Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Impact of Tomato Pomace, Enzymes and/or Amino Acids on Blood Parameters, Serum Biochemicals, Antioxidants, Immune Status and Expression of Immune-Related Genes in Nile tilapia Fish (*Oreochromis nilotius*)

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Abstract

THIS STUDY clarified the significance of including tomato pomace with or without enzymes and/or amino acids in the diet of Nile tilapia on blood parameters, serum biochemicals, antioxidants, and expression of immune- related genes in liver for 8 weeks. Five groups of diet were formulated with protein content (28%): (G1) a control diet without tomato pomace (TP), (G2) TP included by 15%, (G3) TP 15% with lysine (1.79%) and methionine (0.94%), (G4) TP 15% with gallizyme® (0.2%) and (G5) TP 15% with lysine, methionine and gallizyme®. The results of red blood cells (RBCs), white blood cells (WBCs) count and the value of hemoglobin and hematocrit showed non-significant difference in all groups *(p>0.05)*. While, levels of serum creatinine, urea and ALT were significantly decreased in G3, G4 and G5 compared to G2 (*P<0.05).* There was a significant difference in the levels of serum super-oxide dismutase (SOD), glutathione peroxidase (GPX) and malondialdehyde (MDA) in the last three groups compared to G1 and G2 (*P=0.02),* (*P=0.01),* (*P<0.0001)* respectively. However, phagocytic activity and phagocytic index was non significantly differed in the five groups, but lysozyme activity showed a higher value in G3 and G4 compared to G2 *(p<0.05)*. The relative expression levels of SOD and interleukin-12 in the liver of tilapia were significantly differed *(p<0.05)* in the last three groups, while expression levels of catalase showed no significant difference in all groups. Finally, inclusion of dietary TP with enzymes or amino acids could enhance antioxidant status and immune parameters in Nile tilapia fish.

Keywords: TP, Gallizyme®, SOD, MDA, Creatinine, ALT, GPX.

Introduction

Aquaculture is an acknowledged technique of raising animals which helps to meet the world's increasing demand for protein due to population growth [1]. The cost of nutrition, of which protein (specifically fishmeal) is the most expensive ingredient is a significant issue for the aquaculture business [2, 3]. Feed production has a significant impact on land occupation, water reliance, acidification, and climate change; fishmeal and soy meal are particularly connected to these effects [4, 5]. Thus, an effort is being made to use less of the meals listed above. The appropriateness of substitute protein sources in aquaculture diets has been the subject of numerous studies

Globally, tomatoes (Solanum lycopersicum L.) are one of the most widely grown vegetables. Large amounts of residue from tomato processing are called tomato pomace, and they make up 2–10% of the weight of all fresh tomatoes [6, 7]. Typically, tomato pomace is composed of seed, peel, and a small quantity of pulp [7, 8]. Tomato pomace is an excellent natural supply of lipids (5–10%), protein (10–20%), and fiber (60–70%). Lycopene and beta-carotene, two bioactive substances included in tomato waste, have been shown to have strong antioxidant properties. It is thought to be a potential natural source of antioxidants due to its high level of phytochemical composition [9] [10]. Despite these advantages, there are a few issues with plant protein components in feeds that need to be considered. These issues include the presence of ANFs, imbalanced essential amino acids, and complex carbohydrates, which may restrict the use of plant protein in feed composition [11, 12]. The development of processing systems capable of efficiently removing or degrading these ANFs from plant feed components is essential to improve fish

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(Received 17 May 2024, accepted 12 August 2024)
DOI: 10.21608/EJVS.2024.290519.2096

utilization of plant-based protein. Exogenous enzymes that have the potential to specifically break down many ANFs in fish diets that contain high levels of plant protein are of great interest. This might significantly increase the nutritional value of plant-based protein components in fish diets [13, 14]. Furthermore, exogenous enzymes may change the substrate that some populations of gut microorganisms may access, enhancing the digestibility of feeds [15] . It is believed that the addition of xylanase and glucanase to diets containing non-starch polysaccharides (NSPs) in Nile tilapia aquafeeds will improve the fish's ability to digest nutrients, particularly crude protein and crude lipids, and will encourage the growth of specific beneficial bacteria [16, 17]. Tomato pomace has large levels of fiber and non-starch polysaccharides; therefore, adding amino acids or high-quality protein sources with better digestibility and an AA balance can help minimize these effects [5].

Thus, our main goal was to assess the impact of tomato pomace with or without enzymes and/or amino acids on blood parameters, antioxidant and immune status in serum and their genes expression in Nile tilapia fish.

Material and Methods

Tomato Pomace (TP)

We purchased tomato pomace (TP) from a local supplier and allowed it to dry for five days, four hours a day in the sun. It was powdered and subjected to a chemical composition analysis upon drying, in order to prepare the diet as indicated in Table (1).

Exogenous enzymes

Gallizyme, an exogenous enzyme utilized in the experiment, was acquired from Tex Bioscience Company in India. These enzymes include pectinase (30000 U/kg), protease (400000 U/kg), alpha-Galactosidase (10000 U/kg), beta-Glucosidase (10000 U/kg), arabinase (7000 U/kg), phytase (500000 U/kg), lipase (10000 U/kg), and wheat bran up to 1 kg. According to [18], enzyme combinations were added to this investigation at a rate of 0.2%.

Fish and experimental diets

In this investigation, 225 monosexual fingerlings Nile tilapia was used. The fish were kept in glass tanks for two weeks to acclimate them before the trial started. The experimental fish were acquired from a private farm and had an initial weight of 21.2±.08g/fish. The fish basal diet, shown in Table (2), was created in compliance with NRC (2011) [19]. Fish were split into five groups at random, with 45 fish each aquarium and three duplicates of 15 fish each replication. G1 was a control diet that contained no TP; G2 was a TP 15% (TP); G3 was a TP 15% supplemented with lysine (1.79%) and methionine (0.94%) (TPA); G4 was a TP 15% diet that included exogenous enzymes (0.2% of the diet) (TPE); and G5 was a TP 15% diet that included lysine (1.79%), methionine (0.94%), and (0.2%) exogenous enzymes (TPAE). After the trial started and for the next eight weeks, every day, dechlorinated fresh water was used to refill roughly half of each tank's water level, and fish wastes and excreta were extracted by siphoning. Throughout the investigation, the following parameters were recorded: temperature between (24°C–27°C), dissolved oxygen 6.5 ± 0.5 mg l-1, pH 7.1 ± 0.8, EC 219 ± 2 µ mho/cm, and ammonia adjusted to the average permissible limits (<0.1 mg total ammonia). Assessments of the water quality were carried out in compliance with [20].

Sampling

Five fish were randomly chosen from each group at the ending of the feeding trial. Using clean syringes, blood was extracted from the caudal vein of five fish for each group and divided into two halves with clean syringes [21]. In order to assess hemoglobin (Hb), hematocrit (Htc), red blood cells (RBCs), and white blood cells (WBCs), the first half was collected using the anticoagulant ethylenediaminetetraacetate (EDTA; 10%), according to [22] . The blood sample was allowed to clot overnight at 4 °C before the second half was centrifuged for 10 minutes at 3000 rpm. The serum without hemolysis was stored at a temperature of -20°C before to use.

Haematological indices

Erythrocytic and leukocytic counts determination:

A hemocytometer and Natt-Herrik solution were used to count the erythrocytes and leukocytes in accordance with [23].

Hemoglobin concentration determination:

According to [23], the cyano-methemoglobin technique Drabkin's solution was used to calculate the hemoglobin concentration. Using ferricyanide and cyanide ions, the cyano-methemoglobin technique changes all hemoglobin derivatives into methemoglobin. Methemoglobin is a colorless, stable red substance that can be quantified via colorimetry.

Packed cell volume determination:

The PCV% was estimated using the micro hematocrit method, [24].

Determination of differential leukocytic count (DLC):

After obtaining thin blood films, they were air dried, fixed for three to five minutes with methanol, then stained for eight to ten minutes with Gimsa stain. They were then rinsed with distilled water and allowed to dry. According to [23], the white blood cells were counted among a hundred blood smears. According to [25], the absolute DLC was computed

using the following formula: Total leukocytic count x number of individual white cells / 100 equals absolute DLC.

Biochemical indices

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activities were measured colorimetrically at 505 nm wavelength by Bio diagnostic Co., Egypt, in accordance with [26].

Serum creatinine and urea were determined using Bio-diagnostic co. Egypt according to [27].

Glucose, triglyceride and cholesterol were measured using the protocols provided in commercial kits purchased from Vitro-Scient co. and Bio-diagnostic co. Egypt.

Antioxidants

Serum Superoxide dismutase (SOD), Catalase, Lipid peroxide, Malondialdehyde (MDA) and Glutathione peroxidase (GPx) activities were determined colorimetrically using the protocols in commercial kits from Bio-diagnostic co. Egypt according to [28-31] respectively.

Immune indices

Lysozyme activity

The fish lysozyme ELISA kit (Sunlong Biotech Co., China) was used to measure the serum lysozyme activity using the ELISA micro-well technique at a wavelength of 450 nm. The micro plate ELISA reader was used in accordance with the manufacturer's instructions.

Phagocytic activity

The bacterial strain Aeromonas hydrophila (1.2 × 106 CFU) was supplied by department of fish diseases and management, Sakha Aquaculture Research Unit, Central Lab, Egypt.

Leukocyte phagocytic function followed the method of [32] slightly modified as follows: after blood collection, 0.5 ml of blood was dropped into centrifuge tubes, to which was added 0.25 ml of 1 × 106 Aeromonas hydrophila suspension before shaking. The tubes were kept at 28°C in a water bath for 30 min, and shaken every 10 min. After this time, in order to centrifuge ,the blood smears were done in duplicates just after incubation and stained by Giemsa /May-Grunwald [33].

The number of leukocytes that engulfed bacteria was counted as percentages in relation to total leukocyte number in the smear from the phagocytosis assay.

mRNA expression of antioxidants and immune related genes

The Qiagen RNeasy plus Mini kit was utilized to extract RNA from the liver tissue. The concentration and purity of every RNA sample were measured using the Spectrophotometer Nano Drop ND-1000 (Nano Drop Technologies, Wilmington, Delaware, USA) at 260 and 260/280 nm ratios. Utilizing a High-Capacity RNA-to-cDNA Master Mix kit, reverse transcription of the RNA samples was carried out to produce cDNA.

For the amplification procedure, Thermo Scientific Maxima ® SYBR Green/ROXqPCR Master Mix (2×) by Rotor-Gene Q (Qiagen, USA) and PCR primers specific to each gene were utilized. Table (3) shows that Primer Express 3.0 (Applied Biosystems, USA) was used to generate G, which were then blasted on NCBI/Blast to confirm the specificity of the required gene. We determined the identity and specificity of each gene by examining the melting curves of the PCR products. The obtained Threshold Cycle (Ct) value made it possible to calculate the $2^{-\Delta\Delta Ct}$ of the mRNA expression of the different genes.

Ethics statement

Based on ethical criteria, the Medical Research Committee at Kafrelsheikh University's Faculty of Veterinary Medicine in Egypt gave approval for this study (approved number: KFS-IACUC/123/2023).

Statistical analysis

One-way analysis of variance (ANOVA) was used to do a statistical analysis on the collected data. Tukey's test was used for intergroup comparisons at ($p < 0.05$). The IBM SPSS 27 statistical software was used to perform all statistical data.

Results

Blood parameters

In this study, it was observed in Table (4) that addition of amino acids or enzymes to tomato pomace diet was non- significantly different in RBCS, Hb and HB derivatives in the experimental groups: G1, control diet without tomato pomace (TP), G2, TP 15%, G3, TP with amino acids (TPA), G4, TP with enzymes (TPE) and G5, TP with AAs and enzymes (TPAE), ($p > 0.05$)

Also, in Table (5), the WBCS and their derivatives showed no significant difference in groups supplemented with TPA, TPE and TPAE compared to the control group and TP ($p > 0.05$).

Serum biochemical indices

Table (6) presented the impact of tomato pomace and or enzymes or amino acids on serum biochemical parameters in tilapia fish, it was observed that supplementation of enzymes alone or with amino acids to tomato pomace (G4 and G5) significantly decreased serum creatinine levels compared to tomato pomace group (G2) ($p = 0.002$). Also, it was found that tomato pomace with amino acids (G3) and tomato pomace with enzymes (G4)

were significantly decreased urea levels in comparison to the other groups (p = 0.02). Serum ALT was significantly decreased in the control group (G1), TP with amino acids (G3), TP with enzymes (G4) and TP with amino acids and enzymes (G5) compared to tomato pomace group (G2) (p = 0.04). it was indicated that supplementation of enzymes, amino acids or both to tomato pomace diet was non significantly differed in the levels of glucose, AST, triglycerides and cholesterol compared to control and tomato pomace groups G1 and G2 ($p > 0.05$).

Antioxidant status

The effect of tomato pomace with or without enzymes and / or amino acids on antioxidant enzymes in tilapia fish was highlighted in Table (7).

The levels of superoxide-dismutase (SOD) and GPX was significantly increased in tomato pomace groups supplemented with amino acids, enzymes and both of them (G3), (G4) and (G5) compared with control group (G1) and tomato pomace (G2), (p=0.02) and (p= 0.01) respectively.

It was also observed that TP with amino acids (G3), TP with enzymes (G4) and TP with amino acids and enzymes (G5) was significantly decreased malondialdehyde (MDA) levels compared with G1 and G2, ($p< 0.0001$). on the other side catalase levels in all groups showed no significance (p = 0.12).

Immune parameters

Figure (1) presented the impact of TP with enzymes and /or AAs on immune indices in Nile tilapia fish.

It was found that, there was no significant difference in the phagocytic activity and phagocytic index in groups supplemented with TP with enzymes, TP with amino acids and TP with both compared to other groups (G1) and (G2) (p = 0.24) and (p = 0.41). while the lysozyme activity showed a significant difference in TPA (G3) and TPE (G4) compared to TP (G2) ($p< 0.0001$).

Gene expression analysis

Figure (2) showed the impact of tomato pomace, with or without enzymes and/or amino acids, on the mRNA expression of Nile tilapia genes associated to antioxidants and immunity.

Expression of superoxide dismutase and catalase enzyme

The results of antioxidant related genes indicated that supplementation of enzymes or amino acids to tomato pomace diet in tilapia fish enhanced the expression level of SOD in TPA, TPE and TPAE compared to TP group (p = 0.01). otherwise, the expression level of catalase showed in significant difference between all groups G1, G2, G3, G4 and G5 (p = 0.48).

Expression of interleukin- 12

The results revealed that supplementation of enzymes to tomato pomace (G4) was significantly decreased the expression level of interleukin-12 compared to control group (G1), TP (G2), TPA (G3) and TPAE (G5), (p = 0.01).

Discussion

Tomato pomace, which is essentially composed of leather, seeds, and the hard tissues of the whole tomato, is an inexpensive byproduct of making tomato paste. Because it contains vital amino acids, fatty acids, and minerals, it has a high nutritional value and may be used as animal feed [8]. Tomato waste, primarily the peel, has a high level of carotenoids [34, 35]. Lycopene, which accounts for 80–90% of all carotenoids, is the most common carotenoid found in tomatoes. Among the dietary carotenoids, it is one of the strongest antioxidants [36]. Hematological indices, such as hemoglobin (Hb) value, hematocrit (Hct), red blood cell (RBC), and white blood cell (WBC) count, are thought to be useful indicators for determining fish health and feed efficiency [37, 38]. our findings presented no significant differences between G1 (control), G2 (TP), G3 (TPA), G4 (TPE) and G5 (TPAE) in all blood parameters and these findings are in agreement with [39] who indicated that common carp supplemented with varying amounts of tomato pomace showed no variations in blood parameters.

Fish health and physiological status can be understood with the use of serum biochemical markers. Fish, for example, utilize glucose as their main energy source and as an indication of physiological stress. Furthermore, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity levels are important markers for the diagnosis of liver integrity and digestive function [40]. The current study found that glucose and AST levels in dietary tomato pomace with or without enzymes or amino acids had no significance with the control group. These findings are consistent with those reported by [41], that tilapia (*Oreochromis niloticus*) exposed to cadmium and provided an experimental meal supplemented with 9 mg of lycopene/kg food showed a significant decrease in blood glucose, AST, and ALT levels. The main component of tomato pomace, lycopene, has been shown to have strong antioxidant effects and to enhance liver function in laying hens [42, 43]. Lycopene benefited liver and kidney and reverse the negative effects of aflatoxin in Peckin duckling [44]. Fish health can be determined by following changes in triglycerides and cholesterol, which can provide insight into the fish's lipid metabolism [45]. Too much focus has been given on how dietary fiber

affects blood cholesterol and lipid metabolism [46-48]. In this experiment, we found that serum triglycerides and cholesterol content was not differed between control group and TP groups and that finding are on the same context with [49] who reported that addition of DTP up to 15% in the diet of broiler lowered serum cholesterol content, and this is in contrast with [50] who indicated that there is a detrimental relationship between broiler fat and fiber digestibility.

Antioxidants are essential for the body and the food chain because they counteract oxidative damage compounds in animal tissues by reducing oxidative processes and the harmful effects of reactive oxygen species (ROS). They also effectively inhibit the initiation or propagation of oxidative chain reactions, which delays or inhibits the oxidation of lipids or other molecules [51-53]. The presence of antioxidant compounds such lycopene, α-tocopherol, vitamins, carotene, folate, and phenolic acids in tomato pomace could perhaps account for the observed increase in antioxidant effects [54-56]. In this study, there was a significant increase in the activity of SOD and GPX and a decrease in the level of MDA in tomato pomace groups with enzymes and amino acids as compared with tomato pomace only. The reason may be presence of exogenous enzymes which may enhance the nutrient digestibility and efficiency of feed and these findings agreed with [57] who found that adding 15% tomato powder to layers diet had no significant on blood GPx activity, MDA, SOD, or antioxidant capacity**.**

There was no data performed on the impact of TP with enzymes on phagocytic and lysozyme activity in Nile tilapia fish. An enzymatic indicator of non-specific immunity in fish is lysozyme activity [58]. One of the primary mediators of innate immunity in fish against pathogens such bacteria, viruses, and parasites is phagocytosis [59]. Phenolic chemicals (flavonoids, phenolic acids), as well as carotenoids, in tomato pomace have have anticancer, antibacterial, antimutagenic, immune-modulatory properties and anti-inflammatory properties. These properties may contribute to an increase in immunity by changing multiple cellular signal transduction pathways and triggering endogenous defensive activities [60, 61]. The current study showed an increase in the lysozyme activity in G3 and G4. But phagocytic activity was not significantly differed between all groups.

There are no research studies performed on the impact of TP with or without enzymes and /or amino acids on the genes related to antioxidant and immune response in Nile tilapia fish. Tomato pomace polyphenols molecularly activate the signal transduction pathway by promoting Keap-1 dissociation from Nrf2, which happens in response to stressors and is linked to the suppression of lipid peroxidation and the elevation of antioxidant enzyme activity [62]. It was discovered that lycopene boosts nuclear Nrf2 expression, which raises the synthesis of antioxidant enzymes [63]. In this experiment the expression level of catalase in tomato groups and the control group showed no difference while in SOD there was a difference in TPA, TPE and TPAE.

One of the key cytokines in the control of inflammation is IL-12, which mainly acts through T cells to promote proinflammatory and pathogenicity [64, 65]. The phenolic compounds in the tomato pomace which have anti-inflammatory properties enhance immunity [60]. It was observed that expression level of IL-12 was significantly differed in G3 and G4 compared to other groups.

Conclusion

Based on the mentioned above, tomato pomace with enzymes and / or amino acids could be included at a level 15% in the diet of Nile tilapia fish with improving antioxidant, immune and health status of fish.

Acknowledgment

To everyone who helped with this study, we would like to thank you

Conflicted interest

There aren't any disclosed conflicts of interest.

Funding statement

Self-sufficient

Author's contributions

Each author contributed something to this work.

TABLE 1. Chemical composition of tomato pomace on dry matter basis.

Nutrient	%
Dry matter	93.0
Crude protein	17.64
Crude fat	8.07
Crude ash	9.5
NDF	47.4
ADF	42.7
Lignin	18.8

TABLE 2. Composition of ingredients and nutrients in Nile tilapia fish

Feedstuff	G1	G2	G3	G4	G5
Corn gluten meal %	10	10	10	10	10
Soybean meal (48) %	35.32	32.19	32.19	32.19	32.19
Corn grains, yellow %	27.28	18.33	18.1	18.33	18.1
Rice polishing %	10	8	8	8	8
Wheat middling %	12	12	12	12	12
Tomato pomace %	¬	15	15	15	15
Soy oil %	2.5	2	2	2	2
Enzyme mixtures[1] %	_	-	-	0.2	0.2
Premix[2] %	0.2	0.2	0.2	0.2	0.2
Lysine Hcl %	0.04	-	0.04	-	0.04
DL- methionine %	0.22	0.04	0.23	0.04	0.23
Mono calcium phosphate %	1.06	1.09	1.09	1.09	1.09
Limestone %	0.88	0.65	0.65	0.65	0.65
CMC binder[3]%	0.5	0.5	0.5	0.5	0.5
Crude protein %	28.05%	28.06%	28.06%	28.06%	28.06%
Starch%	27.04%	21.05	20.85	21.05%	20.85%
Lipid%	6.25%	6.76	6.76%	6.76%	6.76%
DE (kcal/kg diet)	3033	2957	2957	2957	2957
Lysine %	1.43	1.43	1.79	1.43	1.79
Methionine %	0.75	0.75	0.94%	0.75	0.94
Calcium %	0.7	0.7	0.7	0.7	0.7
Available phosphorus %	0.45	0.45	0.45	0.45	0.45

[1]Gallizyme composed of protease (400000 U/kg), alpha-Galactosidase (10000 U/kg), beta-Glucosidase (10000 U/kg), pectinase (30000 U/kg), arabinase (7000 U/kg), phytase (500000 U/kg), lipase (10000 U/kg), and wheat bran up to 1 kg.
[2]premix; contained 1000 mg of copper, 1000 mg of iodine, 100 mg of selenium, 1 mg of cobalt, 100,000 mg of iron, 10,000 mg of manganese, 30,000 mg of zinc, 200,000 IU of vitamin A, 10,000 mg of vitamin E, and 2000 IU of vitamin D3.
1000 mg folic acid, 1000 mg B1 and B2; 4000 mg B6 and 4 mg B12; 20,000 mg niacin; 20 mg biotin; 10,000 mg pantothenic acid; calcium carbonates up to 1000 gm per kilogram
G1 (Control), G2 (Tomato pomace TP), G3 (Tomato pomace with amino acids TPA), G4 (Tomato pomace with enzymes TPE), G5 (Tomato pomace with amino acids and enzymes TPAE)
[3]CMC binder; (carboxy methyl cellulose)

TABLE 3. primers used for PCR analysis

Primers	(5'-3')	Accession number
CAT	F-CCCAGCTCTTCATCCAGAAAC	JF801726.1
	R-GCCTCCGCATTGTACTTCTT	
SOD	F-CCCTACGTCAGTGCAGAGAT	JF801727.1
	R-GTCACGTCTCCCTTTGCAAG	
IL-12	F-GGGTGCGAGTCAGCTATGAG	XM_003437924.4
	R-GGTTGTGGATTGGTTGCGTC	
β-actin	F: CCACACAGTGCCCATCTACGA	EU887951.1
	R: CCACGCTCTGTCAGGATCTTCA	

Cat (catalase), SOD (superoxide dismutase), IL-12 (interlukin-12)

TABLE 4. Effect of tomato pomace with or without enzymes and or amino acids on RBCS and Hb in Nile tilapia fish

	G1	G2	G3	G4	G5	P value
RBCS (10^6/mm^3)	2.11±0.14[a]	3.36± 0.66[a]	3.34±0.81[a]	3.43±0.8[a]	3.31±0.44[a]	0.55
Hb (g/100ml)	6.31±0.41[a]	10.24±2.06[a]	10.16±2.54[a]	10.31±2.18[a]	8.9±1.58[a]	0.50
PCV (%)	19.67±1.45[a]	33±6.66[a]	32±8.33[a]	33±7.51[a]	29±5.57[a]	0.56
MCV	93.25±0.93[a]	98.07±0.46[a]	95.1±1.94[a]	96.57±0.59[a]	97.52±0.86[a]	0.06
MCH	29.96±0.1[a]	30.43±0.12[a]	30.35±0.46[a]	30.37±0.6[a]	30.09±0.48[a]	0.90
MCHC	32.13±0.42[a]	31.03±0.03[a]	31.92±0.31[a]	31.45±0.46[a]	30.86±0.42[a]	0.13

G1 (Control), G2 (Tomato pomace TP), G3 (Tomato pomace with amino acids TPA), G4 (Tomato pomace with enzymes TPE), G5 (Tomato pomace with amino acids and enzymes TPAE)

TABLE 5. Effect of tomato pomace with or without enzymes and or amino acids on WBCS parameters in Nile tilapia fish

	G1	G2	G3	G4	G5	P value
WBCS (10^3/mm3)	6.14±0.78 [a]	9.93±0.59 [a]	11.28±0.97 [a]	9.04±1.09 [a]	10.63±2.35 [a]	0.13
Heterophil %	13±1.15 [a]	10.67±1.2 [a]	10.67±0.33 [a]	10.67±1.2 [a]	11±2.08 [a]	0.67
Heterophil (10^3/mm3)	0.81±0.14 [a]	1.05±0.11 [a]	1.17±0.12 [a]	0.98±0.2 [a]	1.08±0.06 [a]	0.42
Lymphocyte %	79±1.53 [a]	81±1.53 [a]	80.33±0.88 [a]	80.33±1.76 [a]	80±3.06 [a]	0.96
Lymphocyte (10^3/mm3)	4.84±0.56 [a]	8.06±0.62 [a]	9.18±0.85 [a]	7.24±0.79 [a]	8.6±2.07 [a]	0.13
Monocyte %	6±1 [a]	7±1 [a]	7±1 [a]	7.33±0.88 [a]	8±1.15 [a]	0.73
Monocyte (10^3/mm3)	0.37±0.6 [a]	0.69±0.06 [a]	0.73±0.06 [a]	0.66±0.12 [a]	0.83±0.19 [a]	0.12
Eosinophil %	1.33±00.88 [a]	0.33±0.33 [a]	1±0 [a]	0.67±0.33 [a]	0.67±0.33 [a]	0.68
Eosinophil (10^3/mm3)	0.09±0.07 [a]	0.03±0.03 [a]	0.18±0.05 [a]	0.07±0.03 [a]	0.07±0.04 [a]	0.62
Basophil %	0.67±0.33 [a]	1±0 [a]	0.67±0.33 [a]	1±0.58 [a]	0.33±0.33 [a]	0.68
Basophil (10^3/mm3)	0.04±0.02 [a]	0.1±0.01 [a]	0.1±0.06 [a]	0.09±0.06 [a]	0.05±0.05 [a]	0.82

G1 (Control), G2 (Tomato pomace TP), G3 (Tomato pomace with amino acids TPA), G4 (Tomato pomace with enzymes TPE), G5 (Tomato pomace with amino acids and enzymes TPAE)

TABLE 6. Effect of tomato pomace with or without enzymes and or amino acids on serum biochemical indices in Nile tilapia fish

	G1	G2	G3	G4	G5	P value
Creatinine (mg/dL)	1.9±0.17[ab]	2.19±0.2[a]	1.3±0.21[bc]	1.09±0.07[c]	1.14±0.04[c]	0.002
Urea (mg/dL)	1.99±0.08 [ab]	2.4±0.37 [a]	1.41±0.25[b]	1.35±0.06 [b]	1.58±0.06 [ab]	0.02
ALT (U/L)	21.57±0.95 [ab]	23.34±3.43[a]	18.53±1.49 [ab]	17.08±2.17 [ab]	12.91±1.28 [b]	0.04
AST (U/L)	22.75±1.33 [a]	21.41±1.1 [a]	18.91±1.56 [a]	17.93±2.62 [a]	19.15±0.81 [a]	0.27
Glucose (mg/dL)	9.87±0.7 [a]	10.93±1.36 [a]	11.41±0.93 [a]	10.84±0.78 [a]	10.68±0.52 [a]	0.82
Triglycerides (mg/dL)	95.88±2.77 [a]	93.45±3.90 [a]	82.7±3.47[a]	93.25±4.45 [a]	92.73±1.96 [a]	0.6
Cholesterol (mg/DL)	97.39±5.42 [a]	98.13±1.92 [a]	91.33±4.89 [a]	96.1±3.05 [a]	90.41±4.71 [a]	0.60

G1 (Control), G2 (Tomato pomace TP), G3 (Tomato pomace with amino acids TPA), G4 (Tomato pomace with enzymes TPE), G5 (Tomato pomace with amino acids and enzymes TPAE)
ALT (alanine aminotransferase), AST (aspartate aminotransferase)

TABLE 7. Effect of tomato pomace with or without enzymes and or amino acids on antioxidant status in Nile tilapia fish

	G1	G2	G3	G4	G5	P value
SOD (U/g)	20.616±0.3[b]	24.764±0.99 [ab]	28.998±1.44 [a]	25.852±1.71 [ab]	25.89±2.56 [ab]	0.02
GPX (U/g)	7.472±0.62 [b]	7.16±0.68 [b]	9.114±0.47 [ab]	10.42±0.84 [a]	9.538±0.34 [ab]	0.01
MDA (nmol/g)	28.942±0.82 [a]	28.66±1.07 [a]	17.924±2.23 [b]	18.628±1.51 [b]	18.12±1.68 [b]	<0.0001
Catalase (U/g)	10.29±0.25[a]	10.516±1.16 [a]	10.604±0.28 [a]	12.694±0.77 [a]	10.828±0.44 [a]	0.12

G1 (Control), G2 (Tomato pomace TP), G3 (Tomato pomace with amino acids TPA), G4 (Tomato pomace with enzymes TPE), G5 (Tomato pomace with amino acids and enzymes TPAE)
SOD (superoxide dismutase), GPX (glutathione peroxidase), MDA (malondialdehyde)

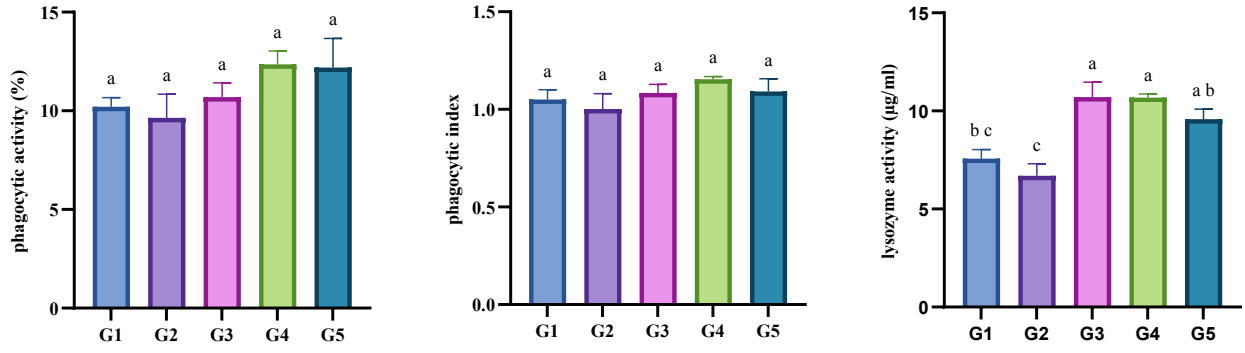

Fig. 1. Effect of tomato pomace with or without enzymes and or amino acids on immune indices in Nile tilapia fish

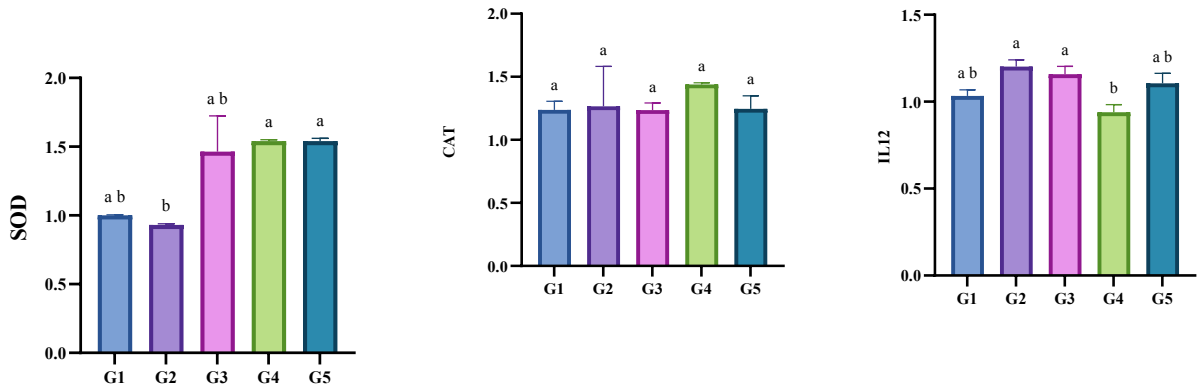

Fig. 2. Effect of tomato pomace with or without enzymes and or amino acids on expression levels of SOD, Cat and IL-12 in Nile tilapia fish

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تأثير ثفل الطماطم مع أو بدون الإنزيمات و/أو الأحماض الأمينية على مؤشرات الدم والكيمياء الحيوية في الدم ومضادات الأكسدة والحالة المناعية والتعبير عن الجينات المضادة للأكسدة والمناعية في أسماك البلطي النيلي (Oreochromis nilotius)

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الملخص

أوضحت هذه الدراسة أهمية تضمين ثفل الطماطم مع أو بدون إنزيمات و/أو أحماض أمينية في علیقة اسماك البلطي النيلي من حيث مؤشرات الدم والكيمياء الحيوية في الدم ومضادات الأكسدة والحالة المناعية والتعبير عن الجينات المضادة للأكسدة والمناعية في الكبد لمدة ثمانية أسابيع. تمت صياغة خمس مجموعات من النظام الغذائي بمحتوى بروتين مماثل28) ٪ :((G1)علیقة ظابطة بدون ثفل الطماطم(TP) ، TP (G2)مع معدل تضمين 15٪، 15 TP (G3)٪ مع ليسين (1.79٪) والميثيونين(0.94%) ، 15% TP (G4)مع الجاليزيم (0.2%) و 15% TP (G5) مع اللايسين والميثيونين والجاليزيم. أظهرت نتائج فحص عدد خلايا الدم الحمراء (RBCs) وعدد خلايا الدم البيضاء (WBCs) وقيمة الهيموجلوبين والهيماتوكريت اختلافاً غير معنوي في جميع المجموعات .(P>0.05) بينما انخفضت مستويات الكرياتينين واليوريا وناقلة أمين الألانين (ALT) والدهون الثلاثية بشكل ملحوظ في G3 و G4و G5مقارنة بـ .(P<0.05) G2 كان هناك اختلاف كبير في مستويات ديسموتاز فائق الأكسيد (SOD) والجلوتاثيون بيروكسيديز (GPX) والمالونديالدهيد (MDA) في المجموعات الثلاث الأخيرة مقارنة بـ G1 وG2 (P=0.02)، (P <0.0001) .(P=0.01)على التوالي. ومع ذلك، لم يختلف نشاط البلعمة ومؤشر البلعمة بشكل كبير في المجموعات الخمس، ولكن نشاط الليزوزيم أظهر قيمة أعلى في G3 و G4مقارنة بـ P) G2 .(0.05>اختلفت مستويات التعبير النسبي لـ SOD و (IL-12) interleukin-12في كبد البلطي بشكل كبير (P <0.05) في المجموعات الثلاث الأخيرة، في حين لم تظهر مستويات التعبير عن الكاتالاز أي اختلاف كبير في جميع المجموعات. وأخيرًا، فإن إدراج TP الغذائي مع الإنزيمات و/أو الأحماض الأمينية يمكن أن يعزز حالة مضادات الأكسدة والعوامل البيوكيميائية والمناعية في أسماك البلطي النيلي.

الكلمات المفتاحية : جاليزيم، SOD، MDA، الكرياتينين، الكوليسترول، IL12 ، TP.