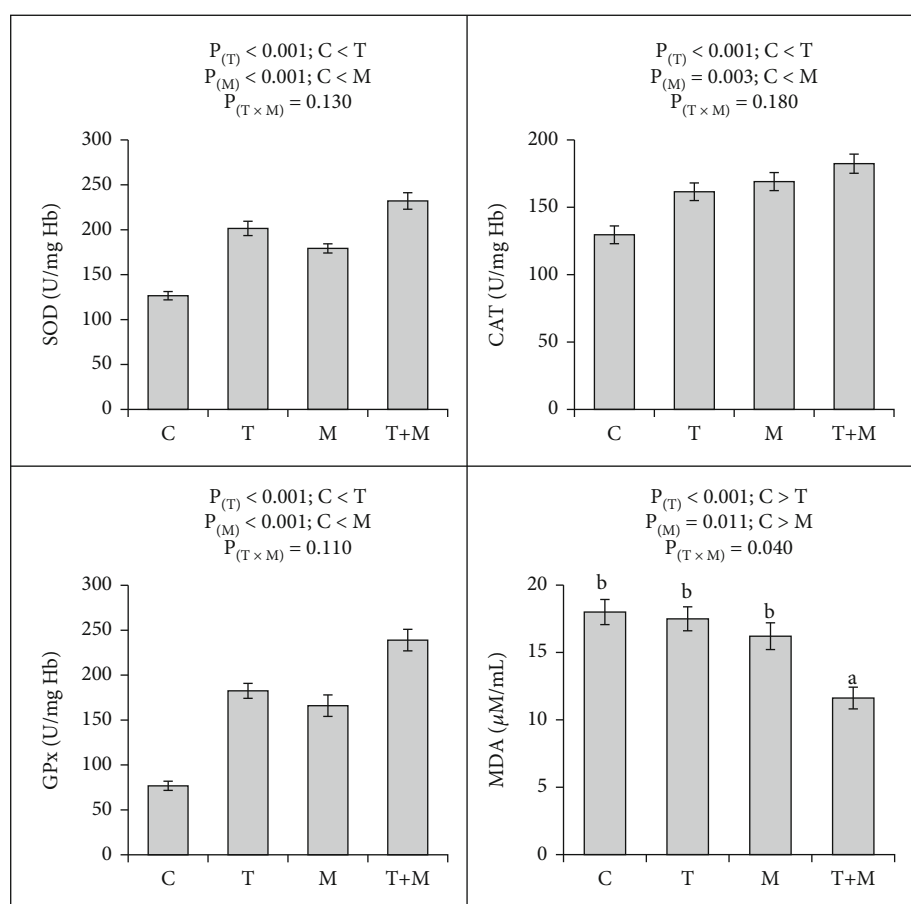
to sulfur amino acid shortage, which must be covered by exogenous supplementation to support maximum growth and welfare of the fish [35]. The requirement for these amino acids has not been determined in Persian sturgeon, and indeed, such data are available only in white sturgeon, among the sturgeons, but limited to dietary methionine (2.2 g/100 g pr), not taurine [36]. Besides, fish requirement for dietary taurine is 1.5-6 g/100 g pr [37], and poor growth performance and feed efficiency in C treatment are believed to be related to sulfur amino acid shortage (1.6 g methionine/100 g pr and 0.1 g taurine/100 g pr). Either taurine or methionine supplementation was capable to boost the fish growth performance. In this regard,
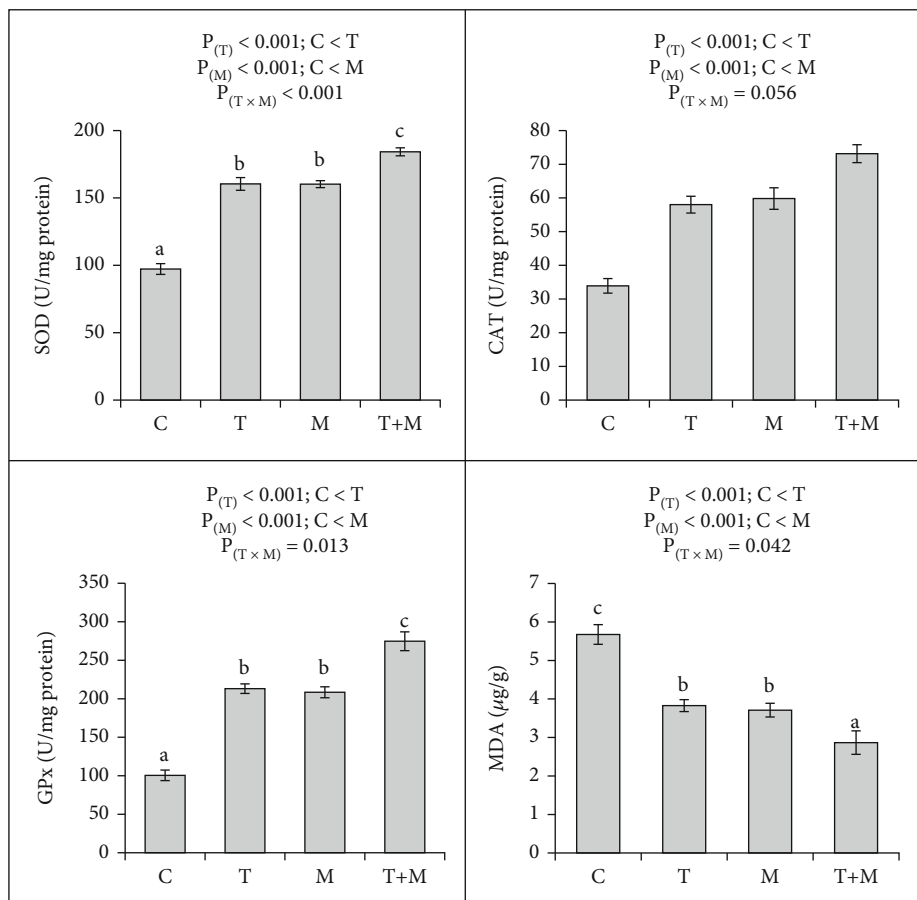
FIGURE 2: Hepatic antioxidant parameters of Persian sturgeon during 70 d feeding with taurine- and/or methionine-supplemented diets (mean ± SE; $n = 6$). Different letters above the bars show significant differences among the treatments based on multiple comparison (Duncan's test). C: control treatment without taurine and methionine supplementation; T: supplemented with 1 g/kg taurine; M: supplemented with 2 g/kg methionine; T+M: supplemented with 1 g/kg taurine and 2 g/kg methionine.

variable results have been obtained in different studies. Some species like Asian seabass [18] and largemouth bass [17] need dietary methionine supplementation to have the best growth performance, when fed a plant-based diet; conversely, meagre [19] and California yellowtail [20] need dietary taurine supplementation to exhibit the highest growth performance. However, combination of taurine and methionine inclusion in Persian sturgeon diet supports the highest growth and feed efficiency, which is partially similar to the results obtained in Atlantic salmon [21] and Nile tilapia [22].

Taurine and methionine are known as immunostimulant amino acids in fish. Dietary methionine effects on fish blood leukocyte count have been consistent, as researchers reported an increase in blood leukocyte count in European seabass (*Dicentrarchus labrax*) [6], Jian carp (*Cyprinus carpio*) [7], and ruho carp (*Labeo rohita*) [8] fed methionine-supplemented diets. However, the effects of dietary taurine supplementation on fish leukocyte count have been variable. Dietary taurine supplementation has shown mixed effects on blood leukocyte count in beluga, *Huso huso*, depending on supplemental levels and water salinity [38]. Taurine has produced no significant effects on blood leukocyte count in Nile tilapia, *Oreochromis niloticus*, but mitigated leukemia after exposure to toxic substances [39]. Similar results have been

observed when crucian carp, *Carassius auratus*, is intoxicated with ammonium acetate [40]; however, taurine has mitigated leukocytosis in yellow catfish, *Pelteobagrus fulvidraco*, intoxicated with ammonium acetate [41]. Methionine is a methyl donor and participates in polyamine synthesis, which is essential in cell proliferation [6], whereas taurine is concentrated in leukocytes, forming up to 50% of amino acid pool of the cells [14]. Therefore, increase in the number of leukocytes in the present study may be due to higher leukocyte synthesis and/or viability caused by taurine and/or methionine administration.

Plasma innate immune components are reliable markers of fish immunocompetence and are monitored in various researches. Lysozyme is an important bactericidal enzyme [42], and the complement proteins are important in lysis of foreign germs and increase phagocytosis by opsonization [43]; both lysozyme and complement proteins protect fish at an early phase of infection. The higher lysozyme and ACH50 activities have been reported in different fish, orally administered with sulfur amino acids. For example, taurine supplementation improved lysozyme and/or ACH50 activities in yellowfin sea bream, *Acanthopagrus latus* [44], European seabass [45], and yellow catfish [46]. Moreover, taurine administration has been found to mitigate declines in

lysozyme and ACH50 activities in fish exposed to toxic substances [39–41]. Similarly, dietary methionine administration significantly increased lysozyme and ACH50 activity in European sea bass [6], Jian carp [7], and ruho carp [8]. In the present study, higher lysozyme activity in T, M, and T+M treatments might be due to a higher number of blood neutrophils, which are responsible for lysozyme secretion. Moreover, higher ACH50 activity might be due to improved hepatic health (see below) and/or complement protein production. Higher plasma Ig levels in the present study were in line with previous studies on yellowfin sea bream [44], yellow catfish [46], Jian carp [7], and ruho carp [8]. The higher total Ig in the present study might be due to higher leukocyte (lymphocyte) count, which increases Ig production (as observed in the above-mentioned studies).

Sulfur amino acids are postulated to have antioxidant effects in animals. There are several studies assessing antioxidant activity of taurine and methionine in fish. Such benefits are related to enhanced antioxidant enzymes' activity as well as direct radical scavenging [4]. Previous studies have shown both taurine and methionine reduce lipid peroxidation by modulating SOD, CAT, and GPx activities in different fish species, including ruho carp [11], Jian carp [7], yellow catfish [10, 46], and yellowfin sea bream [44]. Moreover, dietary taurine supplementation has been shown to counteract oxidative conditions caused by toxic substances in fish [39–41]. Therefore, improved antioxidant capacity and reduced lipid peroxidation in the liver of Persian sturgeon may explain lower plasma ALT, AST, and ALP activities, as these enzymes are indicators of hepatic health in fish [47]. Despite the aforementioned studies that focused on antioxidant responses of fish internal organs, the present study demonstrated that sulfur amino acids are potent to improve the antioxidant system in erythrocytes. The only study on this topic has demonstrated that dietary taurine supplementation improves osmotic fragility of erythrocytes in grass carp, *Ctenopharyngodon idella* [48]. Moreover, taurine deficiency has been found to cause anemia in yellowtail, *Seriola quinqueradiata* [49]. Such effects may be related to both osmoregulatory and antioxidant effects of taurine that protect erythrocyte and reduce hemolysis [37]. Antioxidant effects of sulfur amino acids on erythrocytes have not been studied in fish, but mammalian studies have approved that sulfur amino acids protect erythrocytes and hemoglobin against oxidation [50–55]. Based on this, higher RBC, HB, and Hct in the taurine- and/or methionine-treated Persian sturgeon might be due to improvement in antioxidant capacity and lower hemolysis in these fish.

In conclusion, dietary sulfur amino acid supplementation is necessary to support maximum growth, immunocompetence, and hepatic and erythrocyte antioxidant capacity in Persian sturgeon fed a low fish meal diet. Antioxidant improvement protects fish against hepatic damage and hemolysis, which improves the fish health. Although 1 g/kg taurine and 2 g/kg methionine improve the fish growth performance, immune responses, and antioxidant capacity, the maximum responses have been observed when the fish diet was simultaneously supplemented with both sulfur amino acids.

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