Egyptian Journal of Veterinary Sciences

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

Nutritional Effect of Using a Bioactive Mixture (Lemon, Onion and Garlic) on Growth Performances, Feed Utilization, Immune Status and Gene Expression of Nile tilapia (*Oreochromis niloticus*)

Hesham Abozaid[1*], Ali S. M. Elnadi[1], Hayam Mansour[2], Dalia M. Aboelhassan[2], Elham Awad[3], Yasser A. A. El-Nomeary[1], Hamed A. A. Omar [1], Wafaa T. Abbas[3] and Ibrahim M. Farag[2]

[1] *Department of Animal Production, Agricultural and Biology Institute, National Research Centre, 33 El-Bohouth Street, P.O:12622, Dokki, Giza, Egypt.*
[2] *Department of Cell Biology, Biotechnology Research Institute, National Research Centre, 33 El-Bohouth Street, P.O:12622, Dokki, Giza, Egypt.*
[3] *Department of Hydrobiology, Veterinary Research Institute, National Research Centre, 33 El-Bohouth Street, P.O:12622, Dokki, Giza, Egypt.*

Abstract

THIS study investigated the effect of a lemon, onion, and garlic (LOG) juice mixture on the Nile tilapia health and productivity including; growth performance, feed utilization, body composition, immune status and gene expression of IGF-1, IGF-2, and GH genes. 120 fish were randomly distributed among 12 tanks into four groups before fed with different iso-nitrogenous and iso-caloric diets, containing 0%, 0.5%, 1.0%, or 1.5% of LOG juice for 56 days. The results revealed significant improvements in growth performance, especially specific growth rate in all LOG groups compared to the control. Moreover, a significant increase has been recorded in crude protein percentages in fish body composition. Energy retention (ER)% and protein productive value (PPV)% displayed substantial increases in all experimental groups. Moreover, AST and cholesterol levels were significantly higher in the 1.5% LOG group than the other groups. Immune parameters including lysozyme activity, antiprotease activity and total protein increased in 0.5% LOG and 1% LOG groups compared to the control. Furthermore, the dietary interventions led to a significant up-regulation of growth-related genes expression, compared to the control group, especially for IGF-1 and GH. This study demonstrates the efficacy of the bioactive mixture (LOG) in improving the Nile tilapia health; growth performance, and growth-related gene expressions and immunity. The study results recommended using LOG as a growth promoter, especially with a dose of 1.5% LOG.

Keywords: Nile tilapia growth, body composition, blood parameters, immune status, gene expressions.

Introduction

Fish serve as a crucial dietary protein source for humans, supplying over 20% of the average per capita intake of animal proteins globally [1]. The exploration of herbs, spices, and medicinal plants as potential growth promoters and additives has garnered increased attention [2]. Additionally, these substances exhibited antioxidative [3], antimicrobial [4], and digestion-enhancing properties by stimulating endogenous enzymes [5]. Among the natural additives, garlic, onion, and lemon juice have shown positive effects on animal health and production [6]. However, using of some substances such as antibiotics by heavy and continuous way in animal food that could be have negative effect on human health through its residues in milk and meat, which could be transferred from animal to human [6, 7]. Lemon is a rich source of potassium, calcium, and vitamins [7]. Additionally, Lemon peel contains phenolic compounds [8]. These compounds have various health benefits, such as antimicrobial [9], anti-cancer [10], and antioxidant properties [11].

Melvin *et al.* [12] reported that onion bulbs have a wide range of organic sulfur compounds, flavonoids, phenolic acids, sterols, saponins, sugars, and traces of volatile oil compounds. Various parts of the plant have antibacterial, antiviral, antiparasitic, and antifungal properties. Additionally, these components have diverse health

*Corresponding author: Hesham Abozaid, E-mail: g_hesham@yahoo.com . Tel.:00201153046771
(Received 31/01/2024, accepted 26/02/2024)
DOI: 10.21608/EJVS.2024.266900.1814

benefits, such as antihypertensive, hypoglycemic, antithrombotic, anti-hyperlipidemic, anti-inflammatory, and antioxidant effects.

Garlic (*Allium sativum*) is a widely used herb for preventing and treating various diseases, as it has many bioactive components that can reduce risk factors [13]. Among these components are sulfur-containing compounds that could contribute to garlic's benefits [14]. These compounds have health-promoting properties, including antibacterial [15], antifungal, antiparasitic, antiviral [16], antioxidant [17] and anticancer activities [18].

Previous studies have demonstrated the inhibitory effects of garlic, onion, and lemon juice on molds and fungi [19]. Moreover, adding natural additives to feed has increased the antioxidant content, suggesting their potential as natural antioxidants to prevent unwanted oxidation processes [20-23]. Lemon, onion, garlic, and their derivatives have shown beneficial effects as antioxidants, anticancer, anti-inflammatory, antibacterial, antiviral, and antifungal agents, contributing to the prevention and treatment of various diseases, including hypertension, hyperlipidemia, cardiovascular, atherosclerosis and other metabolic diseases [2, 24, 25].

Recent studies have indicated the growing interest in lemon, onion, and garlic in aquaculture for their potential as natural bioactive compounds to enhance growth performance and disease resistance in aquatic animals [25, 26].

Additionally, several growth factors play pivotal roles controlling somatic growth in vertebrates, including fish, and are influenced by nutritional status in aquaculture [27].

Therefore, this study aims to investigate the nutritional and biological impacts of using a bioactive natural juice (LOG) on the growth performances, feed utilization, body composition, biochemical parameters, immune parameters and the expressions of IGF-1, IGF-2 and GH genes in Nile tilapia fish.

Material and Methods

Experimental fish

The mono-sex fish used in this experiment were obtained from the Abbassa Fish Hatchery, Sharkia Government, Egypt. After acclimatization, 120 Nile tilapia fish (179 g±0.96) assigned to 12 glass aquaria (80×40×30cm) filled with 60L of dechlorinated tap water. The aquaria were randomly divided into four experimental groups (0%, 0.5%, 1.0%, or 1.5% of LOG), each with three replicates of 10 fish per aquarium. They were acclimated to a control diet for 2 weeks before starting our trial.

Experimental diets

LOG juice was prepared as 100, 100, and 12.5 g/L of lemon, onion, and garlic, respectively, as described by Bassuony *et al*. [2]. The bioactive mixture (LOG) was sprayed at four levels: 0, 0.5, 1.0, and 1.5% of the basal diet that consisted of 35% ground yellow corn, 20% soybean meal, 6% wheat bran, 10% corn starch, 7.5% fish meal, 15% poultry by-products, 5% vegetable oil, and 1.5% vitamins and minerals mixture, as shown in Table (1). The first group of fish is the control (0% LOG) which received a basal diet without LOG. The other three experimental groups were provided with the basal diet sprayed with LOG at concentrations of 0.5%, 1.0%, and 1.5%, respectively, equivalent to 5, 10, and 15 ml per kg of feed (volume/weight). They were formulated to be isonitrogenous & isocaloric with a diameter ranging from 2 to 3 mm. The fish were fed manually at a rate of 3% of their weight, three times daily for 56 days. The preparation and application of the juice onto the basal diet followed the method described by [23, 24].

Feed efficiency (FE)

The following equations were used in calculating the feed efficiency.

FE% = WG (g) / FI (g)

Protein productive value (PPV%) = [PR_1 (Final fish protein) – PR_0 (Initial fish protein) / PI (Protein intake)] 100.

Energy retention (ER%) = E (Final carcass energy) - E_0 (Initial carcass energy) / E_F (Feed intake energy) X 100

Body Composition assessment

The assessment of fish body composition was conducted before and after the feeding experiment, initially using 8 fish at the beginning of the trial and subsequently involving 6 fish from each treatment group later. This assessment aimed to determine values related to both energy and protein retention.

Energy Calculations

GE of the basal rations and the body composition in the experimental groups, were calculated to determine the energy retention percentages. The calculations utilized specific values: 5.65 Kcal/g CP, 9.40 Kcal/g EE, 4.15 Kcal/g CF and NFE [28, 29]. Furthermore, the metabolizable energy was derived using values of 4.50, 8.15, and 3.49 Kcal for protein, fat, and carbohydrate, respectively. The protein energy ratio (mg CP/Kcal ME) was calculated following the guidelines outlined in NRC [30].

TABLE 1. Composition of basal diet (BD) and the experimental design

Basal Diet	
Ingredients	**%**
Ground yellow corn	35.0
Soybean meal	20.0
Wheat bran	6.0
Corn starch	10.0
Fish meal	7.5
Poultry by-products	15.0
Vegetable oil	5.0
Vitamins and minerals mixture	1.5

Experimental Diets			
(Control) 0% LOG	**0.5%LOG**	**1.0%LOG**	**1.5%LOG**
BD	BD+5ml LOG/Kg feed	BD+10 ml LOG/Kg feed	BD+15 ml LOG/Kg feed

LOG: Bioactive mixture of lemon, onion and garlic juice

Growth performances

The parameters of growth performance determined as:

BWG = W1 - W0.

SR% = (final fish number / initial fish number) X 100.

SGR = [In final weight (g) - In initial weight (g)] / period X 100.

FCR = total dry matter intake (TDMI) (g) / weight gain (TBWG) (g).

Crude protein efficiency ratio (CPER) = TBWG (g) / total crude protein intake (TCPI) (g).

Table 2 showed the chemical analysis for different rations.

TABLE 2. The chemical analysis of experimental rations

Item	**Experimental diets**			
	0% LOG	**0.5% LOG**	**1.0% LOG**	**1.5% LOG**
Moisture	8.15	9.11	9.32	9.53
Dry matter (DM)	91.85	90.89	90.68	90.47
Chemical analysis on DM basis				
Organic matter (OM)	92.49	92.80	92.88	93.00
Crude protein (CP)	32.28	32.77	32.86	33.00
Crude fiber (CF)	5.72	5.75	5.80	5.84
Ether extract (EE)	3.00	3.02	3.06	3.08
Nitrogen free extract (NFE)	51.49	51.26	51.16	51.08
Ash	7.51	7.20	7.12	7.00
Gross energy kcal/ kg DM	4480.00	4500.13	4500.81	4510.62
Metabolizable energy kcal/ kg DM	349.41	350.98	351.36	351.87
Protein energy ratio (mg CP/ Kcal ME)	92.38	93.37	93.52	93.78

Gross energy (kcal/ kg DM) was calculated according to Blaxter, [28] and MacRae and Lobley, [29].
Metabolizable energy and protein energy ratio calculated according to Reitman and Frankel [30].

Blood samples

The samples of blood are collected from the caudal vein of 5 fish using insulin syringe. The blood was left until clot (for two hours), then it centrifuged, and the collected serum is kept at -20°C till used.

Biochemical assays

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [30], cholesterol [31] and glucose concentrations [32] were estimated using commercial biochemical kits (Bio-diagnostics, Egypt). The previous parameters were analyzed colorimetrically following the instructions of the manufacturer protocol using an Agilent Cary UV-Vis spectrophotometer (100/300 Series).

Immune parameters

Lysozyme activity

The turbidimetric assay for lysozyme was carried out according to Parry *et al*. [33]. Thus, 40

µl of serum was added to 2 ml of a suspension of *Micrococcus lysodeikticus* (Sigma-Aldrich, 0.2 mg/ml) in a 0.05 M sodium phosphate buffer (pH 6.2). This reaction was carried out at 25^{o}C and absorbance was measured at 530 nm after 0.5 and 4.5 min on a spectrophotometer. A unit of lysozyme activity was defined as the sample amount causing a decrease in absorbance of 0.001/min.

Antiproteases activity

The serum anti-trypsin activity was measured by established methods [34, 35]. Thus, 20 µl of standard trypsin solution (Sigma-Aldrich, 5 mg/ml) was incubated with 20 µl of serum for 10 min at 22˚C. Subsequently, 200 µl of 0.1 M PBS (PH 7.2) and 250 µl of 2% azocasein solution (20 mg/ml PBS) were added before incubated for 1 h at 22˚C. The reaction was stopped with the addition of 500 µl of 10 % (v/v) trichloro acetic acid (TCA) and incubated for 30 min at 2˚C. The mixture was centrifuged at 6000 x g for 5 min and 100 µl of the supernatant was transferred to a 96 microwell flat bottom plate containing 100 µl of 1 N NaOH/well. The absorbance was read in the spectrophotometer at 410 nm. Positive control (100%) was prepared by replace the serum with buffer. For a negative control, buffer replaced both serum and trypsin. The percentage inhibition of trypsin activity was calculated by comparing with a positive control sample.

Total protein, albumin and globulin:

Total protein [36] and albumin [37] were estimated using commercial biochemical kits (Bio-diagnostics, Egypt). Globulin obtained by subtracting albumin concentration from total protein concentration. Each biochemical parameter was colorimetrically analyzed according to its manufacturer's instructions using an Agilent Cary UV-Vis spectrophotometer.

Gene expression analysis

Total RNA was extracted from liver tissue using TRIzol Reagent (Invitrogen; Thermo Fisher Scientific). The extracted RNA was quantified by NanoDrop 2000 (Thermo Fisher Scientific), and reverse transcribed using a High Capacity cDNA synthesis Kit (Applied Biosystems; Thermo Fisher Scientific) according to the manufacturer's instructions. Real-time quantitative PCR (qPCR) reactions were applied (Quant Studio^{TM} 5 Real-time PCR System) using β-actin gene as an internal reference gene for normalizing of the expression data. Briefly, qPCR of 20 µl reaction contained 10 µl of SYBR Green I Master (Roche Diagnostics GmbH), 1 µl each of forward and reverse primers, 2 µl of cDNA template, and 6 µl of nuclease-free water. The thermocycling program consisted of the denaturation step at 95°C for 10 min followed by 40 cycles of 95°C for 15s, annealing at 58°C for 15 seconds, and extension at 72°C for 30 seconds. Expression levels of the studied gene relative to β-actin were assessment using $2^{-\Delta\Delta Ct}$ formula [38]. The sequences of each primer that used for gene expression analysis are shown in Table (3).

TABLE 3. Primer sequences used for gene expression analysis

Gene	Primer Sequences	Reference
GH	F: CTGTCTGTCTGTCTGTCAGTCGT R: AGAGGAGACGCCCAAACAC	**Rentier-Delrue *et al.*** [39].
IGF-1	F: CCCGAACTTCCTCGACTTGA R: CCTCAGCCAGACAAGACAAAAA	**Wang *et al.*** [40].
IGF-2	F: CCCCTGATCAGCCTTCCTA R: GACAAAGTTGTCCGTGGTGA	**Wang *et al.*** [40].
β-actin	F: ACCCACACAGTGCCCATC R: CAGGTCCAGACGCAGGAT	**Monteiro** [41].

Ethics approval

This study was approved according to Ethics of Medical Research Committee of National Research Centre, Al Buhouth st. Dokki- Cairo Egypt under the number 07451223.

Statistical analysis

Obtained data were statistically analyzed using one-way analysis of variance (ANOVA) [42]. Duncan's Multiple Range test [43] was selected for inter group comparisons.

Results and Discussion

Chemical analysis

The data shown in Table (2) indicates that all tested rations formulated to sufficiently meet requirements for fish. Crude protein percentages ranged from 32.28% to 33% across the four experimental diets. The gross energy content varied between 4480 to 4510.62 kcal/kg DM, while the metabolizable energy ranged from 349.41 to 351.87 kcal/kg DM. Additionally, the protein energy ratio ranged from 92.38 to 93.78 mg CP/Kcal ME across

the four experimental diets. These values are considered adequate to fulfill the dietary needs of Nile tilapia fish.

Growth performance and survival ratio of fish

The results depicted in Table (4) indicate a significant enhancement (P<0.05) in growth performance, specific growth rate, and survival ratio of the experimental diet groups. Notably, no fish mortalities were observed in the fish fed with diets containing LOG. Our results align with observation of Mohammady *et al*. [25], whose found that, quadratic improvement in FBW, TBWG, SGR, and survival rate (P<0.0006) when fish fed diets containing 10, 20, and 30 ml of LOG/kg of feed. Specifically, the group of fish receiving a diet containing 20 ml of LOG/kg feed exhibited the most substantial growth response, reaching 35.50 g for FBW, 31.2 g for TBWG, 3.02% for SGR, and 99.33% for survival rate. However, studies by Heuer *et al*. [44] and Baba *et al*. [45] have highlighted the potential risks associated with the use of antibiotics in aquaculture, including issues related to toxicity, organism sensitivity to antibiotic residues, and environmental contamination. Therefore, these factors have prompted aqua culturists to explore and seek sustainable alternatives to antibiotics.

Recent research endeavors focused on the evaluation of phytogenic components, such as limonene, as antioxidants and growth promoters across various fish species. Yet, there remains a significant gap in understanding the effects of combined phytogenic compounds such as LOG [46, 47].

Maniat *et al*. [48] observed positive growth effects in fish fed phytogenic-enriched diets, such as fenugreek meal, chamomile flowers meal, and garlic, phytogenic-enriched diets, whereas, Takaoka *et al*. [49] found no significant impacts on growth. Additionally, recent research highlights the beneficial effects of LOG juice in other animal species. For instance, Ahmed *et al*. [50] reported that rabbits supplemented with varying doses of LOG juice (5, 10, 15, and 20 ml/kg feed) showed increased average daily gains of 20%, 29%, 36.1%, and 19.3%, respectively, compared to the control group. Moreover, reduced mortality rates were observed in rabbits provided with LOG juice in their drinking water at concentrations of 3 ml/L (10.90%) and 2 ml/L (12.73%), respectively, compared to the control group (21.818%) [51].

Grasping the optimal dosages of phytogenic compounds for different fish species, and the underlying physiological mechanisms responsible for promoting growth and stimulating the immune system remains crucial [52]. The observed heightened growth response in Nile tilapia might be attributed to the inherent biological properties of the bioactive compounds found in the LOG mixture. These compounds are believed to stimulate various physiological responses, including increased feed intake, enhanced secretion of digestive enzymes, and improved antioxidant status [25]. Additionally, onion and garlic have been associated with increased growth by facilitating higher glucose flow into tissues and exhibiting activity similar to thyroid hormones [53]. Moreover, their sulfur compound contents, recognized as active antimicrobial agents, are known to fortify immunity [54, 55].

TABLE 4. Growth performance, specific growth rate, and survival ratio of the experimental Groups

Item	Experimental diets				SEM	Sign. P<0.05
	Control 0% LOG	0.5% LOG	1.0% LOG	1.5% LOG		
Number of fish	30	30	30	30	-	-
Initial weight (IW)	182	177	180	177	0.96	NS
Final weight (FW)	406^{b}	415^{b}	491^{a}	492^{a}	12.63	*
Total body weight gain (TBWG)	224^{b}	238^{b}	311^{a}	315^{a}	12.69	*
Duration experimental period	**56 days**					
Average daily gain, g (ADG)	4.00^{b}	4.25^{b}	5.55^{a}	5.63^{a}	0.23	*
Specific growth rate (SGR)	0.63^{c}	0.67^{b}	0.78^{a}	0.79^{a}	0.02	*
No. starter fish	30	30	30	30	-	-
No. final fish	28	30	30	30	-	-
Survival rate (SR)	93.33^{b}	100^{a}	100^{a}	100^{a}	1.12	*
Number of dead fish	2	-	-	-	-	-
Mortality rate percentages	6.67	Zero	Zero	Zero	-	-

a and b in the same row are means that having different superscripts differ significantly (P<0.05).

Feed utilization

The data presented in Table (5) highlights the significant impact (P<0.05) of dietary treatments on FI, FCR, CPI, and PER compared to the control group. The highest values for FI, FCR, CPI, and PER were observed in the group of fish fed with 1.5% LOG, equivalent to 15 ml LOG/kg feed. These findings align with Badr *et al*. [51], who noted a significant elevation (P<0.05) in the daily feed intake of rabbits with increasing levels of a natural mixture juice (NMJ) containing lemon,

onion and garlic in drinking water. They also observed that rabbits supplemented with 1, 2, and 3 ml/L NMJ showed improved feed conversion compared to the control.

Ahmed *et al*. [50] mentioned that supplementation of rabbit diets with LOG juice at varying levels (0, 5, 10, 15, and 20 ml/kg feed) did not significantly affect dry matter intake. However, they noted a significant improvement (P<0.05) in feed conversion compared to the control. Contrarily, Kim *et al*. [56] and Takaoka *et al*. [49] observed no significant effects on feed efficiency when utilizing *Scutellaria baicalensis* Georgi extract or medicinal herbs in catfish (*Silurus asotus*) or at the early juvenile stage of Red seabream (*Pagrus major*).

TABLE 5. Feed utilization of the different experimental groups

Item	Experimental diets				SEM	Sign. P<0.05
	Control 0 % LOG	0.5% LOG	1.0% LOG	1.5% LOG		
Total body weight gain, g (TBWG)	224^{b}	238^{b}	311^{a}	315^{a}	12.69	*
Feed intake (FI), g	593.32^{b}	587.12^{b}	649.12^{a}	642.54^{a}	9.09	*
Feed conversion ratio (FCR)	2.65^{c}	2.47^{b}	2.09^{a}	2.04^{a}	0.08	*
Crude protein%	32.28	32.77	32.86	33.00	-	-
Crude protein intake (CPI), g	191.52^{b}	192.40^{b}	213.30^{a}	212.04^{a}	3.31	*
Protein efficiency ratio (PER)	1.17^{c}	1.24^{b}	1.46^{a}	1.49^{a}	0.04	*

a, b and c in the same roware means that having different superscripts differ significantly (P<0.05).
FCR: Expressed as g of DM intake / g gain PER:Expressed as g of g gain / g CP intake.

Impact of dietary inclusion of LOG on fish body composition

The body composition of fish in different experimental groups (Table 6), changed significantly upon being fed diets containing LOG. Notably, there was a significant (P<0.05) increase in crude protein percentages, while there were significant reductions in ether extract and gross energy (P<0.05). Conversely, the content of organic matter and ash remained unaffected (P>0.05) in the fish diets containing LOG.

In a study by El-Dakar *et al*. [57], significant differences were observed in fish body composition, particularly in crude protein, lipids, ash, and gross energy. However, they reported no significant differences between treatments (P>0.05). Similarly, Ali and El-Feky [58] and Abo-State *et al*. [59] indicated no statistical variations in whole body moisture, ether extracts, and ash content when prebiotics, manna oligosaccharides, or β-glucan were utilized in commercial diets for Nile tilapia fingerlings.

TABLE 6. Fish body composition of the experimental groups

Item	Initial body composition	Experimental diets				SEM	Sign. P<0.05
		Control 0% LOG	0.5% LOG	1.0% LOG	1.5% LOG		
Moisture	70.99	71.59^{d}	75.59^{a}	74.68^{b}	73.43^{c}	0.46	*
Dry matter (DM)	29.01	28.41^{a}	24.41^{d}	25.32^{c}	26.57^{b}	0.46	*
Chemical analysis on DM basis							
Organic matter (OM)	80.36	85.23	84.99	85.32	84.94	0.09	NS
Crude protein (CP)	53.72	56.15^{c}	56.82^{b}	58.52^{a}	57.02^{b}	0.27	*
Ether extract (EE)	26.64	29.08^{a}	28.17^{b}	26.80^{d}	27.92^{c}	0.25	*
Ash	19.64	14.77	15.01	14.68	15.06	0.09	NS
Gross energy kcal/ 100g	554	591^{a}	586^{b}	583^{b}	585^{b}	1.04	*
Gross energy cal/ g DM	5.54	5.91^{a}	5.86^{b}	5.83^{b}	5.85^{b}	0.01	*

a, b, c and d in the same row are means that having different superscripts differ significantly (P<0.05).
GE calculated according to [28, 29].

Energy retention and protein productive value percentages:

Table (7) revealed a significant increase in both energy retention (ER)% and protein productive value (PPV)% among the Nile tilapia fish fed diets containing LOG. Specifically, compared to the control group (considered 100%), the protein ER% saw enhancements of 104.95%, 122.03%, and 125.09% in groups 0.5% LOG, 1% LOG, and 1.5% LOG, respectively. Correspondingly, PPV% values showed improvements of 107.71%, 131.21%, and

128.94% for the same groups compared to the control.

These findings parallel those reported by Abo-State *et al*. [59], indicating notable variations (P<0.05) in ER% and PPV% among treatments. They observed superior ER% and PPV% in groups receiving diets supplemented with β-glucan and mannan oligosaccharide (MOS) at 2 and 4g/kg, followed by the 6g/kg diet. Interestingly, no significant were noted among varying levels of β-glucan and MOS concerning PPV and ER percentages.

TABLE 7. Energy retention (ER)% and protein productive value (PPV)% of different experimental groups

Item	Experimental diets				SEM	Sign. P<0.05
	Control 0% LOG	0.5% LOG	1.0 % LOG	1.5% LOG		
Initial weight (IW), g	182	177	180	177	0.96	NS
Final weight (FW), g	406^{b}	415^{b}	491^{a}	492^{a}	12.63	*
Calculation the energy retention (ER)%						
Energy content in final body fish (cal / g)	5.91^{a}	5.86^{b}	5.83^{b}	5.85^{b}	0.01	*
Total energy at the end in body fish (E)	2399^{b}	2432^{b}	2863^{a}	2878^{a}	69.21	*
Energy content in initial body fish (cal / g)	5.54 k cal					
Total energy at the start in body fish (E_0)	1008	981	997	981	5.49	NS
Energy retained in body fish ($E-E_0$)	1391^{b}	1451^{b}	1866^{a}	1897^{a}	70.35	*
Energy of the feed intake (Cal / g feed)	4.480	4.500	4.501	4.511	-	-
Quantity of feed intake	593.32^{b}	587.12^{b}	649.12^{a}	642.54^{a}	9.09	*
Total energy of feed intake (EF)	2658^{b}	2642^{b}	2922^{a}	2898^{a}	41.35	*
Energy retention (ER)%	52.33^{b}	54.92^{b}	63.86^{a}	65.46^{a}	1.75	*
Calculation the protein productive value (PPV)%						
Crude protein% in final body fish	56.15	56.82	58.52	57.02	-	-
Total protein at the end in body fish (PR_1)	228^{b}	236^{b}	287^{a}	281^{a}	8.29	*
Crude protein% in initial body fish	53.72					
Total protein at the start in body fish (PR_2)	97.77^{a}	95.08^{c}	96.70^{b}	95.08^{c}	0.35	*
Protein Energy retained in body fish (PR_3) = ($PR_1 - PR_2$)	130.23^{b}	140.92^{b}	190.30^{a}	185.92^{a}	8.38	*
Crude protein in feed intake (CP%)	32.28	32.77	32.86	33.00	-	-
Total Protein intake (PI), g	191.52^{b}	192.40^{b}	213.30^{a}	212.04^{a}	3.14	*
Protein productive value (PPV)%	68.00^{b}	73.24^{b}	89.22^{a}	87.68^{a}	2.95	*

a, b and c in the same row are means that having different superscripts differ significantly (P<0.05).

Biochemical parameters

The biochemical parameters observed in Table (8) displayed various changes across the experimental groups. Serum ALT levels exhibited no significantly differ in compared to control. However, AST levels in the group which received a diet containing 1.5% LOG, showed a notable and significant elevation (P<0.05) compared to other groups.

In terms of serum glucose and cholesterol concentrations, the dietary treatments did not significantly impact their levels (P>0.05) across the experimental groups, except in the 1.5% LOG group. The 1.5% LOG group demonstrated a significantly higher cholesterol level (P<0.05) of 241.97 mg/dl compared to the other groups. Where, values ranged from 120.23 to 167.79 mg/dl.

TABLE 8. Biochemical parameters of the experimental groups

Item	Experimental diets				SEM	Sign. P<0.05
	Control 0% LOG	0.5% LOG	1.0 % LOG	1.5% LOG		
AST (Unit/l)	45.49^{a}	57.26^{a}	50.88^{a}	169.37^{b}	10.90	*
ALT (Unit/l	77.39	74.44	90.14	100.32	8.20	NS
Glucose (mg/dl)	94.69	132.92	179.86	174.02	14.81	NS
Cholesterol (mg/dl)	120.23^{a}	145.31^{a}	167.79^{ab}	241.97^{b}	11.73	*

a and b in the same row are means that having different superscripts differ significantly (P<0.05).
ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

Biochemical analysis and hematological parameters are fundamental in monitoring the physiological condition of fish, particularly following the introduction of new dietary additives, allowing for the evaluation of potential adverse effects [60-62]. Liver enzymes like ALT and AST are reflective of the liver's health and function, while blood glucose and cholesterol serve as key

indicators of physiological stress. Stressors can prompt the release of hormones like adrenaline and cortisol in fish, triggering glycogenesis and glycogenolysis pathways that increase glucose production to meet the energy demands induced by stress [63].

Mohammady *et al*. [25] observed a quadratic response in fish hematological parameters with increasing levels of LOG. As the inclusion of LOG in diets increased, there was a significant linear decrease in ALT, AST, cholesterol, and triglyceride levels. This trend aligns with findings in the Nile tilapia fed with sweet orange peel (*Citrus sinensis*), which showed lower cholesterol and triglyceride levels [64]. This reduction could be attributed to compounds such as flavones, flavonoids, or short-chain fatty acids present in the dietary additives [65, 66]. Moreover, Mohammady *et al*. [25] found a polynomial correlation between total protein, albumin, and globulin and different levels of LOG. Additionally, LOG improved RBC and Hb I all treatments. Similar improvements in hematological noted in fish supplemented with various bioactive compounds and phytogenic components [67, 68].

Omer *et al*. [70] reported that including LOG in the diets of growing rabbits at different concentrations did not significantly impact total protein and globulin levels. However, it notably affected albumin levels and the albumin-to-globulin ratio at certain LOG concentrations. Conversely, Ahmed *et al*. [23] found no significant change in serum total protein when LOG was added to calves' rations, indicating no noticeable impact on liver function's protein synthesis. Low protein levels may be attributed to decreased protein absorption and synthesis, coupled with increased protein losses. Additionally, Hassan and Abdel-Raheem [70] observed higher serum concentrations of globulin in calves fed diets containing garlic. Previous investigation has reported that the blood parameters do not always follow the same trend across experimental fish, so it is difficult to draw a firm conclusion about which parameter should be considered [71]. Younes *et al.* [72] confirmed the enhancing role of using onion as a growth promoter in *Oreochromis niloticus*, recorded a significant increase in glucose and cholesterol levels in the highest onion concentration level, which agreed with our results of using 1.5% LOG. On the other hand, fish with higher growth rates as we recorded in 1.5% LOG treatment were more sensitively to any changes in physiological status that might cause changes in the blood parameters. Finally, there was an interpretation of blood data of fish because it must be done on light of the specific individual experiment condition because of absence of referenced ranges for fish haematological and biochemical measurements [73].

Immune parameters

Highest antiproteases activities reported in the group fed with 1% LOG (85.01%) (Fig.1), followed by 0.5% LOG juice (83.79%), while the lowest activity was in 1.5% LOG without significant differences ($P>0.05$). Similarly, the group that consumed 1% LOG exhibited the highest lysozyme activity at 688.54%, (Fig. 2) followed by the group that ingested 0.5% LOG (651.88%). In contrast, the group fed with 1.5% LOG recorded the lowest activity (439.79%). No significant differences among treatments, although, 1% and 0.5% LOG showed the highest values of total protein (Fig. 3) and globulin (Fig. 4), The impact of using of LOG juice on the immune status of the Nile tilapia revealed an increase in the lysozyme, antiproteases, total protein, and globulin in the groups fed with 1% LOG and 0.5% LOG, respectively, more than other groups. Previous studies in other fish showed an enhancement in immune parameters after the administration of diets that contain either lemon or garlic or onion but separately. For example, dietary 1.5% of lemon (powder) increased some of the immune parameters like; lysozyme and total immunoglobulin of rainbow trout fed for 45 days [74]. Also, *Labeo rohita* fed a diet enriched with dried lemon (especially 2.5 g/Kg of fish) for 60 days, showing elevations in phagocytic, complement, and lysozyme activities and total protein compared to the control [75]. Using dietary 1% onion powder in the beluga juvenile diet for 8 weeks, showed a significant increase in lysozyme activity, respiratory burst activity, and total protein [76]. Moreover, Nile tilapia administered dietary supplements with either crude or extracts of onion showed an enhancement in immune parameters like antiprotease and lysozyme activities Myeloperoxidase, total protein, albumin, and globulin [77]. The use of garlic as a dietary supplement has been documented to have immunomodulatory effects in various fish species, including rainbow trout [78], hybrid tilapia [79], Asian sea bass [80], and Caspian roach [81]. The research indicates an increase in immune parameters such as total protein, lysozyme, antiprotease, and bactericidal activities, but 1.5% dose recorded the lowest value (without a significant difference). In contrast, the dose of 1.5 % of the mixture recorded the highest albumin value comparing to other groups (Fig. 5).

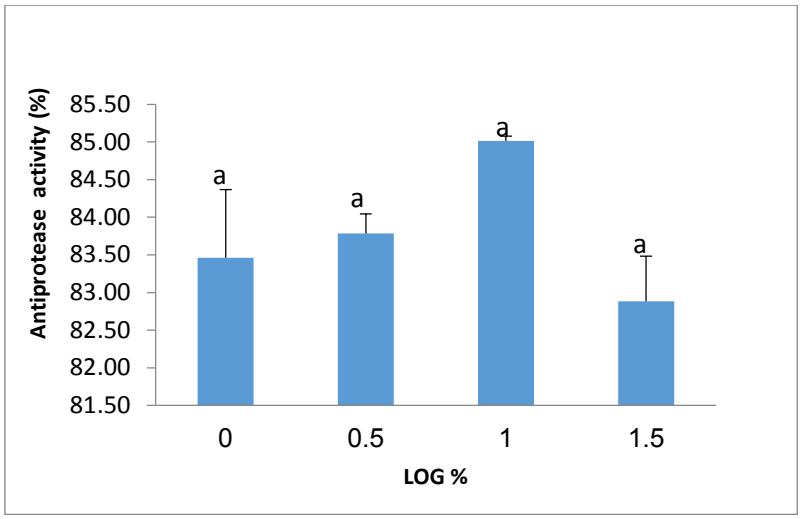

Fig.1. Antiprotease activity of Nile tilapia fed diets supplemented with 0%, 0.5%, 1%, and 1.5% of the LOG juice. The same letter is not significantly different (P>0.05). Bars=mean ± S.E.

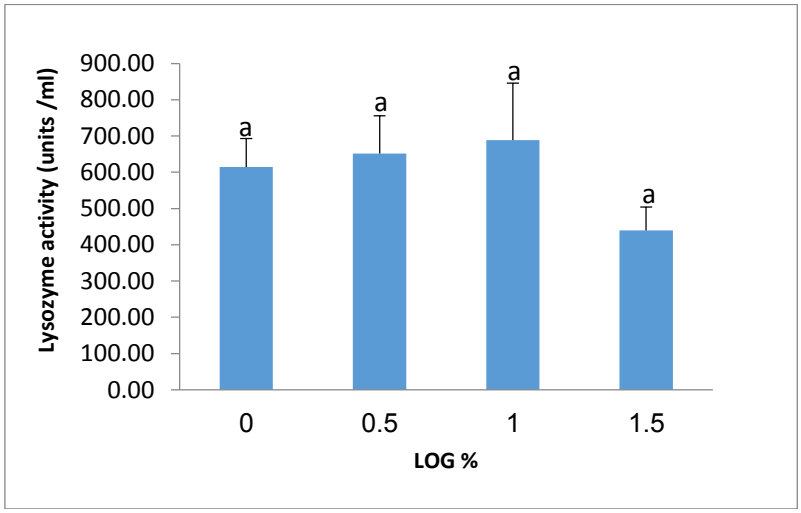

Fig. 2. Lysozyme activity of Nile tilapia fed diets supplemented with 0%, 0.5%, 1%, and 1.5% of LOG juice. The same letter is not significantly different (P>0.05). Bars=mean ± S.E.

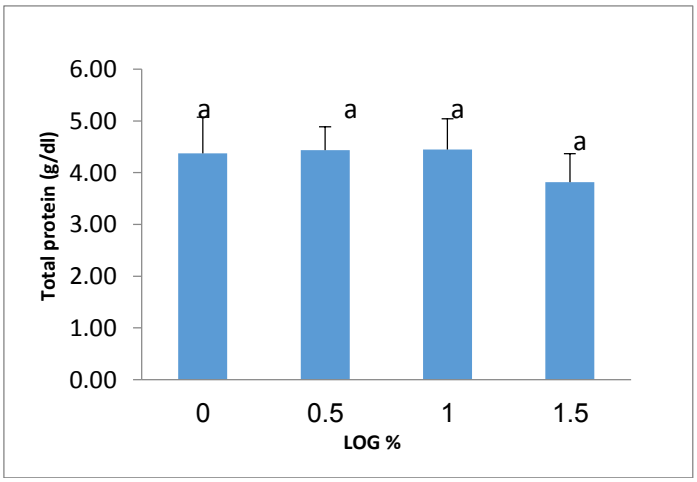

Fig. 3. Total protein of Nile tilapia fed diets supplemented with 0%, 0.5%, 1%, and 1.5% of the LOG juice. The same letter is not significantly different (P>0.05). Bars=mean ± S.E.

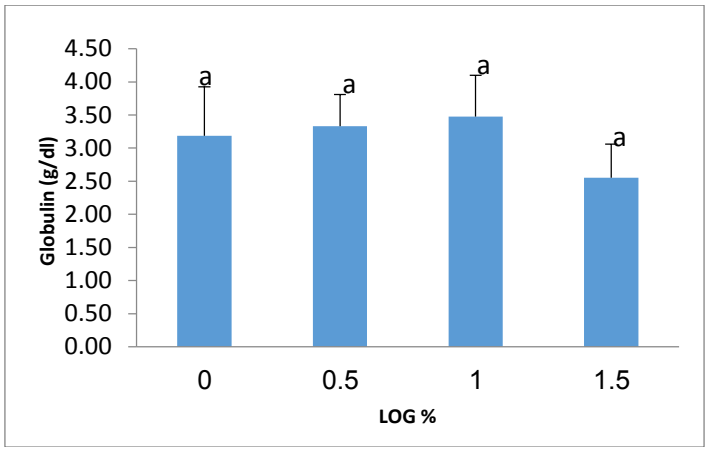

Fig. 4. Globulin of Nile tilapia fed diets supplemented with 0%, 0.5%, 1%, and 1.5% of the LOG juice. The same letter is not significantly different (P>0.05). Bars=mean ± S.E.

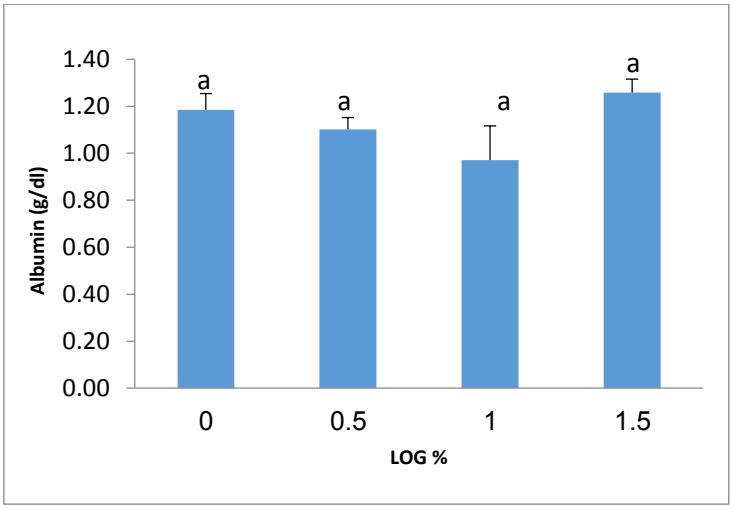

Fig. 5. Albumin of Nile tilapia fed diets supplemented with 0%, 0.5%, 1%, and 1.5% of the LOG juice. The same letter is not significantly different (P>0.05). Bars= mean ± S.E.

Gene expressions of growth-associated genes

The analysis of gene expressions in liver tissue of the Nile tilapia fed different concentrations of LOG is illustrated in Figure (6).

IGF-1 Gene Expression

The mRNA levels of IGF-1 significantly increased in fish fed 0.5% (P<0.05), 1.0% (P<0.01), and 1.5% (P<0.001) LOG compared to the control (0% LOG). This showed a clear dose-dependent pattern, where higher LOG doses corresponded to elevated IGF-1 mRNA expressions. The highest expression occurred at 1.5% LOG, while the lowest was observed at 0.5% LOG.

IGF-2 Gene Expression

For IGF-2 mRNA, levels significantly elevated (P<0.05) in fish fed diets with 0.5% or 1.0% LOG compared to the control. Additionally, a marked up-regulation was seen in fish fed 1.5% LOG compared to the control (P<0.01), 0.5% LOG (P<0.05), and 1.0% LOG (P<0.05).

GH Gene Expression

The analysis revealed up-regulation of GH mRNA in fish fed with all three LOG levels: 0.5% (P<0.05), 1.0% (P<0.01), and 1.5% (P<0.001) compared to the control. Similar to IGF genes, GH mRNA levels increased as LOG concentration increased, with the highest expression noted at 1.5% LOG and the lowest at 0.5% LOG.

The observed results demonstrated significant up-regulation of three growth-related genes (IGF-1, IGF-2, and GH) compared to the control, indicating that gene expression levels increased in tandem with escalating levels of lemon grass oil (LOG). Mohammady *et al*. [25] reported significant up-regulations of IGF-2, SOD, CAT, and GH gene expressions in the Nile tilapia fish fed diets containing varying amounts of LOG (10, 20, and 30 ml LOG/ Kg) compared to the control group, with the highest gene expressions observed in fish fed a diet containing 20 ml LOG Kg. Aanyu *et al*. [46] similarly noted significant enhancements in the expression of the IGF-1 gene in Nile tilapia fish fed

diets supplemented with limonene. Moreover, Jahanbakhshi *et al*. [82, 83] demonstrated that diets supplemented with garlic significantly up-regulated immune deficiency (IMD) and heat shock proteins (HSP70) gene expressions in giant freshwater prawn fish compared to the control group. Conversely, Abu-Elala [84] reported significant up-regulation of related immune genes in the Nile tilapia (*O. niloticus*) fed a diet containing *Spirulina platensis* and garlic. Bassuony *et al*. [2] and Mohammady *et al*. [25] proposed that the observed up-regulation or improvements in gene expressions in various fish types due to LOG or lemon and garlic extracts might be attributed to numerous bioactive compounds, particularly polysaccharides, vitamin C, β-carotene, and flavonoids present in these extracts. These constituents act as antimutagenic agents, interrupting the initial chain reaction of oxidation or scavenging and neutralizing free reactive radicals (ROS), thereby reducing DNA oxidative damage and promoting genomic stability, which in turn influences animal gene expressions [85-88]. Notably, these compounds have demonstrated various effects: polysaccharides from oyster mushroom extracts improved gene expressions related to insulin and coagulation factor in liver tissue of diabetic rats [89], vitamin C from *Moringa oleifera* extract ameliorated specific gene expressions in liver tissue of rats injected with carbon tetrachloride (CCL4) [89], β-carotene from *Spirulina platensis* enhanced antioxidant gene expressions in rainbow trout fish [88] and Nile tilapia [90] farmed in aquaculture and flavonoids found in *Citrus reticulata* "chachi" peels caused significant alterations in numerous gene expressions related to salt stress response [91, 92].

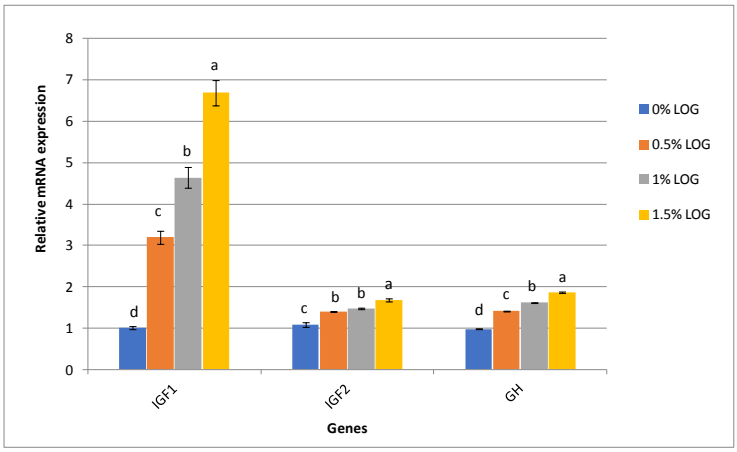

Fig. 6. Relative expression levels of IGF-1, IGF-2 and GH genes were determined by Real-time PCR in the liver of Nile tilapia fed with four LOG diets (0%, 0.5%, 1% and 1.5%).

Conclusion

The incorporation of the bioactive mixture comprising lemon, onion, and garlic juice (LOG) in Nile tilapia diets showed a notable enhancement in growth performance, feed utilization, growth-related genes, biochemical parameters and immune parameters. Therefore, the study recommended using LOG as a growth promoter and as an immunostimulant for the best productivity. It was worth to mention that, the optimal dose of LOG supplementation regarding to the growth performance seems to be 1.5% LOG, equivalent to 15 ml of essential oil per kg of feed.

References

1. Zhang, F., Man, Y.B., Mo, W.Y. and Wong, M.H. Application of Spirulina in aquaculture: a review on wastewater treatment and fish growth. *Reviews in Aquaculture,* **12**(2), 582-599 (2020).

2. Bassuony, N.I., Hafez, L.M., Ibrahim, A.F., El-Sebeay, A.S. and Omer, H.A. Effect of using natural bioactive mixture composed of lemon, onion and garlic juices on weight and lipid profiles for overweight rats. *European Journal of Applied Sciences,* **13**(1), 9-17 (2021).

3. Hui, Y.H. Oleoresins and essential oils. In: Hui, YH, editor. Bailey's industrial oil and fat products. New York, Wiley-Interscience Publication, Cap. 6. pp: 145-153 (1996).

4. Dorman, H.J.D. and Deans S.G. Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *Journal of Applied Microbiology,* **88**, 308-316 (2000).

5. Brugalli, I. Alimentacaoalternativa: An utilizacao de fitoterapicos ou nutraceuticos como moduladores da imunidade e desempenho animal. Anais do SimposiosobreManejo e Nutri cao de Aves e Suinos; Campinas, Sao Paulo. Brasil. Campainas (BNA), pp: 167-182 (2003).

6. Wagr, A., Quaratulain, S., Altaf, H., Ahmad, G.M. and Ashhar, Z. Evaluation of different garlic extracts for antibacterial activity. *Science International,* **5**, 385-386 (1994).

7. Chevallier, A. Encyclopedia of medicinal plants. DK Publishing, New York, pp: 81 (1996).
8. González-Molina, E., Domínguez-Perles R., Moreno D.A. and García-Viguera C. Natural bioactive compounds of citrus Limon for food and health. *Journal of Pharmaceutical and Biomedical Analysis,* **51**, 327-345 (2010).
9. Dhanavade, M.J., Jalkute, C.B., Ghosh, J.S. and Sonawane, K. D. Study antimicrobial activity of lemon (Citrus lemon L.) peel extract. *British Journal of pharmacology and Toxicology*, **2**(3), 119-122 (2011).
10. Rawson, N.E., Ho C.T. and Li S. Efficacious anti-cancer property of flavonoids from citrus peels. *Food Science Human Wellness,* **3**, 104-109 (2014).
11. Wilmsen, P.K., Spada D.S. and Salvador M. Antioxidant activity of the flavonoid hesperidin in chemical and biological systems. *Journal of Agricultural and Food Chemistry,* **53**, 4757-4761 (2005).
12. Melvin, J.M., Jayochitra J. and Vijayapriaya M. Antimicrobial activity of some common spices against certain human pathogens. *Journal of Medicinal Plants Research,* **3**, 1134-1136 (2009).
13. Thomson M., Al-Amin Z. M., Al-Qattan K. K., Shaban L. H., Ali M. Anti-diabetic and hypolipidaemic properties of garlic (Allium sativum) in streptozotocin-induced diabetic rats. *International Journal of Diabetes and Metabolism,* **15**,108–115(2007)..
14. Amagase, H., Petesch B.L., Matsuura H., Kasuga S. and Itakura Y. Intake of garlic and its bioactive components. *The Journal of Nutrition,* **131**(S3), 955S-962S (2001).
15. Sivam, G.P. Protection against Helicobacterpylori and other bacterial infections by garlic. *The Journal of Nutrition,* **131**(3S), 1106S-1108S (2001).
16. Ankri, S. and Mirelman D. Antimicrobial properties of allicin from garlic. *Microbes and Infection,* **1**, 125-129 (1999).
17. Prasad, G., Laxdal V.A., Yu M. and Raney B.L. Antioxidant activity of allicin, an active principle in garlic. *Molecular and Cellular Biochemistry,* **148**, 183-189 (1995).
18. Agarwal, K.C. Therapeutic actions of garlic constituents. *Medicinal Research Reviews,* **16**, 111-124 (1996).
19. Elgayyar, M., Draughon, F.A., Golden, D.A. and Mount, J.R. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *Journal of Food Protection,* **64**(7), 1019-1024 (2001).
20. Wangensteen, H., Sanulsen, A.B. and Malterud, K.E. Antioxidant activity in extracts from coriander. *Food Chemistry,* **88**, 293-297 (2004).
21. Khir, A.A. and Ibrahim Nany, S. Effect of coriander and anise as feed additives on performance of buffalo calves. *Egyptian Journal of Nutrition and Feeds,* **10**(2), 453-460 (2007).
22. Aiad, A.M., Neamat I. Bassuony, Afifi, A.A. and Abo-Donia, F.M. Adding natural juice of vegetables and fruitage to ruminant diets: (A). Lemon, onion and garlic juice supplemented to diets fed to suckling buffalo calves and its effect on digestibility, growth performance and fungi count. *World Journal of Agricultural Science,* **4** (2), 149-156 (2008).
23. Ahmed, A.A., Bassuony, N.I., Awad, SE-HS., Aiad, A.M. and Mohamed, S.A. Adding natural juice of vegetables and fruitage to ruminant diets (B). Nutrients utilization, microbial safety and immunity, Effect of diets supplemented with lemon, onion and garlic juice fed to growing buffalo calves. *World Journal of Agricultural Science,* **5**(4), 456-465 (2009).
24. Williams, C.A. and Lamprecht, E.D. Some commonly fed herbs and other functional foods in equine nutrition: a review. *The Veterinary Journal,* **178**(1), 21-31 (2008).
25. Mohammady, E.Y., Soaudy, M.R., A. Elashry, M.A., EL-Erian, M.M.A., Farag, A., Badr, A.M.M., Bassuony, N.I., Ragaza, J.A., El-Haroun, E.R. and Hassaan, M.S. Can dietary phytogenic mixture improve performance for growth, digestive enzyme activity, blood parameters, and antioxidant and related gene expressions of Nile tilapia, *Oreochromis niloticus*?. *Animal Feed Science and Technology,* **290**, 115369 (2022),
26. Yildiz, H.Y. and Yilmaz, B.H. Dietary citric acid decreased the theront number of Cryptocaryon irritans (Ciliata) in seawater adapted tilapia (*Oreochromis niloticus*). *Journal of Fish Diseases,* **47**(2),e13881 (2023).
27. Herkenhoff, M.E., Ribeiro, A.O., Costa, J.M., Oliveira, A.C., Dias, M.A.D., Reis Neto, R.V., Hilsdorf, A.W.S. and Pinhal, D. Expression profiles of growth- related genes in two Nile tilapia strains and their crossbred provide insights into introgressive breeding effects. *Animal Genetics,* **51**(4), 611-616 (2020).
28. Blaxter, K.L. The energy metabolism of ruminants. 2nd ed. Charles Thomas Publisher. Spring field. Illinois, USA (1968).
29. MacRae, J.C. and Lobley, G.E. Some factors which influence thermal energy losses during the metabolism of ruminants. *Livestock Production Science,* **9**(4), 447-456 (1982).
30. Reitman, S. and Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, **28**(1), 56-63 (1957).
31. Ellefson, R.D. and Caraway, W.T. Fundamentals of clinical chemistry. Ed Tietz, N.W. Philadelphia, WB Saunders. p: 506 (1976).
32. Caraway, W.T. and Watts, N.B. Carbohydrates In: Fundamentals of clinical chemistry. 3ry ed. Edited by Tietz, N.W. Philadelphia, WB Saunders. p: 422-47 (1987).
33. Parry Jr, R.M., Chandan, R.C. and Shahani, K.M. A Rapid and Sensitive Assay of

Muramidase. Proceedings of the Society for Experimental Biology and Medicine. *Society for Experimental Biology and Medicine* (New York, N.Y.), **119**, 384-386 (1965).

34. Ellis, A.E. Inhibition of the Aeromonas salmonicida extracellular protease by α2-macroglobulin in the serum of rainbow trout. *Microbial Pathogenesis,* **3**(3), 167-177 (1987).

35. Lange, S., Guđmundsdottir, B.K. and Magnadottir, B. Humoral immune parameters of cultured atlantic halibut (Hippoglossushippoglossus L.). *Fish and Shellfish Immunology,* **11**(6), 523-535 (2001).

36. Cannon, D.C., Olitzky, I.G. and Inkepen, J.A. Proteins. In R.J. Henry, D.C. Cannon, and J. W. Winkelma (Eds.), In clinical chemistry-principles and techniques 2nd ed., Harper and Row Publishers. pp: 405-503 (1974).

37. Tietz, N.W. Clinical guide to laboratory tests. W. B. Saunders. pp: 1096 (1995)

38. Livak, K.J. and Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods,* **25**(4), 402-408 (2001).

39. Rentier-Delrue, F., Swennen, D., Philippart, J.C., L'hoir, C., Lion, M., Benrubi, O. and Martial, J.A. Tilapia growth hormone: molecular cloning of cDNA and expression in Escherichia coli. *DNA*, **8**(4), 271-278(1989).

40. Wang, B., Xu, Y., Liu, X., Liu, Q., Liu, Y., Zhang, Y. and Shi, B. Molecular characterization and expression profiles of insulin-like growth factors in yellowtail kingfish (*Seriola lalandi*) during embryonic development. *Fish Physiology and Biochemistry*, **45**, 375-390 (2019).

41. Monteiro, S.M., dos Santos, N.M., Calejo, M., Fontainhas-Fernandes, A. and Sousa, M. Copper toxicity in gills of the teleost fish, Oreochromis niloticus: effects in apoptosis induction and cell proliferation. *Aquatic Toxicology*, **94**(3), 219-228 (2009).

42. SPSS. Statistical Package for Social Science (Software version: 22.0) (2020). IBM. https://www.ibm.com/support/pages/spss-statistics-220-available-download

43. Duncan, D.B. Multiple range and multiple F tests. *Biometrics,* **11**, 1-42 (1955).

44. Heuer, O.E., Kruse, H., Grave, K., Collignon, P., Karunasagar, I. and Angulo, F.J. Human health consequences of use of antimicrobial agents in aquaculture. *Clinical Infectious Diseases,* **49**(8), 1248-1253 (2009).

45. Baba, E., Acar, Ü., Öntas, C., Kesbiç, O.S. and Yılmaz, S. Evaluation of Citrus limon peels essential oil on growth performance, immune response of Mozambique tilapia Oreochromis mossambicuschallenged with Edwardsiellatarda. *Aquaculture,* **465**, 13-18 (2016).

46. Aanyu, M., Betancor, M.B. and Monroig, O. Effects of dietary lemone and thymol on the growth and nutritional physiology of Nile tilapia (Oreochromis niloticus). *Aquaculture,* **488**, 217-226 (2018).

47. Al-Sagheer, A.A., Mahmoud, H.K., Reda, F.M., Mahgoub, S.A. and Ayyat, M.S. Supplementation of diets for Oreochromis niloticuswith essential oil extracts from lemongrass (Cymbopogon citratus) and geranium (Pelargonium graveolens) and effects on growth, intestinal microbiota, antioxidant and immune activities. *Aquaculture Nutrition,* **24** (3), 1006-1014 (2018).

48. Maniat, M., Ghotbeddin, N. and Ghatrami, E.R. Effect of garlic on growth performance and body composition of benni fish (*Mesopotamichthys sharpeyi*). *International Journal of Biosciences,* **5**, 269-277 (2014).

49. Takaoka, O., Ji, S.C., Ishimaru, K., Lee, S.W., Jeong, G.S., Biswas, A. and Takii, K. Dietary medicinal herbs and enzyme treated fish meal improve stress resistances and growth performance at early juvenile stage of red sea bream Pagrus major. *Aquaculture Research*, **47**, 390-397 (2016).

50. Ahmed, S.M., Omer, H.A.A. Badr, A.M., Bassuony, N.I. and Baker, A.A. Natural bioactive mixture composed of lemon, onion andgarlic juice for feeding rabbits. *International Journal of Chem Tech Research,* **9**(6), 121-130 (2016).

51. Badr, A.M.M., Bassuony, N.I., Easa, F.M. and Hanna, M.F.S. Effect of adding natural mixture juice composed of onion, garlic and lemon (NMJ) in filter drinking water on 1. Performance of growing rabbits. *Egyptian Journal of Rabbit Science,* **23** (1), 55-75 (2013).

52. Yang, C., Chowdhury, M.A.K., Hou, Y. and Gong, J. Phytogenic compounds as alternatives to in-feed antibiotics: Potentials and challenges in application. *Pathogens,* **4**, 137-156 (2015).

53. Habbak, M.M., Saleh, K., Arbid, M.S. Hegazi, A.G. and Sofy, H. Influence of garlic (*Allium Sativum* L.) on some biological and biochemical changes in Japanese quail with special reference to itshypocholesterolemic activity. *Archive-fur Gefluelkunde,* **53** (2), 73 (1989).

54. Dedi, H. and Elssenuwenger, T. The effect of phytogenic feed additives on performance and carcass quality of pigs. *International Pig Topics,* **15**(6), 11-13 (2000).

55. Ibrahiem, A.I., Adel, K.I., Talib, E.A., Fathi, F.M. and Awadalla, S.A. Hematological and immunological changes associated with feeding of onion and/or garlic in broiler Muscovy ducks. *Egyptian Journal of Animal Production,* **41**(2), 259-266 (2004).

56. Kim, K.T., Jeon, G.H., Cho, S.H., Lim, S.G., Kwon, M. and Yoo, J. Effects of dietary inclusion of various concentrations of ScutellariabaicalensisGeorgi extract on growth, body composition, serum chemistry and challenge test of far eastern catfish (*Silurusa sotus*). *Aquaculture Research*, **44**, 1502-1510 (2013).

57. El-Dakar, A.Y., Mouhmed, A.Y., Elgamal, A., Mohamed Abd ElBaky Amer, Shalaby, S.M. and

Abdel-Aziz, M.F. Effect of re-feeding regime under different stocking density of Nile tilapia, Oeochromisniloticuson growth performance, nutritional efficiency and fish body composition.Mediterranean *Aquaculture Journal,* **10**(1), 1-9 (2023).

58. Ali B.A. and El-Feky, A. Enhancing growth performance and feed utilization using prebiotics in commercial diets of Nile Tilapia (*Oreochromis niloticus*) fingerlings. *Egyptian Journal of Nutrition and Feeds,* **22** (1), 219-225 (2019).

59. Abo-State H.A., El-Monairy M.M., Hammouda Y.A. and Hassan H.M.A. Effect of dietary supplementation of manna oligosaccharide and β-glucan on the performance and feed utilization of Nile tilapia fingerlings. *Current Science International,* **10**(1), 226-233 (2021).

60. Rashidian, G., Bahrami Gorji, S., Farsani, M.N., Prokić, M.D. and Faggio, C. The oak (Quercus brantii) acorn as a growth promoter for rainbow trout (Oncorhynchus mykiss): Growth performance, body composition, liver enzymes activity and blood biochemical parameters. *Natural Product Research,* **34**(17), 2413-2423 (2020).

61. Hassaan, M.S., Mohammady, E.Y., Soaudy, M.R. and Abdel Rahman, A.A. Exogenous xylanase improves growth, protein digestibility and digestive enzymes activities in Nile tilapia, Oreochromis niloticus, fed different ratios of fish meal to sunflower meal. *Aquaculture Nutrition,* **25**(4), 841-853 (2019a).

62. Hassaan, M.S., Mohammady, E.Y., Soaudy, M.R., El-Garhy, H.A., Moustafa, M.M., Mohamed, S.A. and El-Haroun, E.R. Effect of *Silybum marianum* seeds as a feed additive on growth performance, serum biochemical indices, antioxidant status, and gene expression of Nile tilapia, *Oreochromis niloticus* (L.) fingerlings. *Aquaculture,* **509**, 178-187 (2019b).

63. Authman, M.M., Abbas, W.T., Abbas, H.H., Kenawy, A.M., Ibrahim, T.B., and Abd El-Hady, O.K. Ameliorative effect of the dietary Egyptian leek (*Allium ampeloprasum* L. var. Kurrat) on zinc toxicity of the African catfish *Clarias gariepinus* (Burchell, 1822). *Aquaculture Research,* **52**(11), 5656-5672 (2021).

64. Acar, Ü., Kesbiç, O.S., Yılmaz, S., Gültepe, N. and Türker, A. Evaluation of the effects of essential oil extracted from sweet orange peel (*Citrus sinensis*) on growth rate of tilapia (*Oreochromis mossambicus*) and possible disease resistance against *Streptococcus iniae*. *Aquaculture,* **437**, 282-286 (2015).

65. Youssef, M.K.E., Youssef, H.M.K.E. and Mousa, R.M.A. Evaluation of antihyperlipidemic activity of citrus peels powders fortified biscuits in albino induced hyperlipidemia. *Food Public Health,* **4**, 1-9 (2014).

66. Zhao, Y., Liu, J., Hao, W., Zhu, H., Liang, N., He, Z., Ma, K.Y. and Chen, Z.Y. Structure-specific effects of short-chain fatty acids on plasma cholesterol concentration in male Syrian hamsters. *Journal of Agricultural and Food Chemistry,* **65**(50), 10984-10992 (2017).

67. Hassaan, M.S. and Soltan M.A. Evaluation of essential oil of fennel and garlic separately or combined with Bacillus licheniformis on the growth, feeding behavior, hemato-biochemical indices of *Oreochromis niloticus* (L.) fry. *Journal of Aquaculture Research and Development,* 422-429 (2016).

68. Ngugi, C.C., Oyoo-Okoth, E. and Muchiri, M. Effects of dietary levels of essential oil (EO) extract from bitter lemon (Citrus limon) fruit peels on growth, biochemical, haemato-immunological parameters and disease resistance in Juvenile *Labeo victorianus* fingerlings challenged with Aeromonas hydrophila. *Aquaculture Research,* **48** (5), 2253-2265 (2017).

69. Omer, H.A.A., Ahmed, S.M, Bassuony, N.I. and Elalawy H.M.H. Response of growing rabbits to diets containing bioactive mixture composed of lemon, onion and garlic juice. *Advances in Environmental Biology,* **9**(24), 312-319 (2015).

70. Hassan, E.H. and Abdel-Raheem, S.M. Response of growing buffalo calves to dietary supplementation of caraway and garlic as natural additives. *World Applied Sciences Journal,* **22**(3), 408-414 (2013).

71. Abdelhamid, A.M., Refaey, M.M., Salem, M.F. and El-Kattan, M.A.M. Factors Affecting Fish Blood Profile: A-Effect of Nutritional Treatments. *Egyptian Journal of Aquatic Biology and Fisheries*, **23**(2), 517-526 (2019).

72. Younes, A.M., Gaafar, A.Y., Abu- Bryka, A.E.D.Z., Awad, E.S., Abo- Aziza, F., Abd El- Aziz, T.H., Darwish, D.A., Authman, M.M. and Abbas, W.T. Effects of Onion (Allium cepa) in diets of Oreochromis niloticus: Growth improvement, antioxidant, anti- inflammatory and disease resistance perspectives. *Aquaculture Research*, **52**(5), 2324-2334 (2021).

73. Esmaeili, N. Blood performance: a new formula for fish growth and health. *Biology*, **10**(12), 1236 (2021).

74. Chekani, R., Akrami, R., Ghiasvand, Z., Chitsaz, H. and Jorjani, S. Effect of dietary dehydrated lemon peel (*Citrus limon*) supplementation on growth, hemato-immunological and antioxidant status of rainbow trout (*Oncorhynchus mykiss*) under exposure to crowding stress. *Aquaculture,* **539**, 736597 (2021).

75. Harikrishnan, R., Thamizharasan, S., Devi, G., Van Doan, H., Kumar, T.T.A., Hoseinifar, S.H. and Balasundaram, C. Dried lemon peel enriched diet improves antioxidant activity, immune response and modulates immuno-antioxidant genes in *labeo rohita* against *aeromonas sorbia*. *Fish and Shellfish Immunology,* **106**, 675-684 (2020).

76. Akrami, R., Gharaei, A., Mansour, M.R. and Galeshi, A. Effects of dietary onion (*allium cepa*) powder on growth, innate immune response and hemato-biochemical parameters of beluga (*Huso huso* Linnaeus, 1754) juvenile. *Fish and Shellfish Immunology,* **45**(2), 828-834 (2015).

77. Younes, A.M., Gaafar, A.Y., Abubryka, A.E.Z., Awad, E.S., Aboaziza, F., Abd Elaziz, T.H., Darwish, D.A., Authman, M.M. and Abbas, W.T. Effects of onion (*Allium cepa*) in diets of *Oreochromis niloticus*: Growth improvement, antioxidant, anti-inflammatory and disease resistance perspectives. *Aquaculture Research,* **52**(5), 2324-2334 (2021).

78. Nya, E.J. and Austin, B. Use of garlic, *allium sativum*, to control *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (walbaum). *The Journal of Fish Diseases,* **32**(11), 963-970 (2009).

79. Ndong, D. and Fall, J. The effect of garlic (allium sativum) on growth and immune responses of hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*). *Journal of Clinical Immnunology and Immunopathology Research*, **3**(1), 1-9 (2011).

80. Talpur, A.D. and Ikhwanuddin, M. Dietary effects of garlic (*Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection in asian sea bass, *Lates calcarifer* (bloch). *Aquaculture,* **364**, 6-12 (2012).

81. Ghehdarijani, M.S., Hajimoradloo, A., Ghorbani, R. and Roohi, Z. The effects of garlic-supplemented diets on skin mucosal immune responses, stress resistance and growth performance of the caspian roach (*Rutilus rutilus*) fry. *Fish and Shellfish Immunology,* **49**, 79-83 (2016).

82. Jahanbakhshi, A., Pourmozaffar, S., Adeshina, I., Mahmoudi, R., Erfanifar, E. and Ajdari, A. Selenium nanoparticle and selenomethionine as feed additives: Effects on growth performance, hepatic enzymes' activity, mucosal immune parameters, liver histology, and appetite-related gene transcript in goldfish (*Carassius auratus*). *Fish Physiology and Biochemistry,* **47**, 639-652 (2021).

83. Nasirov, A.M., Rzayev, F.H., Seyidli, Y.M., Gasimov, E.K., Bunyatova, K.I., Ibrahimova, N.E. and Seyidbeyli, M.I. The Effect of ZnO Nanoparticles to Paradilepis Scolicina Rudolphi, 1819 (Cyclophyllidea: Dilepididae) Cestode Observed First in Common Carp (*Cyprinus Carpio L.,* 1758) in Azerbaijan. *Egyptian Journal of Veterinary Sciences,* **55**(1), 83-99 (2024).

84. Abu-Elala, N.M., Abd-Elsalam, R.M., Marouf, S., Abdelaziz, M. and Moustafa, M. Eutrophication, ammonia intoxication, and infectious diseases: interdisciplinary factors of mass mortalities in cultured Nile tilapia. *Journal of Aquatic Animal Health,* **28**(3), 187-198 (2016).

85. Gad, A.S., Khadrawy, Y.A., El-Nekeety, A.A., Mohamed, S.R., Hassan, N.S. and Abdel-Wahhab, M.A. Antioxidant activity and hepatoprotective effects of whey protein and Spirulina in rats. *Nutrition,* **27**(5), 582-589 (2011).

86. Eshak, E.S., Iso, H., Mizoue, T., Inoue, M., Noda, M. and Tsugane, S. Soft drink, 100% fruit juice, and vegetable juice intakes and risk of diabetes mellitus. *Clinical Nutrition,* **32**, 300-308 (2013).

87. Aboelhassan, D.M., Hafiz, N.A., Darwish, H.R., Shabana, M.E., Eshak,M.G., Hassanane, M.M., Farag, I.M. and Abdalla, A.M. Enhancing effects of *Moringa oleifera* leaf extract on carcinogenic Aflatoxin B1-induced genetic alterations, haematoxicity and histological changes in liver and kidney of rats. *Bioscience Research,* **15**(2), 814-833 (2018).

88. Teimouri, M., Yeganeh, S., Mianji, G.R., Najafi, M. and Mahjoub, S. The effect of *Spirulina platensis* meal on antioxidant gene expression, total antioxidant capacity, and lipid peroxidation of rainbow trout (*Oncorhynchus mykiss*). *Fish Physiology and Biochemistry,* **45**, 977-986 (2019).

89. Eshak, M.G., Hassanane, M.M., Farag, I.M., Shaffie, N.M. and Abdalla, A.M. Evaluation of Protective and Therapeutic Role of *Moringa oleifera* leaf extract on CCL4-induced genotoxicity, hemotoxicity and hepatotoxicity in rats. *International Journal of Pharm Tech Research,* **7**(2), 392-415 (2015).

90. Abozaid, H., Elnady, A.S., Aboelhassan, D.M., Mansour, H., Elmeged, A., Abedo, H.M., Ghaly, I.S., Radwan, H.A., Abbas, W.T. and Farag, I.M. Impact of Spirulina platensis as a Dietary Supplement on Growth Performance, Blood Biochemical Parameters, and Expression of Growth-Related Genes in Nile tilapia (*Oreochromis niloticus*). *Egyptian Journal of Veterinary Sciences,* **54**(6), 1265-1277 (2023).

91. Su, J., Peng, T., Bai, M., Bai, H., Li, H., Pan, H., He, H., Liu, H. and Wu, H. Transcriptome and metabolome analyses provide insights into the flavonoid accumulation in peels of *Citrus reticulata* 'Chachi'. *Molecules,* **27**(19), 6476 (2022).

92. Yousef, A.F., Moawad, A.A. and Abdou, M.S. Molecular Characterization Of Antimicrobial Resistance In Edwardsiella Tarda Isolated From Diseased Tilapia Fish In Egypt. *Egyptian Journal of Veterinary Sciences,* **54**(7), 21-32 (2023).

تأثير خليط نشط حيوياً من عصير الليمون والبصل والثوم على أداء النمو واستخدام العلف وتكوين الجسم والحالة المناعية والتعبير الجيني لسمك البلطي النيلي (Oreochromis niloticus).

هشام ابوزيد[1]، على سليمان محمد النادى[1]، هيام منصور[2]، داليا محمد ابوالحسن[2]، إلهام عوض[3]، ياسرأحمد عبدالفتاح النميرى[1]، حامدعبدالعزيز على عمر[1]، وفاء توفيق عباس[3] و ابراهيم محمد فرج[2]

[1] قسم الانتاج الحيوانى- معهد البحوث الزراعية والبيولوجية -المركز القومي للبحوث - الدقى- الجيزة- 12622- مصر.
[2] قسم بيولوجيا الخلية- معهد بحوث التقنيات الحيوية- المركز القومي للبحوث - الدقى- الجيزة- 12622- مصر.
[3] قسم الأحياء المائية - معهد البحوث البيطرية -المركز القومي للبحوث - الدقى- الجيزة- 12622- مصر.

أجريت هذه الدراسة لمعرفة تأثير خليط عصير الليمون والبصل والثوم (LOG) على صحة وإنتاجية البلطي النيلي (*Oreochromis niloticus*) بما في ذلك؛ أداء النمو، وإستخدام الأعلاف، وتكوين الجسم، والتعبير الجيني لهرمونات النمو والحالة المناعية. وبعد التأقلم، تم توزيع حوالي 120 سمكة بلطي نيلية (متوسط وزن الجسم الأولي 179 جم ± 0.96) بشكل عشوائي على 12 حوضًا. تم تقسيم أحواض السمك إلى أربع مجموعات (30 سمكة لكل منها) قبل إطعامها بنظام غذائي مختلف متساوي النيتروجين ومتساوي السعرات الحرارية، يحتوي على 0%، 0.5%، 1.0%، أو 1.5% من عصير LOG لمدة 56 يومًا. أظهرت النتائج تحسنا معنويا في أداء النمو وخاصة معدل النمو النوعي في جميع مجموعات عصير LOG مقارنة بالتحكم، مع عدم وجود وفيات مسجلة. كما أن استهلاك العلف، ونسبة التحويل الغذائي، وتناول البروتين الخام، ونسبة كفاءة البروتين زادت معنويا في مجموعات عصير LOG مقارنة بالسيطرة. علاوة على ذلك فقد تم تسجيل زيادة معنوية في نسب البروتين الخام في تركيب جسم السمكة. أظهر الاحتفاظ بالطاقة (ER)٪ والقيمة الإنتاجية للبروتين (PPV)٪ زيادات كبيرة في جميع المجموعات التجريبية. علاوة على ذلك، كانت مستويات AST والكوليسترول أعلى بشكل ملحوظ في مجموعة LOG بنسبة 1.5% مقارنة بالمجموعات الأخرى. أظهرت التحليلات المناعية، بما في ذلك نشاط الليزوزيم ونشاط مضاد الأنزيم البروتيني والبروتين الكلي، زيادة في المجموعات التي تم تغذيتها بـ 0.5% LOG و1% LOG مقارنةً بالتحكم (ولكن بدون اختلافات كبيرة) علاوة على ذلك، أدت التدخلات الغذائية إلى تنظيم كبير لثلاثة جينات مرتبطة بالنمو. و IGF1 و IGF2 و GH، مقارنة بالمجموعة الضابطة. ارتبطت مستويات التعبير الجيني بشكل إيجابي مع جرعة LOG، خاصة بالنسبة لـ IGF1 وGH، والتي أظهرت تعزيزًا أكبر من IGF2. توضح هذه الدراسة فعالية الخليط النشط بيولوجيا (LOG) في تحسين صحة البلطي النيلي؛ أداء النمو، والتعبيرات الجينية المرتبطة بالنمو والمناعة. أوصت نتائج الدراسة باستخدام LOG كمحفز للنمو ومنشط للمناعة، خاصة بجرعة 1.5% LOG (أي ما يعادل 15 مل من LOG / كجم علف)، والتي سجلت الإضافة المثالية.

الكلمات الدالة: البلطى النيلى، معدلات النمو، خصائص الدم ، الحالة المناعية ، التعبير الجينى