EFFECT OF CINNAMALDEHYDE AND YEAST ON GROWTH PERFORMANCE, FEED UTILIZATION AND ITS ANTIBACTERIAL ACTIVITY AGAINST FISH PATHOGENS OF NILE TILAPIA FINGERLINGS

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ABSTRACT

This experiment was conducted to study the effects of cinnamaldehyde and yeast with or without combination of optimal dose of phytase and citric acid on growth performance, feed utilization, body composition, economical evaluation and its antibacterial activity against fish pathogens of Nile tilapia fingerlings. Five experimental diets (Treatments) were composed of T1 as the control with no additives, T2 was supplemented with 1g/kg of Biotronic® Top3; T3 also supplemented with (1g/kg of Biotronic® Top3 in addition to 1000 FTU/Kg phytase and 30 g/kg citric acid). While T4 was supplemented with 4g/kg of Levabon® Aquagrow E; T5 also supplemented with (4g/kg of Levabon® Aquagrow E in addition to 1000 FTU/Kg phytase and 30 g/kg citric acid). The results indicated that the best growth performance and feed utilization of Nile tilapia fingerlings fed on T3 while, the lowest group fed on control. Antibacterial activity showed that the combination between Biotronic® Top3, citric acid and phytase had a potent activity against all tested isolates compared with antibiotics. It could be concluded that using combination of Biotronic® Top3 as cinnamaldehyde, phytase and citric acid was recommended to improve growth performance, feed utilization, body composition and the immune response of Nile tilapia fingerlings under these experimental conditions and could reduce the feed cost.

Keyword: Cinnamaldehyde, yeast, phytase, citric acid, Nile tilapia, fingerlings, growth performance, feed utilization, Antibacterial Activity.

INTRODUCTION

Cinnamaldehyde is component of Biotronic® Top3 and it is phytochemical derived from the essential oils of cinnamon (Michiels *et al.,* 2007) which can be found in the bark or leaves (Davidson *et al.,* 2013). Biotronic® Top3 has triple action for improved performance. A formula of synergistically acting organic acids (combination of formic, propionic and lactic acids), phytochemical (cinnamaldehyde) and Biomin® Permeabilizing Complex, a proprietary complex with a synergetic effect: it disrupts and permeabilizes the outer-membrane of Gram negative bacteria, increasing their sensitivity to the other compounds within the supplement mixture (Menanteau-Ledouble *et al.,* 2017). Biotronic® Top3 and these substances have been proven to have antimicrobial properties and to inhibit bacterial growth (Knarreborg *et al.,* 2002). Two main mechanisms of action have been suggested for this antimicrobial activity: it has been suggested that lowering of the pH hindered bacterial growth (Riemensperger *et al.,* 2012).

Cinnamaldehyde (CA) has antimicrobial effects, as it targets the FtsZ protein, which plays an important role in the cell division of pathogenic bacteria. The CA binds to the FtsZ, inhibits its assembly and perturbs the formation of the Z-ring thus inhibiting the process of cell division (Domadia *et al.,* 2007). Therefore, it is not surprising that research found a strong antibacterial effect of cinnamaldehyde on bacteria at low inclusion levels (Michiels *et al.,* 2007). However, even though natural replacements for AGPs are known, fighting Gram-negative bacteria is still a challenge. Cinnamaldehyde and several of its derivatives have been shown to have antibiotic (Li *et al.,* 2015; Nair *et al.,* 2014) as well as quorum quenching abilities (Niu *et al.,* 2006) and addition of cinnamaldehyde resulted in a significant reduction in the mortality of burbot larvae exposed to either *A. salmonicida* or *A. hydrophila* (Natrah *et al.,* 2012). Moreover, organic acids have been shown to cross the bacterial membrane in an unionized form before separating into H^+ and $HCOO^-$ ions inside the cytoplasm where they interfere

with protein synthesis (Roth and Kirchgessner, 1998). However, this subject has still to be thoroughly investigated (Ng and Koh, 2016).

The inclusion of yeast of genus *Saccharomyces* on feed improved the growth of several species of fish (Abdel-Tawwab *et al.*, 2008; Chiu *et al.*, 2010; Ahmad *et al.*, 2014, 2015; Samir *et al.*, 2017; El-Mousallamy *et al.*, 2015), and despite the fact that *Saccharomyces* yeast are able to produce phytase when the phytic acid is present (Nayini and Markakis, 1984) so farther are not reports of its use on high contents of soybean diets. For the example, Levabon® Aquagrow E is composed of autolyzed *Saccharomyces cerevisiae*, produced by an internal process technology for standardized autolytic degradation of the yeast cell. This yeast product is rich in bioactive ingredients and nutrients such as nucleotides, essential amino acids, peptides, cell wall carbohydrates and B vitamins which is well established as probiotic and prebiotic feed supplement (Martínez Cruz *et al.*, 2012) and is known to have an stimulatory effect on the immune system of fish and other organisms (Volman *et al.*, 2008).

Medicated feeds are used in fish diets to therapy of pathogenic bacteria and control the bacterial outbreaks which have a vital effect on development and sustainability of the aquaculture industry (Ranjan *et al.*, 2017). Biotronic® Top3 (Cinnamaldehyde) and Levabon® Aquagrow E (yeast) are commercially available and has been applied both in shrimp and fish farming settings. For this reason, it was decided to investigate the effect of Biotronic® Top3 and Levabon® Aquagrow E as a feed supplement in Nile tilapia yeast with or without combination of optimal dose of phytase and citric acid on growth performance feed utilization, body composition, economical evaluation and its antibacterial activity against fish pathogens of Nile tilapia fingerlings.

MATERIAL AND METHODS

Experimental tank and fish:

The present study was conducted at Fish Laboratory, Department of Animal Production and Fish Resources in Faculty of Agriculture, Suez Canal University, Ismailia- Egypt. Nile tilapia fingerlings (*Oreochromis niloticus*)

with an average initial body weight is (20.48 ±0.27g) were used in this experiment. The fish were obtained from Central Laboratory for Aquaculture Research, Abbaasa, Abu-Hamaad, Sharkia, Egypt. Fish were acclimated to laboratory conditions for 2 weeks prior the experimental study. After that, three hundred and seventy five fingerlings Nile tilapia were stocked in 15 V-shaped fiber tanks with capacity around 120 liter (25 fish/ tank/3 replicate each). Each fiber tanks was aerated by using small air-bumps. Settled fish wastes along with a half of fiber tanks water was siphoned daily, and replaced by well-aerated and dechlorinated tap water from a storage tank. Fish in each fiber tanks were weighted every 10 days throughout of experimental period (60 days). Dead fish were daily recorded and removed. At the end of the study, fish were individually weighed.

Water quality parameters:

The tanks were supplied with air blowers continuously aerated. Photoperiod was 12h light/ 12h dark regulate. The part of water tank was exchanged daily and totally with fresh water (dechlorinated tap water) every 10 days. Water temperature and dissolved oxygen were measured by mettler Toledo, model 128.s/No1242 instrument and recorded 27.2 ± 1°C and 5.3 ± 0.5 mg/l, respectively. pH was measured by Orion model 720A,s/no 13062 (7.5 ± 0.3) and ammonia was measured by Hanna ammonia meter (0.002±0.005 mg/l).

Experimental diet:

Five treatments diets were designed as: T1 as the control with no additives; T2 was supplemented with 1g/kg of Biotronic® Top3 (Biomin Australia Pty Ltd., Carlingford, Australia) is a combination of (formic, propionic and lactic acids alongside cinnamaldehyde) according recommended level (Biomin, 2017); T3 also supplemented with (1g/kg of Biotronic® Top3 in addition to 1000 FTU/Kg phytase and 30 g/kg citric acid). While T4 was supplemented with 4g/kg of Levabon® Aquagrow E (Biomin Australia Pty Ltd., Carlingford, Australia) composed of autolyzed (*Saccharomyces cerevisiae*) according to (Batista *et al.*, 2016); T5 also supplemented with (4g/kg of Levabon® Aquagrow E in addition to 1000 FTU/Kg

phytase and 30 g/kg citric acid). Phytase used was a 6-phytase (EC 3.1.3.26) obtained from *Buttiauxella sp*. expressed in *Trichoderma reesei* (Axtra PHY, Danisco Animal Nutrition, DuPont Industrial Biosciences, Marlborough, UK). Citric acid was obtained from (El Nasr Pharmaceutical Chemicals Company) in a powder form. Dose of combination of phytase and citric acid was chosen according to (Nehad *et al.*, 2019).

The dry ingredients of each diet were thoroughly mixed, and 100 ml of water was added per kg diet. Afterwards, the mixture (ingredients and water) was blended using a kitchen blender to make a paste of each diet. Diet ingredients were ground and thoroughly mixed and the oil was slowly added at the same time of mixing with warm water (45°C) until the diets began to clump. Dose of phytase enzyme was mixed first into warm water (Rachmawatia *et al.*, 2017) then added to feeds. Noodle-like feed pellets, which were then broken to make 2-mm die pellets, were prepared using kitchen mincer. The pellets were dried by the sun for 2 days with keeping Ventilation and flipping then stored in plastic bags when completed drying in a deep freezer at -2°C until use. The experimental diets were contained 30 % protein and gross energy 454.07 Kcal/100g. The ingredients and chemical composition of experimental diets are shown in Table 1. Fish were hand fed two times at 09:00 am and 02.30 pm per day to apparent satiation.

Table 1. Ingredients and chemical composition of experimental diets.

Ingredients (g/Kg)	Treatment no.				
	1	2	3	4	5
Fishmeal (60% CP)	50.0	50.0	50.0	50.0	50.0
Soybean meal (CP 47%)	480.0	480.0	480.0	480.0	480.0
Wheat bran	120.0	120.0	120.0	120.0	120.0
Rice bran	80.0	80.0	80.0	80.0	80.0
Yellow corn	220.0	219.0	188.9	216.0	185.9
Sunflower oil	30.0	30.0	30.0	30.0	30.0
Vitamin[1]	10.0	10.0	10.0	10.0	10.0
Mineral[2]	10.0	10.0	10.0	10.0	10.0
Biotronic® Top[3]	0.0	1.0	1.0	0.0	0.0
Levabon®Aquagrow E[4]	0.0	0.0	0.0	4.0	4.0
Phytase[5]	0.0	0.0	0.1	0.0	0.1
Citric Acid	0.0	0.0	30.0	0.0	30.0
Total	1000	1000	1000	1000	1000
Proximate chemical analysis (%)					
Dry matter	90.94	90.64	90.40	90.60	90.55
Crude protein (Cp)	30.05	30.30	30.25	30.30	30.35
Ether extract (EE %)	8.84	8.05	8.05	8.40	8.40
Total ash	7.03	7.50	7.63	7.42	7.41
Crude fiber (CF)	5.08	5.15	5.58	5.39	5.84
NFE[6]	48.73	49.00	48.49	48.49	48.00
P	0.84	0.80	0.83	0.83	0.80
Ca	0.55	0.52	0.54	0.50	0.53
Gross energy (GE) Kcal/ Kg[7]	454.07	449.14	446.75	450.34	448.61
P/E ratio (mg $kcal^{-1}$)	66.18	67.46	67.71	67.28	67.65

1. Each Kg vitamin premix contained Vitamin A, 4.8 million IU, D3, 0.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin,20 mg
2. Each Kg mineral premix contained Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.
3. Biotronic® Top3, Biomin Australia Pty Ltd., Carlingford, Australia
4. Levabon®Aquagrow E, Biomin Australia Pty Ltd., Carlingford, Australia
5. (Axtra® PHY, sourced from a Buttiauxella species bacterium and is expressed in a *Trichoderma reesei* fungus **(Danisco Animal Nutrition)**.
6. Nitrogen free extract=100-(protein+ lipid+ ash+ fiber)
7. Gross energy, Based on 5.65 Kcal/g protein, 9.45 Kcal/g fat and 4.12 carbohydrate Kcal/g **(NRC, 2011)**

Experimental Methodology:

Growth performance parameters.

Average Weight Gain (AWG) = Average final weight (g)–Average initial weight (g)

Weight gain (WG %) = 100 x (Final weight–initial weight)/initial weight

Specific Growth Rate (SGR %/day) = 100 [(Ln final weight – Ln initial weight)/ time]

Feed utilization parameters.

Feed Intake (FI) = Amount of consumed feed per period (g)

Feed Conversion Ratio (FCR) = Total feed consumption (g)/ weight gain (g)

Feed Efficiency Ratio (FER) = weight gain (g) / Total feed consumption (g)

Protein Efficiency Ratio (PER) = body weight gain (g)/ protein intake (g

Protein productive value (PPV%) = 100 (protein gain in fish (g) /protein intake in diet (g))

Survival Rate (SR %).

Survival Rate (SR %) = 100 × (final number of fish survived in tank/initial number of fish stocked in tank)

Chemical composition of diet and fish:

At the beginning and end of the experiment, 5 fish sample was taken randomly from each experimental group for chemical analysis of body composition. The experimental diets and whole body composition of fish samples were analyzed for moisture, crude protein (CP %), total lipid % and ash (%) except crude fiber (CF %) in experimental diets. The nitrogen free-extract (NFE %) was calculated by differences, by deducting the sum of percentages of CP%, EE%, CF % and ash% from 100. Gross energy (kcal/g DM,GE) contents of the experimental diets was calculated by using factors of 5.65, 9.45 and 4.12 kcal/g of protein, lipid

and carbohydrates, respectively (NRC, 2011). Mineral was estimated by measuring phosphorus (P) and calcium (Ca) in experimental diets and whole body. All chemical analyses were carried in three replicate according to (AOAC, 2019).

Antibacterial activity test:

Bacterial strains: *Aeromonas hydrophila, Pseudomonas fluorescens, Enterococcus faecalis, Vibrio alginolyticus and Proteus mirabilis* were used in this study, these bacteria were isolated from Nile tilapia fish and identified phenotypically and genotypically according to (Quinn *et al.* 2011). All tested strains were kindly obtained by Prof. Mohamed Enany, professor of fish microbiology, bacteriology department, faculty of veterinary medicine, SCU.

Assessment of antibacterial activity:

Powder samples of Biotronic® Top3 and Levabon® Aquagrow E with or without combination of phytase and citric acid (Dose of combination of phytase and citric acid was chosen according to (Nehad *et al.*, 2019), and 4 antibiotic (nalidixic acid (30 µg), erythromycin (15 µg), amoxicillin (25µg) and cefadroxil (30µg) were used because of its wide antibacterial spectrum and high potency, and most commonly used antibiotic against various diseases caused by Gram-negative and Gram-positive bacteria in fish farming. A powder samples of Biotronic® Top3 and Levabon® Aquagrow E with or without combination of phytase and citric acid were suspended in sterile distilled water with a powder/solvent ratio 1:1(w/v) (Bonilla and Sobral, 2017). Each tested materials were mixed well by vortex, then putted in water bath at 40 C for 4 hours (modified) (Choi *et al.*, 2016), then mixed well again by vortex (modified) (Menezes-Blackburn *et al.* 2015 and Nassan *et al.*, 2015) and the supernatant was collected. A prepared sterilized filter paper discs, 6 mm in diameter were thoroughly wet by immersing it in the obtained supernatant of tested samples. After incubation of the plates for 18 hours at 37°C the degree of sensitivity was calculated by measuring the clear zones of inhibition of growth produced by

diffusion of the antibacterial agent from the discs into the surrounding medium. The results were interpretated according to (CLSI, 2014).

Economical Evaluation:

The cost of feed required to produce a unit of fish biomass was estimated using economic evaluation (Samir *et al.*, 2017). The estimation was based on the local retail sale market price of all the dietary ingredients at the time of the study. These prices (in LE/kg) were as follows: 25 L.E for fish meal; 6.5 L.E for soybean meal; 3.0 L.E for wheat bran; 3.0 L.E for Rice bran; 4.0 L.E for yellow corn , 20 L.E for sunflower oil; 15 L.E for Vit; 15 L.E for Minerals; 104 L.E. for Biotronic® Top3, 175 L.E. for Levabon® Aquagrow E, 400 L.E. for phytase and 150 L.E. for citric acid.

Feed cost of kg: Calculated from the price of feed ingredient and the cost per kg gain (FCR ×price of kg feed).

Reduction % of feed cost of Kg gain was calculated as a percentage from the highest value.

Statistical analysis:

One way ANOVA according to Steel and Torrie (1981) was used to compare between groups for each variables. Tukey test was used as a post hoc to compare mean differences at significant level 0.05 or 0.01 (P value). A Computer program software CoStat version 6.311 was used to analysis the data of experiments.

RESULTS AND DISCUSSION

Table 2 showed that Growth performance, feed utilization and survival rate parameters of Nile tilapia fingerlings fed different experimental diets for 60 days. Data indicated that there was a significant difference ($p<0.05$) among the experimental groups. The highest significant difference ($p<0.05$) in final body weight, weight gain, weight gain%, specific growth rate, FCR, FER, PER and PPV parameters were observed on fish fed T3 in comparison to the control and the other treatments. While, there were no significant differences between T2

and T3 in FI and between T2 and T4 in PPV. Growth performance and feed utilization were improved due to the supplementation of the diets with the acidifier Biotronic Top3 which composed of three active additives as phytase and citric acid which contribute as bioactive on growth performance and feed utilization. Moreover, related to the presence of phytase enzyme supplementation which played a significant role in increasing growth performance. Phytase supplementation in ingredient-based diet may result in release of these chelated nutrients by hydrolysing the phytase and makes them available to the fish (Liu *et al.,* 2013). Also, Citric acid is the organic acids which have been reported to improve the growth and feed utilization of rainbow trout (Hernández *et al.* 2012). Similarly, several studies in tilapia suggested that organic acids might be protective against infections (Ng and Koh, 2016). However, this subject has still to be thoroughly investigated (Ng and Koh, 2016). Furthermore, Nehad *et al.,* (2019) who reported that there was significant differences in interaction between phytase and citric acid (1000 FTU/Kg+ 30 g/Kg) and recorded the highest growth performance and feed utilization parameters. This is in agreement with other studies on different species including Nile tilapia (Abo-State and El-Deen, 2017 and Amer *et al.,* 2018) and *L. rohita* (Bano and Afzal, 2017). In contrast, Menanteau-Ledouble *et al.* (2017) reported that rainbow trout fed on Biotronic® Top3 supplement showed an improvement in weight gain, final weight but not significant in WG, FW and FCR. This is may be due to difference between fish species or experimental conditions. Phytase supplementation significantly improved FI and FCR of *O. niloticus* fingerlings fed on a diet increasing the palatability and conversion rate of diet may be due to enhanced release of nutrients of diets by breaking down the bonds between phytase-protein and phytase minerals (Vielma *et al.,* 2004). Further phytase may chelate with amino acids in the stomach of different fish species and reduces the availability of amino acid (Usmani and Jafri, 2002). There was no significant difference ($p>0.05$) in survival rate among experimental fish groups. This is in agreement with

previous studies on different species including carp (Omar, 2017) and catfish (Setiawati *et al.*, 2016).

Also data in Table (2) showed that fish fed T2 diet had higher significant than T4 diet. While, fish fed on T4 had higher significant than the group of fish fed on T5. This is may be related to the beneficial effect on food digestion, increasing the pancreatic discharges, reducing the passage rate, and promoting changes in the intestinal microbiota (Petrolli *et al.*, 2012) and the chemical compounds that compose it, such as the active ingredient cinnamaldehyde, which is responsible for the smell, as well as the antioxidant, antimicrobial, and antifungal activities (Singh *et al.*, 2007). This is in agreement with other studies on different species including (Ávila *et al.*, 2015) who reported that addition of phytase and yeast in rainbow trout diets with high content of soybean meal for first time. This is may be due to yeast and yeast with phytase might be related to a better nutrient digestibility, particularly the protein fraction (Lara-Flores *et al*., 2003; Waché *et al*., 2006 and Ávila *et al.* 2015).

Table 2. Growth performance, feed utilization and survival rate parameters of Nile tilapia fingerlings fed different experimental diets for 60 days.

T	IBW (g)	FBW (g)	W. Gain (g)	W. Gain (%)	SGR (% g/day)	FI (g feed/fish)	FCR	FER	PER	PPV	SR %
1	20.75 ± 0.01	41.13 ± 0.30^{d}	20.38 ± 0.29^{d}	98.21 ± 1.37^{d}	1.14 ± 0.01^{d}	41.97 ± 0.60^{d}	2.06 ± 0.00^{a}	0.49 ± 0.00^{e}	1.62 ± 0.00^{e}	33.85 ± 0.02^{bc}	90 ± 0.05
2	20.64 ± 0.35	49.85 ± 1.30^{b}	29.21 ± 1.65^{b}	141.52 ± 10.37^{b}	1.47 ± 0.07^{b}	51.46 ± 2.18^{ab}	1.76 ± 0.02^{d}	0.57 ± 0.01^{b}	1.87 ± 0.03^{b}	31.06 ± 0.02^{c}	96 ± 0.02
3	20.41 ± 0.17	54.93 ± 0.45^{a}	34.52 ± 0.28^{a}	169.13 ± 0.01^{a}	1.65 ± 0.00^{a}	54.81 ± 0.74^{a}	1.59 ± 0.01^{e}	0.63 ± 0.00^{a}	2.08 ± 0.01^{a}	39.78 ± 0.04^{a}	97 ± 0.01
4	20.29 ± 0.15	46.54 ± 0.02^{c}	26.25 ± 0.12^{bc}	129.37 ± 1.55^{bc}	1.38 ± 0.01^{bc}	48.35 ± 0.18^{bc}	1.84 ± 0.02^{c}	0.54 ± 0.00^{c}	1.79 ± 0.02^{c}	30.74± 0.02^{c}	96 ± 0.04
5	20.21 ± 0.20	44.21 ± 0.66^{c}	24.00 ± 0.86^{c}	118.75 ± 5.39^{c}	1.30 ± 0.04^{c}	46.68 ± 1.78^{c}	1.94 ± 0.00^{b}	0.51 ± 0.00^{d}	1.69 ± 0.00^{d}	34.86 ± 0.05^{b}	93 ± 0.01
P value	0.47	0.00 **	0.00 **	0.00 **	0.00 **	0.00 **	0.0002 **	0.00 **	0.00**	0.00**	0.20

**Significant p-value ≤0.05, **highly significant p-value ≤0.01, using ANOVA test.*
Different letters at the same column mean significant different

Data in Table 3 showed Chemical composition of whole body (% dry matter basis) of Nile tilapia fingerlings fed different experimental diets for 60 days. There were a significant difference (p<0.05) among all experimental treatments. It was observed that there were no significant differences (P>0.05) in body moisture content among the experimental groups. The greatest improvements in body CP, ash and Ca contents were significantly (p<0.05) enhanced in fish fed the diet containing T3. While, the lowest CP and Ca contents were observed in fish fed T1. Also, there was no significant differences (p<0.05) in P content among the experimental groups while, it was improved and increased in fish group fed on T3. The group of fish fed on T 3 had highest significant lipid content in comparison to the control and the other treatments. Theses improvement may be related to that T3 composed of the three active additives as Biotronic® Top3, phytase and citric acid which contribute as bioactive compound which phytase supplementation positively affected chemical composition of body. On the other hand, citric acids can contribute in nutritional ways, because they are components in several metabolic pathways for energy generation (da Silva *et al.,* 2012). These results suggested that, High level of Ca in fish feed will chelate with phytase forming an insoluble complex or complete with phytase for the binding site at the myoinositol ring and thus block the site of phytase mediated substrate hydrolysis (Qian *et al.,* 1996). These results showed that Biotronic® Top3 supplementation plays a role in enhancing feed intake with a subsequent enhancement of fish body composition. Moreover, due to the high feed intake, nutrients utilization, and digestibility, the high changes in protein and lipid content in fish body could be linked with changes in their synthesis and deposition rate in muscles. This is in agreement with other studies on different species (Loh *et al.,* 2008). Also, Nehad *et al.,* (2019) who reported that there was significant differences in interaction between phytase and citric acid (1000 FTU/Kg+ 30 g/Kg) and recorded the highest in crude protein, ash, P and Ca of fish bodies increased and total lipid was decreased significantly (p<0.05). Also, they were observed that there were no significant differences (P>0.05) in body

moisture content. In contrast, Abo-State and El-Deen (2017) who found that there were no significant difference (p>0.05) noticed among all treatments in dry matter (DM), crude protein (CP), lipid, and ash content of body composition of Nile tilapia. Amer *et al.* (2018) reported that that the body proximate composition of Nile tilapia was not significantly different (P > 0.05) in moisture, crude protein, lipid and ash contents of the whole body of fish among the control group and other groups**.** This is may be due to different experimental conditions or experimental diets contained to Biotronic® Top3, phytase and citric acids. This inconsistency in the outcome of different authors may be attributed to differences in feed ingredients, nutritional quality of ingredient, water quality, fish spices and size and culture or experimental conditions.

Table 3. Chemical composition of whole body (% dry matter basis) of Nile tilapia fingerlings fed different experimental diets for 60 days.

T	Moisture (%)		Crude protein (CP %)	Total lipids (%)	Ash (%)	P	Ca
Initial	73.40 ± 0.03	%on the DM basis	55.09 ± 0.11	23.88 ± 0.65	21.03 ± 0.42	1.40 ± 0.23	0.57 ± 0.10
1	71.26 ± 0.05		58.55 ± 0.35^{d}	19.26 ± 0.41^{a}	22.19 ± 0.05^{b}	1.57 ± 0.09^{b}	0.47 ±0.01^{e}
2	76.14 ± 0.02		62.35 ± 0.35^{b}	20.00 ± 0.90^{a}	17.65 ± 0.05^{c}	1.72 ± 0.17^{ab}	0.76 ±0.01^{c}
3	75.05 ± 0.04		65.35 ± 0.05^{a}	10.55 ± 0.05^{c}	24.10 ± 0.10^{a}	2.13 ± 0.08^{a}	1.00 ±0.02^{a}
4	75.58 ± 0.01		62.10 ± 0.70^{b}	19.80 ± 0.10^{a}	18.10 ± 0.80^{c}	1.68 ± 0.17^{ab}	0.70 ±0.01^{d}
5	72.01 ± 0.02		60.10 ± 0.30^{c}	17.60 ± 0.40^{b}	22.30 ± 0.10^{b}	1.85 ±0.04a^{b}	0.82 ±0.02^{b}
P Value	0.21		0.00 **	0.00 **	0.00 **	0.19	0.00 **

**Significant p-value ≤0.05, **highly significant p-value ≤0.01, using ANOVA test.*
Different letters at the same column mean significant different

Results in Table 4 showed that there were different diameters of inhibition zone (mm) as antibacterial activity against different isolated bacteria from fish. Biotronic® Top3 had a wide antibacterial activity against all tested bacteria except *Vibrio alginolyticus*. The highest inhibition zone was recorded

in *Pseudomonas fluorescens* then *Entercoccus faecalis* and *Aeromonas hydrophila* followed by *Proteus mirabilis*. This is in agreement with previous studies on different species including shrimp (Mercy and Gopalakannan, 2018), Nile tilapia (Abo-State and El-Deen, 2017) and rainbow trout (Menanteau-Ledouble *et al.*, 2017).

Biotronic® Top3, phytase and citric acid had antibacterial activity against all tested bacteria. The highest inhibition zone in *Pseudomonas fluorescens* and *Entercoccus faecalis* then *Aeromonas hydrophila* and *Vibrio alginolyticus* followed by *Proteus mirabilis* was recorded. This is may be due to role of these additives as Biotronic® Top3, phytase and citric acid which can effect on fish pathogen. (He *et al.*, 2017) concluded that the tested organic acids and essential oils mixture beneficially affects intestinal microflora and improves immune response and disease resistance of *L. vannamei*.

Levabon®Aquagrow E did not have antibacterial activity against tested bacteria except *Vibrio alginolyticus* that recorded mild inhibition zone. Abdelhamid and El-Barbary (2013) reported that Bio-Mos® showed sensitivity against *Bacillus sp.*, *Enterobacter sp.*, *Klebsiella* and *Staphylococcus epidermidis*. In contrast, European seabass (Abdelmalek *et al.*, 2015) and rainbow trout (Huyben *et al.*, 2017). Prebiotics selectively stimulate growth of beneficial bacteria and these may compete for adhesion sites with pathogenic bacteria, hence excluding them (Pérez-Sánchez *et al.*, 2014). Levabon®Aquagrow E, phytase and citric acid had antibacterial activity against tested bacteria except *Vibrio alginolyticus* and *Proteus mirabilis*. It was recorded the highest inhibition zone in *Pseudomonas fluorescens* then *Aeromonas hydrophila* followed by *Entercoccus faecalis* while *Vibrio alginolyticus* and *Proteus mirabilis* were highly resistance. Goda *et al*. (2018) concluded that addition of Garlen®; Diamond V XPC®, and Bactozyme® individually or mixed alternately as immune stimulants in early weaning larval diets of European sea bass under hatchery conditions led to decrease intestinal bacterial load. The results revealed that all tested antibiotics (Amoxicillin,

Erythromycin and Cefadroxil) were detected resistance against *Aeromonas hydrophila, Pseudomonas fluorescens, Vibrio alginolyticus, Proteus mirabilis* and *Enterococcus faecalis.* While Nalidixic acid had antibacterial sensitivity to all tested bacteria but resistance against *Proteus mirabilis.*

Table 4. Inhibition zone diameter of (mm) of antibacterial activity against different isolated bacteria from fish.

	Aeromonas hydrophila	*Pseudomonas fluorescens*	*Vibrio alginolyticus*	*Proteus mirabilis*	*Entercoccus faecalis*
Biotronic® top3	1.8	2.8	R	1.3	1.8
Biotronic® top3 + Citric acid + Phytase	1.8	2	1.8	1.4	2
Levabon®Aquagrow E	R	R	0.8	R	R
Levabon®Aquagrow E + Citric acid + Phytase	1	1.1	R	R	0.8
Nalidixic acid	1.3	1.4	1.9	R	2.4
Amoxicillin	R	R	R	R	R
Erythromycin	R	R	R	R	R
Cefadroxil	R	R	R	R	R

R, Resistance

Economic Evaluation:

Table 5 showed that the economic evaluation of experimental diets used in the study. The hieghest feed cost to produce one kg fish gain was recorded in group of fish fed on T5. It was found that the group of fish fed T3 had the lowest reduction in feed cost percent by 78.01% . The Biomin perforizing substance in Biotronic Top3 is a substance unique in acidifier products on the global acidifier market and a “revolution” as it presents a different strategy to act against bacteria. The synergisms caused by the inclusion of the Biomin perforizing substance, allows a reduction in inclusion level, resulting in economical benefits for the end experiment.

Table 5. Economic Evaluation of experimental diets used in the study.

T	Price/kg feed P.T	FCR	Feed cost/kg gain P.T	Reduction% of feed cost of Kg gain
1	7.05	2.06	14.52	61.59
2	7.15	1.76	12.58	53.37
3	11.57	1.59	18.40	78.01
4	7.73	1.84	14.23	60.35
5	12.15	1.94	23.58	100.00

CONCLUSION

It concluded that T3 the combination of dietary (1g Biotronic® Top3+ 1000 FTU/kg phytase+ 30g citric acid) was the best in term of growth performance, feed utilization and antibacterial activity against Nile tilapia fingerlings pathogens under these experimental conditions and could be recommended to reduce the feed cost.

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تأثير السينمالدهيد والخميرة على النمو وكفاءة الاستفادة الغذائية ونشاطهما ضد البكتريا الممرضة في اصبعيات البلطى النيلى

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الملخـــــص العربـــــي

اجريت هذه التجربة لدراسة تأثير السينمالدهيد والخميرة مع او بدون المعدل الامثل من انزيم الفيتيز وحمض الستريك على أداء النمو وكفاءة الاستفادة الغذائية وتحليل مكونات الجسم والتقييم الاقتصادى ونشاطهما ضد البكتريا الممرضة في اصبعيات البلطى النيلى. تم تكوين خمس علائق تجريبية (معاملات): المعاملة الاولى (كنترول) بدون اضافات، تم اضافة منتج Biotronic® Top3 بمعدل ١جم/كجم للمعاملة الثانية وكذلك اضيف الى المعامله الثالثة (منتج Biotronic® Top3 بمعدل ١جم/كجم واضافة انزيم الفيتيز بمعدل ١٠٠٠ وحدة/كجم و٣٠جم/كجم من حمض الستريك) ؛ كما تم اضافة منتج Levabon® Aquagrow E الى المعاملة الرابعة بمعدل ٤جم/كجم ؛ وكذلك اضيف الى المعاملة الخامسة (منتج Levabon® Aquagrow E بمعدل ٤جم/كجم و انزيم الفيتيز بمعدل ١٠٠٠ وحدة/كجم و٣٠جم/كجم من حمض الستريك).

اظهرت النتائج تفوق المعامله الثالثه فقد اظهرت تحسن معنوي ملحوظ علي معدلات النمو والاستفاده الغذائيه واظهر اختبار النشاط ضد البكتريا الممرضة ان مخلوط منتج Biotronic® Top3 وحمض الستريك مع انزيم الفيتيز ذو نشاط قوى ضد البكتريا المختبرة مقارنة بالمضادات الحيوية. ويتستنتج من ذلك ان استخدام مخلوط منتج Biotronic ® Top3 وحمض الستريك مع انزيم الفيتيز هو الموصى به لقدرته على تحسين نمو الاسماك وذلك عن طريق تحسين الاستفاده من العلف ومحتوى الجسم وبالتالى رفع من مناعه اصبعيات اسماك البلطى النيلي تحت هذه الظروف التجريبية وبذلك امكنه تقليل تكلفه الغذاء.