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The Protective Effects of Korill Product on Carp Fingerlings Reared in High Densities and Challenged with Albendazole Treatment

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Abstract: The objectives of the present study were to evaluate the potential of the Korill (KO), a product based on krill oil, supplemented in fish feed (5 g/kg feed) to alleviate density-induced stress in carp fingerlings, and its protective capacity in case of anthelmintic drug administration (ABZ—albendazole). Thus, the fish were divided into four groups: LD (low density with normal feed), LD-KO (low density with KO supplementation feed), HD (high density with normal feed), and HD-KO (high density with KO supplementation feed). During the first trial, the fish held under different densities were fed normal feed and KO feed for two months, following a 2 × 2 factorial experimental design. In the second trial, seven fish per tank were subjected, for one week, to albendazole treatment (administered daily by an oral dose of 5 mg/kg body weight). For both trials, blood and plasma samples were used to quantify hematological and biochemical parameters. The results showed that the KO diet alleviated the negative impact of ABZ treatment on liver function and the metabolic profile of carp fingerlings reared in high densities. In addition, KO feeding improved lysozyme activity (LZM) and therefore the immune status of the fish, and reduced oxidative damage in the liver, demonstrated by a decrease of malondialdehyde (MDA) content and an increase of total antioxidant capacity (TAC).

Keywords: density stress; albendazole; krill oil; oxidative stress; hepatotoxicity

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1. Introduction

Over the past two decades, the aquaculture industry faced major changes toward the diversification of cultivated species and sustainable production intensification, contributing significantly to the world food sector [1].

In intensive aquaculture systems, economic efficiency is dictated by unitary production, which is dependent on the growth performance of the fish and the level of stocking density. However, high stocking density (HD) has been evaluated as a chronic stressor responsible for different physiological stress responses, putting at susceptibility or risk the homeostasis and health of the fish [2,3]. The principal corticosteroid triggered by HD is cortisol, which, during crowding stress, rises at high concentrations in blood inducing immunosuppressive and catabolic actions [4]. Therefore, humoral immune parameters [5], serum metabolites (osmolality, albumin, and globulin values), liver antioxidant-related parameters (SOD, CAT, GPx, and MDA levels), lipid [6] and liver proteome patterns are affected [7–9].

In order to counteract the negative effects induced by the high densities practiced in intensive production systems, various solutions to increase the immune response of fish have been already tested in numerous studies. In this sense, the main approach was the nutritional path, which aimed to supplement the diet with antioxidant compounds such

as herbal compounds and their extracts [10,11], essential oils [12,13] or functional feed additives [14] to stimulate the fish immunity to cope with the stress.

Besides stress induced by HD, cultured fish are also exposed to other types of stressors such us transport, manipulation (fishing, sorting), sudden changes in water quality (water safety, oxygenation, composition), or diseases and, implicitly, the treatments applied for their control. It is well known that disease prevention is more desirable than medical treatment. However, in practice, treatments are still applied sometimes since stimulation of immune response does not necessarily offer total protection against pathogens [15]. High stocking density practiced in fish farms favors the dispersion and transmission of the parasites, especially in the open systems where the fish are exposed to opportunistic species from the wild [16,17]. Thus, in some cases, broad-spectrum antiparasitic agents are used to control the infestation.

Albendazole (ABZ) is a broad-spectrum anthelmintic drug used in the zootechnic sector [18]. Recently, ABZ became a potential drug for use in aquaculture; however, it is not specifically regulated for aquaculture [19]. Previous studies concerning the impact of ABZ on fish highlighted its efficiency on pathogen control [20,21] but also its toxic effect on rainbow trout (*Oncorhynchus mykiss*) infected with *Gyrodactylus* spp. [22], teratogenic and embryotoxic effects as well as developmental abnormalities in zebrafish, *Danio rerio* [23,24], and oxidative stress, elevated lipid peroxidation, or altered hematological parameters in African Catfish [25]. In order to counteract the negative effects of ABZ, a nutritional strategy must be found in the sense of using functional ingredients that increase fish immunity and reduce oxidative stress. Such diets are viewed as important complementary component of fish health management in aquaculture.

Phospholipids (PLs) are lipidic biomolecules constituting the main structural elements in the formation of cell membranes, being also essential for the normal functioning of cells and organs. For example, they can act as secondary messengers in cell signaling, this process being essential, among others, in the regulation of cellular growth and differentiation, ion transport, or nutrient absorption metabolism. Fish cannot synthesize phospholipids at an optimal rate to meet their nutritional requirements and, therefore, it is recommended to include enough phospholipids in the fish diet to ensure adequate growth as well as stress resistance [26]. Krill oil is a unique lipid consisting of diverse lipid classes and is characterized by a high concentration (39.29% to 80.69%) of PLs associated with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Krill oil also contains tocopherols, vitamin A, and astaxanthin, which help to support the health of the organisms and that may enhance the immune function [27–29]. In mice studies, it has been already demonstrated that krill oil can improve the absorption and delivery of DHA inside the body when compared with fish oil [30].

High content of n-3 polyunsaturated fatty acids (n-3 PUFA) and PLs, but also the richness in valuable nutrients such as vitamins, flavonoids, minerals, and antioxidants [31,32], has put krill oil on the list of candidates with multiple benefits such as immunomodulation [33], hepatoprotection against the effect of toxic drugs [34], analgesic and anti-inflammatory effects [35], along with antioxidant response [36,37]. However, even though the functionality of krill oil has been proven, very few studies have provided detailed information about the molecular mechanisms to elucidate how exactly krill oil exerts its beneficial role. It is widely recognized that the functionalities of krill oil are corelated with its chemical composition [32].

In the aquaculture industry, krill was explored rather as a protein source [38–40] and as carotenoids for pigmenting fish flesh [41]. Few studies addressed the potential of KO as a functional ingredient for stress and health management of intensively cultured fish [42,43]. According to our knowledge, there are no reported studies regarding the effect of KO diet supplementation on the oxidative stress parameters of carp reared under high stocking density or treated with ABZ.

This research aims to investigate the capacity of Korill product to alleviate stocking density stress in carp fingerlings and its hepatoprotective potential in case of anthelmintic treatment with ABZ.

2. Materials and Methods

2.1. Experimental Design and Fish Maintenance

The present study was carried out in the recirculating aquaculture system (RAS) at the Romanian Center for Modelling Recirculating Aquaculture Systems from the "Dunărea de Jos" University of Galați, Romania. The RAS was previously described [44].

Common carp (*Cyprinus carpio*) fingerlings were purchased from a local farm and transported to the laboratory facilities at Dunărea de Jos University of Galați. All fish were acclimatized for 2 weeks during which were fed with a commercial diet (43% protein, 12% fat, 4% fiber, 6% ash. (Skretting, Vignetto, Italy) twice a day (2% of biomass).

Feeding trail (Trial 1). After the acclimation period, a total number of 300 fish (mean weight 113.58 $\pm$ 11.09 g) were stocked in 12 fiberglass tanks (water volume 500 L) to create four experimental groups with triplicates for each group: LD (low density fed with normal feed), LD-KO (low density fed with 5% Korill product-supplemented feed), HD (high density fed with normal feed), and HD-KO (high density fed with 5% Korill product-supplemented feed). For LD treatments, 15 fish per tank were used (3.5 kg/m^3), while HD treatments, there were 35 fish per tank (8 kg/m^3). For LD-KO and HD-KO, the feed was supplemented with 5 g/kg Korill product from Sanience company (containing 500 mg pure krill oil, 200 mg phospholipids, 90 mg Omega-3 EPA/DHA fatty acids, and 400 µg astaxanthin), while for LD and HD, the feed was supplemented with fish oil. To incorporate the oils, the basal diet was ground then mixed with the specified oil and transformed into isoenergetic, isolipidic, and isoproteic pellets. After being dried, the pellets were transferred to plastic bags and stored in a freezer at -20 °C before feeding. The fish were fed with the above-mentioned diets within 2 months; feed was administrated manually to avoid feed competition and fighting among fish. When uneaten feed was observed, the feed administration was stopped, and the remaining pellets were quantified after being removed from the tank using a small net and dried. A photoperiod of 16 h light and 8 h dark was maintained. The biomass in each tank was weighed biweekly to adjust the feed amount.

ABZ exposure trial (Trial 2). After 2 months, 7 fish from each tank were randomly selected and retained for albendazole treatment. Therefore, the fish were moved in an experimental RAS system with 12 glass aquaria of 130 L individual volume. The ABZ treatment was administered daily by an oral dose of 5 mg/kg body weight for 7 consecutive days. The fish received the same diet (adjusted to biomass) under the same feeding regime.

The procedures presented in this study were approved by the Ethics Committee of the university in accordance with the Experimental Certificate of Animal Use (no. 783/2022). All animals were handled according to the principles stated by the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

2.2. Water Quality Parameters

During the trial, water quality parameters were monitored daily. Water temperature, pH, dissolved oxygen, ammonium, and nitrate were measured automatically with an Endress+Hauser monitoring system, Endress+Hauser AG, Switzerland, (probes of oxygen and temperature are placed in each tank while the RAS system is provided with two probes for pH, ammonium, and nitrate), while the nitrite concentrations were quantified weekly with a Skalar SAN++ analyzer, Skalar Analytical, Breda, The Netherlands.

2.3. Sampling Protocol and Blood Analysis

The fish blood was sampled (seven fish per tank) for hematological, biochemical, and antioxidant assays at the end of the 60-day feeding trial and after the 7-day exposure to albendazole. The fish were gently caught and placed in an anesthetic chamber (0.7 mL/L of 2- phenoxyethanol until deep anesthesia) before manipulation [45].

Blood was taken from the caudal vein using a heparinized syringe and transferred to sterilized tubes. The procedure was performed on ice until samples were transferred to the laboratory for further analysis. Each sample taken was split into two Eppendorf tubes: the first part was transferred into a sterile 2 mL Eppendorf tube with anticoagulant added for the hematological analysis, while the second part was stored in a 2 mL Eppendorf tube which was used for plasma separation. Plasma was obtained by centrifugation of blood at 3500 rpm (1166× *g*) for 10 min and used for further biochemical analyses.

The hematological profile was determined using the routine methodology of fish hematology [46]. The red blood cells count (RBC $\times 10^6/\mu L$) were determined using glass blood diluting pipette and Vulpian diluting solution. Calculation of RBC ($10^6/\mu L$) was performed using the number of counted cells, number of squares in which they were counted, square volume, and blood dilution [47]. For the hematocrit or packed cell volume (PCV, %) determination, we used the microhematocrit method, after centrifugation of blood at 12,000 rpm (13,709× *g*) for 5 min. Hemoglobin concentration (Hb, g/dL) was measured by cyanmethemoglobin method using Drabkin's reagent (DIALAB, Wiener Neudorf, Austria), and then the absorbance was read at a wavelength of 540 nm [48] using a Specord 210 UV–Vis spectrophotometer (Analytic Jena, Jena, Germany). The hematological indices, mean corpuscular volume (MCV, fL), mean corpuscular hemoglobin (MCH, pg), and mean corpuscular hemoglobin concentration (MCHC, g/dL) were calculated from the PCV, Hb, and RBC [49,50].

For the biochemical determinations, we used the VetTest® Chemistry Analyzer and IDEXX VetTest kits (IDEXX Laboratories, Inc., Westbrook, ME, USA) for albumin (ALB, g/dL), globulin (GLOB, g/dL), total serum protein (TP, g/dL), glucose (GLU, mg/dL), alanine aminotransferase (ALT, U/L), aspartate aminotransferase (AST, U/L), alkaline phosphatase (ALP, U/L), gamma-glutamyltransferase (GGT, U/L), direct bilirubin concentration (BIL D, mg/dL), cholesterol (CHOL, mg/dL), high-density lipoprotein (HDL, mg/dL), low-density lipoprotein (LDL, mg/L), and triglycerides (TG, mg/dL).

Lipid peroxidation measured by malondialdehyde (MDA, nmol/mL) concentrations were determined according to the Ohkawa (1979) method [51], and the optical density of samples was read at 532 nm. The results were expressed as nmol of MDA per mL of plasma or, per g of liver homogenate (nmol/g liver). Serum lysozyme activity (LZM, U/mL) was measured based on the turbidimetric assay, Enzymatic Activity of Lysozyme Protocol (Sigma, EC 3.2.1.17, Sigma-Aldrich, St. Louis, MO, USA). Total antioxidant capacity (TAC, mMol Trolox) was measured spectrophotometrically, at an optical density of 734 nm, using the ABTS—(2,2-azinobis 3-ethylbenzothiazoline-6sulphonic acid) according to the method described by [52].

2.4. Data Analysis

All the statistical analyses were performed using the SPSS statistical software for Windows, Version 16.0, United States, Chicago, SPSS Inc. Hematological and serum parameters were expressed as means ± S.E. of the replicates, considering each tank as the experimental unit. Data were analyzed by two-way ANOVA analysis having stocking density and feed as independent variables. Before statistical analyses, both normality and homogeneity of variance were confirmed by Shapiro–Wilk, and Levene's tests, respectively. All two-way ANOVA analyses were followed by a Ducan's post hoc test when significant differences were detected. T dependent test was used to compare the mean difference. The level of significance was set at $p < 0.05$ for all analyses.

3. Results

3.1. Physicochemical Parameters of Water

During the feeding trial, the mean values of water quality did not differ significantly ($p > 0.05$), and remained in the optimum range for carp culture [53]: temperature 22.4 ± 2.04 °C; pH = 7.25 ± 1.24; dissolved oxygen 7.56 ± 1.23 mg/L; ammonium 0.16 ± 0.09 mg/L; nitrate < 0.20 mg/L; and nitrites < 0.010 mg/L.

3.2. Hematological and Biochemical Parameters

At the end of the feeding trial (after 60 days) and after the ABZ challenge (after 7 days of exposure), fish hematological parameters such as hemoglobin (Hb), red blood cells count (RBC), hematocrit (PCV), and erythrocytes indices (MCV, MCH, MCHC) were studied/computed (Table 1).

Table 1. Hematological parameters of *C. carpio* (Lin.) in different treatments after 60 days.

Parameters	Before ABZ Challenge				After ABZ Challenge			
	LD	**LD-KO**	**HD**	**HD-KO**	**LD**	**LD-KO**	**HD**	**HD-KO**
RBC ($\times 10^6/\mu L$)	$1.24 \pm 0.14^{a*}$	$1.31 \pm 0.16^{a*}$	$1.14 \pm 0.14^{a*}$	$1.28 \pm 0.05^{a*}$	$1.40 \pm 0.12^{a*}$	$1.67 \pm 0.15^{a*}$	$1.36 \pm 0.21^{a*}$	$1.41 \pm 0.11^{a*}$
Hb (g/dL)	$8.42 \pm 0.63^{a*}$	$8.83 \pm 0.69^{a*}$	$9.71 \pm 0.51^{a*}$	$9.34 \pm 0.27^{a*}$	$8.44 \pm 0.31^{c*}$	$9.48 \pm 0.39^{d*}$	$7.09 \pm 0.73^{a**}$	$7.28 \pm 0.72^{b**}$
PCV (%)	$28.22 \pm 2.10^{a*}$	$29{,}13 \pm 1.31^{a*}$	$31.59 \pm 1.80^{a*}$	$27.93 \pm 2.65^{a*}$	$31.30 \pm 2.17^{c*}$	$31.80 \pm 1.77^{c*}$	$23.00 \pm 2.62^{a**}$	$28.87 \pm 2.02^{b*}$
MCV (fl)	$238.59 \pm 36.97^{a*}$	$233.77 \pm 34.33^{a*}$	$286.63 \pm 12.3^{a*}$	$220.36 \pm 14.88^{a*}$	$231.23 \pm 12.30^{a*}$	$196.57 \pm 9.74^{a*}$	$194.52 \pm 16.87^{a**}$	$213.77 \pm 11.01^{a*}$
MCH (pg)	$71.71 \pm 11.45^{a*}$	$70.68 \pm 10.97^{a*}$	$88.01 \pm 10.31^{a*}$	$73.46 \pm 4.46^{a*}$	$62.40 \pm 6.37^{a*}$	$58.68 \pm 6.07^{a*}$	$56.48 \pm 9.06^{a**}$	$53.01 \pm 7.38^{a*}$
MCHC (g/dL)	$30.02 \pm 2.05^{a*}$	$30.21 \pm 1.21^{a*}$	$30.93 \pm 2.02^{a*}$	$34.42 \pm 3.44^{a*}$	$27.33 \pm 1.57^{a*}$	$30.35 \pm 2.52^{a*}$	$30.55 \pm 5.63^{a*}$	$25.56 \pm 2.71^{a**}$

Values are Mean ± S.E., $n = 3$. Values with different superscripts in a row differ significantly (ANOVA, $p < 0.05$). Values with different symbols */** in a row differ significantly after treatment (T dependent, $p < 0.05$). RBC—red blood cells (erythrocytes) count; Hb—hemoglobin; PCV—packed cell volume; MCV—mean corpuscular volume; MCH—mean corpuscular hemoglobin; MCHC—mean corpuscular hemoglobin content.

At the end of the feeding experiment, although no significant differences ($p > 0.05$) were detected, the data showed a slight decrease in values of RBC in higher densities and higher values in variants fed with supplemented KO diet. The stocking density or diet had a non-significant effect on Hb, PCV, MCV, MCH, and MCHC. However, after ABZ treatment, values of Hb showed a significant decrease in both HD and HD-KO groups ($p < 0.05$) with the lowest value in HD. PVC, MCV, and MCH dropped only in HD groups. The ABZ treatment also induced a significant decrease ($p < 0.05$) of MCHC values only in the HD-KO group.

3.3. Serum Metabolic Profile

At the end of the trial and after the ABZ challenge, the metabolic profile of the fish was analyzed; the values (mean ± SE) for albumin (ALB), globulin (GLOB), albumin/globulin ratio (A/G), total serum protein (TP), glucose (GLU), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), direct bilirubin (BIL D), total bilirubin (BIL T), high-density lipoprotein (HDL), low-density lipoprotein (LDL), cholesterol (CHOL), triglycerides (TG), and total lipids (TL) are presented in Table 2.

Before the ABZ challenge, fish kept under higher stocking density and fed a normal diet registered a significant ($p < 0.05$) increase in GLU, AST, ALT, and GGT compared with groups fed the KO diet. KO did not induce significant changes in ALP, BIL D, BIL T, TL, and lipid fraction, except HDL, which had higher values in LD-KO and HD-KO variants.

After ABZ treatment ALB, GLOB, and TP increased in all experimental variants, with both factors, density and diet, showing a significant interaction ($p < 0.005$) for these parameters. GLU increase significantly after ABZ with the highest value in the HD variant and the lowest value in the LD-KO variant followed by HD-KO and LD. Treatment also induced an increase in ALT and AST values in all variants, with a significantly higher mean value for HD groups. HDL values increased after ABZ in all variants showing no significant differences ($p > 0.05$) among treatments. LDL was not influenced by diet or density during the feeding trial but increased significantly after ABZ treatment when the highest mean value was registered for HD groups.

Table 2. Serum metabolic profile parameters of *C. carpio*.

Parameters	Experimental Groups				Interaction
	LD	LD-KO	HD	HD-KO	Feed × Density
			Before ABZ challenge		
ALB (g/dl)	$1.09 \pm 0.03^{a*}$	$1.00 \pm 0.05^{a*}$	$1.13 \pm 0.10^{a*}$	$1.03 \pm 0.07^{a*}$	$p = 0.084$
GLOB(g/dl)	$2.76 \pm 0.25^{c*}$	$2.96 \pm 0.10^{c*}$	$2.33 \pm 0.06^{a*}$	$2.56 \pm 0.15^{b*}$	$p = 0.034$
A/G	$0.39 \pm 0.03^{b*}$	$0.33 \pm 0.02^{a*}$	$0.48 \pm 0.03^{c*}$	$0.40 \pm 0.01^{b*}$	$p = 0.020$
TP (g/dl)	$3.83 \pm 0.18^{b*}$	$3.96 \pm 0.25^{b*}$	$3.36 \pm 0.05^{a*}$	$3.59 \pm 0.10^{b*}$	$p = 0.235$
GLU (mg/dl)	$48.33 \pm 3.79^{a*}$	$43.67 \pm 2.42^{a*}$	$68.67 \pm 3.21^{c*}$	$51.50 \pm 5.03^{b*}$	$p = 0.967$
ALT (U/L)	$81.33 \pm 3.59^{a*}$	$54.33 \pm 3.46^{a*}$	$110.00 \pm 4.94^{c*}$	$96.00 \pm 3.58^{b*}$	$p = 0.560$
AST (U/L)	$120.67 \pm 8.36^{a*}$	$106.67 \pm 5.16^{a*}$	$189.67 \pm 4.98^{c*}$	$150.33 \pm 6.53^{b*}$	$p = 0.872$
ALP (U/l)	$129.20 \pm 4.44^{a*}$	$103.60 \pm 4.50^{a*}$	$135.33 \pm 4.31^{a*}$	$113.50 \pm 6.79^{a*}$	$p = 0.403$
GGT (U/L)	$0.50 \pm 0.71^{a*}$	$0.50 \pm 0.71^{a*}$	$3.00 \pm 0.83^{c*}$	$1.33 \pm 1.15^{b*}$	$p = 0.457$
BIL D(mg/dl)	$0.20 \pm 0.07^{a*}$	$0.22 \pm 0.08^{a*}$	$0.20 \pm 0.05^{a*}$	$0.18 \pm 0.04^{a*}$	$p = 0.213$
BIL T (mg/dl)	$0.29 \pm 0.10^{a*}$	$0.25 \pm 0.11^{a*}$	$0.32 \pm 0.09^{a*}$	$0.27 \pm 0.06^{a*}$	$p = 0.187$
HDL (mg/dl)	$79.73 \pm 6.85^{a*}$	$90.07 \pm 6.38^{b*}$	$74.63 \pm 5.03^{a*}$	$87.73 \pm 8.24^{b*}$	$p = 0.578$
LDL (mg/L)	$27.67 \pm 6.00^{a*}$	$26.33 \pm 2.65^{a*}$	$25.67 \pm 4.85^{a*}$	$27.67 \pm 12.22^{a*}$	$p = 0.858$
CHOL (mg/dl)	$211.67 \pm 15.57^{a*}$	$204.67 \pm 8.50^{a*}$	$203.50 \pm 5.69^{a*}$	$181.33 \pm 8.02^{a*}$	$p = 0.961$
TG (mg/dl)	$449.33 \pm 9.29^{a*}$	$435.00 \pm 33.51^{a*}$	$440.00 \pm 4.43^{a*}$	$421.33 \pm 27.74^{a*}$	$p = 0.642$
TL (mg/dl)	$1026.33 \pm 63.01^{a*}$	$983.00 \pm 74.64^{a*}$	$999.00 \pm 9.81^{a*}$	$931.33 \pm 43.25^{a*}$	$p = 0.734$
			After ABZ challenge		
ALB (g/dl)	$1.28 \pm 0.06^{b**}$	$1.18 \pm 0.06^{a*}$	$1.42 \pm 0.05^{c**}$	$1.21 \pm 0.03^{a**}$	$p = 0.044$
GLOB (g/dl)	$3.02 \pm 0.04^{b**}$	$3.22 \pm 0.22^{c**}$	$2.75 \pm 0.18^{a**}$	$3.11 \pm 0.13^{b**}$	$p = 0.026$
A/G	$0.40 \pm 0.02^{a*}$	$0.36 \pm 0.03^{a*}$	$0.51 \pm 0.01^{b**}$	$0.38 \pm 0.01^{a*}$	$p = 0.312$
TP (g/dl)	$4.30 \pm 0.15^{b**}$	$4.40 \pm 0.22^{c**}$	$4.17 \pm 0.32^{a**}$	$4.32 \pm 0.15^{b**}$	$p = 0.031$
GLU (mg/dl)	$86.80 \pm 4.95^{b**}$	$75.20 \pm 3.11^{a**}$	$97.60 \pm 3.80^{c**}$	$85.60 \pm 4.73^{b**}$	$p = 0.751$
ALT (U/L)	$92.40 \pm 2.40^{b**}$	$65.00 \pm 2.47^{a**}$	$148.50 \pm 1.44^{d**}$	$122.00 \pm 2.99^{c**}$	$p = 0.011$
AST (U/L)	$233.50 \pm 10.32^{b**}$	$197.75 \pm 6.79^{a**}$	$367.25 \pm 7.25^{d**}$	$281.00 \pm 7.06^{c**}$	$p = 0.012$
ALP (U/l)	$53.20 \pm 0.73^{a*}$	$69.60 \pm 4.33^{a**}$	$74.80 \pm 2.19^{a*}$	$75.53 \pm 3.95^{a**}$	$p = 0.494$
GGT (U/L)	$2.40 \pm 2.30^{a**}$	$2.50 \pm 1.29^{a**}$	$4.00 \pm 1.00^{b**}$	$2.40 \pm 1.14^{a**}$	$p = 0.148$
BIL D(mg/dl)	$0.11 \pm 0.04^{a**}$	$0.11 \pm 0.05^{a**}$	$0.13 \pm 0.04^{a**}$	$0.12 \pm 0.03^{a**}$	$p = 0.830$
BIL T (mg/dl)	$0.19 \pm 0.06^{a**}$	$0.19 \pm 0.09^{a**}$	$0.21 \pm 0.06^{b**}$	$0.24 \pm 0.05^{c**}$	$p = 0.860$
HDL (mg/dl)	$75.40 \pm 3.15^{a**}$	$82.10 \pm 6.50^{a**}$	$70.16 \pm 7.45^{a**}$	$80.00 \pm 6.08^{a**}$	$p = 0.571$
LDL (mg/L)	$89.00 \pm 3.76^{a**}$	$81.60 \pm 5.66^{a**}$	$118.40 \pm 9.07^{c**}$	$95.20 \pm 2.52^{b**}$	$p = 0.459$
CHOL (mg/dl)	$228.40 \pm 9.58^{a*}$	$214.80 \pm 7.29^{a*}$	$247.60 \pm 6.89^{b**}$	$229.00 \pm 4.16^{a**}$	$p = 0.771$
TG (mg/dl)	$263.80 \pm 8.94^{a**}$	$266.60 \pm 7.04^{a**}$	$334.40 \pm 6.89^{b**}$	$285.80 \pm 9.05^{a**}$	$p = 0.252$
TL (mg/dl)	$899.00 \pm 10.23^{a**}$	$959.40 \pm 7.87^{b**}$	$871.60 \pm 12.46^{a**}$	$903.00 \pm 15.23^{a**}$	$p = 0.268$

Values are Mean ± S.E., $n = 3$. Values with different superscripts in a row differ significantly (ANOVA, $p < 0.05$). Values with different symbols */** in a column differ significantly after treatment (T dependent, $p < 0.05$).

In the first trial, neither stocking density nor diet influenced CHOL, TL, and TG, but the ABZ caused a significant decrease in TG and TL and a significant increase in CHOL in all variants. Thus, after treatment, the highest mean values for CHOL and TG and the lowest mean value for TL were registered for HD groups.

3.4. Oxidative Stress Parameters and Lysozyme Activity

The effects of stocking density, feeding, and ABZ treatment on the oxidative stress parameters and lysozyme activity of common carp were shown in Table 3.

Before the ABZ challenge, the MDA concentrations from plasma and liver were significantly increased ($p < 0.05$) in the groups fed with the normal diet and held in high density. Significant higher ($p < 0.05$) MDA concentrations were registered after the ABZ challenge in all variants; however, the lowest concentration was observed in LD-KO and the highest in the HD variant. Furthermore, TAC values in Cyprinus carpio serum were significantly (interaction $p < 0.05$) influenced by stocking density and feeding. Before the ABZ challenge, TAC significantly ($p < 0.05$) increased in fish from the LD-KO group.

After the ABZ challenge, significant decreases were observed in the TAC values, the lower values being registered in the HD group. The lysozyme activity in the blood serum of Cyprinus carpio fingerlings was significantly higher ($p < 0.05$) after 60 days in groups fed KO-supplemented diets. After ABZ, the lysozyme activity significantly increases ($p < 0.05$) in all groups except LDKO; the lowest values were registered in the HD variant.

Table 3. Oxidative stress parameters and Lysozyme activity of *C. carpio* in different treatments (after 60 days of feeding different diets and after the ABZ challenge).

Parameters	Before ABZ Challenge				After ABZ Challenge			
	LD	LD-KO	HD	HD-KO	LD	LD-KO	HD	HD-KO
MDA (nmol/mL)	$2.10 \pm 0.09^{b*}$	$1.67 \pm 0.13^{a*}$	$2.67 \pm 0.16^{c*}$	$2.00 \pm 0.15^{b*}$	$2.44 \pm 0.11^{b**}$	$1.88 \pm 0.17^{a**}$	$2.98 \pm 0.15^{c**}$	$2.28 \pm 0.13^{b**}$
MDA (nmol/g liver)	$8.12 \pm 0.17^{b*}$	$5.49 \pm 0.10^{a*}$	$8.47 \pm 0.21^{c*}$	$8.12 \pm 0.22^{b*}$	$10.77 \pm 0.19^{c**}$	$6.61 \pm 0.19^{a**}$	$11.84 \pm 0.18^{c**}$	$9.12 \pm 0.14^{b**}$
TAC (mM Trolox)	$21.78 \pm 0.25^{b*}$	$24.64 \pm 0.29^{c*}$	$19.31 \pm 0.26^{a*}$	$21.48 \pm 0.31^{b*}$	$18.97 \pm 0.22^{a**}$	$20.95 \pm 0.24^{a**}$	$14.37 \pm 0.21^{b**}$	$19.21 \pm 0.23^{a**}$
LZM (U/mL)	$7.70 \pm 0.29^{b*}$	$9.10 \pm 0.12^{c*}$	$7.33 \pm 0.14^{a*}$	$9.03 \pm 0.11^{c*}$	$9.18 \pm 0.18^{b**}$	$9.33 \pm 0.16^{c*}$	$8.54 \pm 0.11^{a**}$	$9.58 \pm 0.14^{d**}$

Values are Mean ± S.E., $n = 3$. Values with different superscripts in a row differ significantly (ANOVA, $p < 0.05$). Values with different symbols */** in a column differ significantly after treatment (T dependent, $p < 0.05$). MDA—malondialdehyde; TAC—Total antioxidant capacity; LZM—lysozyme activity.

4. Discussion

In aquaculture farms, the fish species are exposed to different stress conditions that can maintain their influence on the physiological mechanisms either for a limited period of time (acute stress) or for extended periods (chronic stress). In the first case, depending on the stressor type, the organisms experience transitory changes and therefore the recovery of normal immunocompetence occurs after a few hours or days [54]. In the second case, chronic stress involves complex physiological changes, including alteration of energetic metabolism pathways, necessary to cope with the demands of the stress [55]. Moreover, in chronic situations, keeping the low but persistent intensity of the stressor could lead to depression or even suppression of the immunity system. In aquaculture, stocking density represents a factor of welfare impairment even if a moderate, acceptable limit, combined with good management practices, is applied [56]. In these cases, although biological markers are not showing dramatic changes, the situation can quickly worsen when other stressors, such as chemical treatment, are applied. The present study evaluated the protective potential of KO on the welfare of carp held under different densities when challenged with ABZ treatment.

In general, stocking density can compromise the health of farmed animals [57] and cause hematological and metabolic changes in fish [58]. Thus, increased values of Hb, Hct, and RBC in higher stocking densities were reported for species such as gilthead seabream (*Sparus aurata*) [59], rainbow trout (*Oncorhynchus mykiss*) [3], or African catfish (*Clarias gariepinus*) [60]. Our results revealed that the levels of non-specific stress responses such as blood RBC and Hb or the hematological indices were not affected by moderate stocking density or the KO-supplemented diet. Nevertheless, after albendazole treatment, a significant decrease ($p < 0.05$) of hemoglobin values were observed in carp fingerlings held in both HD and HD-KO variants, with the lowest value for HD groups.

It has been wildly accepted that the blood profile of fish could be easily altered by external stressful factors since it represents the link between the surrounding and internal environment. Reduction in hemoglobin concentration in both HD groups after ABZ exposure could be due to the cumulative inhibitory effect of both stress factors, density and ABZ, on the enzyme systems which are responsible for Hb synthesis [58]. Similar results were observed in hematocrit and hemoglobin in tambaqui (*Colossoma macropomum*) treated with albendazole [61]. The significantly higher Hb in the HD-KO variant compared with the HD variant emphasizes the beneficial role of KO on the Hb synthesis. In fact, it is already

demonstrated that due to its antioxidant property, astaxanthin stimulates the immune system and functions of organs related to hematopoiesis [62]. The significant reduction of PVC in the HD variant after ABZ treatment could be due to drug-induced hemolysis via membrane lipid peroxidation or decreased red cell size [25], confirmed also by MCV reduction. Nevertheless, in HD-KO groups, the PVC was not significantly affected after ABZ treatment; this situation could be due to the intake of fatty acids n-3 PUFAs, which has been shown to have crucial importance at cellular levels for maintaining membrane homeostasis [30].

Determination of blood indices have particular importance in describing anemia in fish [63], MCV, MCH, MCHC being widely used for determining the size, content, and density of Hb in red blood cells [64]. In our study, before ABZ treatment, the differences in MCV, MCH, and MCHC values were non-significant among variants, supporting the assumption that increasing stocking density did not pose many challenges to the erythrocytes and did not indicate any pathological condition in the studied fish. However, after ABZ treatment, values of MCV and MCH showed a significant decrease in HD groups ($p < 0.05$) and, respectively, a significant decrease of MCHC values in HD-KO groups as a result of a decrease in Hb but not in PVC for this variant. Other studies reported similar effects of pharmaceuticals on PVC, Hb, and MCV in fish, as a result of anemia and due to the oxidative stress arising from the metabolic interactions between the drug and the cell environment [65].

In our study, the values of hematological parameters (Table 1) showed a variation range consistent with the life stage of the carp [66]. Nevertheless, the HD groups receiving the KO diet presented a less impaired hematological status due to the beneficial effect of KO compounds in special omega 3 fatty acids, which are mostly bound in phospholipid form and easily absorbed by cell membranes, contributing to the increased stress tolerance [28].

The content of total protein, albumin, and globulin in the serum is often used to assess the nutritional and metabolic status of the fish held under different dietary regimes [67] or different stressful conditions, these biomarkers also reflecting, indirectly, the welfare status [68]. Under high stocking density, a drop in serum protein, albumin, or globulin has been previously reported for species such as *Labeo rohita* [69], *Cyprinus carpio* [11], or *Oreochromis niloticus* [70].

In our study, after the feeding trial, the fish reared under HD conditions showed the lowest content of serum protein and globulin. Although groups receiving the KO diet had higher protein and globulin serum concentrations compared with groups fed with normal diet and held in the same density, significant differences were registered only for high-density groups; albumin was not affected by density or feeding regime. A similar pattern of biochemical parameter alteration has been reported by other authors [71–73] in response to the high stocking density of the fish. However, other authors [7] showed no changes in total proteins but significantly lower globulin values and higher albumin levels in *Oncorhynchus mykiss* reared at high stocking density. Globulin is a diagnostic biomarker with significant value in fish. Thus, the increase in serum globulin and the decrease in the levels of the albumin–globulin ratio represents an important indicator of non-specific immunity and enhanced protective mechanisms for fish [74]. In the present study, after the feeding trial, A/G ratio was influenced by both factors—diet and density—and by their interaction ($p < 0.05$). The high A/G ratios were found in HD groups while the lower values were observed in groups receiving the KO diet.

After ABZ treatment, the serum total proteins and globulin levels increased in all experimental groups ($p < 0.05$); however, total protein and globulin registered significantly higher values in groups fed with the KO diet and held in low densities. A significant increase in serum protein and globulin level was also found in fish treated with other similar drugs, such as praziquantel [75] and levamisole [76]. Nevertheless, based on present results, the fish held under density stress and ABZ treatment, fed with a normal diet, reveal a more affected non-specific immune response than the fish held in the same

conditions but fed a diet containing KO; the highest A/G ratio value was registered in the HD group ($p < 0.05$).

Glucose is also considered a good indicator of fish stress (acute or chronic), being often used as a welfare biomarker [77]. Data from the present study confirmed that carp serum glucose was significantly higher in both HD groups, regardless of the diet. From this point of view, the KO diet lowered glucose, especially in high-density groups. After the ABZ challenge, the glucose increased in all experimental variants, registering the highest value in the HD group fed with a normal diet, suggesting an overwhelming physiological response to stress induced by the combined factors represented by high stocking density and ABZ toxicity.

In addition to glucose, the CHOL and TG in the blood are considered reliable parameters for fish stress as they are related to energy and lipid metabolism [78,79]. It is acknowledged that in fish kept under high stocking densities, in order to cope with the increased energy demand, energy reserves are affected either through consumption or reallocation [59]. Thus, depending on the specie or density tested, reported results are controversial: CHOL and TG were markedly increased at high stocking density in *Carassius gibelio* [80], *Ictalurus punctatus* [81], or *Megalobrama amblycephala* [82], while for species such as *Oncorhynchus mykiss* [83], *Micropterus salmoides* [8], or *Trachinotus ovatus* [84], CHOL and TG reserves were depleted to cope with increased energy demand generated by stressful conditions. For carp, after the feeding trial, total cholesterol was not significantly affected by density ($p > 0.05$) or diet ($p > 0.05$), although lower values were observed for higher densities and for groups fed the KO diet. No differences were induced by feeding regime or density in LDL concentration ($p > 0.05$), while HDL values registered higher values for fish fed the KO diet. However, ABZ treatment induced a significant increase in LDL in all experimental groups, among which the highest value was quantified in HD and the lowest in LD-KO, suggesting that KO alleviated the negative impact of ABZ on the lipid profile. The HDL decreased significantly ($p < 0.05$) after ABZ treatment but without differences among experimental variants.

Chronic stress also altered the liver function, assessed here through biomarkers such as ALT, AST, ALP, and GGT. Normally, these enzymes have constant variation unless environmental stress or some pathological challenges are addressed. For teleost fish, ALT and AST enzymes are the main aminotransferases related to amino acid metabolism in the liver [85]. For carp reared in the present experiment, under crowding and stressful conditions, the AST and ALT levels, measured after the feeding trial, increased significantly ($p < 0.05$) in higher density variants, while for ALP, no significant ($p > 0.05$) changes were induced by either feeding regime or stocking density. Other authors reported increased values for AST, ALT, and ALP for species such as gibel carp, *Carassius gibelio* [80], or grass carp, *Ctenopharyngodon idella* [11], held in higher densities, but they found no significant differences in serum ALT, AST, and ALP for largemouth bass, *Micropterus salmoides* [8]. KO-supplemented diet induced significantly lower values for AST ($p < 0.05$) measured in groups from high densities and significant changes in ALT in both tested densities. ALP was not affected by density or feeding regime.

After the ABZ challenge, ALT and AST liver enzyme concentration increased for all variants with the highest levels in HD groups. However, it is worth noticing the significantly lower values ($p < 0.05$) registered for both density groups receiving the KO diet. For these parameters, a significant diet x density interaction ($p < 0.05$) was found. Alkaline phosphatase (ALP) values measured on carp exposed to ABZ registered a significant decrease ($p < 0.05$) for groups fed with the normal diet, regardless the density. In the current study, the decrease in ALP activity in fish after ABZ exposure may result from disruption of the membrane transport system since ALP is an enzyme directly involved in the activities of membrane transport [86].

Direct bilirubin among other biomarkers could be used as an indicator of liver impairment and/or nutritional status. Total bilirubin concentrations in fish serum are considered almost negligible in comparison with mammals due to the lower activity of biliverdin

reductase [87]. In the present study, bilirubin was not affected by density or KO diet but was significantly reduced after ABZ treatment in all cohorts, BIL T showing higher values in HD groups. GGT activity is also low in healthy fish, with significant changes occurring in diseases or toxicological challenges [88]. GGT activity was enhanced by high density and by ABZ treatment, indicating, along with AST and ALT, liver malfunction or damage. Using KO in the carp diet significantly reduced the GGT level in the serum of the fish held in high densities.

As a result of metabolic processes at the cellular level, reactive oxygen species (ROS) are produced and eliminated, through complex physiological mechanisms which are designed to maintain a continuous dynamic balance. When ROS are in excess, free radicals cause lipid peroxidation. The main component of lipid peroxides, malondialdehyde (MDA), affects the structure and cellular functions, being characterized by biotoxicity [89]. Increased levels of MDA in different fish tissues have been associated with cellular injury after fish exposure to various stressors. In our study, the results showed that both density and ABZ treatment increased plasma MDA. Nevertheless, the KO show a protective role demonstrated by lower MDA values in LD-KO and in HD-KO variants. Thus, the following increasing MDA profile was emphasized among variants, both before and after ABZ treatment: LD-KO < HD-KO < LD < HD. The same pattern variation was also revealed in the liver but with higher values of MDA explained by the strategic function of the liver in detoxification and protection against ROS [90].

These results indicate that high densities and ABZ treatment induced oxidative stress in the animals, particularly in the liver. This is also supported by the increased ALT enzyme activity in the HD groups. The high level of oxidative stress could be reduced by feeding the fish a diet supplemented with KO, due to its high content of astaxanthin, which has a high antioxidant capacity [3]. Moreover, phospholipids can act as an enhancer of antioxidant capacity [91]. Therefore, the KO containing astaxanthin and phospholipids contribute to ROS scavenging and antioxidant activities.

After ABZ treatment, the total antioxidant capacity (TAC) was reduced in all experimental variants. Similar results were reported for African catfish exposed to ABZ, which showed higher levels of lipid peroxidation and, correspondingly, greater inhibition of antioxidant activities, putting the liver at a higher risk of damage [25]. In the present trial, however, the lower reduction of TAC in variants supplemented with KO may indicate that tissues avoid depleting the total antioxidant reserves to neutralize oxidative stress. Thus, the high content of n-3 PUFAs and astaxanthin from KO may be responsible for the activation of protection mechanisms and/or for the antioxidant action of these molecules limiting the use of the internal TAC reserves.

LZM is a biomarker commonly accepted for the assessment of the immune status of fish after adverse stimuli since it plays an important role in the biodefense system [92]. Decreased LZM plasma concentration has been correlated with an impairment of the immune system of different fish species held at a high stocking density [5,93]. Similarly, in the present work, the LZM level decreased in the HD groups fed with a normal diet, revealing that the density had an adverse effect on the immunity status. However, in cohorts fed the KO diet, LZM activity was enhanced, and no significant differences were detected between the tested stocking densities for these variants. After the ABZ challenge, LZM concentration increased in all variants, showing the highest value in LDKO and the smallest in HD, suggesting that the combination of the anti-inflammatory and antioxidant agents found in krill oil stimulated the production of the molecules involved in immunity, such as lysozyme, and enhanced the organism's capacity to strengthen the defense system when challenged.

5. Conclusions

The present study was conducted to investigate the potential of KO as a functional ingredient for intensive aquaculture. The results showed that KO alleviated density stress and albendazole liver toxicity, improved lipid metabolism, and improved the capacity to

scavenge free radicals. Thus, KO could be regarded as a complementary component of fish health management in aquaculture and could be used to develop nutraceutical products. However, the exact mechanism behind the positive effects of the dietary inclusion of KO needs to be addressed in future studies.

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Institutional Review Board Statement: The procedures presented in this study were approved by the Ethics Committee of the university in accordance with the Experimental Certificate of Animal Use (no. 783/2022). All animals were handled according to the principles set out by the EC Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

Data Availability Statement: All the data are available from the first author, and can be delivered if required.

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References

1. Naylor, R.L.; Hardy, R.W.; Buschmann, A.H.; Bush, S.R.; Cao, L.; Klinger, D.H.; Little, D.C.; Lubchenco, J.; Shumway, S.E.; Troell, M. A 20-year retrospective review of global aquaculture. *Nature* **2020**, *591*, 551–563. [CrossRef] [PubMed]
2. Iguchi, K.; Ogawa, K.; Nagae, M.; Ito, F. The influence of rearing density on stress response and disease susceptibility of ayu (*Plecoglossus altivelis*). *Aquaculture* **2003**, *220*, 515–523. [CrossRef]
3. Yarahmadi, P.; Miandare, H.K.; Hoseinifar, S.H.; Gheysvandi, N.; Akbarzadeh, A. The effects of stocking density on hemato-immunological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). *Aquac. Int.* **2015**, *23*, 55–63. [CrossRef]
4. Barton, B.A. Stress in fishes: A diversity of responses with particular reference to changes in circulating corticosteroids. *Integr. Comp. Biol.* **2002**, *42*, 517–525. [CrossRef]
5. Liu, B.L.; Fei, F.; Li, X.T.; Wang, X.Y.; Huang, B. Effects of stocking density on stress response, innate immune parameters, and welfare of turbot (*Scophthalmus maximus*). *Aquac. Int.* **2019**, *27*, 1599–1612. [CrossRef]
6. Jia, R.; Wang, L.; Hou, Y.; Feng, W.; Li, B.; Zhu, J. Effects of Stocking Density on the Growth Performance, Physiological Parameters, Redox Status and Lipid Metabolism of *Micropterus salmoides* in Integrated Rice–Fish Farming Systems. *Antioxidants* **2022**, *11*, 1215. [CrossRef]
7. Naderi, M.; Keyvanshokooh, S.; Salati, A.P.; Ghaedi, A. Effects of chronic high stocking density on liver proteome of rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol. Biochem.* **2017**, *43*, 1373–1385. [CrossRef]
8. Wang, Y.; Xu, G.; Nie, Z.; Li, Q.; Shao, N.; Xu, P. Effect of Stocking Density on Growth, Serum Biochemical Parameters, Digestive Enzymes Activity and Antioxidant Status of Largemouth Bass, *Micropterus salmoides*. *Pak. J. Zool.* **2019**, *51*, 1509–1517. [CrossRef]
9. Andrade, T.; Afonso, A.; Pérez-Jiménez, A.; Oliva-Teles, A.; de las Heras, V.; Mancera, J.M.; Serradeiro, R.; Costas, B. Evaluation of different stocking densities in a Senegalese sole (*Solea senegalensis*) farm: Implications for growth, humoral immune parameters and oxidative status. *Aquaculture* **2015**, *438*, 6–11. [CrossRef]
10. Sahin, K.; Yazlak, H.; Orhan, C.; Tuzcu, M.; Akdemir, F.; Sahin, N. The effect of lycopene on antioxidant status in rainbow trout (*Oncorhynchus mykiss*) reared under high stocking density. *Aquaculture* **2014**, *418–419*, 132–138. [CrossRef]
11. Adineh, H.; Naderi, M.; Nazer, A.; Yousefi, M.; Ahmadifar, E. Interactive effects of stocking density and dietary supplementation with Nano selenium and garlic extract on growth, feed utilization, digestive enzymes, stress responses, and antioxidant capacity of grass carp, *Ctenopharyngodon idella*. *J. World Aquac. Soc.* **2021**, *52*, 789–804. [CrossRef]
12. Souza, C.D.F.; Baldissera, M.D.; Baldisserotto, B.; Heinzmann, B.M.; Martos-Sitcha, J.A.; Mancera, J.M. Essential oils as stress-reducing agents for fish aquaculture: A review. *Front. Physiol.* **2019**, *10*, 785. [CrossRef]
13. Shourbela, R.M.; El-Hawarry, W.N.; Elfadadny, M.R.; Dawood, M.A.O. Oregano essential oil enhanced the growth performance, immunity, and antioxidative status of Nile tilapia (*Oreochromis niloticus*) reared under intensive systems. *Aquaculture* **2021**, *542*, 736868. [CrossRef]
14. Hoseini, S.M.; Taheri Mirghaed, A.; Iri, Y.; Hoseinifar, S.H.; Van Doan, H.; Reverter, M. Effects of dietary Russian olive, *Elaeagnus angustifolia*, leaf extract on growth, hematological, immunological, and antioxidant parameters in common carp, *Cyprinus carpio*. *Aquaculture* **2021**, *536*, 736461. [CrossRef]

15. Lieke, T.; Meinelt, T.; Hoseinifar, S.H.; Pan, B.; Straus, D.L.; Steinberg, C.E. Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. *Rev. Aquac.* **2020**, *12*, 943–965. [CrossRef]
16. Mortuza, M.G.; Al-Misned, F.A. Prevalence of ectoparasites in carp fry and fingerlings of Rajshahi district, Bangladesh. *J. Parasit. Dis.* **2015**, *39*, 130–133. [CrossRef] [PubMed]
17. Mwainge, V.M.; Ogwai, C.; Aura, C.M.; Mutie, A.; Ombwa, V.; Nyaboke, H.; Oyier, K.N.; Nyaundi, J. An overview of fish disease and parasite occurrence in the cage culture of *Oreochromis niloticus*: A case study in Lake Victoria, Kenya. *Aquat. Ecosyst. Health Manag.* **2021**, *24*, 43–55. [CrossRef]
18. Bártíková, H.; Vokřál, I.; Skálová, L.; Lamka, J.; Szotáková, B. In vitro oxidative metabolism of xenobiotics in the lancet fluke (*Dicrocoelium dendriticum*) and the effects of albendazole and albendazole sulphoxide ex vivo. *Xenobiotica* **2010**, *40*, 593–601. [CrossRef]
19. Busatto, Z.; de França, W.G.; Cyrino, J.E.P.; Paschoal, J.A.R. Assessment of elimination profile of albendazole residues in fish. *Food Addit. Contam. Part A Chem. Anal. Control. Expo. Risk Assess.* **2018**, *35*, 77–85. [CrossRef]
20. Cordeiro, R.P.; Braga, P.A.D.C.; Jonsson, C.M.; Brandão, F.R.; Chagas, E.C.; Reyes, F.G.R. Therapeutic efficacy and bioaccumulation of albendazole in the treatment of tambaqui (*Colossoma macropomum*) parasitized by acanthocephalan (*Neoechinorhynchus buttnerae*). *Aquac. Res.* **2022**, *53*, 1446–1455. [CrossRef]
21. Orobets, V.; Lisovets, E.; Zabashta, S.; Ermakov, A. Control of fish parasites in aquaculture. *IOP Conf. Ser. Earth Envirn. Sci.* **2019**, *403*, 012065. [CrossRef]
22. Tojo, J.; Santamarina, M.; Ubeira, F.; Estevez, J.; Sanmartin, M. Anthelmintic activity of benzimidazoles against *Gyiodactylus* sp. infecting rainbow trout *Oncorhynchus mykiss*. *Dis. Aquat. Org.* **1992**, *12*, 185–189. [CrossRef]
23. Carlsson, G.; Patring, J.; Ullerås, E.; Oskarsson, A. Developmental toxicity of albendazole and its three main metabolites in zebrafish embryos. *Reprod. Toxicol.* **2011**, *32*, 129–137. [CrossRef] [PubMed]
24. Mattsson, A.; Ullerås, E.; Patring, J.; Oskarsson, A. Albendazole causes stage-dependent developmental toxicity and is deactivated by a mammalian metabolization system in a modified zebrafish embryotoxicity test. *Reprod. Toxicol.* **2012**, *34*, 31–42. [CrossRef] [PubMed]
25. Nwani, C.D.; Odo, G.E.; Nwadinigwe, A.O.; Onyeke, C.C.; Atama, C.I.; Ngwu, G.; Oluah, S.N.; Ukonze, J.A.; Ezeibe, B.C.A. Short-term effects of albendazole on the oxidative stress markers and hematological parameters in tissues of African Catfish *Clarias gariepinus*. *J. Aquat. Anim. Health* **2016**, *28*, 222–228. [CrossRef] [PubMed]
26. Kanazawa, A. Effects of docosahexaenoic acid and phospholipids on stress tolerance of fish. *Aquaculture* **1997**, *155*, 129–134. [CrossRef]
27. Gigliotti, J.C.; Davenport, M.P.; Beamer, S.K.; Tou, J.C.; Jaczynski, J. Extraction and characterisation of lipids from Antarctic krill (*Euphausia superba*). *Food Chem.* **2011**, *125*, 1028–1036. [CrossRef]
28. Kaur, K.; Kortner, T.M.; Benitez-Santana, T.; Burri, L. Effects of Antarctic Krill Products on Feed Intake, Growth Performance, Fillet Quality, and Health in Salmonids. *Aquac. Nutr.* **2022**, *2022*, 3170854. [CrossRef]
29. Berge, R.K.; Ramsvik, M.S.; Bohov, P.; Svardal, A.; Nordrehaug, J.E.; Rostrup, E.; Bjørndal, B. Krill oil reduces plasma triacylglycerol level and improves related lipoprotein particle concentration, fatty acid composition and redox status in healthy young adults-a pilot study. *Lipids Health Dis.* **2015**, *14*, 163. [CrossRef]
30. Burri, L.; Berge, K.; Wibrand, K.; Berge, R.K.; Barger, J.L. Differential effects of krill oil and fish oil on the hepatic transcriptome in mice. *Front. Gen.* **2011**, *2*, 45. [CrossRef]
31. Phleger, C.F.; Nelson, M.M.; Mooney, B.D.; Nichols, P.D. Interannual and between species comparison of the lipids, fatty acids and sterols of Antarctic krill from the US AMLR Elephant Island survey area. *Comp. Biochem. Physiol. B Biochem. Mol. Biol.* **2002**, *131*, 733–747. [CrossRef]
32. Xie, D.; Gong, M.; Wei, W.; Jin, J.; Wang, X.; Wang, X.; Jin, Q. Antarctic krill (*Euphausia superba*) oil: A comprehensive review of chemical composition, extraction technologies, health benefits, and current applications. *Compr. Rev. Food Sci. Food Saf.* **2019**, *18*, 514–534. [CrossRef] [PubMed]
33. Ramsvik, M.S.; Bjørndal, B.; Bruheim, I.; Bohov, P.; Berge, R.K. A phospholipid-protein complex from Krill with antioxidative and immunomodulating properties reduced plasma triacylglycerol and hepatic lipogenesis in rats. *Mar. Drugs* **2015**, *13*, 4375–4397. [CrossRef]
34. Ali, K.H.; Al-Jawad, F.H.; Kadhim, H.M. The Possible Hepatoprotecive Effects of "Krill Oil and Silymarin against Carbon Tetrachloride (CCl_4)-Induced Rats Model of Liver Fibrosis: In Vivo Study". *Res. J. Pharm. Technol.* **2021**, *14*, 5953–5958. [CrossRef]
35. Zadeh-Ardabili, P.M.; Rad, S.K. Anti-pain and anti-inflammation like effects of Neptune krill oil and fish oil against carrageenan induced inflammation in mice models: Current statues and pilot study. *Biotechnol. Rep.* **2019**, *22*, e00341. [CrossRef] [PubMed]
36. Mellouk, Z.; Ramirez, M.; Pena, K.; Arivalo, J.; Agustina, M. P2-12: Cell-based anti-oxydant activity of dietary krill oil enrichment in high fat-fed rats target tissues. In *Annales de Cardiologie et d'Angéiologie*; Elsevier Masson: Amsterdam, The Netherlands, 2019; Volume 64, p. S27.
37. Li, X.; Yuan, Y.; Jin, M.; Wang, X.; Hu, X.; Zhao, M.; Zhou, Q. Growth performance, antioxidant capacity, tissue fatty acid composition and lipid metabolism of juvenile green mud crab *Scylla paramamosain* in response to different dietary n-3 PUFA lipid sources. *Aquac. Rep.* **2021**, *19*, 100599. [CrossRef]
38. Olsen, R.E.; Suontama, J.; Langmyhr, E.; Mundheim, H.; Ringø, E.; Melle, W.; Hemre, G.I. The replacement of fish meal with Antarctic krill, *Euphausia superba* in diets for Atlantic salmon, *Salmo salar*. *Aquac. Nutr.* **2006**, *12*, 280–290. [CrossRef]

39. Choi, J.; Lee, K.W.; Han, G.S.; Byun, S.G.; Lim, H.J.; Kim, H.S. Dietary inclusion effect of krill meal and various fish meal sources on growth performance, feed utilization, and plasma chemistry of grower walleye pollock (*Gadus chalcogrammus*, Pallas 1811). *Aquac. Rep.* **2020**, *17*, 100331. [CrossRef]
40. Mørkøre, T.; Moreno, H.M.; Borderías, J.; Larsson, T.; Hellberg, H.; Hatlen, B.; Krasnov, A. Dietary inclusion of Antarctic krill meal during the finishing feed period improves health and fillet quality of *Atlantic salmon* (*Salmo salar* L.). *Br. J. Nutr.* **2020**, *124*, 418–431. [CrossRef]
41. Arai, S.; Mori, T.; Miki, W.; Yamaguchi, K.; Konosu, S.; Satake, M.; Fujita, T. Pigmentation of juvenile coho salmon with carotenoid oil extracted from Antarctic krill. *Aquaculture* **1987**, *66*, 255–264. [CrossRef]
42. Taylor, J.F.; Martinez-Rubio, L.; del Pozo, J.; Walton, J.M.; Tinch, A.E.; Migaud, H.; Tocher, D.R. Influence of dietary phospholipid on early development and performance of Atlantic salmon (*Salmo salar*). *Aquaculture* **2015**, *448*, 262–272. [CrossRef]
43. Li, Y.; Zeng, Q.H.; Liu, G.; Peng, Z.; Wang, Y.; Zhu, Y.; Wang, J.J. Effects of ultrasound-assisted basic electrolyzed water (BEW) extraction on structural and functional properties of Antarctic krill (*Euphausia superba*) proteins. *Ultrason. Sonochem.* **2021**, *71*, 105364. [CrossRef]
44. Dediu, L.; Docan, A.; Crețu, M.; Grecu, I.; Mogodan, A.; Maereanu, M.; Oprea, L. Effects of stocking density on growth performance and stress responses of bester and bester♀× beluga♂juveniles in recirculating aquaculture systems. *Animals* **2021**, *11*, 2292. [CrossRef] [PubMed]
45. Jahanbakhshi, A.; Shaluei, F.; Baghfalaki, M.; Ramazi, F.G.; Ahmadvand, S. Efficacy of 2-phenoxyethanol as an Anesthetic for Two Size of Persian Sturgeon, *Acipenser persicus*. *J. Walailak.* **2012**, *9*, 31–36. [CrossRef]
46. Blaxhall, P.C.; Daisley, K.W. Routine hematological methods for use with fish blood. *J. Fish Biol.* **1973**, *5*, 771–781. [CrossRef]
47. Svobodova, Z.; Pravda, D.; Modra, H. Metody hematologickeho vysetrovani ryb. In *Unified Methods of Fish Haematological Investigations, Edice Metodik*; Research Institute of Fish Culture and Hydrobiology: Vodňany, Czech Republic, 2012; 29p.
48. Hesser, E.F. Methods for routine fish hematology. *Progress. Fish Cult.* **1960**, *22*, 164–171. [CrossRef]
49. Ghergariu, S.; Pop, A.; Kadar, L. *Veterinary Clinical Laboratory Guide*; Ceres Publishing House: Bucharest, Romania, 1985; pp. 82–90. (In Romanian)
50. Svobodova, Z. Stress in fishes (a review). *Bull Vurh Vodnany* **2001**, *4*, 169–191.
51. Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **1979**, *95*, 351–358. [CrossRef] [PubMed]
52. Roberta, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
53. Goran, S.M.A.; Omar, S.S.; Anwer, A.A. Water Quality and Physiological Parameters of Common Carp Fingerling Fed on Jerusalem artichoke Tubers. *Polytechnic* **2016**, *3*, 502–516.
54. Ortuño, J.; Esteban, M.A.; Meseguer, J. Effects of short-term crowding stress on the gilthead seabream (*Sparus aurata* L.) innate immune response. *Fish Shellfish Immunol.* **2001**, *11*, 187–197. [CrossRef] [PubMed]
55. Tort, L. Stress and immune modulation in fish. *Dev. Comp. Immunol. D* **2011**, *35*, 1366–1375. [CrossRef] [PubMed]
56. Saraiva, J.L.; Rachinas-Lopes, P.; Arechavala-Lopez, P. Finding the "golden stocking density": A balance between fish welfare and farmers' perspectives. *Front. Vet. Sci.* **2022**, *9*, 1099. [CrossRef]
57. Baldwin, L. The effects of stocking density on fish welfare. *Plymouth Stud. Sci.* **2010**, *4*, 372–383.
58. Ahmed, I.; Reshi, Q.M.; Fazio, F. The influence of the endogenous and exogenous factors on hematological parameters in different fish species: A review. *Aquac. Int.* **2020**, *28*, 869–899. [CrossRef]
59. Montero, D.; Izquierdo, M.S.; Tort, L.; Robaina, L.; Vergara, J.M. High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead seabream, *Sparus aurata*, juveniles. *Fish Physiol. Biochem.* **1999**, *20*, 53–60. [CrossRef]
60. Dai, W.; Wang, X.; Guo, Y.; Wang, Q.; Ma, J. Growth performance, hematological and biochemical responses of African catfish (*Clarias gariepinus*) reared at different stocking densities. *Afr. J. Agric. Res.* **2011**, *6*, 6177–6182. [CrossRef]
61. Farias, C.F.S.; Brandão, F.R.; de Alexandre Sebastião, F.; de Melo Souza, D.C.; Monteiro, P.C.; Majolo, C.; Chagas, E.C. Albendazole and praziquantel for the control of *Neoechinorhynchus buttnerae* in tambaqui (*Colossoma macropomum*). *Aqua. Int.* **2021**, *29*, 1495–1505. [CrossRef]
62. Lim, K.C.; Yusoff, F.M.; Shariff, M.; Kamarudin, M.S.; Nagao, N. Dietary supplementation of astaxanthin enhances hemato-biochemistry and innate immunity of Asian seabass, Lates calcarifer (Bloch, 1790). *Aquaculture* **2019**, *512*, 734339. [CrossRef]
63. Witeska, M.; Kondera, E.; Ługowska, K.; Bojarski, B. Hematological methods in fish—Not only for beginners. *Aquaculture* **2022**, *47*, 73749. [CrossRef]
64. Ambili, T.R.; Saravanan, M.; Ramesh, M.; Abhijith, D.B.; Poopal, R.K. Toxicological Effects of the Antibiotic Oxytetracyclineto an Indian Major Carp *Labeo rohita*. *Arch. Environ. Contam. Toxicol.* **2013**, *64*, 494–503. [CrossRef]
65. Nwani, C.D.; Omah, M.C.; Ivoke, N.; Nwamba, H.O.; Ani, C.; Ogbonna, S.U. Biochemical, haematological and morphological variations in juvenile *Clarias gariepinus* exposed to Carbendazim®fungicide. *Afr. J. Aquat. Sci.* **2015**, *40*, 63–71. [CrossRef]
66. Baghizadeh, E.; Khara, H. Variability in hematology and plasma indices of common carp *Cyprinus carpio*, associated with age, sex and hormonal treatment. *Iran. J. Fish. Sci.* **2015**, *14*, 99–111.

67. Tahmasebi-Kohyani, A.; Keyvanshokooh, S.; Nematollahi, A.; Mahmoudi, N.; Pasha-Zanoosi, H. Effects of Dietary Nucleotides Supplementation on Rainbow Trout (*Oncorhynchus mykiss*) Performance and Acute Stress Response. *Fish Physiol. Biochem.* **2012**, *38*, 431–440. [CrossRef]
68. Brucka-Jastrzębska, E.; Kawczuga, D. Antioxidant Status and Lipid Peroxidation in Blood of Common Carp (*Cyprinus carpio* L.). *Pol. J. Environ. Stud.* **2011**, *20*, 541–550.
69. Swain, H.S.; Das, B.K.; Upadhyay, A.; Ramteke, M.H.; Kumar, V.; Meena, D.K.; Sarkar, U.K.; Chadha, N.K.; Rawat, K.D. Stocking density mediated stress modulates growth attributes in cage reared *Labeo rohita* (Hamilton) using multifarious biomarker approach. *Sci. Rep.* **2022**, *12*, 9869. [CrossRef] [PubMed]
70. Tammam, M.S.; Wassef, E.A.; Toutou, M.M.; El-Sayed, A.F.M. Combined effects of surface area of periphyton substrates and stocking density on growth performance, health status, and immune response of Nile tilapia (*Oreochromis niloticus*) produced in cages. *J. Appl. Phycol.* **2020**, *32*, 3419–3428. [CrossRef]
71. Kpundeh, M.D.; Xu, P.; Yang, H.; Qiang, J.; He, J. Stocking densities and chronic zero culture water exchange stress' effects on biological performances, hematological and serum biochemical indices of gift tilapia juveniles (*Oreochromis niloticus*). *J. Aquac. Res. Dev.* **2013**, *4*, 2. [CrossRef]
72. Abdel-Tawwab, M.; Hagras, A.E.; Elbaghdady, H.A.M.; Monier, M.N. Dissolved oxygen level and stocking density effects on growth, feed utilization, physiology, and innate immunity of Nile Tilapia, *Oreochromis niloticus*. *J. Appl. Aquac.* **2014**, *26*, 340–355. [CrossRef]
73. Mahmoud, H.K.; Reda, F.M.; Alagawany, M.; Farag, M.R. Ameliorating deleterious effects of high stocking density on *Oreochromis niloticus* using natural and biological feed additives. *Aquaculture* **2021**, *26*, 340–355. [CrossRef]
74. Opiyo, M.A.; Jumbe, J.; Ngugi, C.C.; Charo-Karisa, H. Dietary administration of probiotics modulates non-specific immunity and gut microbiota of Nile tilapia (*Oreochromis niloticus*) cultured in low input ponds. *Int. J. Vet. Sci.* **2019**, *7*, 1–9. [CrossRef] [PubMed]
75. Soltanian, S.; Vazirzadeh, A.; Akbary, P. Effect of Praziquantel on Hemato-Immunological Indices in Common Carp (*Cyprinus carpio*). *Iran. J. Sci. Technol. Trans. A Sci.* **2018**, *42*, 1015–1025. [CrossRef]
76. Aly, S.; Abd-Allah, O.; Mahmoud, A.; Gafer, H. Efficiency of Levamisole in Improving the Immune Response of Catfish (*Clarias gariepenus*) to *Aeromonas hydrophila* Vaccine: Clinico- Pathological Studies. *Mediterr. Aquac. J.* **2010**, *3*, 8–17. [CrossRef]
77. Martínez-Porchas, M.; Rafael Martínez-Córdova, L.; Ramos-Enriquez, R. Cortisol and Glucose: Reliable indicators of fish stress? *Pan-Am. J. Aquat. Sci.* **2009**, *4*, 158–178.
78. Wu, F.; Wen, H.; Tian, J.; Jiang, M.; Liu, W.; Yang, C.; Yu, L.; Lu, X. Effect of stocking density on growth performance, serum biochemical parameters, and muscle texture properties of genetically improved farm tilapia, *Oreochromis niloticus*. *Aquac. Int.* **2018**, *26*, 1247–1259. [CrossRef]
79. Dong, Y.; Jia, R.; Hou, Y.; Diao, W.; Li, B.; Zhu, J. Effects of stocking density on the growth performance, mitophagy, endocytosis and metabolism of *Cherax quadricarinatus* in integrated rice–crayfish farming systems. *Front. Physiol.* **2022**, *13*, 2517. [CrossRef]
80. Onxayvieng, K.; Piria, M.; Fuka, M.M.; Gavrilović, A.; Liang, X.; Liu, L.; Tang, R.; Li, L.; Li, D. High stocking density alters growth performance, blood biochemical profiles, and hepatic antioxidative capacity in gibel carp (*Carassius gibelio*). *Fish Physiol. Biochem.* **2021**, *47*, 203–212. [CrossRef] [PubMed]
81. Refaey, M.M.; Li, D.; Tian, X.; Zhang, Z.; Zhang, X.; Li, L.; Tang, R. High stocking density alters growth performance, blood biochemistry, intestinal histology, and muscle quality of channel catfish *Ictalurus punctatus*. *Aquaculture* **2018**, *492*, 73–81. [CrossRef]
82. Qi, C.; Xie, C.; Tang, R.; Qin, X.; Wang, D.; Li, D. Effect of stocking density on growth, physiological responses, and body composition of juvenile blunt snout bream, *Megalobrama amblycephala*. *J. World Aquac. Soc.* **2016**, *47*, 358–368. [CrossRef]
83. Suárez, M.D.; Trenzado, C.E.; García-Gallego, M.; Furné, M.; García-Mesa, S.; Domezain, A.; Sanz, A. Interaction of dietary energy levels and culture density on growth performance and metabolic and oxidative status of rainbow trout (*Oncorhynchus mykiss*). *Aquac. Eng.* **2015**, *67*, 59–66. [CrossRef]
84. Yang, Q.; Guo, L.; Liu, B.S.; Guo, H.Y.; Zhu, K.C.; Zhang, N.; Jiang, S.G.; Zhang, D.C. Effects of stocking density on the growth performance, serum biochemistry, muscle composition and HSP70 gene expression of juvenile golden pompano *Trachinotus ovatus* (Linnaeus, 1758). *Aquaculture* **2020**, *518*, 734841. [CrossRef]
85. Coz-Rakovac, R.; Smuc, T.; Topic Popovic, N.; Strunjak-Perovic, I.; Hacmanjek, M.; Jadan, M. Novel methods for assessing fish blood biochemical data. *J. Appl. Ichthyol.* **2008**, *24*, 77–80. [CrossRef]
86. Bernet, D.; Schmidt, H.; Wahli, T.; Burkhardt-Holm, P. Effluent from a sewage treatment works causes changes in serum chemistry of brown trout (*Salmo trutta* L.). *Ecotoxicol. Environ. Saf.* **2001**, *48*, 140–147. [CrossRef]
87. Fang, L.S.; Bada, J.L. Biliverdin reductase activity in marine fishes. *Mar. Biol. Lett.* **1982**, *3*, 121–130.
88. Tripathi, N.K.; Latimer, K.S.; Lewis, T.L.; Burnley, V.V. Biochemical reference intervals for koi (*Cyprinus carpio*). *Comp. Clin. Path.* **2003**, *12*, 160–165. [CrossRef]
89. Ming, J.H.; Ye, J.Y.; Zhang, Y.X.; Xu, P.; Xie, J. Effects of dietary reduced glutathione on growth performance, non-specific immunity, antioxidant capacity and expression levels of IGF-I and HSP70 mRNA of grass carp (*Ctenopharyngodon idella*). *Aquaculture* **2015**, *438*, 39–46. [CrossRef]
90. de la Rosa, L.C.; Goicoechea, L.; Torres, S.; Garcia-Ruiz, C.; Fernandez-Checa, J.C. Role of Oxidative Stress in Liver Disorders. *Livers* **2022**, *2*, 283–314. [CrossRef]
91. Liang, X.; Luo, X.; Lin, H.; Han, F.; Qin, J.G.; Chen, L.; Xu, C.; Li, E. Effects and Mechanism of Different Phospholipid Diets on Ovary Development in Female Broodstock Pacific White Shrimp, Litopenaeus vannamei. *Front. Nutr.* **2022**, *9*, 830934. [CrossRef]

92. Saurabh, S.; Sahoo, P.K. Lysozyme: An important defence molecule of fish innate immune system. *Aquac. Res.* **2008**, *39*, 223–239. [CrossRef]
93. Costas, B.; Aragao, C.; Dias, J.; Afonso, A.; Conceicao, L.E.C. Interactive effects of a high-quality protein diet and high stocking density on the stress response and some innate immune parameters of Senegalese sole *Solea senegalensis*. *Fish Physiol. Biochem.* **2013**, *39*, 1141–1151. [CrossRef]