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ORIGINAL PAPER

Effects of a 1.5-year dietary inclusion of white lupin meal (*Lupinus albus*) on Siberian sturgeon (*Acipenser baerii*) performance

A. Szczepański[1], D. Adamek-Urbańska[1], R. Kasprzak[1], W. Cieśla[1], H. Szudrowicz[1], I. Piotrowska[2], P. Gomułka[3], K. Kawalski[1], J. Martynow[1,4], B. Łosiewicz[1,5], P. Bujarski[1], R. Wild[1], M. Kozłowski[2], J. Śliwiński[1] and M. Kamaszewski[1,*]

[1] Warsaw University of Life Sciences, Institute of Animal Sciences, Department of Biology of Animal Environment, 02-786 Warsaw, Poland
[2] Department of Sturgeon Fish Breeding, National Inland Fisheries Research Institute in Olsztyn, 10-719, Olsztyn, Poland
[3] Department of Ichthyology and Aquaculture, University of Warmia and Mazury, Olsztyn, 10-719, Olsztyn, Poland
[4] The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Department of Animal Physiology, 05-110 Jabłonna, Poland
[5] The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Department of Genetic Engineering, 05-110 Jabłonna, Poland

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* Corresponding author:
e-mail: maciej_kamaszewski@sggw.edu.pl

ABSTRACT. Conventional protein sources in aquafeeds, such as fishmeal and soybean meal, raise environmental and ethical concerns, prompting the search for sustainable alternatives. White lupin meal has yielded promising results, but its long-term effects on Siberian sturgeon remain insufficiently studied. In a 540-day experiment, juvenile sturgeons (initial weight: 3.9 ± 0.3 g) were divided into four dietary groups: a soybean-based reference (R), a fishmeal-based control (L0), and two lupin-supplemented groups (L5: 5%, L10: 10%). Basic rearing indices were assessed, followed by other analyses: body proximate composition, histological and immunohistochemical evaluation of digestive organs, enzymatic activity, and oxidative stress markers. All analyses were performed in triplicate. Despite significantly lower final weight in group L10, meat of this fish contained the highest protein and the lowest fat contents. Histological analyses revealed a significant shortening of intestinal folds in the spiral intestine in group L5, while group R showed the strongest immune activation, evidenced by a high number of intestinal CD3-positive cells. Enzymatic profiles indicated impaired antioxidative mechanisms in lupin-fed groups. The findings suggest that lupin meal can be incorporated into Siberian sturgeon diets, although further studies are required.

Introduction

The rapid development of aquaculture has driven the search for new dietary protein sources for aquatic nutrition (Glencross et al., 2020). Fish meal (FM), the conventional primary protein source, can no longer be used extensively due to economic factors like high costs and limited supply (Rawles et al., 2011; World Bank, 2020), along with ecological concerns including overfishing and ecosystem disruption (Olsen and Hassan, 2012; Béné et al., 2016). Plant proteins, particularly soybean (*Glycine max*), have gained attention as alternatives (Jiang et al., 2018); however, their use in aquaculture is

associated with certain limitations (Byerlee et al., 2014; Zlaugotne et al., 2022). Soybean products are characterised by a high content of anti-nutritional factors, mainly phytoestrogens, which can impair aquatic organism development (Martins et al., 2018). In addition, large-scale cultivation of soybean raises ethical and ecological issues, as soybean production has contributed to deforestation and displacement of other food production systems in tropical and subtropical countries (Byerlee et al., 2014). Hence, alternative plant protein sources are being increasingly pursued.

In this context, plants of the genus *Lupinus* represent a promising alternative to soybean due to their greater environmental tolerance (Abraham et al., 2019). They can be cultivated on less fertile soils worldwide, including temperate regions (De la Peña and Pueyo, 2012; Musco et al., 2017), and contain relatively fewer anti-nutrients compared to soybean, most of which can be further reduced during seed processing (Prusinski, 2017). The four main cultivated lupin species are white lupin (*Lupinus albus*), yellow lupin (*L. luteus*), narrow-leafed lupin (*L. angustifolius*) and pearl lupin (*L. mutabilis*) (Abraham et al., 2019). Importantly, their nutritional composition shows considerable variation depending on species, genotype, and cultivation conditions. Nevertheless, they generally have a high nutritional value, as the protein content of lupin seeds usually exceeds 35% dry weight while maintaining low-fat content (6%), and complete absence of starch (Lemus-Conejo et al., 2023). In addition, their nutritional profile includes bioactive peptides, polyphenols and significant dietary fibre content (Rochfort et al., 2007; Pastor-Cavada et al., 2009). Importantly, lupins are cholesterol-free, but contain a large fraction of unsaturated fatty acids (Duranti et al., 2008), with particularly high levels of αlinolenic acid and a favourable n-3/n-6 fatty acid ratio (Chiofalo et al., 2012). Lupin seeds are particularly rich in essential amino acids and important micronutrients such as iron (Fe), zinc (Zn), copper (Cu), calcium (Ca) and phosphorus (P) (Lemus-Conejo et al., 2023). While lupins contain some anti-nutritional factors, e.g., quinolizidine alkaloids (spartenine and lupanine), the levels of these compounds can be effectively reduced through domestication and post-processing (Nalle et al., 2012; Musco et al., 2017). Notably, white lupin seeds generally contain lower levels of these anti-nutritional compounds compared to other lupine species (Zaworska-Zakrzewska et al., 2023). Additionally, the relatively low concentrations of trypsin and chymotrypsin inhibitors present in lupin seeds may actually exert beneficial effects on digestion (Erbaş et al., 2005; Kouris-Blazos and Belski, 2016).

Considering lupin advantageous composition and low environmental requirements, they have been successfully incorporated into both human diets and livestock feed, including aquaculture applications (Abraham et al., 2019; Glencross et al., 2020). Research has demonstrated the potential of lupin-based feeds for various fish and shrimp species, with successful replacement of 40–50% FM in diets (Szczepański et al., 2022). While recent research has evaluated white lupin meal (*L. albus*) in juvenile Siberian sturgeon (*Acipenser baerii*) diets (Szczepański et al., 2025), its long-term effects remain unknown for these slow-growing, high-value fish. Based on previous findings (Szczepański et al., 2022), we hypothesised that moderate inclusion of white lupin meal would not negatively impact sturgeon growth or health parameters. This study therefore aimed to evaluate the feasibility of white lupin meal supplementation throughout a substantial portion of the Siberian sturgeon production cycle.

Material and methods

Adherence to ethical guidelines

The experiment was conducted in accordance with mandatory ethical standards and complied with both Polish and international guidelines. The rearing was conducted at the Sturgeon Fish Breeding Unit in Pieczarki, part of the Stanisław Sakowicz Inland Fisheries Institute in Olsztyn (Poland), under veterinary permit number WNI-28199201 and animal test permit number 056.

Rearing conditions

The experimental fish were Siberian sturgeons obtained through controlled spawning at the Sturgeon Fish Breeding Unit in Pieczarki, part of the Stanisław Sakowicz Inland Fisheries Institute in Olsztyn. After hatching, the larvae were transferred to a recirculation aquaculture system (RAS) consisting of ten tanks, each with a working volume of 4 m^3 (ST 19-22, SDK, Ostróda, Poland). The RAS was equipped with oxygen generators and a CN 3.2 biofilter (SDK, Ostróda, Poland), a Light Bioelementer plastic filling with a total volume of 1.5 m^3 (RK Plast A/S, Skive, Denmark). Larval and post-larval rearing up to day 60 post-hatching (DPH) was performed according to previously established protocols (Kolman and Kapusta, 2018).

Experimental design and dietary formulation

The feeding experiment began when juvenile Siberian sturgeons reached 61 DPH (days post-hatching) and continued until 600 DPH, lasting for a total of 540 days. The experiment was divided into two stages, feeds adjusted to meet the nutritional requirements of fish at different growth stages. Four feeding groups were established: group R – fed a commercial feed with soybean protein, formulated to sturgeon rearing (Aller Aqua, Golub-Dobrzyń, Poland) (composition: protein 48%, lipids 18%, nitrogen free extract (NFE) 15.5%, ash 8.2%, fibre 2.3%); group L0 – fed a formulated diet based on FM without white lupin meal inclusion; and two groups which were fed diets containing 5% (group L5) and 10% (L10) white lupin meal. Fish from groups L0, L5 and L10 were reared in triplicate tanks, while fish from group R were kept in a single tank. The initial stocking density was 360 individuals per tank, with an average body weight of 3.9 ± 0.3 g, resulting in a total of 3600 fish. The fish were fed every 4 h (6 times/day). Feed intake was monitored, and uneaten feed and excrement were regularly removed. The composition and granulation of the feed were adjusted as the experiment progressed. For the first 125 days, the sturgeons were fed smaller granulate feed (0.2–2 mm) adapted to fish size and its composition to nutritional needs (Feed A, B and C). The composition of the feeds is presented in Table 1, while their effects on growth performance and physiological outcomes were published previously by the authors (Szczepański et al., 2025). As the fish grew, both the granulation and composition were modified accordingly. Similarly, the feed ration was also adjusted during the development of the fish. Feed rations were progressively reduced from 1.2 to 0.5% fish biomass, following established protocols (Kolman and Kaputa, 2018). The experimental pellets (3–4 mm) were prepared at the laboratory of the Department of Ichthyology and Aquaculture, University of Warmia and Mazury in Olsztyn. The composition of the experimental feeds is listed in Table 2.

Physical and chemical water parameters

Throughout the experiment, the physicochemical parameters of water at the reservoir outflow were regularly monitored. Oxygen content and pH were measured using a CyberScan PCD 5500 meter (Eutech Instruments, Vernon Hills, USA). Total ammoniacal nitrogen (TAN = NH_4^+-N + NH_3-N)

Table 1. Proximate composition (g kg^{-1} dry weight) of experimental diets during the first 125 days of the feeding experiment (according to Szczepański et al., 2024)

Raw material, g kg^{-1}	Feed composition								
	Feed A			Feed B			Feed C		
	L0	L5	L10	L0	L5	L10	L0	L5	L10
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
Fibre*	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

* Feed A, B, C – granulate feed (0.2 – 2 mm) adapted to fish size and nutritional needs; estimated based on component suppliers' declarations; ** nitrogen free extract, calculated by subtracting the other four parameters from dry weight

Table 2. Proximate composition (g kg^{-1} dry weight) of experimental diets after 125 days of the feeding experiment

Ingredients, g kg^{-1}	Group		
	L0	L5	L10
White lupin	0	50	100
Wheat flour	180	150	130
Fish meal	460	480	395
Krill meal	90	60	70
Wheat gluten	70	100	50
Poultry blood meal	80	40	150
Fish oil	100	100	85
Premix	20	20	20
Proximate composition, %			
dry matter	93.2	91.7	91.5
crude protein	53.7	52.8	54.7
crude fat	15.9	15.6	14.0
ash	10.7	10.5	10.7
fibre*	2.1	1.8	1.7
NFE**	10.9	10.9	11.1
Gross energy, MJ kg^{-1}	21.2	20.7	20.7

L0 – control group; L5 – 5% lupin meal inclusion group; L10 – 10% lupin meal inclusion group; * estimated based on component suppliers' declarations; ** nitrogen free extract, calculated by subtracting the other four parameters from dry weight

and nitrite nitrogen concentrations (NO_2-N) were determined using a spectrophotometer (Aquamate UV-Vis Plus, Kwun Tong, Kowloon, Hong Kong). Water quality parameters were measured at least twice a week, and water temperature was monitored daily. The average water temperature throughout the study was 18.5 ± 2.4 °C. Oxygen concentration remained above 11.5 mg O_2 l^{-1}, and pH values ranged between 7.3 and 7.7. The maximum concentrations of nitrogen did not exceed 0.286 mg TAN l^{-1} and 0.012 mg NO_2-N l^{-1}, respectively. The water replenishment rate in the tanks was maintained at 13 l min^{-1}.

Sampling and rearing indices

At 600 DPH, fifteen individuals from each group (total n = 60) were euthanized using tricaine methanesulfonate (MS-222 at 130 mg l^{-1}) for biometric measurements and tissue sampling. The following parameters were determined:

$$\text{WG (weight gain, g fish}^{-1}) = (BW_f - BW_i)$$

$$\text{SGR (specific growth rate, \% day}^{-1}) = = 100 \times [(\ln BW_f - \ln BW_i) \times TFI^{-1}];$$

$$\text{FCR (feed conversion ratio)} = TFI \times (B_f - B_i)^{-1};$$

$$\text{CF (condition coefficient)} = (W \times 100) \times TL^{-3};$$

where: BW_i – initial mean body weight (kg); BW_f – final mean body weight (kg); W – individual weight (kg); TL – individual total length (m); TFI – total feed intake (kg); B_f – final stock biomass (kg); B_i – initial stock biomass (kg).

Muscle composition

After skin removal, muscle tissues were excised from the left side of each fish, between the dorsal and caudal fin regions. The collected samples were immediately flash-frozen in liquid nitrogen and stored at −80 °C until processing. For analysis, frozen tissues were thawed and homogenised under controlled conditions. Muscle proximate composition was determined in accordance with AOAC International standards (AOAC International, 1990). Dry matter was assessed using the weight-dry method, crude protein by the Kjeldahl method, crude fat using the Soxhlet method, and crude ash by incineration at 550 °C.

Histological analysis

The experimental material consisted of livers and intestinal sections (anterior and spiral segments), dissected from each sampled fish. Tissues were fixed in Bouin's solution, dehydrated and subsequently embedded in paraffin prior to sectioning on a microtome. The 6-µm-thick sections were stained either with haematoxylin and eosin (HE), or with Alcian blue and periodic acidSchiff reagent (AB/PAS) to differentiate between acidic and neutral mucosal cells in the intestine. In addition, PAS staining was conducted to visualise glycogen and lipofuscin deposition in the liver. All stained sections were analysed qualitatively and quantitatively using a NIKON Eclipse Ni-E microscope, equipped with a camera and NIS Elements image analysis software (Nikon Corporation, Tokyo, Japan). The following histomorphometric measurements were performed separately in the anterior (A-) and spiral (S-) intestines: fold length (AFL and SFL, µm), epithelial height (AEH and SEH, µm), supranuclear region height (ASH and SSH, µm), and lamina propria width (AWLP and SWLP, µm). For hepatic evaluation, morphometric analysis included hepatocyte area (HA, μm^2), hepatocyte nuclear area (HNA, μm^2), and the hepato-nuclear index (HNI), calculated as the ratio of nuclear area to total hepatocyte area (HNA/HA).

Biochemical analysis

For biochemical analysis, liver, anterior intestine, and spiral intestine samples were collected, frozen in liquid nitrogen, and stored at −80 °C until processing. Prior to analysis, tissues were thawed and homogenised in distilled water at 4 °C, and then centrifuged at 14 000 × g for 10 min. The resulting supernatant was collected, aliquoted and refrozen in liquid nitrogen, and stored at −80 °C. Enzymatic activity was determined using the previously described methodology (Kamaszewski et al., 2014; Palińska-Żarska et al., 2021). In the anterior and spiral intestine, the activity of digestive enzymes was evaluated, including alkaline phosphatase (ALP) acid phosphatase (ACP), lipase (Lip) amylase (Amyl), trypsin (Tryp), and chymotrypsin (Chym). In the liver, the activities of ALP, ACP, glutathione reductase (GR), glutathione peroxidase (GPX) and superoxide dismutase (SOD) were evaluated.

Statistical analysis

Statistical analyses were conducted using Statistica software (TIBCO Software Inc., Palo Alto, California, USA). Data distribution normality was verified using the Shapiro-Wilk test. For normally distributed data (rearing and histological parameters), ANOVA was followed by either the post-hoc Tukey test or the Fisher test. For data that did not meet normality assumptions (biochemical analysis), the nonparametric Kruskal-Wallis test was applied.

Results

Growth performance and nutrient utilisation

The survival rate during the experimental period was 100%. On day 540 of the feeding experiment, no differences were observed between the groups regarding TL, SL, SGR, and CF. However, significant differences were recorded in body weight, with fish from the L10 group being significantly lighter compared to the other groups. Similarly, WG was also lowest in group L10. Additionally, a significantly higher FCR value was observed in group L5 (Table 3).

Histological analysis

Morphometric analyses showed that fish in group L0 had the most developed intestinal folds in the anterior segment, while those from group L10 in the spiral segment (Table 5). The latter group also had the highest enterocytes in the anterior segment, although no statistically significant differences were observed in the height of the supranuclear part of enterocytes in this section. The lamina propria width remained generally consistent among groups, with the exception of group L10, which showed significantly narrower measurements in both intestinal segments ($P < 0.05$). In the spiral segment, intestinal fold height followed the pattern: L10 > L0 > L5, while enterocyte dimensions were greatest in group L0. Group L5 had the widest lamina propria in the spiral segment, contrasting with its intermediate fold height measurements. (Table 5).

The analysis showed prominent immune cell infiltration within the mucosal layer, consisting primarily of lymphocytes and PAS-positive immune cells containing cytoplasmic granules. These immune cells were significantly more abundant in the spiral intestine compared to the anterior segment. Epithelial detachment from the basement membrane

Table 3. Rearing parameters of Siberian sturgeons on day 540 of the feeding trial

Parameter	Group			
	R	L0	L5	L10
TL, m	0.79 ± 0.05	0.79 ± 0.04	0.80 ± 0.04	0.76 ± 0.05
SL, m	0.60 ± 0.04	0.63 ± 0.03	0.64 ± 0.03	0.60 ± 0.04
BW_i, g	3.9 ± 0.3	3.9 ± 0.3	3.9 ± 0.3	3.9 ± 0.3
BW_f, kg	2.22^a ± 0.45	2.25^a ± 0.27	2.27^a ± 0.34	1.98^b ± 0.37
WG, g kg^{-1}	33.24^a ± 6.82	33.76^a ± 4.05	33.96^a ± 5.09	29.64^b ± 5.60
SGR	1.05 ± 0.04	1.06 ± 0.02	1.06 ± 0.02	1.04 ± 0.03
FCR	0.81^a ± 0.03	0.81^a ± 0.05	0.88^b ± 0.01	0.80^a ± 0.07
CF	0.45 ± 0.04	0.45 ± 0.05	0.44 ± 0.08	0.45 ± 0.07

R – reference group; L0 – control group; L5 – 5% lupin meal inclusion group; L10 – 10% lupin meal inclusion group; TL – total length; SL – standard length; BW_i – initial body weight; BW_f – final body weight, kg; WG – weight gain; SGR – specific growth rate; FCR – feed conversion ratio; CF – condition coefficient; means (± SD) followed by different letters in the same row are significantly different at $P < 0.05$, calculated using the parametric ANOVA test

Table 4. Muscle composition of Siberian sturgeons at day 540 of the feeding trial, %

Parameter	Group			
	R	L0	L5	L10
Dry matter	28.18^b ± 1.14	29.38^{ab} ± 1.16	31.32^a ± 1.24	27.84^b ± 1.13
Crude ash	0.99^b ± 0.02	1.00^b ± 0.02	1.03^b ± 0.02	1.20^a ± 0.02
Crude protein	19.69^a ± 1.00	18.92^b ± 0.94	18.54^b ± 0.96	20.40^a ± 1.03
Crude lipid	7.31^c ± 0.89	9.53^b ± 1.15	12.89^a ± 1.43	7.05^c ± 0.85

R – reference group; L0 – control group; L5 – 5% lupin meal inclusion group; L10 – 10% lupin meal inclusion group; means (± SD) followed by different letters in the same row are significantly different at $P < 0.05$, calculated using the parametric ANOVA test

Proximate muscle composition

Significant differences were recorded in the muscle composition across dietary treatments. The dry matter content was the highest in groups L0 and L5, with L5 showing significantly higher values compared to R and L10. Crude ash content was significantly elevated in group L10 relative to other treatments. The highest crude protein content was observed in groups R and L10; however, these groups showed significantly lower crude lipid content compared to L0 and L5 (Table 4).

Table 5. Histomorphometric analysis of anterior and spiral sections of Siberian sturgeon intestines on day 540 of the feeding trial, μm

Parameter	Groups			
	R	L0	L5	L10
AFL	2301.21^{c} ± 236.15	2870.07^{a} ± 159.80	2350.97^{c} ± 229.13	2566.11^{b} ± 300.79
AEH	47.53^{b} ± 6.81	45.06^{c} ± 5.15	46.13^{bc} ± 5.73	50.03^{a} ± 5.16
ASH	25.91 ± 3.54	26.64 ± 2.94	26.68 ± 4.05	26.01 ± 2.83
AWLP	12.23^{a} ± 2.61	12.69^{a} ± 2.34	12.84^{a} ± 2.63	11.01^{b} ± 3.86
SFL	945.25^{b} ± 161.50	923.09^{b} ± 148.04	649.44^{c} ± 138.10	1057.03^{a} ± 198.11
SEH	44.41^{b} ± 5.53	48.93^{a} ± 6.38	43.97^{b} ± 5.83	46.80^{a} ± 6.01
SSH	25.48^{b} ± 3.57	27.62^{a} ± 3.38	26.05^{b} ± 3.66	25.80^{b} ± 3.34
SWLP	12.25^{b} ± 2.08	12.53^{b} ± 2.47	13.96^{a} ± 2.79	11.01^{c} ± 2.36

R – reference group; L0 – control group; L5 – 5% lupin meal inclusion group; L10 – 10% lupin meal inclusion group. AFL – anterior intestine fold length; AEH – anterior enterocyte height; ASH – anterior intestine supranuclear area of enterocytes; AWLP – anterior intestine width of the lamina propria; SFL – spiral intestine fold length; SEH – spiral enterocyte height ; SSH – spiral intestine supranuclear area of enterocytes; SWLP – spiral intestine width of the lamina propria; means (± SD) followed by different letters in the same row are significantly different at $P < 0.05$, calculated using the parametric ANOVA test

was observed at the apexes of intestinal folds, occurring more frequently in the anterior intestine. Both intestinal segments contained abundant mucous (goblet) cells producing acidic, neutral and mixed acidic/neutral mucins. However, the distribution patterns of these mucous cell types did not differ noticeably between experimental groups (Figure 1). observed in all individuals in terms of storage material: one characterised by the accumulation of glycogen and fat droplets as the primary deposits, and the other predominantly containing glycogen. In all groups, clusters of melanomacrophages were identified, primarily located near blood vessels and associated with focal inflammation (Figure 3

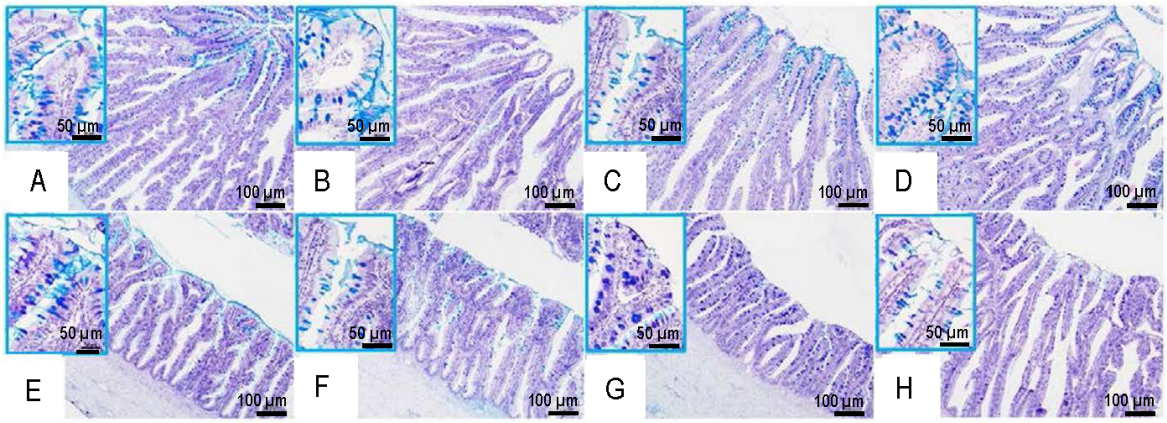

Figure 1. Histological images of cross sections through the anterior (A-D) and spiral (E-G) intestines of Siberian sturgeons

A, E – group R (reference group); B, F – group L0 (control group); C, G – group L5 (5% lupin meal inclusion group); D, H – group L10 (10% lupin meal inclusion group). Insets show folds with numerous mucous cells (blue/purple goblet cells). AB/PAS staining, scale bars: 100 μm; (main images), 50 μm (insets)

CD3-positive cells were found to be sparsely distributed in the intestines, mainly as intraepithelial lymphocytes (IELs) located in the supranuclear area of enterocytes. Small lymphocyte clusters were occasionally present, indicating mild inflammation. However, in the spiral intestine, CD3 labelling was visibly more intense in group R fed the commercial feed, whereas in other groups it was comparable to that in the anterior intestine (Figure 2).

The liver histology of all examined sturgeons showed severe microvesicular steatosis (insets in Figure 3). Two distinct types of hepatocytes were A–D). Melanin was the dominant pigment within these melanomacrophages, accompanied by numerous lipofuscin droplets (Figure 3). Single hemosiderin granules were also visible within the melanomacrophage centres (MMC) in most specimens. Lymphocytes were the main cell type in MMCs, regardless of whether the inflammatory foci were positioned close to blood vessels or within the liver parenchyma (Figure 3). In group R, in addition to severe steatosis and inflammation, localised degenerative changes and necrotic foci were observed.

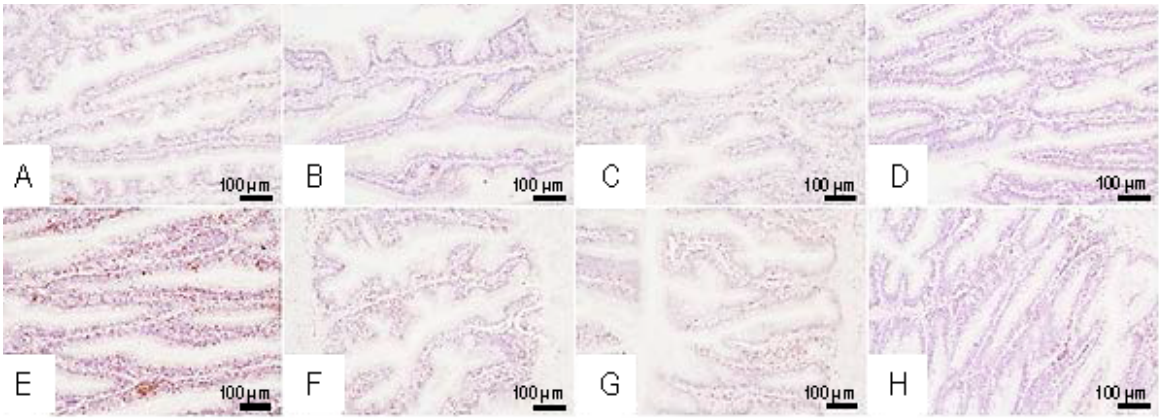

Figure 2. Histological images of cross sections through the anterior (A-D) and spiral (E-H) intestines of Siberian sturgeons

A, E – group R (reference group); B, F – group L0 (control group); C, G – group L5 (5% lupin meal inclusion group); D, H – group L10 (10% lupin meal inclusion group). Anti-CD3 immunohistochemical staining, scale bar: 100 µm

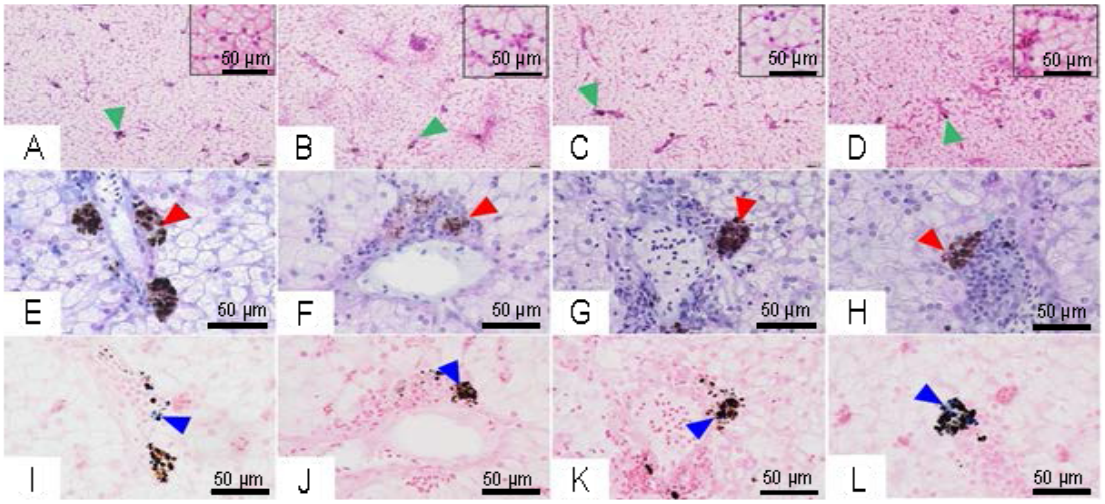

Figure 3. Histological images of the hepatic parenchyma of Siberian sturgeons with clearly visible (magnified insets) hepatocyte steatosis. In melanomacrophage centres, melanin was the main pigment (dark brown), along with lipofuscin (red arrowheads) and a minor presence of hemosiderin (blue arrowheads)

A-D – HE (haematoxylin and eosin) staining; E-H – AB/PAS (Alcian blue and periodic acidSchiff reagent) staining; I-L – Perl's staining. Scale bars: 100 µm (A-D), 50 µm (E-L and all insets)

The largest hepatocytes were observed in fish from groups R and L0 (Table 6), with correspondingly enlarged nuclear areas. These size differences resulted in statistically significant variations in the hepato-nuclear index (HNI), which reached its maximum values in groups R and L10. In contrast, however, hepatocytes in group L10 were characterised by the smallest cellular and nuclear areas (Table 6).

Enzymatic activity

The intestinal enzymatic profiles differed significantly between the groups (Table 7). In the anterior intestine, ALP activity displayed the most notable differences, being highest in group R, and approximately 50% lower in group L5. The highest amylase and chymotrypsin activities were recorded in group L10. Conversely, trypsin showed lowest values in groups L10 and R, while reaching maximal values in group L5. Similar variations occurred in the spiral intestine, where ALP activity again differed substantially between the groups. Chymotrypsin activity remained highest in group L10, while amylase activity showed an inverse pattern – with lowest values in groups L10 and L0, and highest activity in group L5.

Significant differences were also found in ALP activity in the liver (Table 7). The highest

Table 6. Histomorphometric analysis of Siberian sturgeon hepatocytes on day 540 of the feeding trial

Parameter	Groups			
	R	L0	L5	L10
HA, μm^2	475.96^a ± 77.93	491.25^a ± 79.24	441.94^b ± 70.99	372.13^c ± 75.04
HNA, μm^2	41.21^a ± 5.33	40.09^a ± 5.34	36.07^b ± 5.08	33.13^c ± 6.04
HNI, %	9^a ± 1	8^b ± 1	8^b ± 0.8	9^a ± 1

R – reference group; L0 – control group; L5 – 5% lupin meal inclusion group; L10 – 10% lupin meal inclusion group; HA – hepatocyte area; HNA – hepatocyte nucleus area; HNI – hepatonuclear index; means (± SD) followed by different letters in the same row are significantly different at $P < 0.05$, calculated using the parametric ANOVA test

Table 7. Results of biochemical analyses (digestive enzymes and oxidative stress parameters) of Siberian sturgeons on day 540 of the feeding trial, IU g^{-1}

Organ	Groups			
	R	L0	L5	L10
Anterior intestine				
ALP	302^a ± 214	261^b ± 130	152^d ± 104	195^c ± 148
ACP	14 ± 4	17 ± 2	15 ± 4	15 ± 5
Amylase	17^a ± 9	5^b ± 1	8^b ± 6	18^a ± 13
Chymotrypsin	23^c ± 11	20^c ± 11	37^b ± 16	43^a ± 36
Trypsin	48^b ± 27	52^b ± 43	75^a ± 69	49^b ± 40
Spiral intestine				
ALP	98^c ± 73	86^d ± 55	113^b ± 74	149^a ± 86
ACP	9 ± 4	9 ± 4	10 ± 5	10 ± 3
amylase	18^a ± 16	11^b ± 6	18^a ± 14	12^b ± 9
chymotrypsin	12^b ± 9	24^a ± 16	6^c ± 7	28^a ± 14
trypsin	1^c ± 1	1^c ± 1	c3^b ± 2	20^a ± 15
Liver				
ALP	72^a ± 36	42^c ± 14	59^b ± 21	47^c ± 23
ACP	5 ± 2	4 ± 2	4 ± 2	5 ± 3
GR	2^b ± 2	4^a ± 2	2^b ± 1	3^{ab} ± 2
GPX	154^a ± 96	82^b ± 49	162^a ± 64	161^a ± 111
SOD	22^a ± 9	24^a ± 6	3^b ± 1	3^b ± 1

R – reference group; L0 – control group; L5 – 5% lupin meal inclusion group; L10 – 10% lupin meal inclusion group; ALP – alkaline phosphatase; ACP – acid phosphatase; GR – glutathione reductase; GPX – glutathione peroxidase; SOD – superoxide dismutase; means followed by different letters in the same row are significantly different at $P < 0.05$, calculated using the parametric non-parametric Kruskal-Wallis test

activity was found in group R, while the lowest in groups L0 and L10. No significant differences were noted in ACP activity in both intestinal segments and liver tissue. Antioxidant enzyme profiles showed distinct dietary-related patterns. Group L0 demonstrated the highest GR activity but exhibited the lowest GPX activity. Additionally, SOD activity in groups R and L0 was significantly higher compared to the groups fed diets with lupin meal inclusion.

Discussion

In sustainable aquaculture, identifying alternatives to traditional dietary protein and fat sources, such as fish meal and fish oil, is crucial for maintaining environmentally friendly production (Rašković et al., 2011; Glencross et al., 2020). Aquatic feed should combine low carbohydrate and fibre content, minimal anti-nutritional factors, and high digestibility while maintaining an optimal amino acid profile and palatability (Francis et al., 2001). However, despite meeting these criteria, plant-based ingredients cannot entirely replace fish meal in compound aquafeeds (Rašković et al., 2011). For this reason, this feeding trial assessed the feasibility of partial replacement of fish meal with white lupin meal in dietary formulations for Siberian sturgeons, considering all economic and animal welfare aspects of sustainable aquaculture.

Extensive research has been conducted on sturgeon breeding, biology, and ecology (Ruban, 2018). As primarily benthic feeders, their diet varies with ecological niche and body size, progressing from planktonic organisms to invertebrates and even fish (Miller, 2004). While modern sturgeon aquaculture predominantly utilises commercial feeds containing plant proteins (especially soybean meal), alternative

ingredients like white lupin meal have shown promise in other fish species (Szczepański et al., 2022). However, evaluating such substitutions in sturgeons requires particular attention to digestive physiology and development, given their unique biological traits, including late sexual maturation and complex production cycles.

Measurements of total body length showed no significant differences between the experimental groups after 18 months of experimental feeding, indicating that white lupin meal at inclusion levels of 5 and 10% did not impair sturgeon growth. However, differences in fish weight were observed, with sturgeons from group L10 being significantly lighter in relation to the other fish. Body composition analysis showed that the muscles of these fish had the highest protein and the lowest fat content, resembling the composition observed in group R. This suggests that Siberian sturgeon meat produced with a 10% inclusion of white lupin meal can be considered a high-quality product with potential appeal for consumption. Sturgeon meat is renowned for its high nutritional value, being rich in essential amino acids, and protein content exceeding that of common carp (*Cyprinus carpio*), crucian carp (*Carassius carassius*), bighead carp (*Hypophthalmichthys nobilis*), and even pork (Chen et al., 2022). Despite these advantages, sturgeon meat remains underappreciated among consumers, particularly in European countries (Bronzi et al., 2019).

In Teleostei fishes, the anterior part of the intestine plays a primary role in digestion. In sturgeons, however, this function is largely carried out by the distally located spiral intestine, whose spatial structure prolongs digesta passage time while reducing the overall length of the intestines (Rodríguez et al., 2002; Jutfelt, 2011). The elongation of intestinal folds, which increases the absorptive surface, suggests that fish in group L10 likely had difficulties with the absorption of nutrients due to the high dietary white lupin content, despite the feed's reduced fibre content (Aidos et al., 2023). Conversely, groups L5 and R had the shortest intestinal folds in both sections. While this could indicate improved digestive efficiency, similar morphological changes have been associated with adverse effects in soybean-based diets (Hedrera et al., 2013). The introduction of soybean products into the diet of Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), and common carp has been associated with intestinal inflammation and digestive abnormalities. These effects include impaired feed ingestion, shortening of intestinal folds, increased number of goblet cells, and severe immune cell infiltration into the lamina propria (Refstie et al., 2000, 2006; Bakke-McKellep et al., 2007; Krogdahl et al., 2010). In contrast, lupin meal inclusion at levels ≤50% did not elicit such pathological responses in these species (Acar et al., 2018; Anwar et al., 2020; Zhang et al., 2012). In the present study, histological and immunohistochemical analyses of sturgeon intestines revealed only mild abnormalities in group R, mainly manifested by the infiltration of CD3-positive cells in the spiral intestine. The spiral intestine serves not only as the primary digestive site in sturgeons but also plays a crucial immune role (Wei et al., 2019, 2020). The observed CD3-positive cell infiltration in group R suggests that soybean-derived proteins elicit stronger immune stimulation than lupin meal. This aligns with extensive literature documenting soybean-induced enteritis in fish, characterised by lamina propria widening, shortening of intestinal villi, loss of enterocyte supranuclear vacuolisation, severe immune cell infiltration of both lamina propria and submucosa, and disrupted intestinal ion and water transport (Rašković et al., 2011; Kiron et al., 2020; Nimalan et al., 2022). While the increased infiltration of CD3-positive cells might represent a transient, diet-induced immune response (Kasprzak et al., 2023), it could also indicate chronic inflammation (Horn et al., 2023). Therefore, the thinnest lamina propria in group L10 in both the anterior and the spiral intestine may imply minimal immune system activation at higher dietary inclusion levels of white lupin meal. Additionally, the intestinal mucosa of all experimental groups contained abundant mucous cells producing neutral, acidic and mixed acidic-neutral mucins, all of which are essential for proper functioning of the digestive system. Intestinal mucus not only facilitates the activity of digestive enzymes but also plays an active role in immune defence mechanisms (Kim and Ho, 2010; Núñez-Acuña et al., 2016, 2018). Despite these observations, the activity of ACP, a known macrophage marker (Broeg, 2003), showed no significant differences between the groups in any of the examined tissues.

Metabolic processes in the liver are closely linked to nutrition, making the liver's morpho-physiological condition a reliable indicator of nutritional status and potential disorders (Field et al., 2003; Ostaszewska et al., 2005a; Rašković et al., 2011). Therefore, if provided diets are unsuitable, histological changes in hepatic parenchyma can reflect these deficiencies. Common pathological manifestations include hepatocyte vacuolation, necrosis, fatty or glycogen degeneration, and other histopathologies

(Caballero et al., 2003; Tacon, 1992). The cytoplasmic area of hepatocytes is influenced by the quantity of stored glycogen and lipids, while the metabolic and transcriptional activity, primarily concentrated in the nuclei, affects nuclear size. A reduction in nuclear size may signify underlying nutritional deficiencies (Ostaszewska et al., 2005b; Kasprzak et al., 2019). The reduced cytoplasmic and nuclear hepatocyte areas observed in fish from groups L5 and L10 could initially suggest a negative effect of dietary lupin. However, since steatosis was present in the livers of sturgeons from all groups, the variation in hepatocyte size may instead reflect differences in intracellular lipid accumulation rather than a direct lupin-induced pathology. In addition, the livers of R group sturgeons exhibited necrosis and inflammation, with the most heterogeneous structure of all treatments. These findings suggest that dietary soybean may have a more detrimental effect on liver condition and function compared to the inclusion of lupin.

Both a deficiency and excess of dietary lipids can affect the health status of fish (Ahmadi Fackjouri et al., 2011; Zhu et al., 2017). The site of excessive lipid deposition varies among species, potentially impairing organ function (Szendroedi and Roden, 2009; Weil et al., 2013). For instance, in Atlantic salmon, it predominantly affects muscle tissue (Nanton et al., 2007), visceral adipose tissue in Nile tilapia (*Oreochromis niloticus*) (Hanley, 1991), while in red seabream (*Pagrus major*), torafugu (*Takifugu rubripes*) (Kaneko et al., 2013), and Russian sturgeon (*Acipenser gueldenstaedtii*), the liver is the primary site of lipid accumulation (Zhu et al., 2017). Given this species-specific variation, the hepatic steatosis observed in the present study may reflect non-pathological lipid storage rather than a disease state.

Carbohydrates and lipids are closely interconnected in glycol-lipid metabolism, and excessive dietary carbohydrates may cause abnormal lipid deposition in fish (Zhou et al., 2014; 2015; Anand et al., 2017). Although carbohydrates are not essential in fish diets, as fish can synthesise them from fatty or amino acids without affecting growth (NRC, 2011; Qu et al., 2022), they are commonly included in aquaculture feeds due to their cost-effectiveness and potential to improve growth performance by preventing protein catabolism for energy production (Polakof et al., 2012). Additionally, they improve the structural integrity, stability, and palatability of aquafeed pellets (Honorato et al., 2010). For these reasons, evaluating the activity of enzymes involved in carbohydrate breakdown is crucial when introducing new compound feeds into fish diets. The activity of amylase, the enzyme responsible for carbohydrate digestion, is usually higher in herbivorous and omnivorous species than in carnivores (Furné et al., 2008). Additionally, this enzyme is also involved in glycogen metabolism (Łosiewicz and Szudrowicz, 2024). In the present study, relatively high amylase activity was observed in the anterior intestine in group L10 and the spiral intestine in group L5, indicating that the dietary inclusion of lupin did not disrupt this enzyme secretion.

Soybean β-conglycinins are well-documented allergenic proteins known to adversely affect feed intake, growth performance (SGR), and digestive and antioxidant enzyme activities in juvenile Jian carp (*Cyprinus carpio var. Jian*) (Zhang et al., 2013). The structurally similar β-conglutins found in lupin may potentially exert comparable negative effects in sturgeons (Taylor et al., 2015). The activity patterns of trypsin and chymotrypsin, the key enzymes responsible for proper protein digestion (Rombouts et al., 2013), are influenced by multiple dietary factors. High lipid content can impair their function through reduced protein availability (Gawlicka et al., 2002), but their activity can also be impaired when fish receive lowprotein feeds (Zambonino Infante and Cahu, 2007). Paradoxically, excessive dietary protein has also been shown to reduce proteolytic activity in juvenile red tilapia (*Oreochromis sp.*) (Santos et al., 2020). In this context, our results are largely inconclusive, particularly regarding the spiral intestine, where minimal trypsin activity was recorded in groups R and L0. However, considering the generally low trypsin inhibitor content in lupins (Kouris-Blazos and Belski, 2016), further studies should be conducted to assess the specific effects of lupin-derived compounds and other plant-based feed components on trypsin and chymotrypsin activity in sturgeons.

ALP is considered a marker of hepatocyte damage (Sharma et al., 2014; Vetrivel et al., 2014.) and is mainly synthesised in the liver, and, to a lesser extent, in the intestine (Banaee, 2020). The highest hepatic ALP activity observed in group R correlated with the histopathological findings observed in these fish, while the reduced activity in group L10 could indicate a beneficial effect of 10% dietary lupin inclusion on liver function (Wiszniewski et al., 2022). Intestinal ALP, typically expressed in enterocytes, plays a critical role in fatty acid absorption and the maintenance of intestinal barrier integrity (Santos et al., 2022). It also serves as a marker of intestinal

homeostasis and nutrient assimilation (Silva et al., 2010), with higher activity expected in the anterior rather than the spiral intestine (Lallès, 2020). Soybean protein has been reported to inhibit intestinal ALP activity in European seabass (*Dicentrarchus labrax*) (Tibaldi et al., 2006), whereas high protein diets have demonstrated stimulatory effects on ALP activity in both carnivorous and herbivorous prickleback fish (marine family *Stichaeidae*) (Gawlicka and Horn, 2005). However, these patterns are not universal, as soybean proteins have been shown to exert varying effects on ALP activity in different fish species (Lallès, 2020). In the present study, ALP activity in the anterior intestine was notably higher in groups R and L0, while the spiral intestine showed an opposite pattern with maximal activity in group L10. This elevated activity in the spiral intestine of L10 fish could reflect the increased mucosal surface area resulting from previously mentioned elongation of intestinal folds.

The effects of formulated diets on the antioxidant capacity of aquatic organisms remain incompletely understood (Li et al., 2012; Babaei et al., 2017). However, it is well established that the antioxidant defence systems can be affected by nutritional deficiencies (Chowdhury and Saikia, 2020) and elevated lipid and carbohydrate levels in fish diets (Rueda-Jasso et al., 2004). In comparison to other fish taxa, sturgeon livers show relatively low antioxidant enzymatic activity (Tappel et al., 1982; Babaei et al., 2017). SOD and GPX are among the key enzymes involved in detoxifying reactive oxygen species (Slaninova et al., 2009). SOD is the first enzyme that responds to oxygen radicals, demonstrating the most pronounced reaction to oxidative stress (Winston and Di Giulio, 1991; Wiszniewski et al., 2022). While GPX may be crucial in gonadal development (Fu et al., 2012), a disruption in the antioxidant system in fish can lead to alterations in GR activity (Hossen et al., 2023), which plays an important role in early embryonic development (Mora-Lorca et al., 2016). In the conducted study, SOD activity was found to be nearly eight times lower in the lupin-fed experimental groups compared to groups R and L0, while no such pronounced differences were observed for the other two antioxidant enzymes. It should be noted, however, that the reduced SOD activity could be attributed to lupin's phytochemical composition, particularly its polyphenolic compounds (tannins and flavonoids) known for their antioxidant properties (Oomah et al., 2006; Martínez-Villaluenga et al., 2009). Despite extensive research on nutritional impacts on stress responses, data regarding macronutrient effects on oxidative stress and antioxidant defences in sturgeons remain limited (Babaei et al., 2017). For this reason, further research is needed to evaluate the influence of dietary lupin meal on antioxidant status of Siberian sturgeon.

Conclusions

The dietary addition of white lupin meal demonstrated a dual effect. A slight inhibition of growth (weight gain) was observed in group L10 (10% of white lupin meal), which could be attributed to the lower digestibility of lupin protein. However, these fish simultaneously demonstrated superior meat quality and lacked the immune system overstimulation observed with soybean-based commercial feed. Therefore, considering that lupin meal is an environmentally friendly feed ingredient and taking into account the complex biology of sturgeons, further research is needed on the long-term effects of white lupin meal in sturgeon diets.

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Conflict of interest

The Authors declare that there are no conflicts of interest.

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