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HAEMATOLOGICAL AND BIOCHEMICAL CHANGES BROUGHT ON BY USING VICIA PEREGRINA IN THE DIET OF COMMON CARP FISH AS A PARTIAL SUBSTITUTE FOR SOYBEAN MEAL

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Abstract

The study focused on investigating the potential benefits of replacing soybean meal in the diet of the common carp fish Cyprinus carpio with (Culban) Vicia peregrina L. seed meal after improved the nutritional value of culban Vicia peregrina using four different methods: soaking in water for 12 hours, cooking for 40 minutes, roasting for 15 minutes, and germination for 7 days to reduce the effects of anti-nutritional and examining the effects on several blood parameters. Fish with an average weight of 28.5 ± 1.5 g were distributed in 27 plastic aquaria and were distributed for nine treatments. Seven fish were placed in each aquarium before the experiment. Diets with replacement levels of 0 % (control), (20% and 40%) of soybean meal with Culban seed (soaked in water for 12 hours, cooked for 40 minutes; roasted for 15 minutes, and germinated for 7 days) for 8 weeks. At the conclusion of the feeding study, blood samples were taken from three fish in each tank to examine hematological and biochemical characteristics. values of white blood cells, red blood cells, and hemoglobin were high as compared to the control accompanied with significant ($P \leq 0.05$) declines in values of corpuscular haemoglobin concentration MCH in the treatment that contain Culban seed cooking 40 minutes in fish fed diets 40% instead of soybean meal compared to the control. Other blood parameters, including serum glucose, cholesterol, both did not show significant ($P \leq 0.05$) differences between the control and experimental groups. Also, no significant changes were observed in serum (ALP) alkaline phosphatase (GOT) glutamic-oxaloacetic transaminase and (GPT) levels glutamic-pyruvic transaminase activities between the treatments and the control. The results of the present study demonstrated that Vicia peregrina could be used instead of soya bean meal in improving the growth and health of common carp.

Keywords: Hematological characteristics, Culban . Cyprinus carpio, Biochemical parameters

INTRODUCTION

The majority of the aquaculture industry is comprised of coastal mariculture and inland freshwater aquaculture; however, freshwater aquaculture produces around six times as much as coastal mariculture. The production of aquaculture continued to rise quickly towards the start of the twenty-first century. A record-breaking 214 million tons of aquaculture products were produced globally in 2020, an increase of little over 3% from the 213 million tons produced in 2018 (FAO, 2022). The earliest aquaculture species for which historical records are available in the common carp (C. carpio). Since this species has been domesticated for a longer period than any other fish, it has given rise to a variety of variations, including koi, the partially scaled mirror carp (C. carpio var. specularis), the orange-scaled scale carp (C. carpio var. flavipinnis), and the scaleless leather carp (C. carpio var. nudus). Eastern Europe and Asia

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are home to the omnivorous common carp, which eats food originating from plants, animals, and detritus. Generally, it is simplest to wean omnivores to dry specially prepared pellet feed (Kültz, D. 2022). The idea of incorporating affordable legume protein in fish nutrition has gained popularity worldwide. By substituting costly animal protein with legume protein, fish farmers can reduce their production costs and offer more affordable fish products to consumers (Gouveia and Davies 2000), However, using legumes as a protein source is not without its challenges. Legume protein lacks essential amino acids and may contain antinutritional factors that can affect fish growth and development negatively. To overcome these issues, farmers often mix legume protein with small amounts of cereal crops to balance out any nutritional deficiencies (Nleya et al., 2000). One legume that has emerged as a promising protein source for fish is Culban Vicia peregrina. Its seeds contain a high percentage of protein with adequate amino acids and fatty acids, making them suitable for carp diets. While the use of legumes in fish nutrition is not without its limitations, many farmers are still exploring this option as a viable alternative to expensive animal protein. The circulatory system of fish plays a significant role in transporting various components throughout the body. Blood in fish contains a wide range of constituents including nutrients, hormones, minerals, immune components, microorganisms, water, gases, toxins, and waste products (Ciesla, 2007). Nutrients such as amino acids, glucose, and fatty acids are transported to the cells to provide energy and building materials for growth and repair. Oxygen and carbon dioxide are exchanged through the gills and carried by the blood to support respiration, hormones like insulin and growth hormones regulate the bodily functions, while minerals like calcium and magnesium are essential for bone formation and nerve conduction. The immune components in the blood of fish help to protect against pathogens and foreign substances (Ciesla, 2007). As compared to more common terrestrial species, such as humans and domestic animals, fish possess unique cellular components such as nucleated red blood cells and platelets which prohibit the use of most automated analyzers used for mammalian blood counts. Interpreting the blood results of fish can be especially tricky due to these unique features. The effectiveness of using in-house fish blood analyzers has been examined in two recent investigations (Harrenstien et al., 2005 and Palmeiro et al., 2007).

The study of fish hematology has proven to be a valuable tool in assessing the health and well-being of fish populations in aquaculture. An analysis of the hematological profile can provide information about the physiological status of the fish, such as the presence of infection, inflammation, or anemia. This information can be used to diagnose diseases and identify conditions that are causing stress to the fish, which can lower production performance. By combining hematology with other diagnostic tools, such as microbiological and histopathological techniques, a more complete understanding of the fish's health status can be obtained. The use of hematological profiling in fish cultivation has thus become an important method to optimize production performance and maintain overall fish health (Tavares-Dias and Moraes, 2004; Tavares-Dias and Moraes, 2006; Tavares-Dias and Moraes, 2007; Pavlidis et al., 2007). The variations in hematological levels are influenced by a wide range of internal and external factors (Clauss et al., 2008). These characteristics can change depending on a number of factors, including nutritional condition (Svetina et al. 2002; Lim and Klesius, 2003), species (Ranzani-Paiva et al., 2003; Anthony et al., 2010), and age (Orun and Erdeml, 2002; Jamalzadeh and Ghomi, 2009), water quality (Fazio et al., 2012a). The aim of this study was

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to investigate the impact of partially replacing soybean meal with Cuban seed meal in the diet of common carp on various indicators of blood health.

MATERIAL AND METHODS

Experimental Diets Preparation

Reduce the nutritional inhibitors found in Culban grains through various methods of processing. The chosen methods for processing the Culban grains included soaking for 12 hours, cooking for 40 minutes, roasting for 15 minutes, and germination for 7 days. These methods were selected based on the percentage reduction achieved in reducing the antinutritional factors trypsin inhibitor, phytic acid and hydrogen cyanide (Abdulhalim & Mohammad, 2023), through thermal and biological treatments. The aforementioned supreme treatments are substituted 20% and 40% by soybean expensive meal to determine which substituting ratio are much more effective in improving the growth parameters of common carp Cyprinus carpio (Table 1).

Table 1: Feed ingredients containing different percentages of Culban Vicia peregrina seeds treated by different methods as a partial substitute for soybean meal

Diets Ingredients	Treatments								
	control	Soaking for 12 hours		Cooking for 40 minutes		Roasting for 15 minutes		Germination 7 days	
	(0%)	(20%)	(40%)	(20%)	(40%)	(20%)	(40%)	(20%)	(40%)
Fish Meal	10	10	10	10	10	10	10	10	10
Soybean meal	30	24	18	24	18	24	18	24	18
Culban seed	0	10.5	21	10.5	21	10.5	21	10.5	21
Yellow corn	18.5	14.5	10	14.5	10	14.5	10	14.5	10
Local barley	21	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5
Wheat bran	18	18	18	18	18	18	18	18	18
Vit.-min. mix.	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Salt food	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lime stone	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Bentonite	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

Chemical evaluation was conducted using the established procedures of the Association of Official Analytical Chemists to determine the main chemical elements present in experimental diets for fish in table (2)

Table 2. Chemical composition components for the experimental diets (% DM)

Diets Criteria	Treatments								
	Control	Soaking for 12 hours		Cooking for 40 minutes		Roasting for 15 minutes		Germination 7 days	
	(0%)	(20%)	(40%)	(20%)	(40%)	(20%)	(40%)	(20%)	(40%)
Crude protein	26.4	26.5	26.7	26.1	25.8	26	25.8	26.4	26.2
Ether extract	3	2.9	2.8	3.4	3.1	3.3	3	3.1	3
fiber	3.5	3.2	3.5	3.8	4.8	4.5	4.1	4.3	4.7
Ash	3.6	4.7	4.4	4.1	4.2	4.3	4.3	4.4	4.4
(NFE)*	63.5	62.7	62.6	62.6	62.1	61.9	62.8	61.8	61.7
ME (MG/KG)**	14.7	14.5	14.5	14.6	14.4	14.5	14.4	14.5	14.4

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*Nitrogen-Free Extract. ** ME = (Protein x 18.5) + (Fat x 33.5) + (NFE x 13.8).

Fish and feeding experimental procedure

The study took place over a 56-day period in the Fish Laboratory of the Department of Animal Production at the College of Agricultural Engineering Science at the University of Duhok. Fish with an initial weight of 28.5 ± 1.5 g, were acclimated to laboratory conditions for three weeks before being randomly distributed into the aquaria of 70 L capacity. Fish were distributed into 27 experimental aquaria in nine groups of 189 fish each (7 fish per aquarium).

In the table (3) showed the results after feeding experiment in food conversion ratio (FCR), feed efficiency ratio (FER), protein efficiency ratio (PER), and net protein utilization (PPV) when soybean meal was partially replaced with Culban grains in fish feed.

Table 3. Effect of partial replacement of soybean meal with Culban grains on Food intake, FCR, FER, PER and PPV (Rate ± SE)

Diets Parameters	Treatment								
	Control	Soaking for 12 h.		Cooking for 40 min.		Roasting for 15 min.		Germination for 7 days	
	(0%)	(20%)	(40%)	(20%)	(40%)	(20%)	(40%)	(20%)	(40%)
Food intake (g./fish)	38.13± 0.28 a	33.06± 0.26 b	33.21± 0.18 b	36.35± 0.73 ab	33.48± 2.58b	33.48± 0.74 b	36.05± 0.55 ab	34.36 ± 0.44 b	36.07 ± 0.52ab
Feed Conversion Ratio	3.75± 0.29 abc	3.35± 0.13 bcd	3.28± 0.14 bcd	3.32± 0.34 bcd	3.16 ± 0.21 cd	3.17 ± 0.09 cd	3.05 ± 0.10 cd	4.11 ± 0.17 a	3.91 ± 0.23 a
Feed Efficiency Ratio	27.06 ± 2.24 bcd	29.88± 1.18 ad	30.83± 1.08ab	30.73± 2.91 abc	31.94± 2.26ab	32.38 ± 0.59 ab	32.90 ± 1.11 a	24.43 ± 0.99 d	25.77± 1.59 cd
Protein Efficiency Ratio (PER)	1.51 ± 0.037abc	1.44± 0.018 c	1.46± 0.029c	1.53± 0.034a bc	1.49 ± 0.033 bc	1.56 ± 0.030 ab	1.58 ± 0.004 a	1.43 ± 0.003 c	1.44± 0.035c
Protein Productive Value (%)	19.50 ± 0.057 c	19.56± 0.033 c	25.76± 0.466b	25.73± 0.088 b	27.86± 0.033a	27.56 ± 0.120 a	23.26 ± 0.066 c	17.06 ± 0.088 d	14.50± 0.173 e

*Different letters in the same row indicate a significant difference (P≤ 0.05).

Collection of fish blood samples

The blood samples obtained from the fish after the feeding trial. Collected blood samples from fish to assess Complete Blood Count (CBC) parameters and conduct biochemical tests. Three fish per replicate were selected as samples, then cut the caudal peduncle to collect blood. Some of the samples were collected in anticoagulant tubes sodium heparinate (20 U/L) for haematology analysis. The anticoagulant in these tubes helps to prevent the blood from clotting, allowing for proper analysis of blood cells and other haematological parameters. On the other hand, the remaining samples were filled in tubes with no anticoagulant in order to clot to prepare serums for biochemical analyses such as glucose, cholesterol, and liver enzymes.

Serum preparation done for fish blood by a series of steps to separated serum from the cellular components, the collected blood is centrifuged at 5000 × g for 20 min at room temperature to separate serum from the cellular components. The separated serum undergoes a series of

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filtration and purification steps to remove any impurities and ensure its quality. The final product is a clear, yellowish serum that is rich in proteins, lipids, vitamins. Serum biochemical evaluations, such as glucose, triglycerides, total cholesterol, albumin, uric acid, ALP alkaline phosphatase, GOT glutamic-oxaloacetic transaminase and GPT glutamic-pyruvic transaminase. Hematological characteristics that include hemoglobin (HB), erythrocyte counts (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), and differential leukocyte count (WBC) (granulocytes%, lymphocytes%, and monocytes%.). All hematological tests were conducted using a Mindray BC-2800 hematology analyzer that was manufactured in China. This analyzer is a state-of-the-art diagnostic tool that can provide a complete blood count test, comprising of 19 parameters. This device is highly automated, and it has been designed to streamline the process of hematological assessment.

RESULTS AND DISCUSSION

Biochemical parameters glucose, triglycerides, total cholesterol, albumin, uric acid, ALP alkaline phosphatase, GOT glutamic-oxaloacetic transaminase and GPT glutamic-pyruvic transaminase shown in Table (4). No statistically difference was observed in glucose concentrations, uric acid, also no significant changes were observed in serum (ALP) alkaline phosphatase (GOT) glutamic-oxaloacetic transaminase and (GPT) levels glutamic-pyruvic transaminase activities between the treatments and the control.

Table 4: Biochemical parameters of common carp fed with experimental diets for 56 days

parameters	Treatments								
	Control	Soaking for 12 h.		Cooking for 40 min.		Roasting for 15 min.		Germination 7 days	
	0%	20%	40%	20%	40%	20%	40%	20%	40%
Glucose (mg/dl)	58.7± 7.2	62.7± 6.7	76.± 8.6	67.7± 10.10	67± 10.02	69.7± 8.2	62± 5.5	69± 1	60± 5
Triglycerides (mg/dl)	164± 32.7**ab**	141± 26.3**ab**	126± 15.6**ab**	85± 18.7**a**	190± 30.5**b**	186.6± 15.4**ab**	129.7± 36.8**a**	182.3± 18.2**ab**	160.± 5.8**a**
Cholesterol (mg/dl)	77± 9.5 **ab**	74.± 7.8**ab**	92.± 1.5**ab**	74.3± 8.1 **ab**	81.7± 5.5 **ab**	97.3± 0.9**a**	72.7± 11.2 **b**	92.7± 5.8 **ab**	72.3± 7.2 **b**
Albumin (g/dl)	1.9± 0.5**a**	1.05± 0.2**b**	1.4± 0.3**ab**	0.98± 0.2**b**	0.90± 0.08**b**	1.07± 0.07**b**	0.80± 11.2**b**	0.98± 0.14**b**	1.04± 0.26**b**
Uric Acid (mg/dl)	2.4± 0.66	1.5± 0.21	2.6± 0.83	1.7± 0.31	1.4± 0.23	1.7± 0.11	1.35± 0.22	1.23± 0.16	1.39± 0.04
ALP (U/l)	51.33± 10.9	37.6± 11.3	51.65± 10.1	33± 3.2	32.3± 2.4	41.7± 16.9	30.59± 2.3	38.3± 7.8	50.7± 8.4
GOT (U/l)	163± 32.1	139.3± 15.4	152± 26.1	152.3± 15.3	127± 13.8	191± 40.1	138.7± 40.1	132.7± 14.2	125± 22.1
GPT (U/l)	80.6± 20.5 **ab**	63.2± 6.5 **ab**	76.4± 9 **ab**	42.8± 19.2**b**	55.1± 17.7**ab**	106.6±2 7.7 **a**	58.5± 10 **ab**	68.7± 16.7**ab**	88.2± 22.3**ab**

*Different letters in the same row indicate a significant difference ($p \leq 0.05$).

As explained by (Babalola et al., 2009) the levels of hepatic enzymes such as GOT, GPT, ALP, and LDH are essential indicators of hepatic tissue damage. Triglyceride, total cholesterol value

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has not significantly differed in the experimental diet's groups with the control ($p \leq 0.05$). Albumin concentration in the treatment Culban soaking for 12 hours instead of 40% of soybean meal has no significant differences with the control but the other treatments have significant differences with the control treatment ($p \leq 0.05$) and the minimum value was in the diet contain roasting Culban seed for 15 min. (instead of 40% of soybean meal). Serum albumin level is essential in animals for research, because it appear the nutrition state of the animals, cardiovascular system stability, albumin synthesis occurs mainly in the liver, so changes in serum albumin levels can also reflect liver function. (Gopal et al., 1997) and (Januar et al.,2015). Moreover, since albumin binds to various substances, including bilirubin, bile acid salts, hormones, and toxins, it can exert a regulatory effect on metabolic pathways (Mutlu et al., 2015). The decreased in albumin level in roasting treatment is due to its effects seed protein. (Baik and Han 2012) peas, beans, and chickpeas undergo the greatest loss in protein digestibility upon roasting. In lentils, chickpeas, beans, and peas, roasting decreases protein solubility by 21%, 35%, 37%, and 22%, respectively.

Table 5: Haematological parameters of common carp fed with experimental diets for 56 days

parameters	Treatments								
	Control	Soaking for 12 h.		Cooking for 40 min.		Roasting for 15 min.		Germination 7 days	
	0%	20%	40%	20%	40%	20%	40%	20%	40%
RBC (10^{12}/L)	0.8± 0.2	1.55± 0.3	1.11± 0.4	1.81± 0.02	1.47± 0.23	1.3± 0.2	0.96± 0.39	1.8± 0.07	1.01± 0.47
Hb (g/dL)	18.5± 1.1 **ab**	22.6± 2.3 **ab**	21.2± 3.8 **ab**	22± 2.1 **ab**	19.7± 1.3 **ab**	21.4± 1.3 **ab**	17.2± 1.9 **b**	24.4± 1.2 **ab**	23.2± 1.4 **ab**
MCV (cm3/erythrocyte)	115.2± 3.9**ab**	114± 3.2**ab**	119.7± 6.9 **ab**	112.9± 3.3 **ab**	131.2 ±17**a**	107.5± 3.3**b**	113.1± 2.9 **ab**	111.7± 3.1 **ab**	104.6± 2.2**b**
MCH (Pg)	75.3± 2.02**a**	63.33± 8.5**ab**	72.8± 4.9 **ab**	53.3± 8.5 **ab**	38.7± 4.66**b**	55± 9.2 **ab**	71.3± 13.1**ab**	48.7± 7.9 **ab**	40.66± 3.5 **ab**
MCHC (g Hb/dL)	44.7± 2.02	36± 8.5	41± 4.9	29± 8.5	27.3± 4.7	38± 9.2	35.3± 13.1	25.33± 7.9	24.5± 3.5
PLT (10^9 /L)	66.7± 7.1	90.5± 10.3	93.6± 33.9	128.8± 25.4	114.4 ±37.3	90.2± 26.6	81.7± 34.9	144± 2.9	83.7± 28.5

*Different letters in the same row indicate a significant difference ($P \leq 0.05$).

In table (5) no significant differences were observed in RBC′s count, haemoglobin concentration, also MCV, MCHC, and Platelet count. With the control treatment, while the treatment that contain 40% culban cooking 40 min. was significantly less than the control in mean corpuscular haemoglobin MCH. In fish, the average RBC count is 1.56 (×106/mm3), with a typical range of 0.4 to 5.2. Fishes have less blood cells per unit volume as in higher vertebrates (Tandon and Joshi, 1976). Red blood cells count in the fish fed contain of Culban seed processed were higher in values from the control treatment, this could be due to increase the activity of the seeds during processing which reducing the antinutritional factors in the seed. (Raissy et al., 2021) dietary combined herbs extracts in common carp (Cyprinus carpio) feed make the growth better because red blood cells are responsible for carrying oxygen to the various tissues in the body, and therefore, a higher RBC count means a greater supply of oxygen to the tissues. This increased oxygen supply can potentially lead to improved growth rates in

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fish.

Table 6: Differential leukocyte count of common carp fed with experimental diets for 56 days

parameters	Treatments								
	Control	Soaking for 12 h.		Cooking for 40 min.		Roasting for 15 min.		Germination 7 days	
	0%	20%	40%	20%	40%	20%	40%	20%	40%
WBC (10^3cells/ mm^3)	20.1± 2.8**b**	31.7± 3.4**ab**	21± 5.6**b**	28.4± 4.5 **ab**	29.9± 4.5 **ab**	28.6± 4.7 **ab**	19.7± 3.9**b**	38.3± 1.3**a**	20.3± 7.4**b**
LYM%	66.7± 7	90.5± 10.3	93.7±3 3.9	128.8± 25.4	114.4± 37.3	90.22± 26.6	81.7± 34.9	144± 2.9	83.6± 28.5
MON%	25.6±2	25.4± 3	27.5±3	23.5± 0.5	26.2± 2.8	19± 0.88	27.2± 1.3	25± 4.3	24.5± 4.2
GRA%	48.1±3	51.6± 3.2	58±11. 2	59.8±1 5.9	48.9±7. 8	53.2±5. 2	47.2± 6.9	58.4± 4.2	55.4± 12.6

*Different letters in the same row indicate a significant difference ($P \leq 0.05$).

Table (6) showed that the treatment Culban germination for 7 days 20% instead of soybean meal was significantly more than the control in white blood cell count but the others treatments were significantly not different from the control treatment also there were no significant changes in assessed leukocytes (granulocytes, monocytes, and lymphocytes) between the treatment's groups and the control. As germination is active process to reduce the antinutritional substances this done by previous study (Abdulhalim & Mohammad, 2023) trypsin inhibitor, phytic acid and hydrogen cyanide in Culban (Vicia peregrina) seeds are reduced by germination and this agree with (Singh et al., 2014) Germination is domestic ritual that is known to significantly alter the nutritional content of seeds by raising several vitamins and critical amino acids while lowering many anti-nutritional elements. Different studies showed that use plant protein meal instead of fish meal led to change in white blood cell count because these sources contain anti-nutritional factors that effect on fish s immunity and defines system in negative form (Jahanbakhshi et al., 2012 and Potki et al., 2018). For that decreasing these kinds of substances will enhance fish growth. (Mohammad, 2017) growth indices for common carp Cyprinus carpio L. were greatly improved by the germination of Vicia sativa for 5 and 7 days. White blood cells (WBCs) play a critical role in fish immunity by performing several functions such as phagocytosis, antibody production, and the discharge of immune-related molecules. (Magnadóttir 2006; Shoemaker et al. 2015). But, an escalation in the number of WBCs within a fish's body may indicate that its immune system is being triggered or that inflammation is occurring. (Tort 2011).

CONCLUSION

Clearly in this study, fish metabolism was affected when used diet contain Culban seed after processing led to a better growth performance and health. Through this evaluation, researchers aimed to determine the extent to which changes in diet could influence the health of these fish. Ultimately, findings from this study may inform future efforts to optimize fish diets, enhance growth rates, and improve overall fish health and welfare.

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References

1. Abdulhalim, B.A. and M.A. Mohammad .2022. Reducing Antinutritional Hydrogen Cyanide, Phytic Acid, and Trypsin Inhibitor in Rambling vetch, Culban Vicia peregrina. The paper will be Published in "IOP Conference Series: Earth and Environmental Science", Publisher: IOP Publishing Ltd., Volume 2031 at year 2023.
2. Anthony, O., Kechukwu, I., Obinnaya, C., 2010. Haematological profile of the African lungfish, Protopterus annectens (Owen) of Anambra River, Nigeria. Am. J. Sci. 6, 123–30.
3. Babalola, T. O. O., Adebayo, M. A., Apata, D. F., & Omotosho, J. S. (2009). Effect of dietary alternative lipid sources on haematological parameters and serum constituents of Heterobranchus longifilis fingerlings. Tropical Animal Health and Production, 41(3), 371. https://doi. org/10.1007/s11250-008-9199-1
4. Ciesla, B. Hematology in Practice; FA Davis Company: Philadelphia, PA, USA, 2007; p. 230.
5. Clauss, T.M., Dove, A.D.M., Arnold, J.E., 2008. Hematologic disorders of fish. Vet. Clin. North.Am. Exot. Anim. Pract. 11, 445–462.
6. Fazio, F., Faggio, C., Marafioti, S.,Torre, A., Sanfilippo, M., Piccione, G. 2012a. Comparative study of haematological profile on Gobius niger in two different habitat sites: Faro Lake and Tyrrhenian Sea. Cah.Biol. Mar. 53, 213–219.
7. Food and Agriculture Organization (FAO). (2022). The state of world fisheries and aquaculture 2022. https://doi.org/10.4060/cc0461en [Google Scholar]
8. Gopal, V.; Parvathy, S.; Balasubramanian, P.R. Effect of heavy metals on the blood protein biochemistry of the fish Cyprinus carpio and its use as a bio-indicator of pollution stress. Environ. Monit. Assess. 1997, 48, 117–124.
9. Gouveia, A., Davies, S.J., 2000. Inclusion of an extruded dehulled pea seed meal in diets for European sea bass ŽDicentrarchus labrax.. Aquaculture 182, 183–193.
10. Harrenstien, L.A., Tornquist, S.J., Miller-Morgan, T.J., Fodness, B.G. and Clifford, K.E. Evaluation of a point-of-care blood analyzer and determination of reference ranges for blood parameters in rockfish. Journal of the American Veterinary Medical Association 2005;226(2):255–265.
11. Jahanbakhshi, A., Imanpoor, M. R., Taghizadeh, V., & Shabani, A. (2013). Hematological and serum biochemical indices changes induced by replacing fish meal with plant protein (sesame oil cake and corn gluten) in the Great sturgeon (Huso huso). Comparative Clinical Pathology, 22, 1087-1092.
12. Jamalzadeh, H.R., Ghomi, M.R., 2009. Hematological parameters of Caspian salmon Salmo truttacaspius associated with age and season. Mar. Freshwater Behav. Physiol. 42, 81–87.Orun, I., Erdeml, A.U., 2002. A study on blood parameters of Capoeta trutta (Heckel, 1843). J. Biol. Sci. 8, 508–511.
13. Januar, H.I.; Fajarningsih, N.D.; Zilda, D.S.; Bramandito, A.; Wright, A.D. Concentration of fish serum albumin (FSA) in the aqueous extract of Indonesian Perciformes fishes' muscle tissue. Nat. Prod. Res. 2015, 29, 2230–2232.
14. Kültz, D. (2022). A Primer of Ecological Aquaculture. Oxford University Press. ISBN10 0198850239.ISBN13 9780198850236.
15. Lim, C., Klesius, P.H., 2003. Infuence of feed deprivation on hematology, macrophage chemotaxis, and resistance to Edwardsiella ictaluri challenge of channel catfish. J. Aquat. Anim. Health.15, 13–20.
16. Magnadóttir, B. 2006. Innate immunity of fish (overview). Fish & Shellfish Immunology 20 (2):137–51. doi:10.1016/j.fsi.2004.09.006.
17. Mohammad, M. A. 2017. Effect of using germinated for common vetch seeds Vicia sativa on growth performance of common carp Cyprinus carpio L. The Iraqi Journal of Veterinary Medicine.41(1): 118-124.
18. Mutlu, E.; Aydin, S.; Kutlu, B. Alterations of growth performance and blood chemistry in Nile tilapia

DOI 10.17605/OSF.IO/Y58QP

(Oreochromis nuoticus) affected b copper sulfate in long-term exposure. Turk. J. Fish Aquat. Sc. 2015, 15, 481–488.

19. Nleya, T., Vandenberg, A., Araganosa, G., Warkentin, T., Muehlbauer, F. J., & Slinkard, A. E. (2000). Produce quality of food legumes: Genotype (G), environment (E) and (GxE) considerations. In Linking Research and Marketing Opportunities for Pulses in the 21st Century: Proceedings of the Third International Food Legumes Research Conference (pp. 173-182). Springer Netherlands.

20. Palmeiro, B.S., Rosenthal, K.L., Lewbart, G.A. and Shofer, F.S. Plasma biochemical reference intervals for koi. Journal of the American Veterinary Association 2007;230(5):708–712.

21. Pavlidis, M., Futter, W.C., Kathario, P., Divanach, P., 2007. Blood cells of six Mediterranean mariculture fih species. J. Appl. Ichthyol. 23,70-73.

22. Potki, N., Falahatkar, B., & Alizadeh, A. (2018). Growth, hematological and biochemical indices of common carp Cyprinus carpio fed diets containing corn gluten meal. Aquaculture International, 26, 1573-1586.

23. Raissy, M.; Ghafarifarsani, H.; Hoseinifar, S.H.; El-Haroun, E.R.; Naserabad, S.S.; Van Doan, H. The effect of dietary combined herbs extracts (oak acorn, coriander, and common mallow) on growth, digestive enzymes, antioxidant and immune response, and resistance against A. hydrophila infection in common carp, Cyprinus carpio. Aquaculture 2021, 546, 737287.

24. Ranzani-Paiva, M.J.T.E.L., Rodrigues, M.L., Veiga, A.C., Eiras, B.E.S., 2003. Differential leukocyte counts in “dourado” Salminus maxillosus Valenciennes, 1840 from the Mogi- Guaçu River, Pirassununga, SP. Braz. J. Biol. 63, 517–25.

25. Shoemaker, C., D.-H. Xu, B. LaFrentz, and S. LaPatra. 2015. Overview of fish immune system and infectious diseases. Diet nutrients, additives, and fish health, 1–24. Canada: Wiley Blackwell. doi:10.1002/9781119005568.ch1.

26. Singh, P. K., Gautam, A. K., Panwar, H., Singh, D. K., Srivastava, N., Bhagyawant, S. S., & Upadhayay, H. (2014). Effects of germination on antioxidant and anti nutritional factors of commonly used pulses. International Journal of Research in Chemistry and Environment, 4(2), 100-104.

27. Svetina, A., Matasin, Z., Tofant, A.,Vucemilo, M., Fuan, N., 2002. Haematology and some blood chemical parameters of young carp till the age of three years. Acta Vet. Hung. 50, 459–67.

28. Tandon, R.; Joshi, B.D. Total red and white blood cell count of 33 species of fresh water teleosts. Z. Tierphysiol. Tierernahr. Futtermittelkd. 1976, 37, 293–297.

29. Tavares-Dias, M., Moraes, F.R., 2004. Hematologia de peixes teleósteos. Ribeirão Preto, Villimpress Complexo Gráfio.

30. Tavares-Dias, M., Moraes, F.R., 2007. Leukocyte and thrombocyte reference values for channel catfih (Ictalurus punctatus Raf.), with an assessment of morphological, cytochemical, and ultrastructural features. Vet. Clin. Pathol. 36, 49–54.

31. Tavares-Dias, M.; Moraes, F.R., 2006. Hematological parameters for the Brycon orbignyanus Valenciennes, 1850 (Osteichthyes: Characidae) intensively bred. Hidrobiológica. 16, 271–274.

32. Tort, L. 2011. Stress and immune modulation in fish. Developmental and Comparative Immunology 35:1366–75. doi:10.1016/j.dci.2011.07.002.