EFFECT OF DIGESTIVE ENZYMES AND PHYTOADITIVES ON HAEMATOLOGICAL PARAMETERS AND FATTY ACID COMPOSITION OF SIBERIAN STURGEON MEAT *(ACIPENSER BAERI)* REARED IN RECIRCULATING SYSTEM

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Abstract

It is vital for aquaculture to produce high quality, healthy biological material. Diets enriched with active ingredients can contribute to the well-being of sturgeons. For the experiment, 5-month-old specimens of Acipenser baeri were used, with an average weight of 34.07 ± 3.68 g / specimen. For the experimental lot, an experimental feed diet was prepared, supplemented with 0.05% digestive enzymes (protease, lipase, amylase, gluco-amylase, beta-glucanase, lactase, hemi-cellulase, pectinase, cellulase, alpha-galactosidase maltodextrin) and 0.15% phytoadditives consisting of a mixture of herbs (Zingiber officinale, Mentha piperita, Foeniculum vulgare, Pimpinella anisum, in equal proportions). The aim of this study is to determine the effect of digestive enzymes and phytoadditives on the haematological parameters and fatty acid composition of Siberian sturgeon meat (Acipenser baeri). ALA (C18:3n3) (4.66%), EPA (C20:5n3) (4.36%) and DHA (C22:6n3) (6.48%), the most important ω3 type PUFA, are found in the largest quantity in the experimental lot. Immunological and biochemical parameters have been improved by including digestive enzymes and phytoadditives in the diets of Siberian sturgeon.

Key words: *Acipenser baeri*, fatty acids, immunity

INTRODUCTION

Sturgeon rearing in aquaculture has not been done for a long time and has developed steadily. Due to the late maturity of sturgeons, farmers strive to keep the biological material healthy throughout the growing period.

The Organization for Animal Health (OIE) (2014) sets as a guideline the improvement of farm animal welfare, with favourable consequences on productivity, food safety and, consequently, the consumer. There is an increasing emphasis on disease prevention and proper care. Diets enriched with bioactive components contribute to the well-being of sturgeons Simide R, et al. (2016) [12], (Bocioc E. 2011) [2].

In intensive rearing, fish regardless of species is continuously subjected to conditions of discomfort such as handling, density, variations in water quality in its abiotic (pH, dissolved oxygen, temperature, pollutants, etc.) and biotic (plankton, bacteria, etc.) components and last but not least, the composition of feed, which, as a whole, can lead to stress and illness.

In the present experiment *Acipenser baeri* was used, a sturgeon species that is not found in the Danube waters but is one of the most used in aquaculture.

The aim of this study is to determine the effect of digestive enzymes and phytoadditives on the haematological parameters and fatty acid composition of Siberian sturgeon meat (*Acipenser baeri*).

MATERIAL AND METHOD

For the experiment, 2100 specimens of *Acipenser baeri* were used, aged 5 months, with an average weight of 34.07 ± 3.68 g / specimen, distributed in 2 basins with a density of 3.18 kg / m^3 in the control lot and 3.71 kg / m^3 in the experimental lot.

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The experiment was performed in the pilot system, of recirculating type, which belongs to the Institute of Research and Development for Aquatic Ecology, Fishing and Aquaculture of Galati, for a period of 60 days (1.07-30.08.2021).

For the control lot (lot C), Aller Bronze (FS) type feed with a protein content of 45% and a lipid content of 15%, 2 mm granulation was used.

For the experimental lot, an experimental feed diet was prepared, supplemented with 0.05% digestive enzymes (protease, lipase, amylase, gluco-amylase, beta-glucanase, lactase, hemi-cellulase, pectinase, cellulase, alpha-galactosidase incorporated in maltodextrin) and 0.15% phytoadditives consisting of a mixture of herbs (*Zingiber officinale, Mentha piperita, Foeniculum vulgare, Pimpinella anisum*, in equal proportions).

The feed was administered in four meals/day, in a percentage between 0.5%-2.5% of the biomass depending on the temperature of the growing environment.

The composition, ration and granulation of the feed were determined according to the species and the stage of ontogenetic development.

<u>Haematological analysis</u>

To study the haematological parameters, blood was collected on anticoagulant (EDTA - etilen-diamino-tetraacetic-acid), at the beginning and at the end of the experiment, through puncturing the caudal vein.

To determine the number of erythrocytes (mil.cel/µl blood) the Newbauer haemocytometer by direct microscope numbering was used, haemoglobin concentration (Hb, g/dl) was determined by Sahli colorimetric method and haematocrit value (PCV, %) was determined by microhaematocrit method with a Hettich Haematockit 210 centrifuge.

Mean Corpuscular Volume (MCV, µm3), Mean Corpuscular Haemoglobin (MCH, pg) end Mean Corpuscular Haemoglobin (MCHC, (g/dl) were determined as follows:

MCV= Hct*10/no. erythrocyte;

MCH= Hb*10/ no. erythrocyte;

MCHC= Hb*100/Hct.

<u>Fatty acids profiling</u>

The determination of fatty acids in fish meat and fodder was determined by gas chromatography (GC). To extract lipids, the homogenized samples were dried for 1 h at 105°C. The fatty acid methyl esters were analysed with a Clarus-500 gas chromatograph with a Perkin-Elmer mass spectrometry detector, equipped with a system of injection into the capillary column (ratio of 1:100). The change of the fatty acids from the sample to the methyl ester was followed by the separation of the components on the capillary column, the identification by comparison with a chromatography standard.

The relative concentration of fatty acids was calculated as mass percentage of the identified fats.

The working methodology is in accordance with SR CEN ISO / TS 17764-1 / 2008; SR CEN ISO / TS 17764-2 / 2008; EN ISO 661/2005; and SR EN ISO 15304/2003; SR EN ISO 15304 / AC 2005; AOAC 996.01.

HSI = (liver/body in weight) x 100;

VSI = (viscera/body in weight) x 100.

<u>Statistical analysis</u>

All analyses were carried out in triplicate. Statistical analysis was carried out by means of Excel tools. The average values are reported together with standard deviations. The statistical interpretation of the considered data shows a variation within the allowable threshold of $P<0.05$.

RESULTS AND DISCUSSIONS

The chemistry of the living environment has a decisive influence on the development of sturgeon material. The main disease-causing chemicals were monitored hourly (oxygen dissolved in water and saturation), weekly (pH, nitrogen compounds and phenols) or as often as needed.

During the study, in the two experimental basins there were no deviations of the physicochemical parameters in water, outside the optimal spread.

The survival of biological material, an important indicator in any growth system, was monitored throughout the experiment.

The survival of Siberian sturgeon in the experimental lot in which digestive enzymes and phytoadditives were incorporated in feed, was significantly ($p<0.05$) higher, registering a value of 75.5%, compared to control lot where the biological material is fed with standard feed (59%).

The addition of enzymes and phytoadditives influenced the survival rate, the results being more favourable compared to those obtained by Lin L., et al., 2015 [6].

The fatty acid composition of the material involved in the experiment

The concentration of fatty acids in Acipenser baeri meat fed for 60 days with diets supplemented with the addition of enzymes and phytoadditives is illustrated in Table 1.

Table 1 Composition of fatty acids (%), in the meat of *Acipenser baeri* fed for 60 days with diets supplemented with the addition of enzymes and phytoadditives

Fatty Acids, %	***Lot C***	***Lot Exp***
Saturated Fatty Acids	25.46	22.96
Monounsaturated Fatty Acids	38.39	38.78
Polyunsaturated Fatty Acids	35.01	37.06
Other Fatty Acids	1.15	1.20
Eicosapentaenoic Acid (C20:5n3) (EPA)	4.22	4.36
Docosahexaenoic Acid (C22:6n3) (DHA)	6.40	6.48
DHA/EPA	1.52	1.49
Total ω-3 fatty acids	19.59	21.17
Total ω-6 fatty acids	15.42	15.89
ω-3/ω-6	1.27	1.33

Of the total fatty acid content, determined in Siberian sturgeon meat, monounsaturated fatty acids are predominant in both batches: Lot C (38.39%) and Lot Exp (38.78%) Pelic M., et al., 2019 [10].

The concentration of saturated fatty acids (SFA) in the two lots at the end of the experiment varied from 22.96% in the experimental lot to 25.46% in lot C.

The highest saturated fatty acids are - palmitic acid (C16:0), with values between 17.21% in lot C and 15.14% in lot Exp, - myristic acid (C14:0), with values between 3.78% in lot C and 3.60% in lot Exp and - stearic acid (C18:0), with values between 2.96% in lot C and 2.71% in lot Exp.

The most common monounsaturated fatty acid (MUFA) is oleic acid (C18:1n-9cis) which varies between 31.81% in lot C and 32.32% in lot Exp. Along with oleic acid, palmitoleic acid (C16:1n) can also be found, which varies between 5.53% in lot C and 5.54% in lot Exp.

The concentration of polyunsaturated fatty acids (PUFA) in the control lot had a lower value (35.01%) compared to the experimental lot (37.06%), which shows that diets with the addition of enzymes and phytoadditives favourably influenced the accumulation of fatty acids PUFA in Siberian sturgeon meat.

The amount of SFA accumulated in the experimental lot was performed to the detriment of MUFA and PUFA.

Analysing the composition of polyunsaturated fatty acids, we find a higher share of ω3 type fatty acids and a lower share of ω6 type fatty acids in both lots.

The concentration of ω3-type polyunsaturated fatty acids increases simultaneously with the increase of the lipid concentration in Siberian sturgeon meat, while the concentration of ω6 is similar in both lots.

Both the quantity of polyunsaturated fatty acids and their quality are important, highlighted by the ratio between the two constituent groups ω3 and ω6.

In the experimental lot the value of the ω3/ω6 ratio has higher values than in the control lot (1.27 in Lot C and 1.33 in Lot Exp).

The results show that the addition of digestive enzymes and phytoadditives can induce better health and an accumulation of fatty acids in meat, as obtained by Huanhuan

Y. et al., 2020 [3] by the addition of lipids from various sources in feeding diets for *Acipenser baeri*.

ALA (C18:3n3) (4.66%), EPA (C20:5n3) (4.36%) and DHA (C22:6n3) (6.48%), the most important ω3 type PUFA, are found in the largest quantity in the experimental lot.

Clupanodonic acid (docosapentaenoic acid - DPA) which is responsible for the specific odour of fish tissues and oil ranged from 1.21% in lot C to 1.27% in the experimental lot.

Experimental diets with the addition of enzymes and phytoadditives have led to the accumulation of higher amounts of ω3 acids compared to standard feed.

The addition of enzymes and phytoadditives to feed diets had no effect on the EPA / DHA ratio of sturgeon meat, as was influenced for *Acipenser baeri* fed with a full-fat insect-based diet in an aquaponics system Zarantoniello, M., et al., 2021 [8].

Among the ω6 type fatty acids, the most important and with the highest weight is linoleic acid C18:2n6.

In the lots fed with the addition of enzymes and phytoadditives, the concentration of linoleic acid C18:2n6 varied from 12.34% in lot C, to 13.01% in the experimental lot.

The concentration of arachidonic acid C20:4n6 (AA) was found in a higher concentration in lot C (0.89%), compared to the experimental lot (0.82%) fed with the addition of enzymes and phytoadditives.

Acipenser baeri is a valuable fish from a nutritional point of view, due to its fatty acid composition. The high PUFA content comes mainly from feed, so the proper nutrition of farm sturgeon can be a solution for obtaining an optimal fatty acid composition from sturgeon meat.

Haematological analysis

The haematological profile was studied to detect possible physiological disorders of the specimens involved in the experiment.

Biological blood samples (approx. 0.5 - 1 ml, depending on the size of the fish) were taken from 20 specimens at the beginning of experiment and from 10 specimens / basins (1% of the batch specimens), extracted randomly, by the method caudal vein puncture.

Current methods applied in veterinary haematology were used for haematological analyses.

The variation of haematological indices values and erythrocyte constants was analysed, at the beginning and at the end of experiment, in order to highlight the adaptive changes to the diets with enzymes and phytoadditives (table 2).

Blood tests embedded in the leukogram identify elevated values of erythrocytes at the beginning of experiment (8.10 mil/µl) which highlights a state of increased stress. This value decreases during the experiment, finding at the end a slightly increased value for the experimental lot (6.10 mil/µl) compared to the control lot (5.20 mil/µl), values similar to those obtained by Maciej R et. in 2016 [7].

The experimental lot fed with the addition of enzymes and phytoadditives showed higher values of red blood cells, haemoglobin (Hb), white blood cells (WBC) and monocytes compared to those in the control lot ($P < 0.05$), similar to those obtained by Pourgholam M.A., et al., 2017 [11] studying the immune reaction of *Aacipenser beri* fed with an additional diet with *Lactobacillus plantarum*.

Table 2 Variation of haematological indicators at the beginning and at the end of the experiment (after 60 days of experiment)

Parameters	UM	Beginning of the experiment	End of the experiment	
			Lot C	Lot Exp
Haematocrit (Hct)	(%)	84.00	22.00	43.00
Haemoglobin (Hb)	(g/dl)	9.00	6.55	8.20
Erythrocyte	(mil/µl)	8.10	5.20	6.10
Mean Corpuscular Volume (MCV)	(μm^3)	103.80	42.31	70.49
Mean Corpuscular Haemoglobin (MCH)	(pg)	11.11	12.60	13.44
Mean Corpuscular Haemoglobin (MCHC)	(g/dl)	10.71	29.77	19.07
Platelet count (PLT)	($\times 10^9$/l)	3.00	6.00	13.30

The blood test that measures the percentage of red blood cells (Hct) demonstrates high values for the biological material (84%) at the beginning of the experiment. At the end of the experiment, the haematocrit value decreases too much in the control lot and reaches normal values in the experimental lot (Stroe D., 2014).

The values of the haematocrit and the total number of erythrocytes at the end of the experiment correspond to the data found in the literature Kadusa M., et al., 2017 [13].

Lymphocytes, the main types of immune cells that help the body fight bacteria, viruses and other toxins, are found in the largest amount in the experimental lot fed diets enriched with enzymes and phytoadditives (23.94%), compared to the control lot that has values by 8% lower (15.96%) (table 3).

Table 3 Variation of the white blood cell absolute number at the population and at the end of the experiment (after 60 days of experiment)

Parameters	UM	Beginning of the experiment	End of the experiment	
			Lot C	Lot Exp
WBC	($\times 10^9$/L)	332.36	314.37	292.74
Lymph	(%)	11.74	23.94	15.86
Mon	(%)	23.18	5.46	2.15
Neu	(%)	63.28	67.90	80.05
Eos	(%)	1.78	2.48	1.63
Baso	(%)	0.02	0.23	0.32

The biochemical indicators of importance in establishing the blood metabolic profile and on the basis of which a unitary conclusion can be drawn regarding the general physiological state of the specimens involved in the experiment are found in Table 4.

Table 4 The values of biochemical serological analyses at the beginning and at the end of the experiment (after 60 days of experiment)

Parameters		UM	Beginning of the experiment	End of the experiment	
				Lot C	Lot Exp
Total serum protein	TP	g /l	10.45	12.95	12.60
Serum albumin	ALB	g/l	3.45	5.40	6.75
Globulin	GLO	g/l	7.15	7.55	8.25
Albumin / globulin ratio	A/G		0.48	0.72	0.82
Calcium	Ca	mmol/l	1.66	1.76	1.83
Serum glucose (blood glucose)	GLU	mg/dl	2.78	3.94	2.74
Serum phosphorus	P	mmol/l	3.15	3.22	3.31
Serum amylase	AMY	U/L	2.00	21.00	2.00
Total cholesterol	CHOL	mmol/l	1.62	3.29	2.61
Alanine aminotransferase	ALT (TGP)	mmol/l	113.00	90.00	88.00
Aspartate aminotransferase	AST (TGO)	mmol/l	311.00	78.00	75.00
Total bilirubin	TBIL	umol/l	4.89	4.62	2.99
Alkaline phosphatase	ALP	U/L	179.00	258.50	211.00
Serum creatinine	CRE	umol/l	121.00	184.50	270.00
Creatine kinase	CK	U/l	2597.00	1384.50	1819.50

Serum protein values at the beginning and at the end of the experiment vary between 10.45 g / L and 12.60 g / L. These values fall within the normal limits specified in the literature. In the experimental lot, where the diet was supplemented with enzymes and phytoadditives, the value of serum protein showed an increased value that can be correlated with increased appetite for food.

The health of fish influences the variation of proteinemia, both in value and in terms of protein fractions in serum. Increasing the level of total serum protein indicates an adequate intake of protein from food.

The albumin / globulin ratio is well above the 0.3 limit which means a state of stress of the biological material. Decreasing the value of albumin below the normal limit suggests a disturbance of the function of protein synthesis in the liver which is not valid for the biological material involved in the present experiment.

Blood plasma glucose and cholesterol levels change easily under the influence of external or internal factors, which explains its importance as a reference biochemical indicator in assessing the normality of the general physiological state. Blood glucose and cholesterol values for the Siberian sturgeon involved in the experiment remained within normal limits in the experimental lot (GLU - 2.74 mg / dL and CHOL - 2.61 mmol / L) and a slight increase in the control lot (GLU - 3.94 mg / dL and CHOL - 3.29 mmol / L), therefore the mechanisms with the finest homeostatic regulation, in which the hepatopancreas, some extrahepatic tissues and a series of endocrine glands participate, took place normally in the experimental lot fed with improved diets.

The normal values of carbohydrates in the experimental lot create the premises for the normal development of vital processes and there are no imbalances in the metabolic pathways.

Enzyme and phytoadditive feeds were not a stress factor for the biological material involved in the experiment, haematological parameters confirming the adaptability of the biological material to the diets supplemented with enzymes and phytoadditives. The experiment showed that *Acipenser baeri* is a species sensitive to the active components introduced into the food and without haematological reaction to growth densities in the aquaculture systems experienced by Alireza H. Et al. 2013 [1].

Hepatosomatic and viscerosomatic indices, bioindicators of exposure to contaminants and stressors were calculated at the beginning and at the end of the experiment (table 5).

Table 5 Viscerosomatic index (VSI) and Hepatosomatic index (HSI) of *Acipenser baeri* fingerlings fed with diets containing digestive enzyme and phytoadditives after 60 days

Parameters (%)	**Initial**	**After 60 days of experiment.**	
		Lot C	**Lot Exp**
Viscerosomatic index (VSI)	12.85	11.85	10.65
Hepatosomatic index (HSI)	4.80	4.62	3.3

The results (Table 5) indicate a decrease in the value of hepatosomatic (HSI) and viscerosomatic index in the experimental lot compared to the control lot, which highlights a liver and digestive tract with correct functions of digestion, absorption, synthesis and secretion of digestive enzymes, and of carbohydrate metabolism and which has adapted very well to diets enriched with enzymes and phytoadditives. Liver function was stimulated by adding phytoadditives to the diet.

Information on the use of digestive enzymes in sturgeon is quite limited. The VSI and HSI values identified for the experimental lot in the present experiment are comparable to those obtained by Huriye A.K., et al., 2021 [4], who studied the growth of *Acipenser baerii* using diets with added phytase.

CONCLUSIONS

Diets with enzymes and phytoadditives indicate positive changes in haematological values, which are reflected in the improvement of the immune response and disease resistance of juvenile Siberian sturgeon, leading to a decrease in the mortality rate.

Enhanced diets with enzymes and phytoadditives, ensure the obtaining of products with quantities and ratios in PUFA up to the level of functional foods, compared to the usual technologies applied in RAS (recirculating aquaculture system).

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