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Effects of vitamin B_3 and malt extract on growth, blood, immunity, liver enzymes, and gut microbiota of Siberian sturgeon, *Acipenser baerii*

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ABSTRACT

Niacin (B_3) and other vitamins found in malt extract (ME) play a major role in the cellular metabolism and energy production. These nutritional supplements improve feed digestibility and feed conversion ratio (FCR). This study investigated the effects of malt extract and vitamin B_3 on the growth, immunity, liver enzymes, and gut microbiota of the Siberian sturgeon, *Acipenser baerii*. In total, 240 Siberian sturgeons with an initial weight of 23.77 ± 4.66 g were selected and assigned to 8 groups with three replications. During this 8-week trial, the fish were kept in 24 500-liter circular fiberglass tanks equipped with an aerator and a central water outlet (10 fish in each tank). The fish in the control group (T_1) were fed with a certain formulated feed and those in the experimental groups (2-8) with the routine feed containing 1% ME, 1.5% ME, 20 mg kg^{-1} B_3, 30 mg kg^{-1} B_3, 1% ME + 20 mg kg^{-1} B_3, 1.5% ME + 30 mg kg^{-1} B_3, and 96% alcohol, respectively. The highest mean final total weight (49.41 ± 6.79 g) was observed in Group 7. The fish in T_7 exhibited the best performance in weight gain (0.72 ± 0.18), and daily growth (1.28 ± 0.32 % d^{-1}) indicating a significant difference between T_7 and other groups ($p < 0.05$). The highest condition factor (CF) was observed in T_3 with a mean of 1.33 ± 0.069 % ($p < 0.05$). The highest specific growth rate was observed in T_2 with a mean of 0.30 ± 0.75 % ($p < 0.05$). The analysis of gastrointestinal and liver enzymes demonstrated that the highest mean of ALT (34.65 ± 0.49 U L^{-1}) and AST (260.65 ± 17.68 U L^{-1}) were related to Group 6 ($p < 0.05$). The results also showed that the highest number of gut microbiota (6.55 ± 0.84 log CFU g^{-1}) and lactic acid microbiota (2.54 ± 0.41 log CFU mL^{-1}) were observed in T_2 ($p < 0.05$). Based on the study findings, the addition of 1% ME + 20 mg kg^{-1} B_3 and 1.5% ME + 30 mg kg^{-1} B_3 to the certain formulated feed of Siberian sturgeons improved their growth and immunological parameters.

Keywords: Fish, Growth, Immunity, Liver enzymes, Gut microbiota, Siberian sturgeon, *Acipenser baerii*.
Article type: Research Article.

INTRODUCTION

Fish species are a vital source of nutrients and play a critical role in the provision of nutrients to humans (Chrysohoou *et al.* 2007; Balami *et al.* 2019; Thomsen *et al.* 2022). The demand for fishery products has increased, and it is anticipated that the consumption of aquatic organisms will also rise in the future. It is estimated that global requirements for fishery products will surpass their supply by 2030 (Emadi & Rahmanian 2020). Sturgeons are considered the most commercially important fish species due to the high quality of their meat and the value of their caviar (Hung 2017; Williot *et al.* 2018; Raposo *et al.* 2023). The Siberian sturgeon, *Acipenser baerii*, is a non-migratory freshwater species that inhabits the middle and lower reaches of rivers. It is a suitable option for breeding because of its adaptability to low temperatures, resistance to environmental changes, and ability to consume a diverse array of foods (Ruban 2005; Williot *et al.* 2018; Aidos *et al.* 2022). Since sturgeon aquaculture is part of the aquaculture development programs, it is necessary to measure and monitor the physiological status of sturgeon in various breeding environments to optimize the utilization of resources (Hoar *et al.* 1983; Evans &

Claiborne 2005). Disease resistance and growth improvement are two critical factors that influence the performance of cultured aquatic organisms (Lin *et al.* 2007). The addition of immune-stimulating compounds to the feed of fish, particularly in intensive aquaculture, is an appropriate approach to mitigate the occurrence of infectious diseases (Galina *et al.* 2009; Harikrishnan *et al.* 2011). Aquatic nutrition is a newly developed field within nutritional sciences that focuses on the biochemical and physiological aspects of providing the nutritional requirements of aquatic organisms. The growth of aquatic organisms, such as fish, is notably influenced by nutrition (Golden *et al.* 2021). A well-formulated diet not only enhances the ability of fish species to absorb nutrients and fulfil their metabolic requirements, but also reduces aquaculture expenses and minimizes water pollution (Bharathi *et al.* 2019). It is very important to supplement the diet of farmed aquatic animals with vitamins, since vitamins play a crucial role in facilitating vital physiological processes within their body (Wang *et al.* 2017; Khalili Tilami & Sampels 2018). Vitamin B is classified as a water-soluble vitamin (Yaman *et al.* 2021). Seafood is a rich source of B vitamins, which are also essential for human survival. Seafood is also rich in water-soluble vitamins, including pyridoxine, niacin, B_{12}, riboflavin, and pantothenic acid (Yaman *et al.* 2021; Lykstad & Sharma 2023). Niacin, also known as vitamin B_3, plays a vital role in cellular respiration and the release of energy from carbohydrates, fats, and proteins. Additionally, it contributes to the process of glycogen synthesis and plays a major role in the metabolism of fats. In the form of Nicotinamide, it is a vital constituent of two enzymes, NAD and NADP, which play a role in the extraction of energy from nutrients (Penberthy & Kirkland 2020; Pirinen *et al.* 2020; Ahmadian *et al.* 2021). Malt extract (ME) is derived through the process of boiling barley malt in water and subsequently concentrating the resulting liquid under vacuum conditions. The composition of this extract includes 60% maltose, 10% sucrose, and 20% dextrin, along with various nutrients, alpha and beta amylase enzymes, and vitamins B_2 and B_1. ME is utilized as a primary ingredient in the food industry and serves as a versatile food source that provides essential nutrients in the diet while mitigating the negative consequences of consuming sucrose (Kramer 2006; Shaveta *et al.* 2019). ME contains various phenolic compounds, including flavonoids, phenolic acids, diterpenes, and tannins. These compounds are the main contributors to the antioxidant properties of ME (Bonoli *et al.* 2004). In addition, ME contains a high concentration of B-group vitamins, specifically vitamins B_6, B_3, B_2, B_1, and vitamin E (Zubtsov *et al.* 2017; Eremina *et al.* 2021). ME inhibits the decrease in activity of antioxidant enzymes, leading to a drop in the levels of malondialdehyde and carbonyl content in the brain and liver (Qingming *et al.* 2010). Haghparast *et al.* (2016) and Hasehemi *et al.* (2016) studied the effects of adding 30 mg kg^{-1} and 900 mg kg^{-1} niacin (B_3) to the diet of the Siberian sturgeon on their growth and some other physiological parameters and reported a significant difference between the experimental and control groups in body weight increase (BWI), specific growth rate (SGR), and average daily gain (ADG). Many other studies have also reported the positive effects of adding niacin to the diet of fish on their growth and physiological parameters, including 150-200 mg kg^{-1} in Atlantic salmon (*Salmo salar*; Halver 1972), 24.9 mg kg^{-1} in Jian carp (*Cyprinus carpio* var. Jian; Kuang *et al*. 2012), 63.62 mg kg^{-1} in Nile tilapia (*Oreochromis niloticus*) (Huang *et al*. 2013; Jiang *et al*. 2014), 36.86 mg kg^{-1} in *Schizothorax graham* (Zhao *et al*. 2014), 25.5 mg kg^{-1} in grass carp (*Ctenopharyngodon idella*; Feng *et al*. 2016), 90 mg kg^{-1} in common carp (*Cyprinus carpio*; Mohammadi *et al*. 2016), and 29.85-32.25 mg kg^{-1} in golden pompano (*Trachinotus blochii*; Xun *et al*. 2019). Akiyama *et al.* (1995) investigated the effects of adding different levels of ME along with 30% soybean meal (SBM) and 30% malt protein flour (MPF) on growth performance in the rainbow trout (*Oncorhynchus mykiss*). In another study, Ridha *et al.* (2020) examined the effects of three sources of carbohydrates, i.e., sugar, wheat flour, and malt, on the growth of the Nile tilapia. Moreover, Henríquez *et al.* (2023) studied the effects of adding 0.1% ME on nutritional and functional aspects in healthy adult dogs. Nevertheless, it seems that a few studies have addressed the effects of adding both ME and different levels of niacin to the diet of aquatic organisms. The main hypothesis of the present study is that malt extract and vitamin B_3 have a positive and significant effect on growth, immunity, liver enzymes, and intestinal microbial composition in *A. baerii*. The objectives of the study include investigating the effect of these compounds on weight and length growth indices, immune responses, liver enzyme activity, and microbial flora in this aquatic species.

MATERIALS AND METHODS

In this study, 240 *A. baerii* with an average initial weight of 23.77 ± 4.66 g were studied in 8 treatments and three replications in 24 500-L circular fiberglass tanks containing 10 fish each equipped with aerators with central water discharge in a fully controlled manner at the Dr. Keyvan Fisheries and Marine Sciences Research Centre, Islamic

Azad University, Lahijan Branch (in Chamkhaleh City, 5 km from Langrud County, Guilan Province, NW Iran). The control group (T_1) was fed a routine diet (Table 1), while the experimental groups (Treatments 2-8) received the routine feed supplemented with varying concentrations: 1% ME, 1.5% ME, 20 mg kg^{-1} B_3, 30 mg kg^{-1} B_3, 1% ME + 20 mg kg^{-1} B_3, 1.5% ME + 30 mg kg^{-1} B_3, and 96% alcohol, respectively. The fish in each tank were fed with the experimental diets three times a day based on 2-3% of the biomass of each tank. To determine the biomass of each tank, the weight (using a digital scale with an accuracy of 0.01 g) and length (using a biometric ruler with an accuracy of 1 mm) of all fish were measured every 15 days. Following the completion of the experimental feeding, all fish experienced 24 hours of food deprivation. Subsequently, they were anesthetized with 300 mg L^{-1} of clove powder (Fakhrian *et al.* 2021). BWI, SGR, and FCR were also calculated according to the method proposed by Merrifield *et al.* (2010; Table 3).

Table 1. Chemical analysis of the extruded feed provided from Faradaneh Co (Tehran, Iran; https://www.faradaneh.net/).

Chemical composition	**Pre-fattening**	
	FFS1	**FFS2**
Crude protein	48-50	
Crude fat	12-16	
Crude fiber	3.1-5	
Ash	7-10	
Moisture	6-11	
Phosphorus	1-15	
Fish weight	30-100 g	

Preparation of vitamin B_3

About 100 g of powdered vitamin B_3 with 100% purity was obtained from Merck (Germany). After performing the necessary calculations, the amount of vitamin B_3 for each treatment was measured with a precision of 0.001 using a digital scale (Haghparast *et al.* 2016). The amount of vitamin B_3 for each treatment was dissolved in 100 mL 96% medical ethyl alcohol and diluted with 100 mL distilled water. The resulting solution was sprayed on 1 kg feed in the open air and under the shade. The feed was left until the water mixed with the food evaporated slowly (Holden *et al.* 2008; Huang *et al.* 2013; Sharifzadeh *et al.* 2015). Once the feed was dried, it was carefully packed, assigned a number, and then stored in a freezer at -20 °C until it was ready to be consumed. The rations were removed from the freezer and allowed to reach room temperature one hour before feeding. Then, they were measured using a digital scale and provided to the fish.

Preparation of ME

The ME used in this study was purchased from Gorgan Malt Zarin Co (Gorgan, Iran). The ingredients of this product, according to the manufacturer, are shown in Table 2.

Table 2. Chemical analysis of ME.

pH 10% solution	**3.4-8.2**
Water-soluble solids (Brix)	60
Reducing sugars (in g% of maltose)	At least 45
Acidity (lactic acid)	0.6 at most
Protein (%)	1.5
Humidity (%)	38
Total solids (%)	62
Specific gravity at 20 °C	1.3
Refractive index at 20 °C	1.4

Measurement of liver enzymes

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using the IFCC method (International Federation of Clinical Chemistry and Laboratory Medicine) without adding Pyridoxal-5-phosphate. The wavelength used was 340 nm, with a cuvette diameter of 1 cm. The temperature was set to 37 °C, and the spectrophotometer was calibrated using an air blank (Acerete *et al.* 2004).

Gut microbiota assessment

To assess the gut microbiota and lactic acid bacteria at the end of the trial, the fish were transferred to the laboratory for bioassays. At first, the abdominal surface of the fish was disinfected with 70% ethyl alcohol.

Subsequently, incisions were made on their stomach under sterile conditions to take the intestine. After emptying the intestinal contents, it was rinsed three times with the physiological saline solution. The intestinal contents were weighed under sterile containers and then the physiological saline solution was added to prepare the desired dilutions; 0.1 mL of each dilution was then cultured superficially on TSA and MRS culture media. Finally, the plates were incubated at 30-35 °C for 48-72 hours. The number of bacteria was reported in CFU (Mekuria *et al.* 2018).

Statistical analysis

Homogeneity of variance and normality of data were checked using Kolmogorov-Smirnov. The data obtained were organized in Excel and then transferred into SPSS-23 for statistical analysis. The data were described using descriptive statistics, and the mean values were compared between the groups using the one-way analysis of variance (ANOVA) test and Tukey's test. The significance level was considered 0.05.

RESULTS

Growth indices

Results showed a significant difference between the experimental groups in terms of mean total weight and mean length ($p < 0.05$; Figs. 1 and 2). The highest mean total weight (49.41 ± 6.79 g) was observed in T_7, while the lowest (39.85 ± 6.46 g) in the control group. In addition, the highest and the lowest mean total length were observed in T_7 (40.54 ± 6.79 mm) and the control group (31.16 ± 6.46 mm), respectively. The one-way analysis of variance (ANOVA) test also demonstrated a significant difference between the groups in the mean total weight and total length ($p < 0.05$). The study results revealed a significant difference between the experimental groups in BWI, weight gain, K, ADG, and SGR ($p < 0.05$; Table 3). Based on the results, the highest and the lowest mean of BWI were observed in T_7 (20.7 ± 6.69) and T_2 (14.29 ± 3.22), respectively. The highest weight gain (0.72 ± 0.18) was related to T_7, whereas the lowest (0.55 ± 0.11) to T_2. In addition, the highest and the lowest K were observed in T_3 (1.33 ± 0.069) and T_4 (1.18 ± 0.085), respectively. The results also showed that the highest ADG (1.28 ± 0.32) was observed in T_7 and the lowest ADG (1.18 ± 0.085) in T_6. The highest and the lowest SGR were also related to T_2 (0.30 ± 0.075) and T_4 (0.11 ± 0.010), respectively. The one-way analysis of variance (ANOVA) test also demonstrated a significant difference between the groups in growth indices ($p < 0.05$).

Table 3. Growth performance of Siberian sturgeons fed with diets containing different levels of ME and vitamin B_3 in a 56-day trial.

Growth indicators	Experimental diets							
	1	2	3	4	5	6	7	8
Weight (g)	**39.85 ± 6.46^{a}**	**40.14 ± 6.49^{a}**	**43.77 ± 8^{b}**	**44.6 ± 6.34^{b}**	**41.8 ± 4.29^{ab}**	**42.6 ± 3.66^{ab}**	**49.41 ± 6.79^{c}**	**38.65 ± 7.82^{a}**
Length (mm)	**31.16 ± 2.15^{a}**	**32.62 ± 2.41^{a}**	**32.85 ± 2.83^{a}**	**37.7 ± 3.2^{b}**	**33.79 ± 2.67^{a}**	**32.92 ± 2.01^{a}**	**40.54 ± 3.37^{c}**	**32.5 ± 3.72^{a}**
WG (%)	16.08 ± 1.39^{a}	14.29 ± 3.22^{a}	18.11 ± 1.8^{a}	16.64 ± 5.6^{a}	15.27 ± 3.62^{ab}	15.81 ± 5.24^{c}	20.7 ± 6.69^{d}	13.71 ± 1.32^{c}
BWI (%)	0.67 ± 0.04^{a}	0.55 ± 0.11^{a}	0.70 ± 0.03^{a}	0.59 ± 0.16^{b}	0.57 ± 0.10^{b}	0.59 ± 0.16^{c}	0.72 ± 0.18^{a}	0.54 ± 0.03^{b}
FCR (%)	1.27 ± 0.12^{a}	1.21 ± 0.12^{a}	1.33 ± 0.06^{b}	1.18 ± 0.08^{c}	1.23 ± 0.01^{d}	1.29 ± 0.04^{d}	1.21 ± 0.13^{d}	1.18 ± 0.06c
ADG	1.20 ± 0.08^{a}	0.98 ± 0.20^{b}	1.26 ± 0.06^{a}	1.06 ± 0.29^{c}	1.02 ± 0.18^{c}	1.05 ± 0.28^{c}	1.28 ± 0.32^{a}	0.98 ± 0.07^{c}
SGR (%)	0.28 ± 0.03^{ab}	0.30 ± 0.07^{b}	0.27 ± 0.02^{a}	0.11 ± 0.10^{c}	0.15 ± 0.06^{d}	0.12 ± 0.10^{c}	0.14 ± 0.11^{d}	0.21 ± 0.02^{f}
Survival rate (%)	100	100	100	100	100	99	100	98

Note: Values are mean ± standard deviation of three replicates. Significant differences in columns with different letters were determined based on Tukey's test ($p < 0.05$).

Biochemical parameters

The analysis of biochemical parameters indicated that the highest levels of total protein (1.77 ± 0.24 g dL^{-1}), immunoglobulin M (55.8 ± 0.85 g dL^{-1}), total immunoglobulin (0.18 ± 11.03 g dL^{-1}), and active lysozyme (1.77 ± 33.45 U mL^{-1} min^{-1}) were observed in T_2, T_3, and T_5, respectively. The measurement of liver enzymes also demonstrated that the highest mean of ALT (34.65 ± 0.49 U L^{-1}) and AST (260.65 ± 17.68 U L^{-1}) were related to T_6 (Table 4). The one-way analysis of variance (ANOVA) test also demonstrated a significant difference between the groups in the biochemical parameters ($p < 0.05$).

Table 4. Biochemical of Siberian sturgeons fed with diets containing different levels of ME and vitamin B_3 in a 56-day trial.

Biochemical indicators	Experimental diets							
	1	2	3	4	5	6	7	8
Total Protein (g dL^{-1})	1.25 ± 0.21[a]	1.77 ± 0.24[b]	1.72 ± 0.15[b]	1.63 ± 0.11[b]	1.68 ± 0. 1[b]	1.63 ± 0.22[b]	1.71 ± 0.11[b]	1.31 ± 0.22[a]
IgM (mg dL^{-1})	35.10 ± 1.98[a]	53.65 ± 1.63[b]	55.80 ± 0.85[b]	51.30 ± 0.57[b]	49.07 ± 0.18[b]	46.21 ± 0.86[b]	48.30 ± 0.14[b]	35.63 ± 0.1[a]
Total IgM (mg dL^{-1})	7.52 ± 0.59[a]	11.03 ± 0.18[b]	10.38 ± 0.25[b]	10.89 ± 0.59[b]	10.54 ± 0.12[b]	10.28 ± 0.06[b]	10.63 ± 0.05[b]	8.91 ± 0.08[a]
Lysozyme Activity (U mL^{-1} min^{-1})	26.35 ± 1.2[a]	30.20 ± 1.27[b]	32.20 ± 0.71[b]	32.95 ± 0.78[b]	33.45 ± 1.77[b]	30.50 ± 1.41[b]	33.80 ± 1.27[b]	27.55 ± 1.06[ab]
ALT (uL L^{-1})	23.25 ± 1.2	32.20 ± 2.83	2.95 ± 1.21	27.80 ± 1.13	22.75 ± 1.06	34.65 ± 0.49	31.37 ± 0.89	24.50 ± 0.42
AST (uL L^{-1})	192 ± 2.83	211.5 ± 10.61	237.5 ± 10.51	218 ± 11.31	208.5 ± 3.45	260.5 ± 17.68	234 ± 28.282	212.5 ± 7.78

Note: Values are mean ± standard deviation of three replicates. Significant differences in columns with different letters were determined based on Tukey's test ($p < 0.05$).

Gut microbiota assessment

The results of the gut microbiota assessment showed that the highest mean number of total intestinal bacteria (6.55 ± 0.84 log CFU g^{-1}) and acid lactic bacteria (2.54 ± 0.41 log CFU mL^{-1}) were observed in T_2 ($p < 0.05$; Table 5).

Table 5. The mean number of total intestinal bacteria (log CFU g^{-1}) and lactic acid bacteria (Log CFU mL^{-1}) in the intestine of Siberian sturgeons fed with diets containing different levels of ME and vitamin B_3 in a 56-day trial.

Number of bacteria	Experimental diets							
	1	2	3	4	5	6	7	8
Total (log CFU g^{-1})	5.58 ± 0.64[a]	6.55 ± 0.84[b]	5.33 ± 1.07[a]	5.45 ± 1.18[a]	5.38 ± 0.68[a]	5.81 ± 1.34[ab]	6.41 ± 0.52[b]	5.74 ± 0.59[a]
Lactic acid (log CFU mL^{-1})	2.08 ± 0.26[a]	2.54 ± 0.41[b]	1.83 ± 0.29[c]	1.75 ± 0.51[c]	1.78 ± 0.39[c]	2.3 ± 0.61[b]	2.67 ± 0.43[b]	1.74 ± 0.64[c]

Note: Values are mean ± standard deviation of three replicates. Significant differences in columns with different letters were determined based on Tukey's test ($p < 0.05$).

DISCUSSION

The capacity of fish to assimilate and digest nutrients in their diet holds great practical and substantial significance in the aquaculture sector, which strives to maximize the marketability of fish products within the shortest timeframe and at the lowest expense. Given the meticulous balance of ingredients in commercial diets, these diets are expected to provide a high level of absorption efficiency (Prabu *et al.* 2017). The results of this study showed that the diet containing 1.5% ME + 30 mg kg^{-1} B_3 (T_7) had the best effects on growth indices, including mean weight and length, BWI, weight gain, and ADG. Niacin is one of the vitamins that the body requires, crucial for the production and burning of fats (Zubtsov *et al.* 2017). Moreover, feed digestibility can be enhanced by the consumption of ME, which in turn improves FCR through the synthesis of sterols and fatty acids (Enari *et al.* 1963). The carbohydrates, proteins, and fats contained in ME and vitamin B_3 undergo digestion and subsequent absorption through the absorption pathways located in the intestinal wall of the gastrointestinal tract. The body receives the combination of ME and vitamin B_3 through the processes of digestion and absorption in the gastrointestinal tract. This combination enhances the process of digestion and the uptake of nutrients, and it plays a crucial role in the feeding process (O'Brien 1934). Haghparast *et al.* (2016) investigated the effects of different levels of niacin on growth and biochemical parameters of blood and carcass of the sterlet (*Acipenser ruthenus*) and reported a significant difference between the experimental groups in ADG. Hasehemi *et al.* (2016) studied the effects of adding 900 mg kg^{-1} niacin to the diet of the Siberian sturgeon on their growth and some physiological indicators and demonstrated a significant difference between the test and control groups in BWI, SGR, and ADG; these results are consistent with the findings of this study in terms of ADG. ME contains probiotic compounds, including sugar and beta-glucans (Henríquez *et al.* 2023). Beta-glucans are categorized as prebiotics, serving as a nourishing substance for specific microorganisms in the large intestine. These microorganisms can positively affect the host's health (Gibson *et al.* 2017). Beta-glucans are polymeric compounds constituting 75% of the cellular wall of barley endosperm (Šimić *et al.* 2019). Barley seeds typically contain beta-glucans in the range of 2 to 11% (Hughes & Grafenauer 2021). Some sugars found in ME, such as maltotriose and maltotetraose, can serve as a substrate of fermentation in the large intestine of certain species. This process may contribute to the growth of beneficial bacterial populations (Jackson *et al.* 2020). Previous studies have shown that the yeast specimens used to produce ME commonly contain high levels of maltotriose, maltotetraose, and maltose (Zastrow *et al.* 2001). This result is consistent with the findings of the current study regarding the non-significant effect of the diet containing 1% ME on the mean BWI, BW%, and K. However, the diets containing 1% ME + 20 mg kg^{-1} B_3 and 1.5% ME + 30 mg kg^{-1} B_3 caused significant changes compared to the control group. To ensure that fish develop naturally and maintain optimal growth and health, vitamins should be included in their diets, as they cannot synthesize or produce them adequately. Vitamins are essential for normal metabolic functions and growth (Halver 1972). Niacin does not exert a direct influence on the composition of the blood, but it does play a crucial role in maintaining the well-being of the nervous and digestive systems. Niacin, as a constituent of the body's enzymatic activity, plays a role in the breakdown of food and its conversion into energy (Wu *et al.* 2020). The study results indicated that the addition of ME and niacin to the diet of Siberian sturgeons significantly improved total protein, total immunoglobulin, IgM, and lysozyme compared to the control group. The results of Tukey's test also indicated that there was a statistically significant difference between the fish fed with diets containing 30 mg kg^{-1} and 20 mg kg^{-1} niacin compared to other groups ($p < 0.05$). Haghparast *et al.* (2016) investigated the effects of different levels of niacin on hematological indicators of farmed sterlet (*Acipenser ruthenus*) and reported that the addition of 30 mg kg^{-1} niacin to the diet of this species caused a significant difference between the experimental groups in IgM and lysozyme levels, which is consistent with the findings of the current study. The level of protein synthesized in fish liver has a direct effect on the level of plasma protein. Consequently, an increase in protein synthesis in fish liver can lead to an elevation in in the plasma total protein. Furthermore, studies have indicated that higher levels of serum protein in fish are associated with a more robust innate immune response, which may be due to the presence of antibacterial peptides and immunoglobulins in total serum protein (Wiegertjes *et al.* 1996; Uribe *et al.* 2011). In the present study, all groups, except T_8 (96% alcohol), exhibited significantly higher levels of total protein, immunoglobulin M, total, and lysozyme compared to the control group. This result indicates the effects of different levels of ME and niacin on the immune factors of the Siberian sturgeon). Niacin possesses antioxidant properties that enhance cellular antioxidant capacity by inhibiting lipid peroxidation and enhancing cell membrane stability. This feature inhibits the leakage of enzymes into the bloodstream (Salek Yousefi 2000). Niacin is a crucial component in the metabolic processes of lipids, proteins, and carbohydrates. According to certain reports, most animals can produce nicotinic acid from the amino acid tryptophan. Due to the limited conversion of tryptophan to niacin, fish rely on dietary intake of niacin to prevent deficiency and ensure proper growth. Hence, in the case of hybrid fish that utilize glucose and dextrose as a source of carbohydrates, it is essential to include niacin in the diet (De Silva & Anderson 1994; Mohammadi *et al.* 2016). In addition, Feng *et al*. (2016) demonstrated that the deficiency of niacin reduces the levels of lysozyme activity in the grass carp, *Ctenopharyngodon idella*. They also reported that the addition of 25.5 mg kg^{-1} niacin to the diet of grass carps can meet their requirements. According to Mohammadi *et al*. (2019), adding 30 mg kg^{-1} niacin to the diet of fish is enough to keep the hematocrit and leukocyte count at a normal level and prevent malnutrition.

The liver is acknowledged as a crucial component in nutrition due to its role in the metabolism of nutrients. Hence, alterations in liver tissue resulting from the intake of food can be readily observed (Jackson *et al.* 2020). AST and ALT levels are frequently utilized as diagnostic markers for tissue injury, particularly in fish liver. Furthermore, these enzymes can accurately indicate the extent of environmental contamination before the occurrence of hazardous consequences (Öner *et al.* 2009). The results of this study showed the significant ALT alterations in T_2 (1% ME), T_6 (1% ME + 20 mg kg^{-1} B_3), and T_7 (1.5% ME + 30 mg kg^{-1} B_3) and also the significant AST changes in T_3 (1.5% ME), T_6 (1% ME + 20 mg kg^{-1} B_3), and T_7 (1.5% ME + 30 mg kg^{-1} B_3). The variation in ALT and AST observed in this study can be attributed to the elevated metabolic rate of niacin and the strong correlation between protein synthesis in liver tissue and the plasma protein levels (Wiegertjes *et al.* 1996). It is plausible that the elevation in protein synthesis in fish liver leads to an upraise in the plasma total protein levels. Furthermore, there are reports indicating that the upraised levels of serum proteins in fish are associated with a more robust innate immune response. In the current study, we observed an augmentation in the innate immunity of fish, resulting in the enhanced immune response and alignment of liver enzyme activity. The liver's response to the addition of vitamins to the diet includes an alteration in liver enzymes, which in the short term has little effect on liver physiology. However, if this process continues over the long term, the elevation in liver enzymes is not considered an appropriate physiological response. Therefore, for long-term use, it is necessary to reduce the amount of the substance consumed. The intestinal mucosal immune system in fish primarily relies on their immune response, which is associated with antibacterial substances like lysozyme and antimicrobial peptides (Li *et al.* 2015; Gibson *et al.* 2017). The study findings suggest the effect of different levels of niacin and ME on the immunological parameters of Siberian sturgeons. Mohammadi *et al.* (2016) investigated the effects of adding different levels of niacin to the diet of the common carp on the liver tissue, intestine, and some liver enzymes of this fish species, reporting that the upraised level of niacin decreased the activity of liver enzymes, including alanine aminotransferase and aspartate aminotransferase, but caused no significant change in them. This is not consistent with the findings of the current study. According to Feng *et al.* (2021), niacin strengthens the immune system by improving the immunity of the intestinal mucosa. The results of this study demonstrated that the diets containing 1% ME (T_2), 1% ME + 20 mg kg^{-1} B_3 (T_6), and 1.5% ME + 30 mg kg^{-1} B_3 (T_7) caused more significant alterations compared to the other diets. The addition of higher niacin levels to the diet of fish increases the height of the villi, while simultaneously reducing their thickness. The elongation of the villi in the intestine signifies that the intestinal villi are actively functioning. The height of villi is influenced by the nature and digestibility of the food consumed by fish (Mekbungwan & Yamauchi 2004). Nucleotides play a crucial role in the composition of various enzymes, such as nicotinamide adenine dinucleotide (NAD), which is involved in numerous metabolic pathways. The benefits of this substance include enhancing intestinal absorption (Li & Gatlin 2006) and promoting the growth of intestinal villi, which positively affect intestinal health and improve intestinal mucosa microflora (Robertson *et al.* 2000). The findings of Johnston & Weitz (1952), Mohammadi *et al.* (2016) Mohammadi *et al*. (2016), and (Feng *et al.* 2016) corroborate the results of this study.

CONCLUSION

The results of this study indicate that the addition of ME and B_3 to the diet of *A. baerii* has a significant positive impact on growth parameters, immune performance, and overall health. The combination of 1.5% ME and 30 mg kg^{-1} B_3 led to the elevations in weight, length, BWI, and ADG. Furthermore, this combination enhances nutrient absorption and strengthens the immune response by upraising total protein and immunoglobulin levels. Significant alterations in the liver enzyme activity reflect the positive effects of these components on metabolism and liver function. Therefore, it is recommended to incorporate these levels into the diets of Siberian sturgeon to optimize their growth and health.

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REFERENCES

Acerete, L, Balasch, J C, Espinosa, E, Josa, A & Tort, L 2004, Physiological responses in Eurasian perch (*Perca fluviatilis*, L.) subjected to stress by transport and handling. *Aquaculture,* 237: 167-178.

Ahmadian, A, Bouyeh, M & Seidavi, A R 2021, A review of the effects of niacin on broiler productivity. *World's Poultry Science Journal*, 77: 589-604.

Aidos, L, Cafiso, A, Lopez, A, Vasconi, M, Valente, M, Bazzocchi, C & Di Giancamillo, A 2022, Rearing Environment during the Endogenous Feeding Stage of *Acipenser baerii*. *Animals,* 12.

Akiyama, T, Unuma, T, Yamamoto, T, Marcouli, P & Kishi, S 1995, Combinational use of malt protein flour and soybean meal as alternative protein sources of fish meal in fingerling rainbow trout diets. *Fisheries Science,* 61: 828-832.

Balami, S, Sharma, A & Karn, R 2019, Significance of nutritional value of fish for human health. *Malaysian Journal of Halal Research,* 2: 32-34.

Bharathi, S, Antony, C, Cbt, R, Arumugam, U, Ahilan, B & Aanand, S 2019, Functional feed additives used in fish feeds. *International Journal of Fisheries and Aquatic Studies,* 7: 44-52.

Bonoli, M, Verardo, V, Marconi, E & Caboni, M F 2004, Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. *Journal of Agricultural and Food Chemistry,* 52: 5195-5200.

Chrysohoou, C, Panagiotakos, DB, Pitsavos, C, Skoumas, J, Krinos, X, Chloptsios, Y, Nikolaou, V & Stefanadis, C 2007, Long-term fish consumption is associated with protection against arrhythmia in healthy persons in a Mediterranean region—the ATTICA study. *The American Journal of Clinical Nutrition,* 85: 1385-1391.

De Silva, SS & Anderson, TA 1994. *Fish Nutrition in Aquaculture* (Vol. 1): Springer Science & Business Media.

Emadi, MH & Rahmanian, M 2020, Commentary on challenges to taking a food systems approach within the food and agriculture organization (FAO). *Food Security and Land Use Change Under Conditions of Climatic Variability: A Multidimensional Perspective*: pp. 19-31.

Enari, T, Puputti, E & Mikola, J 1963, Fractionation of the proteolytic enzymes of barley and malt. *Proceeding of European Brewery Convention, Brussels*: pp. 36-44.

Eremina, OY, Seregina, NV, Ivanova, TN, Shuldeshova, NV & Zaugolnikova, E V 2021, Micronutrient value and antioxidant activity of malt wheat sprouts. *IOP Conference Series: Earth and Environmental Science,* 677: 022107.

Evans, DH & Claiborne, JB 2005. *The physiology of fishes*. Boca Raton: CRC press.

Fakhrian, M, Jafariyan, M, Pirali Zefrehei, A & Sahraei, H 2021, The effect of dietary medicinal plants on blood parameters of sterlet (*Acipenser ruthenus*). *International Journal of Environmental Research and Education,* 2: 55-59.

Feng, J, Wang, L, Chen, Y, Xiong, Y, Wu, Q, Jiang, Z & Yi, H 2021, Effects of niacin on intestinal immunity, microbial community and intestinal barrier in weaned piglets during starvation. *International Immunopharmacology,* 95: 107584.

Feng, L, Li, S-Q, Jiang, W-D, Liu, Y, Jiang, J, Wu, P, Zhao, J, Kuang, S-Y, Tang, L, Tang, W-N, Zhang, Y-A & Zhou, X-Q 2016, Deficiency of dietary niacin impaired intestinal mucosal immune function via regulating intestinal NF-κB, Nrf2 and MLCK signaling pathways in young grass carp (*Ctenopharyngodon idella*). *Fish & Shellfish Immunology,* 49: 177-193.

Galina, J, Yin, G, Ardó, L & Jeney, Z 2009, The use of immunostimulating herbs in fish. An overview of research. *Fish Physiology and Biochemistry,* 35: 669-676.

Gibson, GR, Hutkins, R, Sanders, ME, Prescott, SL, Reimer, RA, Salminen, SJ, Scott, K, Stanton, C, Swanson, KS, Cani, PD, Verbeke, K & Reid, G 2017, Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nature Reviews Gastroenterology & Hepatology,* 14: 491-502.

Golden, CD, Koehn, JZ, Shepon, A, Passarelli, S, Free, CM, Viana, DF, Matthey, H, Eurich, JG, Gephart, JA, Fluet-Chouinard, E, Nyboer, EA, Lynch, AJ, Kjellevold, M, Bromage, S, Charlebois, P, Barange, M, Vannuccini, S, Cao, L, Kleisner, KM, Rimm, EB, Danaei, G, DeSisto, C, Kelahan, H, Fiorella, KJ, Little, D C, Allison, EH, Fanzo, J & Thilsted, S H 2021, Aquatic foods to nourish nations. *Nature,* 598: 315-320.

Haghparast, R, Khara, H & Farokhroz, M 2016, Effect of vitamin niacin (B3) on growth, blood biochemical and body composition Sterliad (*Acipenser ruthenus*). *Journal of Animal Environment,* 8: 115-122.

Halver, J 1972, The vitamins, in "Fish Nutrition"(ed. by JE Halver) Academic Press. New York, NY, pp. 29-103.

Harikrishnan, R, Balasundaram, C & Heo, M-S 2011, Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. *Aquaculture,* 317: 1-15.

Hasehemi, RA, Bahmani, M & Yousefi, A 2016, Effects of super supplements on growth and some physiological indices in farmed juvenile Siberian sturgeon (*Acipenser baerii*). *International Journal of Fisheries and Aquatic Studies,* 4: 400-406.

Henríquez, LBF, Rentas, MF, Zafalon, RVA, Perini, MP, Marchi, PH, Rodrigues, RBA, Vendramini, THA, Gomes, COMS, Balieiro, JCdC & Brunetto, MA 2023, Effects of Malt Extract on Nutritional and Functional Aspects in Healthy Adult Dogs. *Fermentation,* 9: 870.

Hoar, WS, Randall, DJ & Donaldson, EM 1983. *Fish physiology*: Academic Press.

Holden, JM, Lemar, LE, & Exler, J 2008, Vitamin D in foods: development of the US Department of Agriculture database. *The American Journal of Clinical Nutrition,* 87: 1092S-1096S.

Huang, F, Wen, H, Wu, F, Jiang, M, Liu, W, Tian, J, & Shao, H 2013, The dietary niacin requirement of large GIFT tilapia. *Journal of South China Agricultural University,* 34: 235-240.

Hughes, J & Grafenauer, S 2021, Oat and barley in the food supply and use of beta glucan health claims. *Nutrients,* 13: 2556.

Hung, SS 2017, Sturgeon, *Acipenser* spp. Handbook of nutrient requirements of finfish (1991) (pp. 153-160): CRC Press.

Jackson, MI, Waldy, C, Cochrane, C-Y & Jewell, DE 2020, Consumption of identically formulated foods extruded under low and high shear force reveals that microbiome redox ratios accompany canine immunoglobulin A production. *Journal of Animal Physiology and Animal Nutrition,* 104: 1551-1567.

Johnston, PM & Weitz, EM 1952, The effect of niacin deficiency on the appearance of the Golgi apparatus in the columnar absorbing cells of the duodenum of the albino rat. *Journal of Morphology,* 91: 79-109.

Khalili Tilami, S & Sampels, S 2018, Nutritional Value of Fish: Lipids, Proteins, Vitamins, and Minerals. *Reviews in Fisheries Science & Aquaculture,* 26: 243-253.

Kramer, P 2006, Barley, malt, and malting. *Raw Materials and Brewhouse Operation,* 1: 15-54.

Kuang, S-Y, Xiao, W-W, Feng, L, Liu, Y, Jiang, J, Jiang, W-D, Hu, K, Li, S-H, Tang, L & Zhou, X-Q 2012, Effects of graded levels of dietary methionine hydroxy analogue on immune response and antioxidant status of immune organs in juvenile Jian carp (*Cyprinus carpio* var. Jian). *Fish & Shellfish Immunology,* 32: 629-636.

Li, L, Feng, L, Jiang, W-D, Jiang, J, Wu, P, Kuang, S-Y, Tang, L, Tang, W-N, Zhang, Y-A, Zhou, X-Q & Liu, Y 2015, Dietary pantothenic acid deficiency and excess depress the growth, intestinal mucosal immune and physical functions by regulating NF-κB, TOR, Nrf2 and MLCK signaling pathways in grass carp (*Ctenopharyngodon idella*). *Fish & Shellfish Immunology,* 45: 399-413.

Li, P & Gatlin, DM 2006, Nucleotide nutrition in fish: Current knowledge and future applications. *Aquaculture,* 251: 141-152.

Lin, S, Mai, K & Tan, B 2007, Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, Oreochromis niloticus x O. aureus. *Aquaculture Research,* 38: 1645-1653.

Lykstad, J & Sharma, S 2023, Biochemistry, Water soluble vitamins: StatPearls Publishing, Treasure Island (FL).

Mekbungwan, A & Yamauchi, K 2004, Growth performance and histological intestinal alterations in piglets fed dietary raw and heated pigeon pea seed meal. *Histology and Histopathology*.

Mekuria, B, Gebretekle, GB, Bekele, T, Negussie, M, Kifle, M & Fenta, T G 2018, Bacterial resistance to fluoroquinolones and contributing factors in Addis Ababa, Ethiopia: a mixed methods study. *Ethiopian Pharmaceutical Journal,* 34: 61-72.

Merrifield, DL, Dimitroglou, A, Foey, A, Davies, S J, Baker, RTM, Bøgwald, J, Castex, M & Ringø, E 2010, The current status and future focus of probiotic and prebiotic applications for salmonids. *Aquaculture,* 302: 1-18.

Mohammadi, K, Salati, A & Rajabzadeh, E 2016, Effect of Different Levels of Dietary Niacin on the Gut and Liver Histology and Some Liver Enzymes Activity of Juveniles Common Carp (*Cyprinus carpio*). *Journal of Fisheries,* 69: 253-263.

O'Brien , R 1934, Vitamin B($_3$). *The Biochemical Journal,* 28: 926-932.

Öner, M, Atli, G & Canli, M 2009, Effects of Metal (Ag, Cd, Cr, Cu, Zn) Exposures on Some Enzymatic and Non-Enzymatic Indicators in the Liver of *Oreochromis niloticus. Bulletin of Environmental Contamination and Toxicology,* 82: 317-321.

Penberthy, WT & Kirkland, J B 2020. Niacin, Present knowledge in nutrition (pp. 209-224): Elsevier.

Pirinen, E, Auranen, M, Khan, NA, Brilhante, V, Urho, N, Pessia, A, Hakkarainen, A, Kuula, J, Heinonen, U, Schmidt, MS, Haimilahti, K, Piirilä, P, Lundbom, N, Taskinen, M R, Brenner, C, Velagapudi, V, Pietiläinen, KH & Suomalainen, A 2020, Niacin cures systemic NAD(+) deficiency and improves muscle performance in adult-onset mitochondrial myopathy. *Cell Metabolism,* 31: 1078-1090.e1075.

Prabu, E, Felix, S, Felix, N, Ahilan, B & Ruby, P 2017, An overview on significance of fish nutrition in aquaculture industry. *International Journal of Fisheries and Aquatic Studies,* 5: 349-355.

Qingming, Y, Xianhui, P, Weibao, K, Hong, Y, Yidan, S, Li, Z, Yanan, Z, Yuling, Y, Lan, D, & Guoan, L 2010, Antioxidant activities of malt extract from barley (*Hordeum vulgare* L.) toward various oxidative stress in vitro and in vivo. *Food Chemistry,* 118: 84-89.

Raposo, A, Alturki, HA, Alkutbe, R & Raheem, D 2023, Eating sturgeon: An endangered delicacy. *Sustainability,* 15: 3511.

Ridha, MT, Hossain, MA, Azad, IS & Saburova, M 2020, Effects of three carbohydrate sources on water quality, water consumption, bacterial count, growth and muscle quality of Nile tilapia (*Oreochromis niloticus*) in a biofloc system. *Aquaculture Research,* 51: 4225-4237.

Robertson, PAW, O'Dowd, C, Burrells, C, Williams, P & Austin, B 2000, Use of Carnobacterium sp. as a probiotic for Atlantic salmon (*Salmo salar* L.) and rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Aquaculture,* 185: 235-243.

Ruban, GI 2005. The Siberian sturgeon *Acipenser baerii* Brandt (Vol. 1). Norderstedt (Germany): Demand GmbH.

Salek Yousefi, M 2000, Principle of food nutrition in aquaculture (warm and coldwater fish). Aslani Publication. 320 p. (In Persian).

Sharifzadeh, SA, Khara, H & Ghobadi, S 2015, Effects of vitamins E and Riboflavin (B2) and combinations of them on the hematological parameters of common carp, *Cyprinus carpio* L., fingerlings. *Fisheries & Aquatic Life,* 23: 107-111.

Shaveta, HK, Kaur, S & Kaur, S 2019, Hulless barley: A new era of research for food purposes. *Journal of Cereal Research,* 11: 114-124.

Šimić, G, Horvat, D, Lalić, A, Koceva Komlenić, D, Abičić, I & Zdunić, Z 2019, Distribution of β-glucan, phenolic acids, and proteins as functional phytonutrients of hull-less barley grain. *Foods,* 8: 680.

Talazade, F, Mayahi, M & Soleimankhani, F 2019, Survey on the effect of malt extract on growth performance and carcass characteristics of broiler chickens. *Journal of Animal Environment,* 11: 77-84.

Thomsen, ST, Assunção, R, Afonso, C, Boué, G, Cardoso, C, Cubadda, F, Garre, A, Kruisselbrink, J W, Mantovani, A, Pitter, J G, Poulsen, M, Verhagen, H, Ververis, E, Voet, H v d, Watzl, B & Pires, S M 2022, Human health risk–benefit assessment of fish and other seafood: a scoping review. *Critical Reviews in Food Science and Nutrition,* 62: 7479-7502.

Uribe, C, Folch, H, Enríquez, R & Moran, G 2011, Innate and adaptive immunity in teleost fish: A review. *Veterinarni Medicina,* 56: 486-503.

Wang, L, Xu, H, Wang, Y, Wang, C a, Li, J, Zhao, Z, Luo, L, Du, X, & Xu, Q 2017, Effects of the supplementation of vitamin D3 on the growth and vitamin D metabolites in juvenile Siberian sturgeon (*Acipenser baerii*). *Fish Physiology and Biochemistry,* 43: 901-909.

Wiegertjes, GF, Stet, RM, Parmentier, HK & van Muiswinkel, W B 1996, Immunogenetics of disease resistance in fish: A comparative approach. *Developmental & Comparative Immunology,* 20: 365-381.

Williot, P, Nonnotte, G & Chebanov, M 2018. The Siberian Sturgeon *(Acipenser baerii,* Brandt, 1869). Volume 2: Farming, Springer.

Wu, Y, Li, S, Wang, W & Zhang, D 2020, Associations of dietary vitamin B1, vitamin B2, niacin, vitamin B6, vitamin B12 and folate equivalent intakes with metabolic syndrome. *International Journal of Food Sciences and Nutrition*, 71: 738-749.

Xun, P, Lin, H, Wang, R, Huang, Z, Zhou, C, Yu, W, Huang, Q, Yang, Y, Huang, X & Tan, L 2019, Effects of dietary niacin on growth performance, serum biochemistry, antioxidant ability of liver, intestinal digestion, and absorption in juvenile golden pompano.

Yaman, M, Çatak, J, Uğur, H, Gürbüz, M, Belli, İ, Tanyıldız, S N, Yıldırım, H, Cengiz, S, Yavuz, BB, Kişmiroğlu, C, Özgür, B & Yaldız, M C 2021, The bioaccessibility of water-soluble vitamins: A review. *Trends in Food Science & Technology,* 109: 552-563.

Zastrow, CR, Hollatz, C, de Araujo, PS & Stambuk, BU 2001, Maltotriose fermentation by Saccharomyces cerevisiae. *Journal of Industrial Microbiology and Biotechnology,* 27: 34-38.

Zhao, H, Zhang, L, Deng, Y, Yiang, Y, Zhang, M, Luo, C & Chen, Y 2014, Dietary niacin requirement of Juvenile Kunming. *Fisheries Science* (*Dalian*)*,* 33: 167-170.

Zubtsov, YN, Eremina, OY & Seregina, N 2017, The micronutrient value of byproducts of malting barley. *Voprosy Pitaniia,* 86: 115-120.