Effect Of Natural Growth Promoters In Comparison With Antibiotic In Nile Tilapia diets On Growth Performance, Nutrient Digestibility And Immune Status

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ABSTRACT

The present work was carried out to compare the effect of addition of different natural growth promoters (NGPs) (Essential oils, organic acids, and symbiotic) or antibiotic in the diets of Tilapia Nilotica fingerlings, concering their effects on growth performance, body composition, nutrient digestibility, economic efficiency and immune status. Four hundred eighty Nile tilapia fingerlings with an average body weight, 24.44 ± 0.05 g were used. The fish were randomly divided into 8 equal triplicate groups (each replicate contained 20 fish) and kept in glass aquaria for two weeks to be acclimatized before the start of the experiment. A basal control diet was formulated to contain 30.80% CP and 2940 kcal/kg DE.. For digestibility trials, 0.5% chromic oxide (Cr₂O₃) was included in the diets. Results revealed that the dietary treatments had a highly significant effect (P<0.05) on growth performance parameters. The fish fed diets contained natural growth promoters had a significant higher final body weight, body gain, and body gain % as compared to fish fed other dietary treatments. Moreover, fish fed diets contained natural growth promoters had consumed a significant higher amount of feed as compared to those fed the control or antibiotic supplement diet. Fish fed diets contained natural growth promoters had utilized their feed more efficiently (lower values of FCR) than those fed the control or antibiotics containing diet. Significant higher values of protein utilization parameters were recorded for fish fed diets contained natural growth promoters compared to fish fed the control or antibiotic additive diets. Numerical higher values of dry matter, crude protein, fat and crude fiber digestion coefficient were recorded for fish fed diets contained natural growth promoters. Immunological parameters used showed significant improvement due to supplementation of the diets with natural growth promoters, it could be inferred that supplementation of Nile tilapia fingerlings diets with natural growth promoters had improved growth performance, nutrient digestibility, economic efficiency and immune status of fish as compared to antibiotic supplementation.

INTRODUCTION

Nutrition plays an important role in intensive fish production depending upon the type of feed availability and its cost. Feeding cost exceeds 50% of operation costs (1). Antibiotics as growth promoter feed additives have been banned world wide for some years. The full ban of antibacterial growth promoters might seriously affect post-weaning health, performance and health of peoples who consume that meat or fish containing residues of antibiotics (2). This will necessitate the development of new feeding and health care strategies. Therefore, there is a strong need for natural growth promoters such as probiotics, prebiotics, organic acids as well as herb extracts (essential oils) (3). These feed additives have to

be an efficient, safe, and do not harm the alleviate problems environment (4). То associated with antibiotic resistance, a variety of substances are used as alternatives to antibiotics in animal diets, such as probiotics, prebiotics, organic acids, and plant extracts (essential oils) which used as growth enhancer and immune stimulant in animal diets. These alternatives are necessary because federal regulations prohibit the use of conventional antibiotics and growth promoters in organic production (5). Formic, acetic, and propionic acids reduce the prevalence of Salmonella and Campylobacter bacteria found in the intestines of broilers (6,7).

The present study was aimed to evaluate the effect of addition of different natural growth promoters (NGPs), essential oils, organic acids,

and symbiotic, compared with antibiotic in the diets of Tilapia Nilotica fingerlings and regarding their effects on growth performance, nutrient digestibility, economic efficiency and immune status.

MATERIALS AND METHODS

Experimental fish used

A total of 480 Nile tilapia Fish (Oreochromis niloticus) were procured from private fish farm, Abbassa village, Abu-Hammad district, Sharkia Governorate, Egypt. The fish were nearly of the same size, apparently health and had an average weight of 24.44±0.05 g. The fish were divided into equal 8 triplicate groups (each replicate contained 20 fish) and kept in glass aquaria (30 x 40 x 150 cm) for two weeks to be acclimatized before the start of the experiment. The fish were fed isonitrogenous, isocaloric diet containing different levels of a premixture for each of the additives for 12 weeks. They were supplied with dechlorinated water and air supplied by a small air compressor. Water temperature, dissolved oxygen, PH, ammonium (NH4), nitritev (NO₂), and nitrate (NO₃) were measured three times during the experiment and found to be 28-32°C±2°C, 5.2mg/L, 7.5, 0.4 mg/L, 0.15 mg/L, 10 mg /L Respectively.

The feed additives used

- 1. Essential oils blend contains 0.1 oregano (*Origanum vulgare*), Biomin Holding GmbH, Industriestrasse 21, A-3130 Herzogenburg, Austria.
- 2. Essential oils blend 2: Contains 0.15 oregano (Origanum vulgare), Biomin Holding GmbH, Industriestrasse 21, A-3130 Herzogenburg, Austria.
- 3. Organic acids 1: Contains 6% Formic acid, 1.5% Propionic acid, Biomin Holding GmbH, Industriestrasse 21, A-3130 Herzogenburg, Austria.
- 4. Organic acids 2: Contains 8% Formic acid, 2.5% Propionic acid, Biomin Holding GmbH, Industriestrasse 21, A-3130 Herzogenburg, Austria.
- 5. Synbiotic 1: Contains 20% inactivated yeast (Saccharomyces Cervisiae), 5% inulin, Biomin Holding GmbH, Industriestrasse 21, A-3130 Herzogenburg, Austria.

- 6. Synbiotic 2: Contains 25% inactivated yeast (Saccharomyces Cervisiae), 5% inulin, Biomin Holding GmbH, Industriestrasse 21, A-3130 Herzogenburg, Austria.
- **7-Antibiotic:** Oxytetracycline, "antibiotic" 20 %. Unipharma, universal industrial pharmaceutical Co. El obour City, Cairo, Egypt.

Diets and feeding:-Eight isonitrogenous, isocaloric diet were formulated (8). Proximate chemical composition of feedstuffs used in formulation of the experimental diets is shown in Table 1. All fish groups were fed on the diets at the rate of 3% from body weight during the experiment.

Growth performance parameters

The fish of each group were weighed at the beginning of the experiment and every two weeks intervals (10,11). Weight gain gram and percent (12), Specific growth rate % (13) and feed conversion ratio (FCR) (9) were calculated.

Protein efficiency ratio (PER) (14), Protein retention and Protein productive value (PPV) (13), Condition factor (K) (15) were calculated.

Immune status: Total leukocytic count (TLC) and differential leukocytic count were determined (16,17). Phagocytic % (Number of Neutrophils containing C.albicans / Total number of counted Neutrophils) were determined. Phagocytic index (total number of C. albicans in 100 Neutrophils / 100).

For determination of the apparent digestibility of the diets, chromic oxide (0.5%) was added as a digestibility indicator (18). Chromic oxide content in faeces and in experimental diets was determined (19). Dry matter and nutrients digestibility were calculated (8).

Statistical analysis : The obtained data were statistically analyzed (20) for variance ANOVA, LSD (Least significant difference). Differences among treatment means were compared using Duncan's multiple range tests (21).

Table 1. Physical and chemical composition of the diets.

Ingredient	%					
Yellow corn	35.00					
Wheat flour	10.00					
Soybean meal	18.00					
Fish meal	16.00					
Poultry by-product meal	14.00					
Vegetable oil	5.50					
Vitamins and Minerals mixture*	1.50					
Calculated composition						
DM, %	89.60					
CP, %	30.80					
EE, %	10.26					
CF, %	2.42					
NFE, %	39.00					
Ash, %	7.12					
DE, Kcal/ kg diet**	2940					

*Vitamin and Mineral mixture (alfakema): - Each 1 kg contains: - Vit. A 580000 I.U, vit. D3 8600 I.U, vit. E. 720mg, vit. K3 142mg, vit C 0.1mg, vit B1 58mg, vit B2 34mg, vit. B6 34mg, vit.B12 58mg, Folic acid 86 mg, Pantothenic acid 8mg, Manganese sulfate 65mg, Zinc methionine 3000mg, Iron sulfate 2000mg, Copper sulfate 3400mg, Cobalt sulfate 572mg, Sodium selenite 25mg, Calcium iodide 25mg, Calcium carbonate (Carrier substance) till 1000gm.** digestible energy calculation based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm, NFE 2.5 kcal/gm (9).

RESULTS AND DISCUSSION

Growth performance parameters

The effect of dietary treatments on growth performance of Nile tilapia fingerlings is present in Table 2. Fish groups fed on diet contained organic acids 2 and organic acids 1 achieved the best significant final average body weight (47.07 g and 45.70 g) respectively followed by fish groups fed on diet contained synbiotic 2 (44.80 g), essential oils blend 2 (43.60 g), essential oils blend 1 (43.37 g), and synbiotic 1 (42.32 g), while the lowest values were obtained in fish group fed on diet contained antibiotic (oxytetracycline) (39.08 g) and control group (37.48 g). Concerning the body gain and body gain percent followed a similar trend; Oregano essential oils had positive effects on the growth performance of hybrid catfish (Clarias spp.) (22). The use of organic acid salts or acid blends is an interesting option to promote the performance of a wide variety of aquaculture species worldwide (23). Also, the use of potassium-diformate as organic acid improved the growth performance and feed utilization in Nile tilapia (24).

The results of FCR values were better in fish groups fed on diet contained natural growth promoters in comparison with control and antibiotic (oxytetracycline) groups. The addition of organic acids to the diets improved feed utilization and feed conversion ratio in different fish species (24-26). Also, the evaluation of organic acid blend compared with antibiotic in rainbow trout revealed that feed conversion ratio tended to be lower with increasing the dosages of the organic acid blend compared to the antibiotic group (27).

Protein utilization: The protein efficiency ration (PER), protein retention and productive value (PPV) at the end of the study is showed in Table 2. There were significant differences (P<0.05) between different fish groups, where the Fish groups fed on diet contained organic acids 2 and organic acids 1 gave higher values followed by fish groups fed on diet contained synbiotic 2, essential oils blend 2, synbiotic 1 and essential oils blend 1, while the lowest values were obtained in fish group fed on diet contained antibiotic (oxytetracycline) and control group. Addition of organic acids to the diets improved protein utilization in different fish species (24-26). Also, organic acid

Prot. Eff. ratio

Prot.retention

Prot.Prod.Value

improved the protein utilization in Nile tilapia compared with antibiotic (27).

Condition factor (K): The results of condition factor (k) at the end of the experiment are present in Table 2. There were significant differences (P<0.05) between fish groups. The

highest values were recorded in fish group fed on diet contained natural growth promoters in comparison with control and antibiotic (oxytetracycline) groups. The high condition factor is an indicator to the good nutritional (healthy) state of fish (15).

Items	Experimental diet									
	Control	Antibiotic	Essential oils blend 1	Essential oils blend 2	Organic acids 1	Organic acids 2	Synbiotic 1	Synbiotic 2		
Initial B.Wt.(g)	24.47±0.17	24.35±0.09	24.40±0.09	 24.43±0.04	24.55±0.10	24.40±0.30	24.42±0.19	24.52±0.25		
Final B.Wt. (g)	37.48±0.15 ^g	39.08±0.12 ^f	43.37±0.43de	43.60±0.34 ^{cd}	45.70±0.46 ^b	47.07±0.27ª	42.32±0.34°	44.80±0.76 ^{bc}		
Body gain (g)	13.01±0.05	14.73±0.13 ^e	18.97±0.44 ^{cd}	19.17±0.35 ^{cd}	21.15±0.52 ^b	22.67±0.04ª	17.90±0.51 ^d	20.28±0.82 [∞]		
Body gain %	53.18±0.51 ^f	60.51±0.66°	77.76±1.91 ^{cd}	¹ 78.45±1.46 ^{cd}	86.15±2.36 ^b	92.95±1.24ª	73.35±2.62 ^d	82.76±3.72 ^{bc}		
Sp. gr. rate %	0.52±0.003	0.58±0.01°	0.71±0.01 ^{cd}	0.71±0.01 ^{cd}	0.76±0.02 ^b	0.80±0.01ª	0.67 ± 0.02^{d}	0.74±0.02 [∞]		
Feed intake (g)	42.35±0.56*	38.29±0.53°	41.39±0.46ª	38.14±0.27°	42.58±0.19ª	39.89±0.55 ^b	36.20±0.65d	38.86±0.11 ^{bc}		
FCR	3.29±0.06ª	2.60±0.02 ^b	2.18±0.07 ^c	1.99±0.03 ^d	2.02±0.05 ^d	1,76±0.03 ^f	2.02±0.03 ^d	1.92±0.07 ^d		
Cond.Factor(K)	$1.43 \pm 0.02^{\circ}$	1.50±0.03 ^{bc}	1.69±0.07 [*]	1.66±0.07*	1.63±0.03 ^{ab}	1.66±0.05*	1.59±0.02 ^{ab}	1.65±0.05 ^{ab}		

Table 2. Effect of experimental diets on allover growth performance of fish.

6.03±0.60° abcdef Means within the same row with different superscripts are significantly different (P < 0.05).

1.49±0.05°

0.77±0.07°

1.63±0.02^b 1.61±0.04^b

Immunological parameters: Table 3 shows the results of immunological parameters in different fish groups. There were significant differences (P<0.05) between fish groups. The fish groups fed on diet contained natural growth promoters had a positive effect on Total leukocytic count leukocytic count differential (TLC), and (phagocytic% phagocytic activity and phagocytic index) in comparison with control and antibiotic (oxytetracycline) groups. The use of organic acid salts or acid blends is an interesting option to promote immune response of a wide variety of aquaculture species worldwide (26,27). Also, the use of potassiumdiformate as a non-antibiotic growth promoter improved the immune status in Nile tilapia (24).

1.00±0.01° 1.25±0.01^d

0.50±0.04^d 0.55±0.06^d

3.81±0.28^d 4.68±0.49^d

Digestibility of the diets: The results in Table 4 showed that the highest digestibility coefficient of DM, CP, Fat and CF were recorded in fish groups fed on diets contained organic acid 2 91.30%, 96.31% and 88.91%) (79.20%, respectively followed by synbiotic 2 (78.33%, 90.91%, 96.22% and 88.18%) organic acid 1(78.09%, 90.88%, 96.05% and 88.05%), synbiotic 1 (76.79%, 90.24%, 95.93% and 87.15%), while the lowest digestibility coefficient of DM, CP, fat and CF were observed in antibiotic group (73.50%, 86.92%, and 84.67%) and control group 94.32% 93.44% and 82.84%). (71.35%, 84.28%, Organic acid blend improved nutrient utilization and digestibility of nutrients in Nile Tilapia (23,28). Also, essential oils blend improved nutrient utilization and digestibility of nutrients in aquaculture (22, 29).

0.82±0.06° 1.12±0.03° 1.37±0.01° 0.74±0.06° 0.87±0.05°

6.98±0.47° 8.57±0.28^b 11.18±0.20^a 6.67±0.57^c 7.24±0.41^c

 1.85 ± 0.03^{a} 1.61 ± 0.02^{b} 1.70 ± 0.06^{b}

		Experimental diet										
Item		Control	Antibiotic	Essential oils blend 1	Essential oils blend 2	Organic acids 1	Organic acids 2	Synbiotic 1	Synbiotic 2			
Tot	al leukocytic Count	34.60±0.41°	36.24±0.14 ^d	37.32±0.58 ^{cd}	38.45±0.60 ^{bc}	39.74±0.13	39 .10±0.09 ^{ab}	37.38±0.34 ^{cd}	38.01±0.39 ^{bc}			
2	Neutrophil %	24.87±0.29 ^d	25.57±0.12 ^{∞l}	25.91± 0.38°	26.43±0.44 ^{bc}	27.35±0.24 ^{ab}	27.23±0.17 ^{ah}	27.25±0.30ª	27.43±0.29ª			
Differential leukocytic count 9	Esinophil %	8.76±0.29 ^{hc}	8.36±0.23°	7.98±0.49°	8.61±0.26 ^{bc}	9.98±0.04ª	9.99±0.10ª	8.38±0.21°	9.37±0.27 ^{ab}			
	Basophil %	6.48±0.77°	6.97±0.26 ^{bc}	8.02±0.09ª	8.07±0.07ª	8.36±0.07ª	8.53±0.09ª	7.98±0.01*	7.75±0.14 ^{ab}			
	Lymphocyte %	50.82±0.32 ^d	50.01±0.01°	52.18±0.10 ^{bc}	52.13±0.14 ^{bc}	52.62±0.31 ^{ab}	52.90±0.03ª	51.82±0.09°	51.83±0.06°			
	Monocyte %	2.28±0.16 ^b	2.84±0.04ª	2.68±0.09*	2.72±0.06ª	2.92±0.02"	2.91±0.02ª	2.82±0.06*	2.88±0.04ª			
Phagoc ytic	Phagocytic %	86.82±3.30 ^b	77.49±1.39°	96.32±0.38ª	96.49±0.34ª	98.86±0.06*	99.30±0.10*	99.02±0.28*	98.96±0.14ª			
	Phagocytic index	1.11±0.07°	1.50±0.14 ^d	1.66±0.12 ^{cd}	1.72±0.07 ^{bod}	1.99±0.01**	2.05±0.10 ^a	1.55±0.06 ^d	1.88±0.07 ^{abc}			

Table 3. Effect of experimental diets on some immunological parameters.

abed Means within the same row with different superscripts are significantly different

Ta	able	4.Effec	t of:	experimental	diets on	nutrient	digestibility.

	Experimental diet							
Items	Control	Antibiotic	Éssential oils blend 1	Essential l oils blend 2	Organic acids 1	Organic acids 2	Synbiotic 1	Synbiotic 2
$Cr_2 o_3 \%$ in the diet	0.55	0.53	0.51	0.53	0.55	0.52	0.55	0.52
$Cr_2 o_3 \%$ in the faeces.	1.92	2.00	2.05	2.25	2.51	2.50	2.37	2.40
Crude protein % in the diet	30.80	30.80	30.80	30.80	30.80	30.80	30.80	30.80
Crude protein % in faeces	16.90	15.20	14.10	13.50	12.82	12.89	12.95	12.92
Fat % in the diet	10.26	10.26	10.26	10.26	10.26	10.26	10.26	10.26
Fat % in the faeces	2.35	2.20	1.98	1.92	1.85	1.82	1.80	1.79
C.F % in the diet	2.42	2.42	2.42	2.42	2.42	2.42	2.42	2.42
C.F in the faeces	1.45	1.40	1.38	1.39	1.32	1.29	1.34	1.32
Digestion coefficient of D.M	71.35	73.50	75.12	76.44	78.09	79.20	76.79	78.33
Digestion coefficient of C.P	84.28	86.92	88.61	89.68	90.88	91.30	90.24	90.91
Digestion coefficient of fat	93.44	94.32	95.20	95. 59	96.05	96.31	95.93	96.22
Digestion coefficient of C.F	82.84	84.67	85.81	86.47	88.05	88.91	87.15	88.18

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الملخص العربي

تقيم اضافة منشطات النمو الطبيعية مقارنة بالمضاد الحيوى في علائق البلطي النيلي علي معدلات النمو ومعاملات الهضم والحالة المناعية

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أجريت هذه التجرية لدراسة تأثير اضافة منشطات النمو الطبيعية مقارنة بالمضادات الحيوية في علانق اصبعيات البلطي النيلي وتأثيرها على معدلات النمو وهضم المواد الغذائية والحالة المناعية. تم تقسيم د ٤٨٠ سمكة من اسماك البلطي النيلي بمتوسط وزن ٢٤،٤٤ ±٠٠٠ جرام إلى ثمانية مجموعات تحتوى كل منها على ثلاث مكررات وكل مكرر يحتوى على ٢٠ سمكة ومتقاربة الأوزان. وضعت هذه الأسماك في أحواض زجاجية مزودة بمصدر للتهوية وذات نوعية جيدة من المياه. تم أقلمة الأسماك لمدة أسبوعين قبل بدء التجربة. تم تكوين عليقه أساسيه لتلبيه الاحتياجات الغذائيية لاصبعيات أسماك البلطي النيلي والتبي احتبوت علي ٢٠.٨٠% ببروتين ٢٩٤٠ كيلوكالوري / كيلوجرام عليقه طاقه مهضومه وفي العلائق التجريبيه تم اضافة تركيز ات مختلفة من منشطات النمو الطبيعية بمعدل ١٠ جم /كجم من كل مخلوط والمضاد الحيوى (اوكسى تيتر اسيكلين) بمعدل ١ جم /كجم عليقة. تم تغذيت الاسماك على العلائق المتساويه في الطاقه والبروتين تُلَاتُ مراتَ يوميا بمعدلَ ٣% من الوزن الحي للجسم لمده ١٢ أسبوعا. وأوضحت النتائج أن المعاملات الغذائية كان لها تأثير عالى المعنوية على معدلات النمو في المجموعات المختلفه حيث أن مجموعه الأسماك التي غذيت على عليقه تحتوى على منشطات النمو الطبيعية كمان لها تأثير عالى المعنوبيه على وزن الجسم النهائي. وزيادة الجسم والنسبه المنوب لزياده الجسم مقارنيه بالمجموعات العلاجيه الأخرى وعلاوه على ذلك أن مجموعه الأسماك التي غذيت على عليقه تحتوى على منشطات النمو الطبيعية كان لها تأثير معنوى على استهلاك كميه كبيره من العلف مقارنة بالمجمو عات التي غذيت على المجموعه الضابطه والمضاد الحيوى مجموعه الأسماك التي غذيت على عليقه تحتوى على منشطات النمو الطبيعية كانت تستخدم العلف بشكل أكثر كفاءه (القيم الأقل من معدلات التحويل الغذائي) من تلك التي غذيت على المجموعه الضابطه والمضاد الحيوى. سجلت أعلى قيم معنويه من معاملات استهلاك البروتين للأسماك التي غذيت على عليقه تحتوى على منشطات النمو الطبيعية مقارنه بتلك في الأسماك التي غذيت على العليقه الضابطه أو العلائق التي تحتوى على المضاد الحيوي. و سجلت أعلى قيم عدديه لمعاملات الهضم للماده الجافه والبروتين الخام والدهون والألياف الخام في الأسماك التي غذيت على العليقه التي تحتوى على منشطات النمو الطبيعية . تحسنت بشكل ملحوظ ومعنوى بعض المعاملات المناعيه نتيجه لتدعيم العلائق بمنشطات النمو الطبيعية وخلصت نتائج التجربة الى أن اضافة المنشطات النمو الطبيعية ادت الى تحسين معدلات النمو وهضم المواد الغذائيه والحاله المناعيه في علائق البلطي النيلي.