Ann. Anim. Sci., Vol. 22, No. 4 (2022) 1301–1309 *DOI: 10.2478/aoas-2022-0031*

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EVALUATING LOCAL STRAINS OF SOYBEAN AND CORN CULTIVARS IN THE DIETS OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*): GROWTH AND INSULIN-LIKE GROWTH FACTOR 1, INTESTINAL HEALTH, AND INFLAMMATION FEATURES

Ibrahim I. Al-Hawary[1], Zizy I. Elbialy[1], Dina Basem Barsem[1], Ahmad Abdel-Mawgood[2], Abdallah S. Sallah[3], Tarik S. Rabie[4], Doaa H. Assar[5], Mahmoud A.O. Dawood[6,7♦]

[1]Department of Fish Processing and Biotechnology, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt
[2]Biotechnology Program, Basic & Applied Sciences Institute, Egypt-Japan University of Science and Technology (E-JUST)
[3]Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt
[4]Department of Animal Production and Fish Resources, Faculty of Agriculture, Suez Canal University, Ismailia, 41522, Egypt
[5]Department of Clinical Pathology, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516 Kafrelsheikh, Egypt
[6]Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, 33516, Kafrelsheikh, Egypt
[7]The Center for Applied Research on the Environment and Sustainability, The American University in Cairo, 11835, Cairo, Egypt
♦Corresponding author: mahmoud.dawood@agr.kfs.edu.eg

Abstract

Recently, the high cost of aquafeed affected fish farming feasibility in some countries, including Egypt. The imported soybean meal and corn ingredients consume a large amount of the hard currency, thereby increasing feed prices. Thus, the current study investigated the different sources of soybean and corn on the performances of Nile tilapia. Fish were fed with diet I (based on Egyptian soybean meal cultivar and cornmeal cultivar) or diet II (based on imported soybean meal cultivar and cornmeal cultivar) in a 90-day feeding trial. The results showed no marked effects on the growth performance, protein efficacy ratio, and FCR in the case of fish-fed diet I or diet II. No histological alterations were observed in the skeletal muscle, hepatopancreas, spleen, and intestines, while the diet I-fed group showed normal architecture of the above-listed organs. The expression of liver and muscle IGF-1 showed no changes in fish fed diet I or diet II. No diet-related variations were observed in IL-1β expression in the spleen but increased regulation in the liver of the diet II group compared to the diet I group. Furthermore, significant upregulation of SOD and HSP70 genes was seen in the spleen and liver of the diet II-fed group. We conclude that the inclusion of the Egyptian soybean meal cultivar and cornmeal cultivar (diet I) did not reduce the growth performance and immune-related genes compared with the imported soybean meal cultivar and cornmeal cultivar (diet II).

Key words: Nile tilapia, performance, histopathological findings, growth-related genes, aquafeed, sustainability

Aquaculture is one of the major food contributors globally that provides humanity with cheap animal protein sources (FAO, 2020; Khalidah et al., 2022). Several factors are involved in the success of aquaculture activity, such as high-quality seeds, water source, good management, protection of infectious diseases, and nutritionally balanced aquafeed (Dawood, 2021). Commonly, the ingredients of the aquafeed formulation are imported from specific areas due to the high demand and lack of domestic sources (Galkanda-Arachchige et al., 2020). In some countries, including Egypt, the high prices of farmed fish are related to the high cost of aquafeed, which is attributed to the lack of ingredients (e.g., animal and plant protein sources) (Dawood et al., 2021). The high prices of imported plant protein ingredients imposed aquaculture researchers to look for more sustainable cultivated local plant sources (Kari et al., 2020; Zulhisyam et al., 2020). Soybean meal and corn are among the primary sources of proteins, lipids, and carbohydrates in aquafeed (Kok et al., 2020). Thus, it is necessary to assess the possibility of using alternative cultivated local soybean and corn sources in aquafeed to increase the sustainability and feasibility of the aquaculture industry in Egypt.

Soybean meal (SBM) is commonly used as a plant protein source in aquaculture diets (Abdul Kari et al., 2021; Jones et al., 2020). However, considerable variation could be found in the nutritional value, including nutrient content and anti-nutritional factors among different sources of the same plant ingredient due to genetic variation, different growth conditions, harvesting techniques, processing, and storage (Kari et al., 2022; Urán et al., 2009). SBM was found to cause digestion and absorption problems due to its anti-nutritional content (Zaineldin et al., 2021). The introduction of a high percentage of plant ingredients containing such anti-nutritional factors in fish feed was found to have significant adverse effects on fish health (Dawood and Koshio, 2020). The immune response against such components, stress, and histopathological alterations were noticed (Bandara, 2018). In Atlantic salmon, feeding soybean meal resulted

in enteritis, mainly attributed to the anti-nutritional factor saponin and the alteration in the transcriptome profile of some hepatic genes, lipid metabolism in other fish species (Panserat et al., 2009).

Nile tilapia (*Oreochromis niloticus* L.) is among the most widely grown fish species for its fast growth, high market demand, and resistance to various environments and diseases (Elumalai et al., 2019). Farming Nile tilapia based on properly balanced feed formulations is responsible for high productivity and feasibility. Further, the imported soybean meal and corn consume a large amount of the hard currency, thereby increasing aquafeed prices. Looking for local soybean and corn cultivars could reduce the cost of feeding and increase Nile tilapia sustainability in Egypt. Nevertheless, there was insufficient data on the possibility of replacing familiar plant protein sources with local cultivar plant protein sources (soybean and corn) on the endocrine molecular mechanisms that arbitrate changes in the GH/IGF growth axis, which campaign for muscle growth in Nile tilapia. Thus, the current study aimed at comparing the outcomes of feeding domestic vs. familiar sources of soybean and corn on the growth performance, growth and immune-related genes, and intestinal histological features of Nile tilapia.

Material and methods

Ethical approval

All the procedures, animal treatment, and experimental protocols implemented in the current trial followed applicable guidelines and regulations of the Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University; approval number: IAACUC-KSU-39-2018.

Preparation of the experimental diets

In this experiment, we used two diet formulas: diet I with Egyptian soy cultivar (Giza 111 cultivar) and Egyptian corn cultivar (Giza 321 cultivar), and diet II diet with soybean (Roundup Ready™ soybean, cultivar code: GTS 40-3-2, company name: Monsanto, Saint-Louis, Creve Coeur Missouri, USA) and corn (Agrisure® Duracade™ 5122 maize, Cultivar code: SYN-Ø53Ø7-1 x SYN-IR6Ø4-5 x SYN-BTØ11-1 x DAS-Ø15Ø7-1 x MON-ØØØ21-9, company name: Syngenta, Buenos Aires, Argentina). The ingredients and proximate chemical compositions of the two tested diets are presented in Table 1. The dry ingredients of the formulated diet were mixed with 100 ml water per kg of diet. Subsequently, the mixture (ingredients and water) was mixed to form a paste. The pelleting of each diet was performed via passing the mixed blend through a lab pellet machine set at 1 mm in measurement. The pellets were dried for 24 hours at 52°C in a drying oven and stored in plastic bags at –20°C. The chemical composition and amino acid profile were detected following the standard methods (AOAC, 2012).

Table 1. Ingredients and chemical analysis of experimental diets (on a dry matter basis)

Ingredients (%)	Diet I	Diet II
Egyptian soybean meal cultivar	30	–
Egyptian corn meal cultivar	15	–
Imported soybean meal	–	30
Imported corn meal	–	15
Wheat flour	12	12
Wheat bran	11	11
Rice bran	18	18
Fishmeal	2.5	2.5
Meat meal	5.8	5.8
Corn gluten	4.5	4.5
L-lysine	0.1	0.1
L-methionine	0.1	0.1
Vitamins and minerals mix*	1	1
Total	100	100
Chemical composition		
dry matter	91.6	91.2
crude protein	27.13	27.22
crude lipid	6.32	6.34
crude fiber	6.13	6.11
ash	6.51	6.48
gross energy (Kcal/kg)	4168	4176
digestible energy (Kcal/kg)	3.54	3.61
Amino acid profile (as % in diet)		
cystine	0.38	0.36
histidine	0.61	0.67
isoleucine	0.86	0.84
leucine	1.74	1.69
lysine	1.62	1.58
methionine	0.64	0.68
threonine	1.08	1.11
tryptophan	0.28	0.31

*Vitamin and mineral premix (each 1 kg mix) contains; 4800000 I.U. Vit A, 2000000 IU cholecalciferol (Vit. D), 45.0 g Vit. E, 10.0 g Vit. K, 20.0 g Vit. B_{12}, 6.0 g Vit. B_2, 8 g Vit. B_6, 6.0 g Pantothenic acid, 8.0 g Nicotinic acid, 300 mg Folic acid, 20 mg Biotin, 200 mg Choline, 5 g Copper, 2.0 g Iodine, 15 g Iron, 20 g Manganese, 20 g Zinc, 0.08 g Selenium, 1.5 mg Niacin, 15 mg D-calcium Pantothenate, 28 mg Pyridoxine. HCl, 8 mg Riboflavin, 6.8 mg Thiamin. HCl, 50 mg Manganese sulfate ($MnSO_4$, 36% Mn), 150 mg Zinc sulfate ($ZnSO_4 \cdot 2H_2O$, 40% Zn), 160 mg Copper sulfate ($CuSO_4 \cdot 5H_2O$, 25% Cu), 30 mg Potassium iodide (KI, 24% K, 76% I).

Experimental conditions and design

Healthy one hundred Nile tilapia males with an average weight of 69 ± 1.2 g were acquired from the hatchery unit, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt. Subsequently, fish were acclimated for 14 days on commercially available feeds (30% crude protein manufactured by Skretting, Bilbies, El Sharqia Governorate, Egypt) up to the satiation level twice daily. Then, fish were randomly allocated into equal two groups (50 fish per group) and maintained at ten well-equipped glass aquaria (40 × 60 × 80 cm) with

a stocking density of 10 healthy fish per aquarium. The first group was supplied with diet I, while the second group was fed with the ration supplemented with diet II. All experimental fish were fed twice daily (8:00 and 15:00). Accordingly, they were fed at 3.6% (0–2 weeks), 3.4% (2–4 weeks), 3.2% (4–6 weeks), and 3.0% (6–8 weeks) of their body weight. During this phase, water quality was sustained as dissolved oxygen (DO) (5.89 ± 0.2) mg/l, pH (7.46 ± 0.1), EC 219 + 2 µmho/cm, water temperature (26.0 ± 0.2°C), and photoperiod 12:12h (day and night) (Ghaniem et al., 2022). Successively, a dead fish was eradicated to avoid deterioration of water. After 90 days, all live fish in the experimental groups were anesthetized using MS222 in the concentration of 150 mg/l (Argent Laboratories, Redmond, WA, USA), then weighed to calculate different growth performance parameters as in the following equations:

$$\textit{Weight gain (\%)} = \frac{W2 - W1}{W1} \times 100,$$

$$\textit{Specific growth rate (\% per day)}$$

where: *W1* and *W2* are initial and final body weight (g).

Feed conversion ratio = dry feed intake (g) / live weight gain (g).

Tissue sampling

After the experiments had ended, ten fish were selected randomly from each treatment (2 fish per aquaria), then the liver, muscle, and spleen tissue samples were carefully collected and immediately shocked in liquid nitrogen then stored at –80°C till further analysis.

Histopathological examination

Ten selected fish were deep anesthetized, then the abdomen was opened to collect samples from muscle, liver, spleen, and intestine, which were then fixed in 10% neutral buffered formalin for a duration of 18–24 h. Afterward, samples were dehydrated using ascending grades of ethanol (70–100%), next treated with xylene, then embedded in paraffin wax. Subsequently, 5 µm thick sections were obtained with a rotatory microtome (Leica RM 2125), after that stained by hematoxylin and eosin (H & E) stain and then examined under a light microscope (Leica DM 5000).

RNA extraction and reverse-transcription polymerase chain reaction

RNA extraction from all collected samples was performed using TriZol reagent (iNtRON Biotechnology). The satisfaction of extracted RNA examined via Nano Drop® BioDrop Spectrophotometer enables extraordinarily accurate analyses of extremely small samples with tremendous reproducibility. Furthermore, the absorbance ratio at A260 nm/A280 nm (1.8 to 2.0) is the most frequently used method to determine RNA quality after extraction. For RNA reliability, all extracted RNA samples were electrophoresed on 1.5% agarose gel with 0.5% ethidium bromide (Sigma, Germany) then visualized under a UV transilluminator (Azure c200).

cDNA synthesis and quantitative real-time PCR

Two µg of extracted RNA from all triplicate samples were reverse transcribed using Maxime RT PreMix (Oligo dT primer) (iNtRON Biotechnology, Korea) following the manufacturer's manual. Real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis of mRNA expression of Nile tilapia-specific primers for each of IGF-1, HSP70, SOD, IL-1β, and β-actin as the house-keeping gene was implemented using primers prepared according to Abdelhiee et al. (2021), Elbialy et al. (2021), and Dawood and Koshio (2020).

The cDNAs of examined samples were used as the template for RT-PCR using the SensiFast SYBR No-Rox kit (Bioline) in the mic-PCR Real-time PCR system (Bio-molecular systems). The relative variation in gene expression levels was calculated by using threshold cycle (CT) values which were normalized to the Nile tilapia (*Oreochromis niloticus*), β-actin house-keeping gene using ΔCT values as previously described by Yuan et al. (2006).

Statistical analysis

The Shapiro–Wilk and Levene tests were used to assess the homogeneity and normality of variance. A paired t-test was performed to detect the significances between the two groups. All statistical analyses were performed with GraphPad Prism 5 (San Diego, CA).

Results

Growth performance and feed utilization

The growth performance, feed intake, and protein efficacy rate are demonstrated in Figure 1. The growth performance indicated that no significant ($P \geq 0.05$) effects on final body weight, the weight gain and weight gain %, specific growth rate (SGR), protein efficacy rate, and feed conversion ratio were revealed in the fish group with diet II in comparison with the fish group in diet I.

Histopathological findings

The observed data revealed that fish fed diet I had normal structure; intact muscle bundles of normal parallel muscle fibers (Figure 2 A). The group fed diet II showed atrophy and separation of muscle bundles that are mostly bearing irregular corkscrew fibers (Figure 2 B).

The liver of the fish fed diet I and diet II exhibited normal hepatic architecture with mild vacuolation consistent with glycogen storage and normal hepatopancreas (Figure 2 C and 2 D).

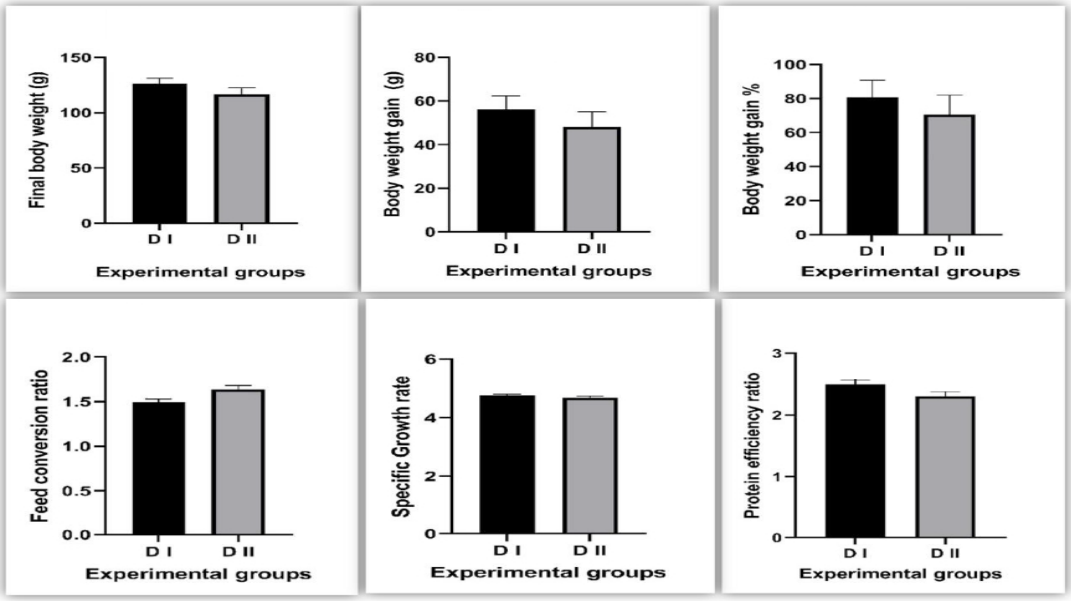

Figure 1. Growth performance parameters of Nile tilapia after 12 weeks in fish groups fed diet I or diet II

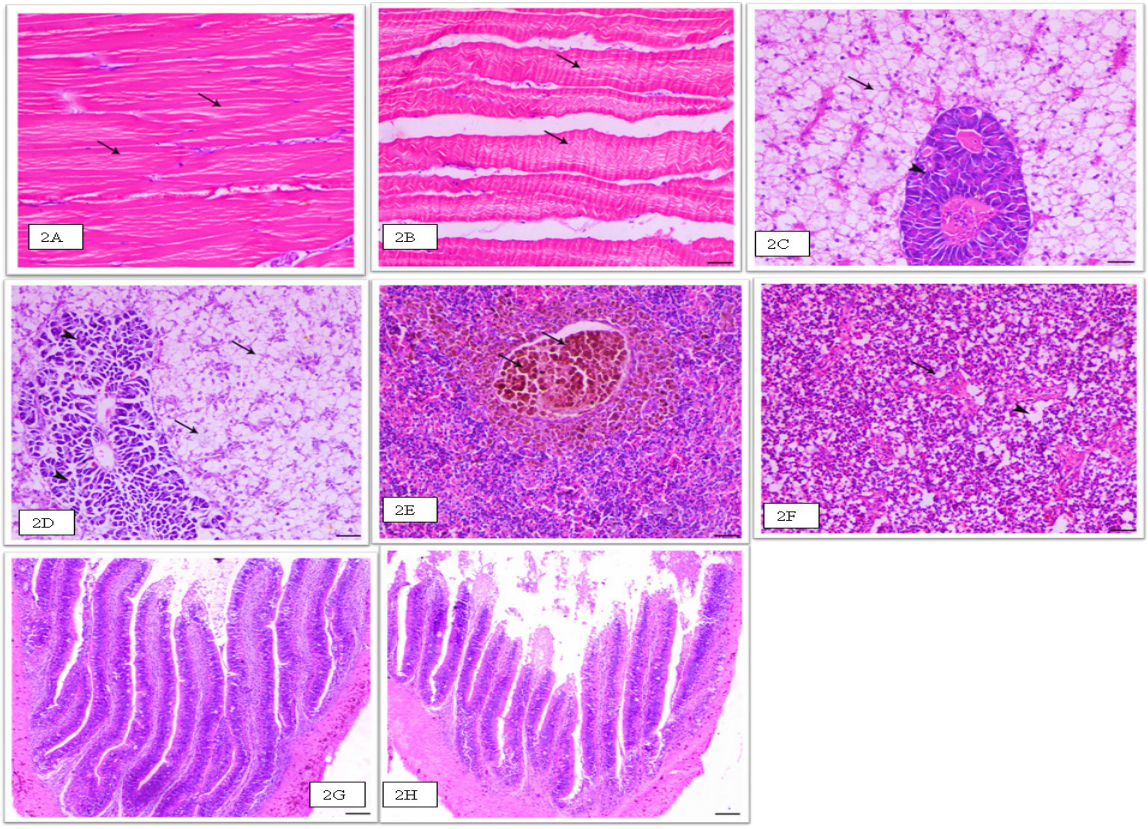

Figure 2. Photomicrograph of Nile tilapia tissues after 12 weeks feeding diet I or diet II. 2 A – muscle samples of fish fed diet I showing normal bundles consisting of normal parallel muscle fibers (arrows), 2 B – muscle samples of fish fed diet II showed atrophy and separation of muscle bundles that mostly showed wavy and irregular fibers (arrows). 2 C – liver samples of fish fed diet I showing normal hepatocytes with mild vacuolation consistent to glycogen sto-rage (arrow) and normal hepatopancreas (arrowhead), 2 D – liver samples of fish fed diet II showed light degree of hepatic vacuolation (arrows) (arrowheads). 2 E and 2 F – spleen of fish fed diet I showed normal capsulated melanomacrophage center with many melanomacrophage cells (arrows) surrounded with plenty of lymphocytes. 2 G – Intestine samples of fish fed diet I showing villi with an increase of their length. 2 H – intestine of fish fed diet II showed normal villi length. H&E,X200, bar – 50 µm

The histopathological assortment based on the spleen of fish fed diet I and diet II revealed a normal capsulated melanomacrophage center with many melanomacrophage cells surrounded with plenty of lymphocytes (Figure 2 E and 2 F). Besides, the inspection of the intestine of the fish fed diet I and diet II revealed a manifestation of normal villi combined with increasing of their length (Figure 2 G). Furthermore, a marked decrease in villi length and goblet cell numbers was detected in the fish fed diet II (Figure 2 H).

Relative mRNA expression levels of growth-related gene

This study demonstrated that liver and muscle IGF-1 were not significantly altered in the fish fed diet I and II (Figure 3).

Gene expression levels of immune and stress response biomarkers

In the current study, the influence of feeding Nile tilapia fish with different sources of soybean and corn was determined; therefore, association analysis of IL-1β, SOD, and HSP70 expression levels in both spleen and liver was presented in Figure 4. The results revealed markedly enhanced up-regulation of these genes in the liver. Moreover, although SOD and HSP70 expression levels were upregulated in the spleen of fish fed diet II compared to diet I, there was no significant difference in IL-1β gene expression in the spleen of both treatments.

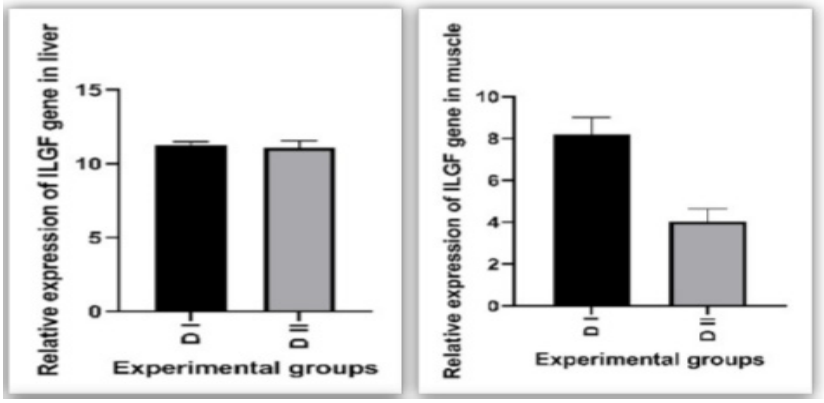

Figure 3. Relative expression of IGF-1 (insulin-like growth factor 1) in liver and muscle tissue of Nile tilapia after feeding two different sources of soybean and corn

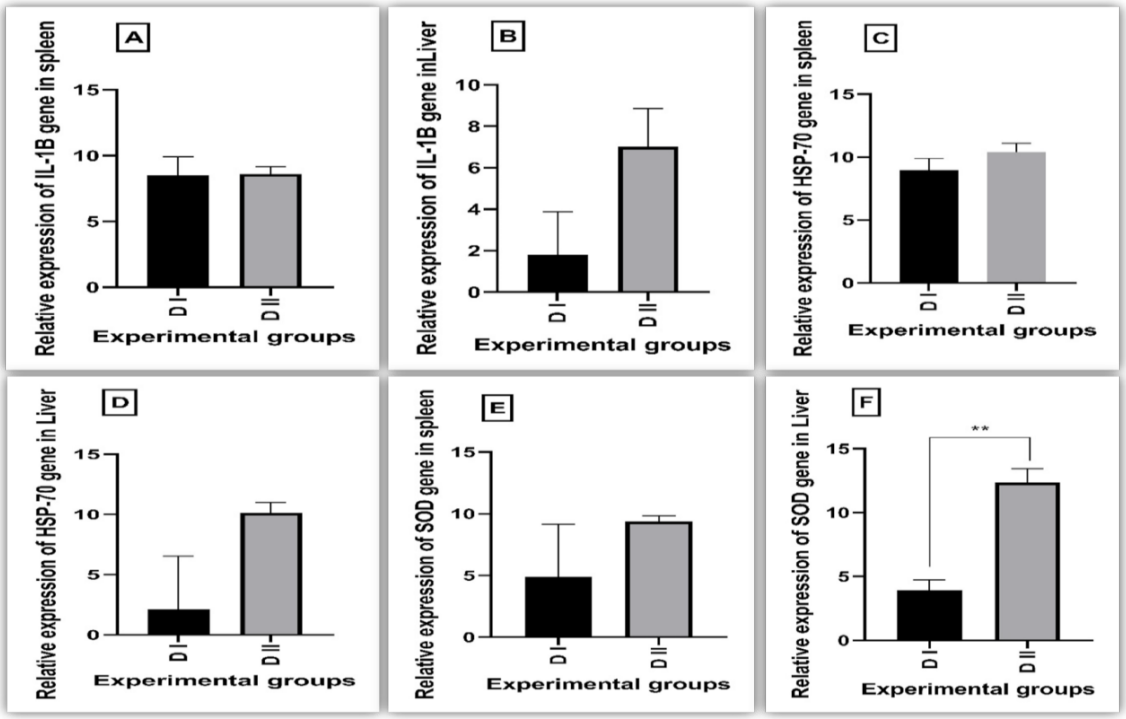

Figure 4. Relative expression of immune-related and stress response genes in spleen and liver tissues: (A) spleen IL-1β gene, (B) liver IL-1β gene, (C) spleen HSP-70, (D) liver HSP70, (E) spleen SOD gene, and (F) liver SOD gene of Nile tilapia after feeding two different sources of soybean and corn. IL-1β – interleukin 1 beta, HSP-70 – Heat shock protein 70, SOD – Superoxide dismutase

Discussion

The fish feed should contain various feed ingredients to satisfy the nutrient requirements of different fish species (Glencross et al., 2007; Hazreen-Nita et al., 2022). Such ingredients are incorporated into the diet according to their nutritional content to meet all nutritional needs of fish (Napier et al., 2020; Roques et al., 2020). In this context, protein concentrates of soybean, wheat gluten, and corn gluten have been used to completely replace fishmeal in aquaculture feeds (Lupatsch and Kissil, 2004). A deep understanding of the relationship between dietary factors and molecular expression levels of physiological processes is urgently required to assess the metabolic steps involved (Moriyama et al., 2000). The results showed similar growth performance without significant differences between fish fed diet I or diet II. The obtained results contradict with Bakke-McKellep et al. (2008), who recorded that feed intake, growth rate, as well as final weight, were all significantly reduced in post--smolt Atlantic salmon, which were fed a different variety of maize (MON810) at levels of 15% and 30% of the whole diet in comparison with fish that fed on other variety of maize. The changes may be related to differences in fish species, feeding habits, and nutritional requirements.

Numerous reports discussed the appearance of specific morphological changes in mammals' small intestine after feeding soybean meal; these reports included a shortening of villi height as well as an increase in intestinal crypt depth, indicated by increasing mucosal cells proliferation (He et al., 2020; Pervin et al., 2020). These features simulate disorders such as human coeliac disease, a syndrome where gluten proteins lead to allergic reactions that trigger intestinal inflammation (Mathan et al., 1986). In a consensus study, the Roundup Ready soybean (Monsanto NV, Brussels, Belgium) was evaluated in the diet of Atlantic salmon; the results demonstrated spleen enlargement as well as potential impaired function of the spleen as a result of the simultaneous increase in the count of smaller-sized RBCs, where the SBM might cause early releasing of immature erythrocytes which might be responsible for spleen enlargement (Hemre et al., 2005). The absence of abnormal features in the intestines, liver, and spleen in Nile tilapia fed diet I or diet II indicates that local soybean and corn cultivars (diet I) could be used as replacers for imported soybean and corn cultivar (diet II).

The skeletal muscle mass regulation constitutes a functional balance between pathways of protein synthesis and degradation, which are dominated by both growth hormone (GH) and insulin-like growth factors (IGFs) (Fuentes et al., 2013; Johnston et al., 2011). In addition, Pérez-Sánchez et al. (1995) indicated that plasma IGF-I and the dietary protein level are positively correlated, elucidating that the growth factor is a strong indicator of the optimal levels of the protein that are requisites for growth. As opposed to circling IGF-I, GH was commonly adversely associated with growth and dietary protein levels. In fish, insulin-like growth factors (IGFs) have been shown to regulate the expansion of skeletal muscle development, which has importance for growth enhancement. Therefore, the somatotropic axis genes (GH and IGF-1) and their regulatory sequences are considered candidate biomarkers to assess growth performance and improve these traits. Also, insulin-like growth factor I (IGF-I) is considered the primary anabolic agent being accountable for tissue growth (Duan, 1998; Thissen et al., 1999); however, there is an apparent confirmation that IGF-II is related to the local paracrine/autocrine regulation mechanism of muscle growth in teleost fishes (Hevrøy et al., 2007; Vong et al., 2003). In this study, no substantial down-regulation of both liver and muscle IGF-1 in the diet II-fed fish compared to the diet I group was observed. The uniqueness among GH and development rate might just be intervened by the predominant degrees of IGF-1 and its adverse input consequences for the pituitary gland, where the plasma IGF-1 increments with higher rates of growth, the levels of GH decrease attributable to an enhanced passive criticism (Picha et al., 2008). Gómez-Requeni et al. (2005) suggested that decreased growth rates accompanied by a supply of plant protein are principally due to lower free plasma IGF availability instead of desensitization of liver GH or disorder in synthesis and release of IGF at both the systemic as well as autocrine–paracrine levels.

The gastrointestinal tract is the leading portal of entry of foreign DNA particles and proteins and the first contact site with molecules (Mohammadi et al., 2021; Palka-Santini et al., 2003). Furthermore, studying the gene expression provided novel opportunities to understand better the essential molecular mechanisms underlying the fish response to diets containing new ingredients (De Santis et al., 2015; Mohammadi et al., 2022 b). In the current study, hepatic gene expression levels (IL-1β, HSP70, and SOD) were significantly increased in diet II compared to diet I, which might be therefore in part due to induction of stress response by the different protein and secondary products resulting from the digestion process of the soybean and its content of anti-nutritional factors. In addition, the gene expression associated with hepatic and muscle growth and the hepatic and spleen genes involved in stress and immune response was observed.

This study found a significant modulation in the expression levels of some immune pro-inflammatory or stress genes in the liver and spleen. The progression of this overtime correlated well with the development of the histopathological signs of the affected organs. The most significant trait was the fundamental up-regulation of the expression level of the hepatic pro-inflammatory cytokine, including IL-1β, HSP70, and SOD. Comparably, immune reactive genes included in the IGF pathway displayed an astonishing co-expression with pro-inflammatory cytokine as well as antiviral genes that are regulated by conserved immune signaling pathways (Alzaid et al., 2016), approving the assumption that mechanisms have

emerged which restrict growth investment as an intrinsic component of host defense.

IL-1β is a pro-inflammatory cytokine that functions on T-helper cells as it provides a co-stimulatory signal for cell activation following the antigen recognition process and acts as part of the acute phase responses (Guarda et al., 2011; Honore et al., 2006; Mohammadi et al., 2022 a). This trial highlighted stress and immune-related biomarkers by focusing on IL-1β, HSP-70, and SOD gene expression levels in the liver and spleen. The liver revealed higher expression levels of IL-1β, HSP-70, and SOD in fish fed diet II than those fed diet I. IL-1β is a cytokine protein secreted by various immune cells and is implicated in inflammation processes (Zou and Secombes, 2016). Also, HSP70 is an effective tool to boost cell survival rate via stress protection, cure, and environmental pressure relief (Ming et al., 2010). What is more, HSP70's relative expression level is a possible stress marker for amphibian creatures (Faggio et al., 2016). The up-regulation of HSP70 mRNA levels detected in the spleen and liver of tilapia fed with diet II demonstrates that this examination can be utilized as an early biomarker for diet prompted stress in the spleen and liver. Stress marker (HSP70) in post-smolt salmon was markedly up-regulated with SBM-induced inflammation (Bakke-McKellep et al., 2008; Gao et al., 2013; Marjara et al., 2012). Similarly, Sagstad et al. (2007) claimed that the possible presence of δ-endotoxin in the MON810 maize might be the reason for the elevated SOD and CAT enzyme activities in the distal part of the intestine in salmon. These results are due to extensive transgenic DNA sequences that resist feed processing and can be isolated from all segments of the alimentary tract, and can also be absorbed (Sanden et al., 2005). Moreover, Sagstad et al. (2007) indicated that taking care of Atlantic salmon, a different variety of maize brought about slight modifications in the activities of CAT and SOD enzyme in the liver and distal digestive system, and the level of HSP70 protein in the liver. These signs were demonstrative of a mellow stress reaction.

In the present study with Nile tilapia fed different soy and corn varieties, histopathological examinations uncovered adverse effects on the skeletal muscle, hepatopancreas, spleen, and intestinal tract, together with its impact on fish growth performance and growth, immune, and stress-related gene expressions.

Conclusion

One of the main points of the current study was to interpret differentiation patterns among Nile tilapia, considering their feed containing different soybean and corn cultivars. The highest disruptions of immune function, which can affect Nile tilapia growth, were observed combined with subtle growth performance, organs histopathological findings, and changes in the GH/IGF-1 axis at the molecular level were recognized. Based on the growth performance and histological study, local soybean and corn cultivars could replace the imported soybean and corn cultivars. The obtained results open the door for future investigations dealing with local feed ingredients as a possible replacer for the common components to reduce the feed cost and sustain the aquaculture industry.

References

Abdelhiee E.Y., Elbialy Z.I., Saad A.H., Dawood M.A.O., Aboubakr M., El-Nagar S.H., El-Diasty E.M., Salah A.S., Saad H.M., Fadl S.E. (2021). The impact of *Moringa oleifera* on the health status of Nile tilapia exposed to aflatoxicosis. Aquaculture, 533: 736110.

Abdul Kari Z., Kabir M.A., Mat K., Rusli N.D., Razab M.K.A.A., Ariff N.S.N.A., Edinur H.A., Rahim M.Z.A., Pati S., Dawood M.A.O., Wei L.S. (2021). The possibility of replacing fish meal with fermented soy pulp on the growth performance, blood biochemistry, liver, and intestinal morphology of African catfish (*Clarias gariepinus*). Aquac. Rep., 21: 100815.

Alzaid A., Castro R., Wang T., Secombes C.J., Boudinot P., Macqueen D.J., Martin S.A.M. (2016). Cross-talk between growth and immunity: coupling of the IGF axis to conserved cytokine pathways in rainbow trout. Endocrinology, 157: 1942–1955.

AOAC (2012). Official Methods of Analysis of AOAC International. 19th edition. AOAC International, Gaithersburg, Maryland, USA. www.eoma.aoac.org.

Bakke-McKellep A.M., Sanden M., Danieli A., Acierno R., Hemre G.I., Maffia M., Krogdahl Å. (2008). Atlantic salmon (*Salmo salar* L.) parr fed genetically modified soybeans and maize: Histological, digestive, metabolic, and immunological investigations. Res. Vet. Sci., 84: 395–408.

Bandara T. (2018). Alternative feed ingredients in aquaculture: Opportunities and challenges. J. Entomol. Zool. Stud., 6: 3087–3094.

Dawood M.A.O. (2021). Nutritional immunity of fish intestines: important insights for sustainable aquaculture. Rev. Aquac., 13: 642–663.

Dawood M.A.O., Koshio S. (2020). Application of fermentation strategy in aquafeed for sustainable aquaculture. Rev. Aquac., 12: 987–1002.

Dawood M.A.O., Metwally A.E.-S., El-Sharawy M.E., Ghozlan A.M., Abdel-Latif H.M.R., Van Doan H., Ali M.A.M. (2020). The influences of ferulic acid on the growth performance, haemato-immunological responses, and immune-related genes of Nile tilapia (*Oreochromis niloticus*) exposed to heat stress. Aquaculture, 525: 735320.

Dawood M.A.O., Ali M.F., Amer A.A., Gewaily M.S., Mahmoud M.M., Alkafafy M., Assar D.H., Soliman A.A., Van Doan H. (2021). The influence of coconut oil on the growth, immune, and antioxidative responses and the intestinal digestive enzymes and histomorphometry features of Nile tilapia (*Oreochromis niloticus*). Fish Physiol. Biochem., 47: 869–880.

De Santis C., Bartie K.L., Olsen R.E., Taggart J.B., Tocher D.R. (2015). Nutrigenomic profiling of transcriptional processes affected in liver and distal intestine in response to a soybean meal-induced nutritional stress in Atlantic salmon (*Salmo salar*). Comp. Biochem. Physiol. Part D Genomics., 15: 1–11.

Duan C. (1998). Nutritional and developmental regulation of insulin-like growth factors in fish. J. Nutr., 128: 306S–314S.

Elbialy Z.I., Salah A.S., Elsheshtawy A., Rizk M., Abualreesh M.H., Abdel-Daim M.M., Salem S.M.R., Askary A.E., Assar D.H. (2021). Exploring the multimodal role of *Yucca schidigera* extract in protection against chronic ammonia exposure targeting: growth, metabolic, stress and inflammatory responses in Nile tilapia (*Oreochromis niloticus* L.). Animals, 11.

Elumalai P., Prakash P., Musthafa M.S., Faggio C. (2019). Effect of alkoxy glycerol on growth performance, immune response and disease resistance in Nile Tilapia (*Oreochromis niloticus*). Res. Vet. Sci., 123: 298–304.

Faggio C., Pagano M., Alampi R., Vazzana I., Felice M.R. (2016). Cytotoxicity, haemolymphatic parameters, and oxidative stress following exposure to sub-lethal concentrations of quaternion-15 in *Mytilus galloprovincialis*. Aquat. Toxicol., 180: 258–265.

FAO (2020). The state of world fisheries and aquaculture, p. 200.

Fuentes E.N., Valdés J.A., Molina A., Björnsson B.T. (2013). Regulation of skeletal muscle growth in fish by the growth hormone – insulin-like growth factor system. Gen. Comp. Endocrinol., 192: 136–148.

Galkanda-Arachchige H.S.C., Wilson A.E., Davis D.A. (2020). Success of fishmeal replacement through poultry by-product meal in aquaculture feed formulations: a meta-analysis. Rev. Aquac., 12: 1624–1636.

Gao C.Q., Ji C., Zhang J.Y., Zhao L.H., Ma Q.G. (2013). Effect of a novel plant phytase on performance, egg quality, apparent ileal nutrient digestibility and bone mineralization of laying hens fed corn-soybean diets. Anim. Feed Sci. Technol., 186: 101–105.

Ghaniem S., Nassef E., Zaineldin A.I., Bakr A., Hegazi S. (2022). A comparison of the beneficial effects of inorganic, organic, and elemental nano-selenium on Nile tilapia: growth, immunity, oxidative status, gut morphology, and immune gene expression. Biol. Trace Elem. Res. https://doi.org/10.1007/s12011-021-03075-5

Glencross B.D., Booth M., Allan G.L. (2007). A feed is only as good as its ingredients – a review of ingredient evaluation strategies for aquaculture feeds. Aquac. Nutr., 13: 17–34.

Gómez-Requeni P., Calduch-Giner J., Vega-Rubín de Celis S., Médale F., Kaushik S.J., Pérez-Sánchez J. (2005). Regulation of the somatotropic axis by dietary factors in rainbow trout (*Oncorhynchus mykiss*). Br. J. Nutr., 94: 353–361.

Guarda G., Braun M., Staehli F., Tardivel A., Mattmann C., Förster I., Farlik M., Decker T., Du Pasquier Renaud A., Romero P., Tschopp J. (2011). Type I interferon inhibits interleukin-1 production and inflammasome activation. Immunity, 34: 213–223.

Hazreen-Nita M.K., Abdul Kari Z., Mat K., Rusli N.D., Mohamad Sukri S.A., Che Harun H., Lee S.W., Rahman M.M., Norazmi-Lokman N.H., Nur-Nazifah M., Firdaus-Nawi M., Dawood M.A.O. (2022). Olive oil by-products in aquafeeds: Opportunities and challenges. Aquac. Rep., 22: 100998.

He M., Li X., Poolsawat L., Guo Z., Yao W., Zhang C., Leng X. (2020). Effects of fish meal replaced by fermented soybean meal on growth performance, intestinal histology and microbiota of largemouth bass (*Micropterus salmoides*). Aquac. Nutr., 26: 1058–1071.

Hemre G.I., Sanden M., Bakke-Mckellep A.M., Sagstad A., Krogdahl Å. (2005). Growth, feed utilization and health of Atlantic salmon *Salmo salar* L. fed genetically modified compared to non-modified commercial hybrid soybeans. Aquac. Nutr., 11: 157–167.

Hevrøy E.M., El-Mowafi A., Taylor R.G., Olsvik P.A., Norberg B., Espe M. (2007). Lysine intake affects gene expression of anabolic hormones in Atlantic salmon, *Salmo salar*. Gen. Comp. Endocrinol., 152: 39–46.

Honore P., Wade C.L., Zhong C., Harris R.R., Wu C., Ghayur T., Iwakura Y., Decker M.W., Faltynek C., Sullivan J., Jarvis M.F. (2006). Interleukin-1αβ gene-deficient mice show reduced nociceptive sensitivity in models of inflammatory and neuropathic pain but not post-operative pain. Behav. Brain Res., 167: 355–364.

Johnston I.A., Bower N.I., Macqueen D.J. (2011). Growth and the regulation of myotomal muscle mass in teleost fish. J. Exp. Biol., 214: 1617–1628.

Jones S.W., Karpol A., Friedman S., Maru B.T., Tracy B.P. (2020). Recent advances in single-cell protein use as a feed ingredient in aquaculture. Curr. Opin. Biotechnol., 61: 189–197.

Kari Z.A., Kabir M.A., Razab M.K.A.A., Munir M.B., Lim P.T., Wei L.S. (2020). A replacement of plant protein sources as an alternative of fish meal ingredient for African catfish, *Clarias gariepinus*: A review. J. Tropic. Res. Sust. Sci., 8: 47–59.

Kari Z.A., Kabir M.A., Dawood M.A.O., Razab M.K.A.A., Ariff N.S.N.A., Sarkar T., Pati S., Edinur H.A., Mat K., Ismail T.A., Wei L.S. (2022). Effect of fish meal substitution with fermented soy pulp on growth performance, digestive enzyme, amino acid profile, and immune-related gene expression of African catfish (*Clarias gariepinus*). Aquaculture, 546: 737418.

Khalidah N., Onas Somdare P., Abdullah Md Harashid K., Ain Othman N., Abdul Kari Z., Seong Wei L., Dawood M.A.O. (2022). Effect of papaya (*Carica papaya*) leaf extract as dietary growth promoter supplement in red hybrid tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*) diet. Saudi J. Biol. Sci., https://doi.org/10.1016/j.sjbs.2022.03.004

Kok B., Malcorps W., Tlusty M.F., Eltholth M.M., Auchterlonie N.A., Little D.C., Harmsen R., Newton R.W., Davies S.J. (2020). Fish as feed: Using economic allocation to quantify the Fish In : Fish Out ratio of major fed aquaculture species. Aquaculture, 528: 735474.

Lupatsch I., Kissil G.W. (2004). Successful replacement of fishmeal by plant proteins in diets for the gilthead seabream, *Sparus aurata* L. Isr. J. Aquac., 56: 20378.

Marjara I.S., Chikwati E.M., Valen E.C., Krogdahl Å., Bakke A.M. (2012). Transcriptional regulation of IL-17A and other inflammatory markers during the development of soybean meal-induced enteropathy in the distal intestine of Atlantic salmon (*Salmo salar* L.). Cytokine, 60: 186–196.

Mathan M.M., Ponniah J., Mathan V. (1986). Epithelial cell renewal and turnover and relationship to morphologic abnormalities in jejunal mucosa in tropical sprue. Dig. Dis. Sci., 31: 586–592.

Ming J., Xie J., Xu P., Liu W., Ge X., Liu B., He Y., Cheng Y., Zhou Q., Pan L. (2010). Molecular cloning and expression of two HSP70 genes in the Wuchang bream (*Megalobrama amblycephala* Yih). Fish Shellfish Immunol., 28: 407–418.

Mohammadi G., Rafiee G., Tavabe K.R., Abdel-Latif H.M.R., Dawood M.A.O. (2021). The enrichment of diet with beneficial bacteria (single- or multi-strain) in biofloc system enhanced the water quality, growth performance, immune responses, and disease resistance of Nile tilapia (*Oreochromis niloticus*). Aquaculture, 539: 736640.

Mohammadi G., Hafezieh M., Karimi A.A., Azra M.N., Van Doan H., Tapingkae W., Abdelrahman H.A., Dawood M.A.O. (2022 a). The synergistic effects of plant polysaccharide and *Pediococcus acidilactici* as a synbiotic additive on growth, antioxidant status, immune response, and resistance of Nile tilapia (*Oreochromis niloticus*) against *Aeromonas hydrophila*. Fish Shellfish Immunol., 120: 304–313.

Mohammadi G., Karimi A.A., Hafezieh M., Dawood M.A.O., Abo-Al-Ela H.G. (2022 b). Pistachio hull polysaccharide protects Nile tilapia against LPS-induced excessive inflammatory responses and oxidative stress, possibly via TLR2 and Nrf2 signaling pathways. Fish Shellfish Immunol., 121: 276–284.

Moriyama S., Ayson F.G., Kawauchi H. (2000). Growth regulation by insulin-like growth factor-I in fish. Biosci. Biotechnol. Biochem., 64: 1553–1562.

Napier J.A., Haslam R.P., Olsen R.-E., Tocher D.R., Betancor M.B. (2020). Agriculture can help aquaculture become greener. Nat. Food., 1: 680–683.

Palka-Santini M., Schwarz-Herzke B., Hösel M., Renz D., Auerochs S., Brondke H., Doerfler W. (2003). The gastrointestinal tract as the portal of entry for foreign macromolecules: fate of DNA and proteins. Mol. Genet. Genom., 270: 201–215.

Panserat S., Hortopan G.A., Plagnes-Juan E., Kolditz C., Lansard M., Skiba-Cassy S., Esquerré D., Geurden I., Médale F., Kaushik S., Corraze G. (2009). Differential gene expression after total replacement of dietary fish meal and fish oil by plant products in rainbow trout (*Oncorhynchus mykiss*) liver. Aquaculture, 294: 123–131.

Pérez-Sánchez J., Martí-Palanca H., Kaushik S.J. (1995). Ration size and protein intake affect circulating growth hormone concentration, hepatic growth hormone binding and plasma insulin-like growth factor-I immunoreactivity in a marine teleost, the gilthead seabream (*Sparus aurata*). J. Nutr., 125: 546–552.

Pervin M.A., Jahan H., Akter R., Omri A., Hossain Z. (2020). Appraisal of different levels of soybean meal in diets on growth, digestive enzyme activity, antioxidation, and gut histology of tilapia (*Oreochromis niloticus*). Fish Physiol. Biochem., 46: 1397–1407.

Picha M.E., Turano M.J., Beckman B.R., Borski R.J. (2008). Endocrine biomarkers of growth and applications to aquaculture: a minireview of growth hormone, insulin-like growth factor (IGF)-I, and IGF-binding proteins as potential growth indicators in fish. N. Am. J. Aquac., 70: 196–211.

Roques S., Deborde C., Richard N., Skiba-Cassy S., Moing A., Fauconneau B. (2020). Metabolomics and fish nutrition: a review in the context of sustainable feed development. Rev. Aquac., 12: 261–282.

Sagstad A., Sanden M., Haugland Ø., Hansen A.C., Olsvik P.A., Hemre G.I. (2007). Evaluation of stress- and immune-response biomarkers in Atlantic salmon, *Salmo salar* L., fed different levels of genetically modified maize (Bt maize), compared with its near-isogenic parental line and a commercial suprex maize. J. Fish Dis., 30: 201–212.

Sanden M., Berntssen M.H.G., Krogdahl Å., Hemre G.I., Bakke-McKellep A.M. (2005). An examination of the intestinal tract of Atlantic salmon, *Salmo salar* L., parr fed different varieties of soy and maize. J. Fish Dis., 28: 317–330.

Thissen J.-P., Underwood L.E., Ketelslegers J.-M. (1999). Regulation of insulin-like growth factor–I in starvation and injury. Nutr. Rev., 57: 167–176.

Urán P.A., Schrama J.W., Jaafari S., Baardsen G., Rombout J.H.W.M., Koppe W., Verreth J.A.J. (2009). Variation in commercial sources of soybean meal influences the severity of enteritis in Atlantic salmon (*Salmo salar* L.). Aquac. Nutr., 15: 492–499.

Vong Q., Chan K., Cheng C. (2003). Quantification of common carp (*Cyprinus carpio*) IGF-I and IGF-II mRNA by real-time PCR: differential regulation of expression by GH. J. Endocrinol., 178: 513–521.

Yuan J.S., Reed A., Chen F., Stewart C.N. (2006). Statistical analysis of real-time PCR data. BMC Bioinformatics, 7: 85.

Zaineldin A.I., Hegazi S., Koshio S., Ishikawa M., El Basuini M.F., Dossou S., Dawood M.A.O. (2021). The influences of *Bacillus subtilis* C-3102 inclusion in the red sea bream diet containing high levels of soybean meal on growth performance, gut morphology, blood health, immune response, digestibility, digestive enzymes, and stress resistance. Aquac. Nutr., 27: 2612–2628.

Zou J., Secombes C.J. (2016). The function of fish cytokines. Biology, 5.

Zulhisyam A.K., Kabir M.A., Munir M.B., Wei L.S. (2020). Using of fermented soy pulp as an edible coating material on fish feed pellet in African catfish (*Clarias gariepinus*) production. Aquacult. Aquarium Conserv. Legis., 13: 296–308.

Received: 9 II 2022
Accepted: 21 III 2022