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THE FEASIBILITY OF USING WHITE LUPIN MEAL IN THE FEED OF JUVENILE SIBERIAN STURGEON (*ACIPENSER BAERII*)*

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Abstract

As with other fishes farming, sturgeon farming depends on the use of well-balanced feed to maintain production. Commercial feeds available on the market are often based on soy protein, which, unfortunately, has many adverse effects on these fish. Therefore, alternative constituents are being sought that could be used in sturgeon fish farming. This study was designed to assess the feasibility of using diets containing white lupin meal at levels of 5% and 10% for juvenile Siberian sturgeon, compared to a formulated control group without lupin inclusion and a reference group fed commercial feed. Histological and immunohistochemical analyses were performed, combined with digestive enzyme activity assays. This study showed that feeding with white lupin meal did not adversely affect the rearing parameters, histology, or enzyme metabolism of sturgeons. An increase in the length of intestinal folds in the anterior intestine was observed in the group with 10% lupin meal inclusion, while this trend was not present in the spiral intestine, which may be indicative of a compensatory physiological mechanism when fish are fed less digestible feed. Antioxidative mechanism impairment was also noted in the two experimental groups. However, further research is still required to determine the possibility of using white lupin meal in the feeding of sturgeon fish, especially for more mature specimens.

Key words: alternative protein sources, aquaculture, fish feeding, sturgeon, white lupin

The Siberian sturgeon (*Acipenser baerii*) is a critically endangered species of freshwater fish of the sturgeon family (Acipenseridae) that naturally occurs in Siberian rivers and Lake Baikal, although it is also traditionally used in the aquaculture industry for meat and caviar. The largest producers are China, Russia, France, Poland, Germany, and Italy (Chebanov and Williot, 2018; IUCN, 2022; FAO, 2022). Acipenseridae, in general, are characterized by several problematic traits, such as late sexual maturation, long intervals between spawning, and high sensitivity to water pollution. Furthermore, overfishing and the destruction of natural ecosystems, the feminization of stocks fed with commercial feeds, and the prices of the feed itself are all factors that affect the quality of stocking material and increase the price of both meat and caviar (Hung, 2017; Kamaszewski et al., 2017; Kolman and Kapusta, 2018).

Sturgeons, like other species kept in aquaculture, need well-balanced feed, in which a sufficient amount of protein is required for proper growth and development. In the past, fish meal was unquestionably the main source of protein used in compound aquafeeds. However, the explosive growth of the aquaculture industry has caused demand for this raw material to outstrip supply, driving up fish meal prices, and leading to the overfishing of natural habitats and disruption of aquatic ecosystems

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(Rawles et al., 2011). Therefore, alternative sources of protein are being sought for inclusion in feed mixtures for aquatic organisms (Kamaszewski et al., 2014 a; Dossou et al., 2018; Potki et al., 2018; Zaretabar et al., 2020).

Research on the use of alternative protein sources in the feeding of fish has been carried out for more than 20 years (Tacon, 1997). Plant-based sources have been of greatest interest, mainly soybeans (Gatlin et al., 2007; Jiang et al., 2018), but research has also been conducted on the use of corn (Potki et al., 2018), wheat gluten (Davies et al., 1997; Tusche et al., 2012; Kamaszewski et al., 2014 a), barley (Morken et al., 2011; Zaretabar et al., 2020), and rapeseed (Luo et al., 2012; Dossou et al., 2018). However, despite their attractive prices, there are some limitations to the use of plant-based feeds in fish nutrition; these arise mostly from the presence of anti-nutritional factors that can have a negative impact on protein digestion and amino acid bioavailability (Gilani et al., 2012; Lazzarotto et al., 2015; Szczepański et al., 2022). Plant proteins are usually less digestible than animal proteins (Gaylord et al., 2008), which, together with reduced absorption, plays a major role in limiting fish growth (Harpaz and Uni, 1999). Therefore, the complete replacement of fish meal with protein alternatives is not a common approach used in present-day aquaculture, as the optimal level of inclusion of dietary plant protein has to be determined experimentally, and is specific for every species (Hua et al., 2019).

Commercial feeds dedicated to sturgeon rearing are often based on soybean meal as the main source of protein. Despite its high protein content and favorable amino acid profile, soybean meal contains many anti-nutritional factors, the most dangerous of which are phytoestrogens, which disrupt sex determination and stimulate the development of intersex gonads (Kamaszewski et al., 2017; Fajkowska et al., 2021). In this context, plants of the genus *Lupinus* may provide an alternative, as they occur over a wide geographic range and exhibit high resistance to both biotic and abiotic factors; which is why they can be successfully grown even in less fertile soils (Abraham et al., 2019). They are likewise characterized by a favorable amino acid profile, but do contain a relatively small number of anti-nutritional factors, which, however, can be almost entirely eliminated by seed processing (Enneking and Wink, 2000; Pieper et al., 2016). The main cultivated lupin species are as follows: white lupin (*Lupinus albus*), yellow lupin (*Lupinus luteus*), narrow-leafed lupin (*Lupinus angustifolius*), and pearl lupin (*Lupinus mutabilis*) (Abraham et al., 2019). Multiple studies conducted on various fish and shrimp species have yielded promising results, where in most cases it was possible to replace fish meal with lupin meal by up to 40–50% (Szczepański et al., 2022). However, there is no published data on the use of lupin in the feeding of sturgeons. Therefore, the aim of this study was to determine the effects of introducing white lupin meal into the diet of juvenile Siberian sturgeons, assessed on the basis of gross production parameters, histological and enzymatic development markers, and the homeostasis of the alimentary tract.

Material and methods

Adherence to ethical guidelines

The experiment was conducted in accordance with mandatory ethical standards, following Polish and international guidelines. Experimental rearing was conducted under conditions of the Sturgeon Fish Breeding Facility in Pieczarki, belonging to the National Inland Fisheries Research Institute in Olsztyn (Poland), under veterinary permit number WNI-28199201 and animal test permit number 056.

Rearing conditions

The experimental animals were juvenile Siberian sturgeons (*Acipenser baerii*) obtained during controlled spawning at the Sturgeon Fish Breeding Facility, Pieczarki. Four females (body weight 18.2–43.5 kg) and five males (body weight 8.6–12.3 kg) were selected as brooders. Egg retrieval and spawning were performed according to previously described procedures (Fopp-Bayat et al., 2007; Feledi et al., 2011). The fertilized eggs were incubated for eight days in McDonald jars, and mass hatching occurred on the ninth day after fertilization. The larvae were then transferred to a recirculation aquaculture system (RAS) consisting of ten tanks with a working volume of 4 m^3 each (ST 19–22, SDK Poland). The RAS was equipped with oxygen generators and a biofilter, type SDK CN 3.2 (SDK, Poland) containing a Light Bioelementer plastic filling having a total volume of 1.5 m^3 (RK Plast A/S, Denmark). Fish rearing was performed up to 60 days post hatching (DPH) according to previously described procedures (Kolman and Kapusta, 2018).

Experimental assumptions and diet preparation

For the experiment, 3,600 Siberian sturgeon juveniles (average body weight 3.9±0.3 g) aged 61 DPH were selected. The fish were placed in 10 tanks with an initial stocking rate of 360 fish per tank. Three experimental groups were set up in which the fish were fed composed feed: a control group with no lupin meal content (L0), and two groups with white lupin meal inclusion at 5% (L5) and 10% (L10). Three batches of each of these feeds were used (sequentially labelled: Feed A, B, C), as their overall composition was slightly adjusted to address the changing nutritional demands of the fish. An additional reference group (R) was fed with a commercial feed dedicated to sturgeon rearing (protein content, 48%; lipid content, 21%; according to the manufacturer's information: Aller Aqua, Poland). This group was established to compare general growth characteristics, and histological and enzymatic parameters. The granularity of the feeds was adapted to the growing fish, while the particle/pellet sizes (0.2–2.0 mm) of the experimental feeds were identical to those of the commercial Aller feed. The composition of the experimental feeds is shown in Table 1.

Table 1. Approximate compositions (g kg^{-1} of dry weight) of the experimental diets

Raw material (g kg^{-1})	Feed composition								
	Feed A			Feed B			Feed C		
	L0	L5	L10	L0	L5	L10	L0	L5	L10
White lupin	0	50	100	0	50	100	0	50	100
Wheat flour	115	90	60	110	70	45	150	110	75
Fish meal	620	600	570	580	570	550	450	455	430
Krill meal	120	95	90	100	100	85	60	40	50
Wheat gluten	50	50	50	50	50	50	50	50	50
Poultry blood meal	65	85	100	90	90	100	150	150	150
Fish oil	0	0	0	40	40	40	110	115	115
Premix	30	30	30	30	30	30	30	30	30
Nutrient (%)									
Dry matter	92.2	91.4	92.1	94.4	94.3	94.5	92.1	92.5	91.6
Crude protein	67.0	65.6	64.7	62.8	62.2	63.1	55.3	56.0	56.1
Crude fat	3.9	4.3	4.4	12.2	11.3	12.3	14.9	15.8	14.9
Ash	10.1	9.9	9.8	11.1	10.8	10.9	11.1	10.7	10.7
Fiber*	1.9	1.6	1.1	1.7	1.5	1.3	1.6	1.3	1.2
NFE**	9.3	10.0	12.1	6.6	8.5	6.9	9.2	8.7	8.7
Gross energy (MJ kg^{-1})	19.4	19.3	19.4	21.2	21.0	21.3	20.9	21.2	20.9

*Fiber was estimated on the basis of the component suppliers' declarations.
**Nitrogen-free extract (NFE) was calculated by subtracting the other four parameters from dry matter.

The L0, L5, and L10 groups were reared in triplicate tanks, while the fish in the R group were maintained in a single tank. The duration of the experiment was 125 days. Fish were fed by hand every four hours (6 times/day). Feed intake was observed (during and between feedings); feces were removed each day; and losses among the reared fish were checked daily.

Physical and chemical water parameters

During the experiment, the physicochemical parameters of the water at the reservoir outflow were monitored. Oxygen content and pH were determined using a Cyber-Scan PCD 5500 meter (Eutech Instruments, USA). Total ammonium nitrogen (TAN = $NH_4^+ + NH_3$) and nitrite nitrogen (NO_2) concentrations were determined using a spectrophotometer (Aquamate UV-Vis Plus). Water quality parameters were measured at least twice a week and water temperature (19.1±2.1ºC) was measured daily. The concentration of oxygen in the water flowing out of the tank did not fall below 9.3 mg O_2 l^{-1}, and the pH values ranged from 7.3 to 7.7. The maximum concentrations of ammonia nitrogen and nitrite nitrogen did not exceed 0.264 mg TAN l^{-1} and 0.011 mg NO_2 l^{-1}, respectively. The water flow rate in the tanks was maintained at 12 dm^3 min^{-1}.

Rearing indices

The biomass was inspected daily. The survival rate during the studied period was 100%. Fish whose visceral tissues were collected for histological and biochemical analyses were euthanized with tricaine methanesulfonate (MS222, 130 mg/dm^3) and then measured and weighed (12 individuals from each group, total $n = 48$), while the experimental feeding setup was continued for other purposes. Based on the measurements taken, it was possible to determine the following indices:

$$WG\ (weight\ gain,\ g fish^{-1}) = (BW_f - BW_i)$$
$$SGR\ (specific\ growth\ rate,\ \%\ d^{-1}) = 100 \times [(\ln BW_f - \ln BW_i) \times t^{-1}];$$
$$FCR\ (feed\ conversion\ ratio) = TFI \times (B_f - B_i)^{-1};$$
$$CF\ (condition\ coefficient) = (BWf \times 100) \times TL^{-3};$$

where BWi = initial mean body weight (g); BW_f = final mean body weight (g); TL = total length (m); TFI = total feed intake (g); t = duration of the experiment (days); B_f = final stock biomass (g); B_i = initial stock biomass (g).

Histological and immunohistochemical analysis

The collected sections of liver, anterior, and spiral intestines were fixed in Bouin's fluid and subjected to standard histological techniques (12 individuals from each group, n=48). Histological sections were stained with hematoxylin and eosin (HE). The neutral and acidic mucosal cells were stained with Alcian blue and periodic acid Schiff (AB/PAS).

The following histomorphometric parameters were studied: anterior intestine fold height (μm) (AFH); anterior enterocyte height (μm) (AEH); supranuclear area of enterocytes of the anterior intestine (μm) (ASH); width of the lamina propria of the anterior intestine (μm) (AWLP); spiral intestine fold height (μm) (SFH); spi-

ral enterocyte height (μm) (SEH); supranuclear area of enterocytes of the spiral intestine (μm) (SSH); width of the lamina propria of the spiral intestine (μm) (SWLP); number of $CD3^+$ cells in the anterior intestine (%); hepatocyte area (μm^2) (HA); and the hepatocyte nuclear area (μm^2) (HNA). The hepatonuclear index was calculated (%) (HNI) based on the values of HA and HNA (Prusińska et al., 2020). The detection of $CD3^+$ immune cells in the anterior intestine was performed immunohistochemically by using an anti-$CD3^+$ antibody (Leica, Bond System), and through visualization using a Leica Novocastra HRP Polymer Kit, according to the manufacturer's protocols. The number of $CD3^+$ cells was calculated within the middle, 100 μm long segment of the intestinal folds and expressed as a percentage of all cells quantified within that area. Microscopic analysis was performed using a Nikon ECLIPSE Ni-E microscope and a computer image analysis system, NIS-Elements AR (Nikon Corporation, Japan).

Enzymatic analysis

Sections of the livers and both intestinal parts were frozen in liquid nitrogen and then stored at −80°C. The collected tissues were then homogenized in deionized water, at 4°C, and centrifuged at 14 000g for 10 min at the same temperature. The supernatant was collected, and the tissues were refrozen in liquid nitrogen and stored at −80°C. Enzymatic activity was determined according to the methodology described by Kamaszewski et al. (2014 b) and Palińska-Żarska et al. (2021). For the livers, the activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) were examined using SPINREACT kits (Spain; REF 1001131 and 1001122, respectively). Analyses of the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were performed using Randox kits (Great Britain; REF SD125 and RS505, respectively). For the anterior and spiral intestines, the activities of ALP and ACP were analyzed as described above, while the activities of amylase (Amyl), trypsin (Trip), lipase (Lip), and chymotrypsin (Chym) were evaluated according to the methods described by Kamaszewski et al. (2014 b). The gross activity results were divided by the protein concentration in the sample and expressed as IU g^{-1}, with the total protein content determined using the methodology presented by Lowry et al. (1951). The assays were conducted following the previous adaptation of the indicated methodologies in 96-well plates (Wiszniewski et al., 2022). Enzymatic activity was determined from absorbance readings at 37°C using a Tecan microplate spectrophotometer (Infinite 200 PRO); determinations were made in triplicates.

Statistical analysis

The study results were statistically analyzed using the program Statistica (TIBCO Software Inc., Palo Alto, California, USA). The Shapiro-Wilk normality test was performed for all tested parameters. Homogeneity of variance was checked using the Levene test, followed either by factor variance analysis ANOVA supported by the Tukey post hoc test and the NIR Fisher test for rearing and histological parameters, or by a non-parametric Kruskal-Wallis test for biochemical analyses.

Results

Growth performance and nutrient utilization

Sturgeons from the R group had the highest average total length compared to the other groups. Furthermore, fish from the R group were statistically larger (SL) and heavier (BW_f) than those from the L5 and L10 experimental groups. No significant differences were observed in the condition coefficient. FCR and SGR differed between groups, reaching their highest values in group R and lowest in groups L5 and L10 (Table 2).

Histology

The effects of white lupin meal on gastrointestinal tract histology are presented in Table 3.

Table 2. Rearing parameters for the studied Siberian sturgeons (total *n*=48)

Parameter	Groups			
	R	L0	L5	L10
TL (m)	0.286 a±0.034	0.254 b±0.027	0.237 b±0.024	0.239 b±0.023
SL (m)	0.218 a±0.026	0.198 ab±0.019	0.185 b±0.0195	0.186 b±0.0189
BW_f (g)	65.64 a±24.36	47.73 ab±15.90	38.81 b±12.05	43.24 b±10.95
WG (g)	62.04 a±24.06	43.83 b±15.60	34.91 b±11.75	39.34 b±10.65
SGR	2.26 a±0.16	2.14 b±0.12	2.04 c±0.13	2.05 c±0.14
FCR	1.92 a±0.24	1.39 b±0.13	1.29 c±0.13	1.23 c±0.13
CF	0.27 nd±0.02	0.28 nd±0.03	0.29 nd±0.03	0.31 nd±0.04

Means (± SD) followed by different letters in the same row are significantly different ($P<0.05$), nd – no differences, calculated using the parametric ANOVA test; TL – total length (m); SL – standard length (m); BW_f – final body weight (g); WG – weight gain (g); SGR – specific growth rate; FCR – feed conversion ratio; CF – condition coefficient.

Table 3. Histological morphometrics of the liver and intestines of the studied Siberian sturgeons

Parameter	Groups			
	R	L0	L5	L10
AFH	1019.21 a±114.21	1056.29 ab±99.67	1063.79 ab±103.81	1073.94 b±146.04
AEH	40.49 a±3.10	39.88 ab±3.04	38.64 b±3.3	39.25 ab±3.44
ASH	20.58 ab±2.35	21.53 a±2.11	19.10 b±2.45	20.25 ab±2.77
AWLP	5.87 a±0.98	5.64 bc±1.22	5.23 b±0.94	5.73 bc±5.73
CD3+	7.37 a±3.98	4.16 b±2.54	6.57 b±8.73	4.12 b±3.11
SFH	462.55 a±58.29	452.60 a±55.97	379.74 b±53.27	366.16 b±45.46
SEH	44.85 a±4.72	42.35 b±5.19	39.17 c±5.01	39.20 c±3.60
SSH	31.42 a±6.39	26.82 b±3.14	25.54 b±6.59	23.02 c±3.00
SWLP	5.29 a±0.88	4.74 b±0.72	4.68 b±1.21	5.84 c±0.98
HA	232.36 a±55.69	334.49 b±127.83	314.13 b±104.18	262.09 a±88.45
HNA	40.41 nd±7.23	41.18 nd±10.18	37.80 nd±6.92	38.14 nd±5.79
HNI	0.164 a±0.03	0.144 b±0.04	0.136 b±0.02	0.149 b±0.02

Means (± SD) followed by different letters in the same row are significantly different ($P<0.05$); nd – no differences, calculated using the parametric ANOVA test.

AFH – anterior intestine fold height (µm); AEH – anterior enterocyte height (µm); ASH – supranuclear area of enterocytes of the anterior intestine (µm); AWLP – width of the lamina propria of the anterior intestine (µm); SFH – spiral intestine fold height (µm); SEH – spiral enterocyte height (µm); SSH – supranuclear area of enterocytes of the spiral intestine (µm); SWLP – width of the lamina propria of the spiral intestine (µm); $CD3^+$ – number of CD3 positive cells in anterior intestine (%); HA – hepatocyte area (μm^2); HNA – hepatocyte nuclei area (μm^2); HNI – hepatonuclear index (%).

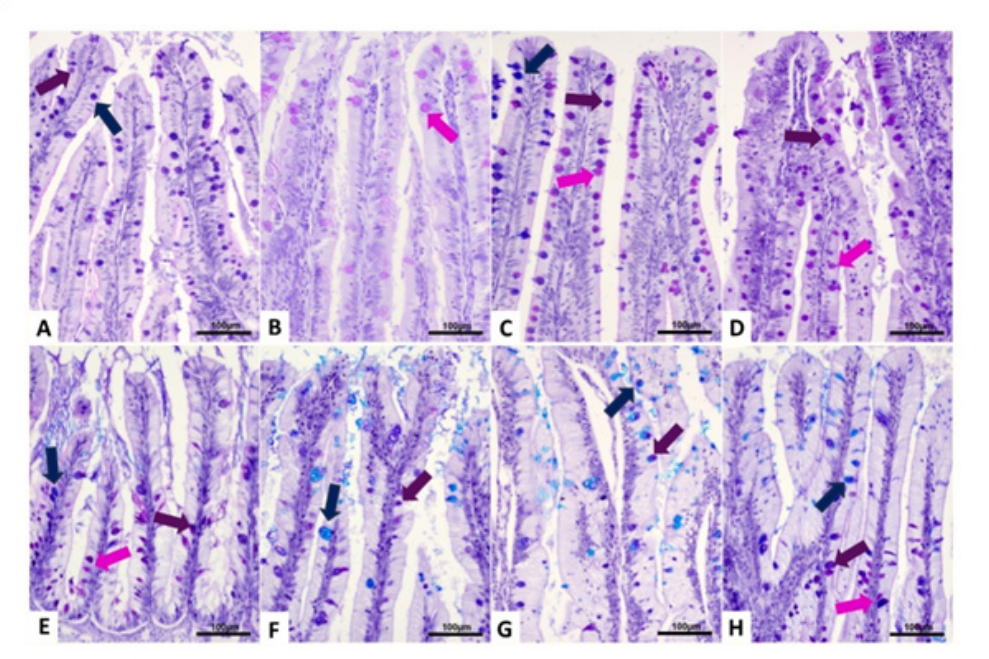

Figure 1. Microscope images of the intestines of the studied Siberian sturgeons. The anterior intestines (A–D) in the groups fed the experimental feeds were dominated by mucus-producing goblet cells that had a neutral reaction. This relationship was not observed in the spiral intestines (E–H), where cells of all three types were visible, except in the L0 group. Group R – A and E; L0 – B and F; L5 – C and G; L10 – D and H. Dark blue arrow – acidic goblet cells; dark violet arrow – neutral or mixed goblet cells; pink arrow – neutral goblet cells. AB/PAS staining; scale 100 µm

In all individuals, the anterior intestinal mucosa was properly differentiated and in groups R, L5 and L10 it contained acidic, mixed and (predominantly) neutral goblet cells, and also immune cells. However, in group L0, only neutral goblet cells were present (Figure 1). Fish in the L10 group showed a statistically higher AFH compared to the R group, and the lowest AEH was noted in the L5 group (Table 3). In the groups fed with the lupin supplementation, there was a local enlargement of the lamina propria at the top of the intestinal folds, along with an epithelial desquamation of minor severity. An increase in the AWLP was also observed in the R group relative to the other groups. The cells labelled as $CD3^+$ that infiltrated the intestinal folds in the anterior segment were mainly observed to be scattered cells, with the highest number being observed in the R and L5 groups.

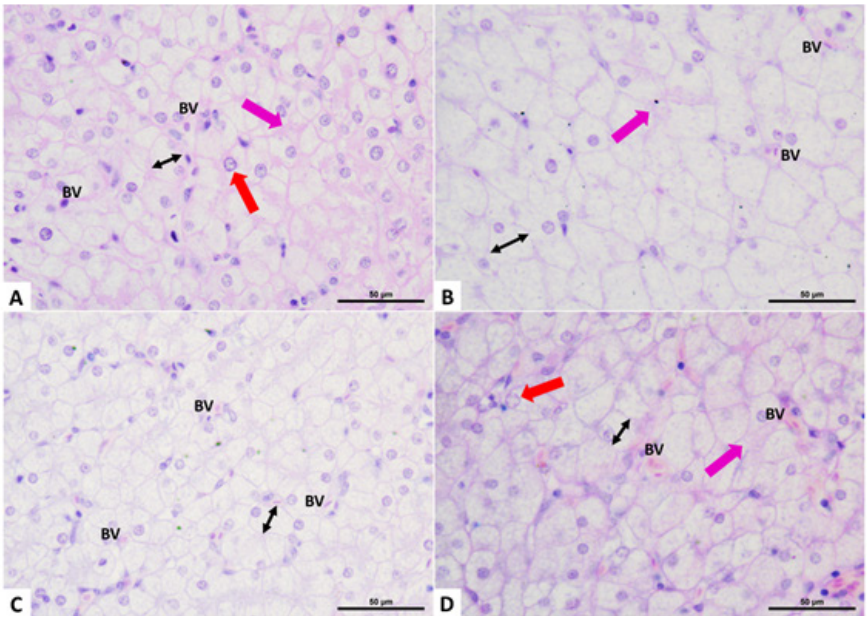

Figure 2. Microscope images of the livers of the studied Siberian sturgeons with clearly visible steatosis. Group R – A; L0 – B; L5 – C; L10 – D. Pink arrow – glycogen deposits; red arrow – enlarged hepatic nuclei; black double arrow – single hepatocyte; BV – blood vessel. AB/PAS staining; scale 50 µm

Table 4. Results of the biochemical analyses (digestive enzymes and oxidative stress parameters) of the studied Siberian sturgeons

Organ	Groups			
	R	L0	L5	L10
Anterior intestine				
ALP	136.4 nd±59	141.2 nd±55	144.7 nd±52	130.0 nd±51
ACP	2.6 nd±1	2.4 nd±1	2.0 nd±0.5	2.1 nd±0.5
Lipase	28.5 a±12	30.7 a±21	40.5 a±26	5.3 b±2
Amylase	16.0 ab±10	33.9 a±23	5.7 b±7	14.1 ab±9
Chymotrypsin	93.4 nd±60	74.9 nd±22	54.8 nd±49	49.9 nd±31
Trypsin	28.0 nd±23	31.1 nd±13	25.0 nd±23	29.1 nd±19
Spiral intestine				
ALP	315.8 nd±160	264.2 nd±109	456.4 nd±236	307.3 nd±107
ACP	1.7 a±0.4	2.5 b±0.9	2.4 ab±0.7	1.8 ab±0.6
Lipase	5.9 a±6	26.4 b±12	26.4 b±8	3.2 a±1
Amylase	18.4 nd±13	14.6 nd±17	8.0 nd±4	16.7 nd±8
Chymotrypsin	64.2 nd±24	56.7 nd±27	77.8 nd±42	56.2 nd±31
Trypsin	35.9 nd±24	25.0 nd±24	48.6 nd±28	37.4 nd±16
Liver				
ALP	242.7 a±93	135.9 a±53	55.0 b±13	50.0 b±13
ACP	3.1 a±0.7	3.0 a±0.7	1.4 b±0.3	1.6 b±0.5
SOD	24.2 a±12	24.3 a±6	4.3 b±2	4.9 b±2
GPx	253.3 ab±54	449.9 a±198	256.7 ab±102	214.2 b±92

Means followed by different letters in the same row are significantly different ($P<0.05$); nd – no differences, calculated using the non-parametric Kruskal–Wallis test.

All displayed parameters (means ± SD) indicate IU g^{-1} of total protein.

The spiral intestine was characterized by enterocytes with a clearly visible supranuclear region and a very bright cytoplasm (Figure 1). Similarly to the anterior intestine, all three types of goblet cells were found, once again with the exception of group L0, where no neutral cells were observed. The greatest SFH and SEH in this intestinal section were observed in the R group. The lowest SFH was found in the L10 group, whereas, individuals in the L5 and L10 groups had the lowest SEH.

The sturgeons' livers were characterized by severe steatosis, with the largest HA observed in the L0 group. There were single glycogen granules in the hepatic parenchyma of groups R and L10 (Figure 2).

Digestive enzymes and oxidative stress

The effects of white lupin meal on digestive and oxidative stress enzymes are shown in Table 4.

No statistically significant differences were observed in ALP, ACP, chymotrypsin, or trypsin activity in the anterior intestine. Sturgeons in the L10 group had the lowest lipase activity. There was a significant difference in amylase activity between the L0 and L5 groups. In the spiral intestine, statistically the highest ACP activity was observed in the L0 group. An increase in lipase activity was observed in the L0 and L5 groups.

Reduced ACP, ALP, and SOD activities were observed in the livers of fish in the L5 and L10 groups. GPx showed the highest activity in the L0 group and the lowest activity in the L10 group. The R and L5 groups showed no statistically significant differences compared to the other groups.

Discussion

Aquaculture, as with other areas of intensive livestock production, relies on appropriately balanced feed to maintain production (Glencross et al., 2020). The main concern is to concurrently preserve animal welfare and maintain high productivity, while also considering all the economic aspects of such practices. In this context, the previously accessible fish meal and fish oil for aquaculture have become expensive raw materials, the sourcing of which also raises ethical issues. For this reason, feeds containing alternative protein sources or other ingredients are formulated (Hodar et al., 2020). However, when introducing new nutrients into fishes' diets, consideration should be given not only to basic production indicators, such as growth or weight, but also to the health and welfare of the fishes. Introducing alternative sources of protein involves risks such as altering the morphology of the digestive tract, and enzyme management (Hung, 2017). All these factors can lead to digestive disorders, which negatively affect production potential, and, as a result, lead to stock losses, both in juvenile and in mature fish.

Based on previous studies of various fish and shellfish species, feeds with a reasonably balanced lupin meal content can be successfully used in aquaculture (Szczepański et al., 2022). In a study by Acar et al. (2018) on the use of white lupin meal in the feeding of salmonid fish, there was a stunting of fish growth at 45% and 60% lupin meal inclusion; however, in the 15% inclusion group there was an improvement both in growth and in feed efficiency ratio. An experiment conducted on blackhead seabream (*Acanthopagrus schlegelii*) showed that it is possible to replace fish meal with lupin meal at a level of 30%, but a 50% replacement resulted in suppressed growth (Zhang et al., 2012). In the present study, coefficients such as FCR and SGR showed significant differences between the experimental and control groups, but the feed was readily taken up by the fish and no deaths were recorded in the stocks. Therefore, the observed differences in fish weight and length were not dependent on the food intake.

It is worth noting that plant-derived feed components may contain anti-nutritional factors that negatively affect feed utilization, especially in carnivorous fish (Lazzarotto et al., 2015). Additionally, it should be indicated that the processing and treatment of the feed (Borreani et al., 2009) and how it is administered all affect its bioavailability. Feeding soy-based products to fish is a common cause of abnormalities and diseases such as enteritis, which is characterized by abnormal intestinal villi and lamina propria, enterocytes lacking supranuclear vacuoles, the lamina propria and submucosa being infiltrated by immune cells, and abnormal intestinal ion and water transport (Refstie et al., 2006; Kiron et al., 2020; Nimalan et al., 2022). The introduction of plant-derived ingredients into the diet of the Amur sturgeon (*Acipenser schrenckii*) caused hepatic dysfunction and also disturbances in the spiral intestinal microbiota (increasing in severity with plant-protein inclusion levels), which, consequently, led to feed absorption and digestibility problems (Wei et al., 2020). In the present study, differences in FCR and SGR values may also have been due to intestinal and hepatic disturbances under the influence of plant-derived compounds. Therefore, it is important to consider the effects of the components contained within the feed on the morphology of the gastrointestinal tract, as well as enzyme-based metabolism.

Intestine health is largely impacted by diet and can be assessed by evaluating histological parameters, which evidence the occurrence of structural changes in the intestine (Aidos et al., 2023). In sturgeons, the anterior and spiral intestines are clearly distinguished (Bucking, 2015). In teleost fishes, the anterior intestine is the main nutrient-absorbing region, while in sturgeons it is the spiral intestine, and is the most important section of the digestive tract, vastly increasing the absorptive area and digestion time while decreasing the overall size of the intestine itself (Jutfelt, 2011; Rodríguez et al., 2002). In aquatic animals, the height of the intestinal folds increases the surface area for nutrient absorption (Moldal et al., 2014). Studies have shown that feeding with soybean meal has a negative effect on the height of intestinal folds in fish (Baeverfjord and Krogdahl, 1996; Bakke-Mc Kellep et al., 2000; Gu et al., 2016). Similarly, a shortening of the intestinal folds was observed with the use of feed containing pea protein concentrate at an inclusion level of 35% (Penn et al., 2011). In the current study, however, different results were recorded, in which the longest intestinal folds were observed in the group with the highest content of white lupin meal, which may indicate good feed absorption or, on the contrary, the action of a compensatory mechanism, i.e., lengthening the intestinal folds to absorb as much nutrient as possible

from the hard-to-digest feed. Meanwhile, no significant differences were observed in the other groups, which may indicate that the use of white lupin meal did not have detrimental effects. However, the results from the anterior intestine did not correlate with those from the spiral intestine, where the shortest intestinal folds were observed in the L5 and L10 groups, while no significant differences were observed in the other groups.

Given that the spiral intestine also has immune functions (Wei et al., 2019), it can be conjectured that, at higher concentrations of plant proteins, compensatory processes occur in the organism to maintain homeostasis, however, this issue requires further research. Similarly, in the case of the intestinal epithelium, the greatest enterocyte height was recorded in the R group for both the anterior and spiral intestine, while lower values were shown for the composed feeds, which also indicates the problematic digestibility of these feeds (Kong et al., 2018). The intestinal epithelium also has immune functions, and the disruption of its integrity or other abnormalities can lead to disorders including enteritis (Sitjà-Bobadilla et al., 2019). The intestinal epithelium also contains cells that secrete mucus, which plays a role in the immune response (Kiron, 2012). Additionally, intestinal mucus is involved in digestive processes by creating an optimal environment for the action of digestive enzymes (Kim and Ho, 2010; Nimalan et al., 2022). Research on Atlantic salmon (*Salmo salar*) showed that fish fed plant-based feed had increased counts of mucous cells due to intestinal inflammation (Nimalan et al., 2022). Similar results have been obtained for red seabream (*Pagrus major*) (Dawood et al., 2015) and Nile tilapia (*Oreochromis niloticus*) (Standen et al., 2013). In the current study, no such trend was observed, and acidic and mixed goblet cells were found.

Like the intestines, the liver plays an important role in the digestion of food (Field et al., 2003), therefore, liver parameters should also be considered when assessing the suitability of feed. The surface area of hepatocytes depends on the amount of stored lipids and glycogen, whereas, the hepatocyte nuclear area may be an indicator of its metabolic activity, thus a reduction in the area of the nucleus may indicate nutritional deficiencies (Kasprzak et al., 2019; Ostaszewska et al., 2005). The hepatocyte area was reduced in the R and L10 groups compared to the other groups, which may have been caused by the higher proportion of plant protein components in the feed, while the lack of differences in nuclear area between any of the groups may indicate the absence of feeding inadequacies.

The physiology of fish nutrition is primarily based on digestive enzyme activity. The presence of enzymes at appropriate locations in the wall and along the lumen of the intestinal tract affects the ability of fish to utilize ingested nutrients (Mir and Channa, 2010). The distribution and activity of digestive enzymes varies with intestinal morphology and feeding habits (Gawlicka et al., 2002; Kolkovski, 2001; Mir and Channa, 2010). Furthermore, the diet and nutritional requirements of sturgeons change depending on their developmental stage (Kolman and Kapusta, 2018). There are three main developmental phases in sturgeons: the yolk sac stage; the actively feeding larval stage; and the metamorphosis stage, in which enzymatic activity reaches values similar to those of juveniles and adults (Babaei et al., 2011; Żółtowska et al., 1999). It is worth noting that the differences in nutritional requirements and enzymatic activity of sturgeons depend not only on age, but also on feed composition, innate feeding habits, and genes (Napora-Rutkowski et al., 2009). In the current experiment, juvenile Siberian sturgeons with an established enzymatic profile were used, so it was expected that any differences in enzyme activity would depend only on the components in the feed. To reliably test the overall ability of sturgeons to digest feed containing a lupin meal inclusion, a variety of digestive enzymes responsible for the digestion of different compounds were selected: alkaline phosphatase, lipase, amylase, trypsin, and chymotrypsin.

Alkaline phosphatase (ALP) is an enzyme of the metalloenzyme family, and is found mainly in cell membranes (Banaee, 2020; Lallès, 2019). This enzyme is primarily involved in phosphate hydrolysis and membrane transfer (Derikvandy et al., 2020). The main organs in which ALP is synthesized are the liver and bones, and, to a lesser extent, the kidneys and intestines (Banaee, 2020). This enzyme is widely used to determine nutrient absorption and intestinal function (Lallès, 2020). Higher protein content in feed also enhances ALP activity (Wiszniewski et al., 2022). Elevated ALP levels may indicate damage to hepatocytes (Sharma et al., 2014; Vetrivel et al., 2014). In the current study, increased ALP concentrations were observed in the livers from the R and L0 groups, which may indicate liver damage; additionally, the R group was characterized by high steatosis. Sturgeons fed with feed supplemented with lupin meal showed a statistically significant lower activity of this enzyme, which may indicate normal absorption of nutrients and the absence of negative effects on liver cells. A similar trend, however, was not observed in the intestines, where no differences in ALP activity were found. Therefore, it can be assumed that feed supplemented with white lupin meal does not adversely affect ALP activity and thus the digestive capacity of sturgeons.

Carbohydrates and lipids are important non-protein components used in aquatic feeds to improve protein utilization and reduce feed prices (Babaei et al., 2016). The addition of carbohydrates to the diet of Siberian sturgeons may have a positive effect on protein utilization and energy retention (Médale et al., 1991); however, some fish species demonstrate difficulties in digesting, metabolizing, and absorbing carbohydrates from the feed (Babaei et al., 2016). Amylase, which is mainly produced by the pancreas, is responsible for carbohydrate digestion. Its main task is to break down complex carbohydrates, such as starch and glycogen, into simple sugars by hydrolyzing glycosidic bonds (Akinfemiwa et al., 2023; Phetlum

and Champasri, 2023). In the conducted experiment there was significantly higher amylase activity in the anterior intestine in the L0 group compared to the fish in the L5 group, likely as a result of the decreasing inclusion of wheat meal in the experimental feed. No such trend was observed in the spiral intestine, which is responsible for slowing the transit rate of digesta through the gut and providing an increased surface area for the absorption of nutrients (Leigh et al., 2021). Amylase activity was relatively similar in the anterior and spiral intestines of sturgeons, in contrast to common carp (*Cyprinus carpio*), in which this enzyme shows greater activity in the distal part (Bilen et al., 2020). In carnivorous species, amylase activity is lower than in herbivorous species; its activity can also be affected by fish age and temperature (Kuz'Mina, 1996, 2002). In this study, feed with a white lupin meal inclusion at a level of 5% decreased amylase activity. This is contrary to what was observed in studies performed on trout (*Oncorhynchus mykiss*) and sea bream (*Sparus aurata*) that were also fed with a feed containing plant protein (Jalili et al., 2012; Vélez-Calabria et al., 2023).

Another enzyme responsible for the proper digestion of food is lipase, which is involved in the transport and digestion of lipids. The primary function of lipase is to break down insoluble fats into soluble forms by hydrolyzing them into fatty acids and glycerol, enabling the digestion and absorption of previously unavailable nutrients (Bhilave, 2019). In carnivorous fishes, most lipid absorption occurs in the anterior intestine (Denstadli et al., 2004). According to Socorro et al. (2000), lipase activity is affected by the fatty acid composition of dietary lipids. In fish, a higher lipase activity is observed when digesting feed containing higher amounts of polyunsaturated fatty acids (PUFA) than monounsaturated fatty acids (MUFA) (Kamaszewski et al., 2014 b; Morais et al., 2005). In lupin meal there are higher amounts of MUFA than of PUFA (Sotelo-Méndez et al., 2023), which may have affected lipase activity in the group with higher dietary lupin meal inclusion.

Trypsin and chymotrypsin are pancreatic enzymes with proteolytic activities (Rombouts et al., 2013). The breakdown of proteins leads to the formation of smaller molecules that can be digested and absorbed by enterocytes. Specifically, trypsin breaks down proteins into smaller peptides, while chymotrypsin further breaks down peptides into amino acids (Ma et al., 2005; Vajda and Szabo, 1976). Trypsin activity can be used to assess feed quality and the nutritional status of fish (Drossou et al., 2006). Abnormalities in trypsin activity in fish can occur during starvation (Napora-Rutkowski et al., 2009), or when fish are administered feed with a high fat content, which affects protein availability and proteolytic enzyme activity (Gawlicka et al., 2002). In the current study, decreased trypsin and chymotrypsin activity may indicate that the feed consumed by sturgeons was low in protein (Zambonino Infante and Cahu, 2007), whereas an increase in the activity of these enzymes may indicate animal malnutrition and an organismal response to maximize the utilization of peptides in the feed (Cara et al., 2007). In both the anterior and spiral intestine, the activity was not significantly different between the groups in the present study, from which it can be concluded that the feed with the addition of lupin meal did not affect the activity of these proteases, and that both the control and experimental feeds met the protein requirements of the sturgeons.

Antioxidant defense systems in fish can be impaired due to some types of nutrients and poor nutritional status (Chowdhury and Saikia, 2020). The effect of a malfunctioning antioxidant system is oxidative stress, which leads to tissue damage and impaired body functions, such as protein phosphorylation, activation of several transcriptional factors, apoptosis, immunity, and the differentiation of cells (Pizzino et al., 2017; Trenzado et al., 2006; Wiszniewski et al., 2022). Disturbances in the antioxidant system were observed in the L5 and L10 groups. According to Hoseini et al. (2022), these disorders may be caused by a deficiency of sulfuric amino acids in lupins. Despite their favorable amino acid profiles, lupins are deficient in methionine and cysteine (Khalid et al., 2016). However, in the case of black carp (*Mylopharyngodon piceus*), supplementation of their diet based on 32% lupin meal with lysine and methionine was not necessary. Fish fed this feed had a lower hepatic lipid content compared to those fed soybean meal feed, but the rearing indices were no different (Yang, 2001). According to Rueda-Jasso et al. (2004), feed containing increased amounts of lipids and carbohydrates can induce stress in fish, thus affecting their health status. When composing feeds, it is important to limit the levels of these compounds in order to support the healthy growth and development of the fish without disrupting their antioxidant system. In the present study, lipase activity was significantly lower in the anterior and spiral intestines of the sturgeons in the L10 group, which may correspond to reduced antioxidant enzyme activity, although further research is needed to confirm this hypothesis.

Histomorphometric analysis did not reveal any stimulation of the immune system in the intestines of the fish from the L0, L5, or L10 groups, which is a positive outcome, because economically important fishes, such as salmonids, have been found to develop inflammation (enteritis) when fed diets containing high levels of legume meal (Aslaksen et al., 2007; Baeverfjord and Krogdahl, 1996). One of the macrophage markers is acid phosphatase, which regulates the ability of these cells to phagocytose pathogens (Broeg, 2003). In addition, acid phosphatase (ACP) in the gastrointestinal mucosa plays a role in protecting against harmful agents, as well as being associated with the absorption and transport of metabolites (Mir and Channa, 2010). According to Gajger et al. (2013) and Kužir et al. (2012), ACP can be localized in the supranuclear area of enterocytes, as well as in the lamina propria, which coincides with the pinocytotic processes and protein digestion (Gisbert et al., 1999).

A decrease in ACP activity was found in the L5 and L10 groups in the livers of the studied fish, however, no such trends were observed in the anterior or spiral intestines. Despite differences in the sizes of the intestinal epithelium in the groups fed the white lupin meal feed compared to the R group, ACP activity was not affected, which may indicate that there were no differences in protein digestion between the studied sturgeon groups. The increased activity of this enzyme in the livers of the groups without lupin meal supplementation may be due to an increased number of immune cells, but it is difficult to clearly assess the cause of these differences and this phenomenon should be further analyzed and investigated. The R group displayed a notable infiltration of $CD3^+$ cells in the anterior intestine, suggesting that the immune system of the fish in this group had been activated. It needs to be mentioned, though, that increased numbers of intestinal $CD3^+$ cells may only be a temporary phenomenon, especially when stimulated because of diet (Kasprzak et al., 2023). Nevertheless, this conclusion was also confirmed by the enlargement of the lamina propria's width in both the anterior and spiral intestines in the sturgeons from the R group. Considering the severe occurrence of hepatic steatosis among the R group fish, it is even more important to find alternative sources of protein that can be used in compound feeds. However, enlargement of the lamina propria's width was also observed in the spiral intestine of L10 fish, which may support the earlier hypothesis that compensatory mechanisms are at work when exposed to increased amounts of plant proteins. Nevertheless, further research is needed to ascertain the effect of white lupin meal on the immune system of Siberian sturgeons.

Conclusions

The current investigation was undertaken during the early developmental stages of juvenile sturgeons, a period of particular importance for the subsequent formation of the digestive system. The morphoanatomical transformations occurring during this period have a long-lasting influence on fish health and growth. Nonetheless, it is not possible to assert with full certainty which level of lupin inclusion in animal feed is more favorable from the standpoint of producers and animal welfare. Taking into account the presence of adverse histopathological alterations in the group receiving commercial feed containing soybean meal and the substantial expense of the feed components utilized, the pursuit of alternative sources of protein components appears to carry even greater significance than previously recognized. The findings are promising, as the fish showed a willingness to consume the feed and no mortalities occurred during the experimental period, while the lupin meal did not adversely affect the histomorphology of the evaluated tissues. It should be noted that lupins are highly tolerant to environmental conditions and can be successfully grown on less fertile soils (Lucas et al., 2015). Given the availability of lupins (Abraham et al., 2019), the rising prices of other ingredients such as fish meal (Rawles et al., 2011), and the environmental and ethical aspects concerning soybean cultivation (Byerlee et al., 2014), lupin may be a promising alternative protein source for compound aquafeeds. Based on the results obtained for other fish species fed with feed supplemented with lupin meal, it can be presumed that long-term feeding with low lupin meal content could also bring positive results for Siberian sturgeons, especially when accounting for the high adaptability of these fish to new, plant-based feeds (Le Boucher et al., 2012). Notwithstanding, further studies are required to evaluate the possibility of using white lupin meal feed in the diets of sturgeon fish for different developmental stages; in particular, studies on the long-term effects of feed containing white lupin meal on Siberian sturgeons.

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