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Tomato Pomace with Exogenous Enzymes and/or Amino Acids Enhanced Growth Performance, Histological Parameters and Gene Expression for Growth in Nile tilapia Fish (*Oreochromis nilotius*)

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Abstract

THIS study evaluated the impact of feeding tomato pomace with or without enzymes and amino acids to Nile tilapia for 8 weeks on growth performance, digestive enzymes, histological parameters and some liver gene expression. Five experimental diets were formulated with similar protein content (28%): (1) a control diet without tomato pomace (TP), (2) TP 15%, (3) TP 15% with lysine (1.79%) and methionine (0.94%), (4) TP 15% with gallizyme (0.2%) and (5) TP 15% with lysine, methionine and gallizyme. The results revealed significant improvements in growth performance, especially body gain by 31.05% and 33.03% and FCR by 21.83% and 23.35% in the 4th and 5th groups, respectively, as compared to the 2nd group. Enzymes alone or enzymes with amino acids significantly increased Villi length and goblet cells number in the intestine of tilapia. There was no significant difference in the activities of lipase and amylase and levels of total protein, albumin and globulin in all groups. The relative expression levels of IGF-1 and GH in the liver of tilapia were significantly increased *($p<0.05$)* in the last three groups. Finally, addition of enzymes with or without amino acids to the Nile tilapia diets improved the growth performance parameters and gene expression.

Keywords: TP, gallizyme, lysine, methionine, GH, IGF-1.

Introduction

For a very long time, the aquaculture industry has provided humans with delicious and safe animal protein [1]. However, more seafood protein is needed due to the ongoing increase in human consumption [2]. Since feed accounts for more than 60% of production costs, the aqua-feed business is essential to the growth of aquaculture [3]. High-quality protein sources are used by the aqua-feed industry to give aquatic animals the energy and protein they need for physiological and metabolic processes [4, 5]. Traditionally, nutritionally balanced feed formulations have been made using fish meal (FM) and plant components (such as soybean meal, maize gluten, wheat middling, sunflower meal, etc.) [6]. Thus, in order to lower feed costs, it is essential to find a new supplier of unconventional feed ingredients [7-13]. Therefore, it is necessary for fish farmers worldwide to investigate the use of alternative, readily available, and reasonably priced plant-based feed ingredients. [14].

One of the more promising substitute sources of protein and energy for the monogastric animal feed business is tomato pomace. [15, 16]. Tomato pomace is a material consisting of the skin, pulp, and broken seeds that are left over after tomatoes are processed to make juice, paste, or ketchup. Protein (17–22%), fat (10–12%), crude fiber (33-57%), NDF

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(50–72%), which is mostly made up of ADF (39–60%), and lignin concentration (20–30%) are all relatively high in tomato pomace [17]. Lycopene levels in tomato pomace, particularly the skin, are notable about five times higher than in pulp (on a moist basis) [18, 19]. Tomato pomace is a rich source of many bioactive substances, including tocopherols, polyphenols, terpenes, and sterols, in addition to carotenoids [20].

Non-starch polysaccharides (NSP) are challenging to digest and have a range of antinutritive effects because of modifications to the gut's morphology, physiology, digesta viscosity, and microbial composition [21]. Recirculating aquaculture systems (RAS) have a higher system waste load due to lower nutrient use, which also causes eutrophication when waste is dumped into nearby water bodies [22]. By adding NSP-degrading enzymes (such as xylanase, β-glucanase, and cellulase) and phytase to diets, it is possible to partially offset the detrimental effects of NSP and phytate on digestion, which in turn enhances growth of fish[23, 24]. The digestion and absorption of feed nutrients as well as the development performance of aquatic animals are markedly inhibited by dietary concentrations of NSPs above 8% [25, 26].

Thus, the purpose of this study was to determine how tomato pomace supplemented with exogenous enzymes and/or amino acids affected growth performance, digestive enzymes, histological parameters, and the expression of genes related to growth.

Material and Methods

Tomato Pomace (TP)

We obtained tomato pomace (TP) from a nearby supplier and let it dry in the sun for 4 hours per day for five days. After drying, it was ground and analyzed for chemical composition before diet preparation as shown in Table (1). Tomato pomace used in this study by inclusion rate of 15% according to previous studies.

Exogenous enzymes

Exogenous enzymes used in the experiment is Gallizyme, it was purchased from (Tex bioscience company, India). The composition of these enzymes is amylase (1000000 U/kg), cellulase (100000000 U/kg), xylanase (1500000 U/kg), phytase (500000 U/kg), lipase (10000 U/kg), pectinase (30000 U/kg), protease (400000 U/kg), alpha-Galactosidase (10000 U/kg), beta- Glucosidase ((10000 U/kg), arabinase (7000 U/kg), and wheat bran up to 1 kg. Enzyme mixtures were added in this study by 0.2% according to [27]

Fish and experimental diets

A 225 monosexual Nile tilapia fingerlings was used in this study. Prior to the beginning of the trial, the fish were housed in glass tanks for two weeks for acclimation. The experimental fish with initial weight (21.2±.08g/fish) were purchased from a private farm. Fish basal diet was developed in accordance with NRC (2011) [28], presented in Table (2). Fish were randomly distributed into 5 groups with 45 fish per aquarium with triplicate of 15 fish per replicate. G1 a control diet without TP, G2 a TP 15% (TP), G3 TP 15% with lysine (1.79%) and methionine (0.94%) supplementation (TPA), G4 TP 15% with exogenous enzymes (0.2% of the diet) (TPE) and G5 TP 15% with lysine (1.79%), methionine (0.94%) and (0.2%) exogenous enzymes (TPAE). After the trial started and for the next eight weeks, fish in each group were weighed every two weeks. Fish were fed 2 times per day (8 am and 4 pm) to apparent satiation. Every two weeks, body weight gain (BWG) was recorded. Then, the total BWG was determined. In a similar manner, FCR was calculated. After that, the total FCR was determined.

The following formulae were used to compute growth indicators:

weight gain (WG, g) = Final body weight (FBW, g) – Initial body weight (IBW, g).

Feed intake (FI, g fish–1) = Consumed feed of each pond / No. of fish.

Feed conversion ratio (FCR) = Feed given (g)/Weight gain (g).

Fish wastes and excreta were removed by siphoning and every day, nearly half of each tank's water level was refilled with dechlorinated fresh water. Temperature between (24°C–27°C), dissolved oxygen 6.5 ± 0.5mg l-1, pH 7.1 ± 0.8, EC 219 ± 2 μ mho/cm and ammonia adjusted to the typical acceptable limits (<0.1 mg total ammonia) were observed during the study. Water quality assessments were conducted in accordance with *Rice, E.W* [29].

Sampling

Blood samples were obtained from five fish per group after finishing the experiment, and caudal vein was used to collect the blood.[30]

Biochemical parameters

Colorimetric analysis was used to determine the total proteins in serum (Spectrum: Egyptian Biotechnology Company, Egypt) at a wavelength of 546 nm [31]. At 630 nm in wavelength, albumins (Bio-diagnostic co. Egypt) were measured colorimetrically [32]. By mathematically deducting albumin from total protein, the globulin content was determined.

Digestive enzymes

The colorimetric determination of lipase (Spectrum, Egyptian firm for Biotechnology, Egypt) carried out at a wave length of 580 nm [33]. At a wave length of 660 nm, amylase (Bio-diagnostic co. Egypt) was measured colorimetrically [34].

Intestine histological examination

After eight weeks, the samples (five fish per replicate) from each group were taken. Freshly deceased fish were used to obtain intestinal tissue samples, which were then kept for at least 24 hours in 10% neutral buffered formalin. The fixed tissue samples were embedded using the conventional paraffin embedding technique. Hematoxylin and eosin (H&E) was then used to cut and stain 5-µm-thick paraffin blocks for histopathological examination [35].

Gene expression analysis

The liver tissue was subjected to RNA extraction using the Qiagen RNeasy plus Mini kit. At 260 and 260/280 nm ratios, the Spectrophotometer Nano Drop ND-1000(Nano Drop Technologies, Wilmington, Delaware, USA) was used to determine the concentration and purity of each RNA sample. In order to create cDNA, Reverse transcription of the RNA samples was performed with a High-Capacity RNA-to-cDNA Master Mix kit.

PCR primers special to each gene and Thermo Scientific Maxima ® SYBR Green/ROXqPCR Master Mix (2×) by Rotor-Gene Q (Qiagen, USA) were used for the amplification process. As indicated in Table (3), G were created using Primer Express 3.0 (Applied Biosystems, USA) and blasted on NCBI/Blast to verify necessary gene's specificity. We analyzed the PCR products' melting curves to show the identity and specificity of each gene. The resulting Threshold Cycle (Ct) value allowed for the determination of the $2^{-\Delta\Delta Ct}$ of the various genes' mRNA expression.

Ethics statement

The Medical Research Committee of the Faculty of Veterinary Medicine at Kafrelsheikh University in Egypt provided an approval for this study based on ethical guidelines (The approval number is KFS-IACUC/123/2023).

Statistical analysis

The acquired data were statistically analyzed using One-way analysis of variance (ANOVA).

For intergroup comparisons, Tukey's test was applied at ($p \leq 0.05$).

Results

Growth performance

Table (4) showed the impact of tomato pomace, with or without enzymes and/or amino acids, on Nile tilapia performance. When comparing G4 (TPE) and G5 (TPAE) to G1 (Control), G2 (TP), and G3 (TPA), the final body weight and body weight gain are significantly higher ($P<0.05$). Between G4 and G5, there was no significance ($P = 0.9$). In G1 and G2, A significant variation in total feed intake ($P=0.03$) which presented in Table (4).

The difference between groups in FCR is presented Table (4). In G4 and G5 the results of total FCR are decreased than other groups ($p<0.002$).

Digestive enzymes

Table (5) presented the impact of tomato pomace with or without enzymes and /or amino acids on digestive enzymes of Nile tilapia. There is no significant difference in the activity of amylase enzymes in all groups except in G3 and G4 ($P=0.02$)

lipase enzymes activity in all groups showed no notable variation ($P=0.98$)

Serum biochemical parameters

Table (6) presented the impact of TP with or without enzymes and /or amino acids on the levels of total protein, albumin and globulin in Nile tilapia.

Serum total protein levels in the five groups showed no significant difference ($p=0.69$).

Also, the levels of serum albumin in the different groups showed no difference ($p=0.7$).

There is no significance between groups ($p=0.58$) in serum globulin levels

Histological parameters

The effects of tomato pomace combined with enzymes and/or amino acids on the morphology of the various intestinal sections in Nile tilapia are shown in Table (7).

It was observed that G5 had significantly more villi length than the G2 in the anterior, middle, and posterior sections of the intestine ($p<0.0001$) Additionally, G5 had a considerably higher number of goblet cells in the anterior and posterior regions than G2 did ($p<0.0001$).

Growth - related genes expression

Figure (1) showed the impact of tomato pomace, with or without enzymes and/or amino acids, on the mRNA expression of Nile tilapia genes associated to growth

Growth hormone gene expression

GH mRNA levels were substantially higher in G5 than in G2 ($p<0.0001$). Additionally, G1, G3, and G4 showed a significant difference ($p<0.01$). There was no discernible difference between G3 and G4 ($P= 0.06$).

Expression of IGF1 gene

IGF1 mRNA levels in G5 were considerably higher than in the other groups ($p<0.0001$). Between G1 and G2, there was no significant difference ($P=$

0.17). Moreover, no obvious difference (*P*= 0.08) in G3 and G4. Otherwise, G3 and G4 had significantly higher *IGF1 mRNA* levels than G1 and G2 (*P*<0.05).

Discussion

Tomato pomace is one of the very healthy by-products that can be utilized as a natural source of lipids (5–10%), protein (10–20%), and fibers (60–70%). Additionally, TP has anti-oxidant properties due to the bioactive substances like ß-carotene and lycopene that are present in it [36]. But, the high fiber diet in monogastric animals can have adverse effect on the performance and health [37, 38]. According to published research, exogenous enzymes in Nile tilapia aquafeed reduce the negative effects of NSPs [24, 39]. As a result, these exogenous enzymes improve the digestion of nutrients, especially crude protein and crude lipids, in fish diets as demonstrated by *Dalsgaard* [40]. The present study revealed an elevation in the BWG and FBW and a decrease in FCR in the groups that contain tomato pomace with exogenous enzymes and amino acids (G4 and G5) as compared with tomato pomace 15% group with no significant difference in the feed intake and these findings are similar to *Adeoye*,[41-43] who highlighted the improved final body weight, specific growth rate, feed conversion ratio, and protein efficiency ratio of tilapia fed a diet supplemented with enzymes. Additionally, numerous studies have demonstrated that exogenous enzymes greatly increase fish development and decrease feed coefficient [44-46]. It was observed that tilapia's growth performance may be enhanced and its protein digestibility and digestive enzyme activity considerably increased when fed diets with multienzyme Natuzyme50® (Bioproton) containing protease, phytase, xylanase, β-glucanase, α-amylase, and cellulase. But, These findings conflict with those of *Amirkolaie,* [47] who found that adding 10% tomato pomace considerably raised the common carp's final weight and specific growth rate. These findings differ from those of *Kavitha* [48] who showed that broiler performance increased when TP was supplemented with enzymes, while nitrogen utilization declined as tomato pomace levels increased. Additionally, Sahin [49] found that adding tomato powder at 2.5 and 5% inclusion rates increased the Japanese quail's growth and feed conversion ratio. *Rahmatnejad* [50] demonstrated that dried TP may be added to broiler chicken diets up to 8% without having a negative impact on performance. supplementation of enzymes with dried TP enhances body weight and feed utilization. The variation could be caused by how tomato pomace is composed, which varies depending on farming and processing methods as well as how much moisture is removed and dried [51], which affect in the % of skin ,pulp and seeds in the tomato and so influences on the available energy of diet with high fiber content as clarified by Squires [37].

Fish's ability to assimilate nutrients is significantly influenced by digestive enzymes [52]. The amounts of the enzymes lipase and amylase did not significantly differ between the groups.The cause may be inclusion of amino acids only or with exogenous enzymes with TP increase the digestive enzymes and these findings are similar with *Liaqat* [53] who clarified that supplementation of amino acids in stripped catfish increase digestive enzymatic activity and *Maas* [39] who claimed that supplementing tilapia diet with phytase and xylanase increased the activity of digestive enzymes.

Our results revealed no significant difference in total protein, albumin and globulin between groups and these outcomes matched with *Elazab,[54, 55]* who found that adding 15% dried tomato pomace to growing rabbit diets raised the amounts of total protein, albumin, and globulins. Fish digestion and absorption is affected by intestinal villi length and goblet cells number [56]. they also affect the absorption area capacity so they considered a good healthy intestine indicators [57]. Goblet cells produce mucous in the different parts of intestine to make a protection of the intestinal mucosal layer [58] . The current study's findings showed that some intestinal villi parts were noticeably longer and had more goblet cells in G5 than in G2 and these results were similar to *Kim,* [59] who reported that comparing dietary supplementary phytase and basal diets, the exogenous multi-enzyme supplement that was added to the diet significantly improved the villus height, crypt depth, and the ratio of villus height to crypt depth.

Mun [60] stated that *GH* and *IGF1* play important function in the growth and health of fish . Few research has been done on how tomato pomace affects the genes linked to growth in Nile tilapia. There are few studies demonstrated impact of TP in tilapia growth- related genes. Our findings present\ed that addition of enzymes and amino acids to TP in tilapia diet has significantly upregulated the mRNA expression of *GH* and *IGF1* and these findings are similar to that of *Hassaan,* [61, 62]who mentioned that tilapia's levels of *IGF1* were raised by adding probiotics and/or exogenous enzymes (protease) to their plant protein diet. *A El-ashry,* [63] indicated that dietary addition of xylanase to plant based diet in tilapia increasing the *GH* levels digestive enzymes and growth performance. *Del Vesco,* [64] shown that adding DL-methionine to tilapia diet raised the amounts of *GH* and *IGF1* in the fish's liver, enhancing performance by promoting the synthesis and release of growth factors and these findings are similar to our results which levels of *GH* and *IGF1* increased in G3, G4 and G5. We can use tomato pomace in fish diet by 15% to improve performance as mentioned by *Abedalhammed* [65]. It was found that including tomato pomace in tilapia diet is lower in cost (9 LE/ kg) than SBM (27 LE

/kg). So, replacing part of SBM by TP in tilapia diet is economic. This work is the first to study inclusion of tomato pomace with lysine and methionine in tilapia fish. Also. Studies on growth related genes with tomato pomace in tilapia fish are very rare

Conclusion

Based on the aforementioned findings, it is possible to conclude that Nile tilapia could make better use of TP up to 15% of the diet when combined with enzymes and/or amino acids. However, adding TP alone to a tilapia's diet negatively impacts both health and performance

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Conflicted interest

There are no conflicts of interest to declare.

Author's contributions

Every author made a contribution to this work.

Funding statement

Self-supporting.

TABLE 1. Chemical composition of dried tomato pomace

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 feed ingredients and nutrients in Nile tilapia fish

Feedstuff	G1	G2	G3	G4	G5
Corn gluten meal	10%	10%	10%	10%	10%
Soybean meal	35.92%	32.29	32.29%	32.29%	32.29%
Corn grains	27.58%	18.81	18.81%	18.81%	18.81%
Rice polishing	10%	8	8%	8%	8%
Wheat middling	12%	12	12%	12%	12%
Tomato pomace	-	15	15	15%	15%
Enzyme mixtures	-	-	-	0.2%	0.2%
Premix[1]	0.2%	0.2	0.2%	0.2%	0.2%
Lysine Hcl	0.43%		0.46%	-	0.46%
Synthetic methionine	0.22%	0.09	0.28%	0.09%	0.28%
Mono calcium phosphate	1.32%	1.35	1.35%	1.35%	1.35%
Limestone	0.59%	0.6	0.6%	0.6%	0.6%
CMC binder	0.5	0.5	0.5	0.5	0.5
Crude protein %	28%	28%	28%	28%	28%
Starch%	27.04%	21.25	21.25	21.25%	21.25%
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 premix; Each kilogram contained 1000 mg of copper, 1 mg of cobalt, 1000 mg of iodine, 100 mg of selenium, 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
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 3. Primers used for real time PCR analysis

Primers	(5'-3')	Accession number
GH	F: GTTGTGTGTTTGGGCGTCTC R: CAGGTGCGTGACTCTGTTGA	HM565014.1
IGF-1	F: GTTTGTCTGTGGAGAGCGAGG R: GAAGCAGCACTC GTCCACG	Y10830.1
β-actin	F: CCACACAGTGCCCATCTACGA R: CCACGCTCTGTCAGGATCTTCA	EU887951.1

TABLE 4. Effect of tomato pomace with enzymes and /or amino acids supplementation on performance of Nile tilapia

	G1	G2	G3	G4	G5	P value
Initial BW (g/fish)	21.2±0.08[a]	21.2±0.08 [a]	21.2±0.08 [a]	21.2±0.08 [a]	21.2±0.08 [a]	0.43
Final BW (g/fish)	42±0.58[b]	41.5±0.58[b]	44.5±0.87[ab]	47.8±0.73[a]	48.2±0.72[a]	0.004
BWG(g/fish)	20.84±0.54[b]	20.26±0.6 [b]	23.3±0.83[ab]	26.56±0.74 [a]	26.96±0.96 [a]	0.002
Feed intake(g/fish)	41.24±0.32[a]	39.71±0.11[a]	40.17±0.14[ab]	40.65±0.38[ab]	40.62±0.51[ab]	0.04
FCR	1.98±0.06 [a]	1.96±0.06 [a]	1.76±0.12 [ab]	1.53±0.05[b]	1.51±0.05[b]	0.002

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 enzymes and /or amino acids supplementation on Digestive enzymes of Nile tilapia

	G1	G2	G3	G4	G5	P value
Amylase (U/ml)	41.93±2.37[ab]	38.74±1.64[ab]	32.13±2.59[b]	48.34±4.4[a]	39.25±3[ab]	0.04
Lipase (U/ml)	32.22±4.01[a]	34.76±3.17[a]	30.56±6.45[a]	32.60±3.78[a]	20.03±0.76[a]	0.98

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 enzymes and /or amino acids supplementation on total protein, albumin and globulin of Nile tilapia

	G1	G2	G3	G4	G5	P value
Total protein (g/dl)	4.59±0.16[a]	5.15±0.64[a]	5.16±0.29[a]	5.17±0.56[a]	5.48±0.28[a]	0.69
albumin (g/dl)	1.63±0.03	1.63±0.1	1.64±0.07	1.56±0.04	1.53±0.06	o.7
Globulin (g/dl)	2.96±0.17	3.51±0.5	3.51±0.54	3.6±0.24	3.94±0.31	0.58

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)

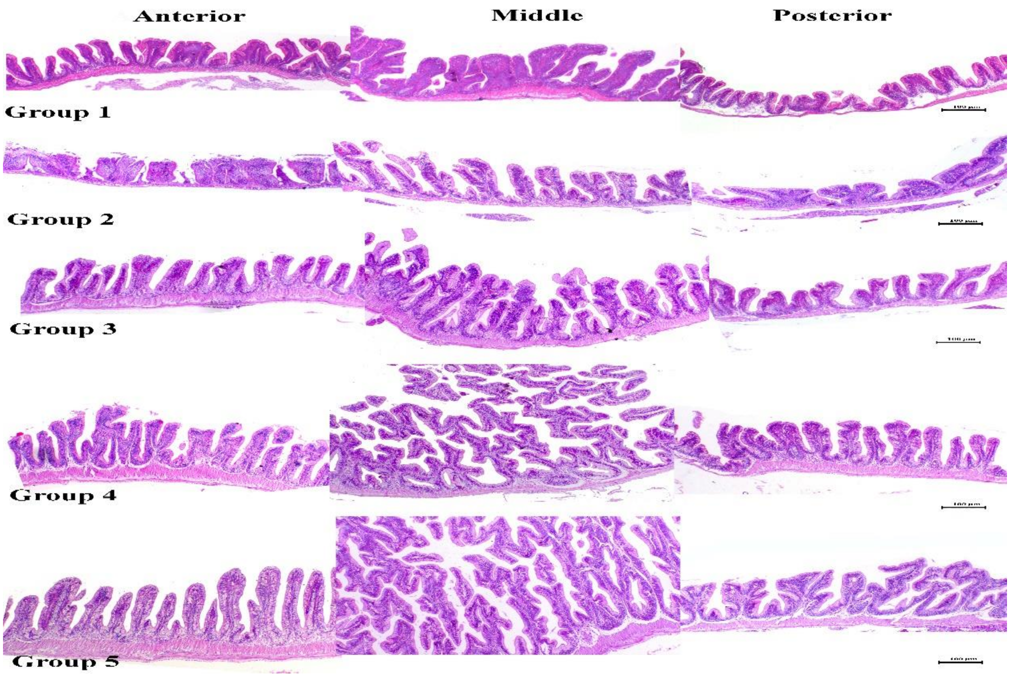

Fig.1. The effect of tomato pomace with enzymes and or amino acids in histological parameters in Nile tilapia

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)

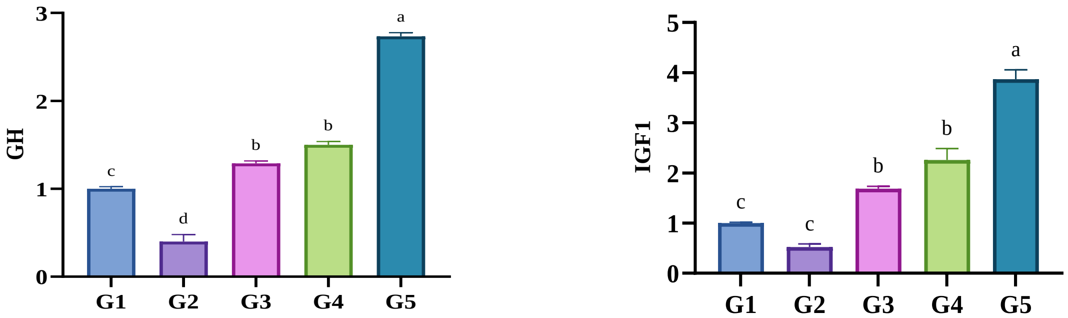

Fig.2. Effect of tomato pomace with enzymes and /or amino acids supplementation on GH and IGF1 of Nile tilapia
G1 (Control), G2 (Tomato pomace TP), G3 (Tomato pomace with amino acids TPA), G4 (Tomato pomace with enzymes TPE), G5 (Tomato pomace with amino acids and enzymes TPAE)

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ثفل الطماطم مع انزيمات خارجية المنشأ و/او أحماض أمينية تعزز أداء النمو، والمعايير النسيجية والتعبير الجيني للنمو في أسماك البلطي النيلي

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الملخص

قامت هذه الدراسة بتقييم تأثير تغذية ثفل الطماطم (TP15%) مع أو بدون إنزيمات وأحماض أمينية لأسماك البلطي النيلي لمدة 8 أسابيع على أداء النمو والإنزيمات الهاضمة والبارامترات النسيجية وبعض التعبيرات الجينية للكبد. تم إعداد خمس وجبات غذائية تجريبية بمحتوى بروتيني مماثل (28%): (1) نظام غذائي ضابطة بدون بروتين TP، (2) بروتين TP 15%، (3) بروتين TP 15% مع ليسين (1.79%) وميثيونين (0.94%)، (4) بروتين TP 15% مع إنزيمات خارجية (0.2%) و (5) بروتين TP 15% مع ليسين وميثيونين وإنزيمات خارجية. وقد كشفت النتائج عن تحسن ملحوظ في أداء النمو، خاصةً زيادة وزن الجسم بنسبة 31.05% و33.03% ومعامل التحويل الغذائي بنسبة 21.83% و23.35% في المجموعتين الرابعة والخامسة على التوالي مقارنةً بالمجموعة الثانية. زادت الإنزيمات وحدها أو الإنزيمات مع الأحماض الأمينية بشكل ملحوظ من طول الزغابات وعدد الخلايا الكأسية في أمعاء أسماك البلطي. لم يكن هناك فرق كبير في مستويات الليباز والأميلاز والبروتين الكلي والألبومين والجلوبيولين في جميع المجموعات. كانت مستويات التعبير النسبي لعامل النمو IGF-1 وهرمون النمو في كبد أسماك البلطي مرتفعة بشكل ملحوظ ($p<0.05$) في المجموعات الثلاث الأخيرة. أخيرًا، أدت إضافة الإنزيمات مع أو بدون الأحماض الأمينية إلى الوجبات الغذائية لأسماك البلطي النيلي إلى تحسين معايير أداء النمو والتعبير الجيني.

الكلمات المفتاحية: ثفل الطماطم، الإنزيمات الخارجية، الأحماض الأمينية، التعبير الجيني.