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Evaluation of Dietary Inclusion of Mango Kernel Meal and Oat Extract on Performance and Immunity of *Oreochromis niloticus*

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Abstract

The aim of the current study was to evaluate the effect of dietary supplementation with mango (*Mangifera indica*) kernel meal (MKM) and oat (*Avena sativa*) extract (OE) on growth performance, some serum biochemical parameters, immune status and protection of *Oreochromis niloticus* against *Aeromonas hydrophila* infections. A total of 180 *Oreochromis niloticus* fingerlings were divided to three experimental groups (each of 60 and each group was divided into four aquaria, 15 fish/aquarium); The first group was fed the control diet (without any additive) and the second group was fed diet supplemented with MKM, while the third group was fed diet supplemented with OE for 60 days. Growth performance and immunological parameters (lysozyme, immunoglobulin M) were examined at 2 months of age. Fish were challenged by *A. hydrophila* and the mortality were recorded after 14 days post-challenge. Fish fed diet contained OE had a higher final body weight, body weight gain and body weight gain percentage when compared with other groups. Immunological parameters estimated in this work were significantly improved ($P<0.05$) in MKM and OE fed groups when compared with the control group. Survivability after 14 days post-challenge was lower in control group (60%) and higher in other dietary groups. In conclusion, MKM and OE improved the growth performance, blood constituents, immune status and enhanced the challenging ability of *Oreochromis niloticus* against *A. hydrophila*.

Keywords: Nile Tilapia, Mango Kernel Meal, Avena Sativa Extract, Performance, Immunity.

Introduction

In Egypt, fish occupy an important economic and ecological niche in agricultural systems. *A. hydrophila* is a pathogen causing hemorrhagic septicemia in fish, which can be controlled by the antibiotics [1]. Nevertheless, antibiotics overuse may result in bacterial resistance or products residues leading to many harmful problems to fish health, consumers, environment and food safety concerns [2]. Alternative feed additives were introduced as potential replacements for antibiotics. Mango kernel is readily available by-products of mango. It has antimicrobial [3], antifungal, antiviral [4], antioxidant activity [5] and immune-modulators [6]. It can be considered as a valuable energy feed due to its rich oil, starch and antioxidants content with low crude protein level [7,8]. Mango kernel is considered as an antibiotic against pathogenic microorganisms [9], which can be utilized as an unconventional product to activate the immune status in fish [10].

Oat *(Avena sativa*) is rich in protein, minerals and vitamins, which contains more crude protein, ether extract, soluble fiber and β-glucan when compared with other cereals. Oat and its constituents have many pharmacological activities like immunomodulatory, antiatherogenic, antioxidant, anticancer and topical anti-inflammatory [11]. The aims of our trial were to study the effect of MKM and OE as natural feed additives at rate of 5 g/kg diets on the growth performance, some blood metabolites, immunity and resistance to *Aeromonas hydrophila* of *Oreochromis niloticus* fingerlings.

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Material and Methods

Preparation of Mango (Mangifera indica) Kernel meal (MKM)

Mango fruits (Zebdia) were purchased from the local markets. The seed kernels were obtained from each fruit, washed, air-dried then manually removed the kernels from the seeds. Kernels were cut into small parts by the grinder and then boiled to remove the most of anti-nutritional factors. They were transferred to the freezer at -80°C and dried in freeze dryer to remove excessive moisture then stored at 4°C [12].

Extract preparation from oat (Avena sativa)

A. sativa samples were obtained from the local markets in Sharkia Governorate. Maceration 100 g of dried ground oat with 100 mL water and put them in a water bath, incubated for 24 h at 60°C and then filtered and repeated the same process three times. Evaporation of water by using a lyophilizator to obtain a dry extract, which stored at 4°C in the refrigerator [13].

Aeromonas hydrophila strain

Aeromonas hydrophila with standard known biochemical and pathogenicity profiles were kindly provided from microbiological archive of the Department of Microbiology, Animal Health Research Institute, Dokki, Egypt. Bacteria were cultured in nutrient broth for 24 h at 37°C and then the cultures were centrifuged at 3000 rpm for 10 min. The supernatants were discarded and the pellets were resuspended in phosphate buffered saline (PBS, pH 7.4) and prepared to 1×10^7 cells/mL. These bacterial suspensions were serially diluted with PBS and used for the challenging experiment.

Fish used and diets and feeding

One hundred and eighty *Oreochromis niloticus* (14-16 g) were purchased from the Central Laboratory for Aquaculture Research, Abbassa. Sharkia., Egypt. The fish were left for 2 weeks for acclimatization before the experimental initiation and then randomly distributed into glass aquaria (80 X 60 X 30 cm) of 80 liter capacity provided with de-chlorinated fresh water, aerator and thermostatically controlled at 22±2°C. Fish were allocated into 3 groups; each group was divided into four aquariums (15 fish/aquarium). Water was partially changed by siphoning method to remove the excreta of fish daily. Dry pellets diets were formulated to meet the nutrient requirements of *O. niloticus* [14] (Table 1). The dietary treatments included: (1) control diet, (2) diet contained MKM (5 g/kg diet) and (3) diet supplemented with OE (5 g/kg diet). The fish were fed diets 4 times per day at a rate of 3% of BW for 2 months. Feedstuffs used in diets formulation was analyzed for dry matter, crude protein and ether extract based on the procedures of AOAC [15].

Growth performance and biochemical analysis

Fish were weighed at the beginning and the end of the trial. Average BW was calculated by dividing the total BW of fish by fish number in each group. Body weight gain, BW gain percentage and specific growth rate (SGR) percentage were determined [16]. At the trial end (60 days), five fish from each aquarium were anaesthetized and the blood samples were collected into Eppendorf tubes without anti-coagulant from the fish caudal peduncle and then centrifuged (3,000 rpm for 15 min) for serum preparation. Samples were stored immediately in freezer until used for biochemical and immunological analysis. Total protein and albumin [17], albumin globulin ratio (A/G ratio) and globulin were estimated. Aspartate aminotransferase (AST) [18] and alanine aminotransferase (ALT) [19] were estimated.

Immunological analysis

The turbidity test was utilized for measuring the lysozyme activity. Chicken egg lysozyme (Sigma) was utilized as a standard and 0.2 mg cc lyophilized genus *Micrococcus lysodeikticus* in 0.04 M sodium phosphate buffer (pH 5.75) was utilized as substrate. Fifty mL of serum was added to 2 mL of the bacterial suspension and the diminution in the absorbance at 540 millimicron was resolved after 0.5 and 4.5 min incubation at 22°C. One unit of lysozyme activity was characterized as a reduction in absorbance of 0.001 min. Serum lysozyme activity was evaluated by the strategy for Ellis [20].

Measure technique for of IgM

ELISA Kit was utilized for determining Immunoglobulin M (IgM). List No. CSB-E12045Fh (96 test). CUSABIO BIOTECH CO., Ltd. [21].

Challenge Test

Following 60 days of feeding and blood samplings, ten fish from each group were infused intra-peritoneally with 0.2 mL ($3X10^7$cell/mL) culture suspension. The groups stayed for perception period of 14 days. The fish were checked routinely for any disease signs. Moreover, the behavioral variations and mortality rates were recorded.

Mortality percent = No. of death in a predefined period / Total population in the course of that period X 100.

Statistical analysis:

The results were analyzed by one way ANOVA [22] and LSD test. Statistical significance statement were based on ($P<0.05$).

Table 1: Ingredients and calculated composition of the experimental diets fed to *O. Niloticus* during experiment

Ingredients	Experimental diets		
	Control	MKM[1]	OE[2]
Yellow corn (kg)	35	34.50	34.50
Mange kernel meal (kg)	-	0.50	-
Oat extract (kg)	-	-	0.50
Wheat flour (kg)	10	10	10
Soybean meal 44% (kg)	18	18	18
Fish meal 60% (kg)	16	16	16
Poultry by-product meal (kg)	14	14	14
Vegetable oil (kg)	5.50	5.50	5.50
Vitamin and mineral mixture* (kg)	1.50	1.50	1.50
Calculated composition			
Dry matter %	91.37	91.39	90.93
Crude protein %	30.79	30.79	30.75
Ether extract %	10.44	10.48	10.42
Crude fiber %	2.42	2.43	2.41
Ash %	7.12	7.13	7.11
Nitrogen-free extract %	38.99	38.96	38.63
Digestible energy (Kcal/ kg diet)**	2944.41	2943.43	2933.46
Analyzed composition			
Moisture %	9.00	8.90	10.00
Crude protein %	29.30	29.25	29.10
Ether extract %	9.40	9.90	9.20

[1]Analyzed composition for mange kernel meal (MKM) includes (92% DM, 7.70% CP, 12.50% EE, 2.90% CF, 2.80% Ash and 66.10% NFE). [2]OE: oat extract. *Vitamin and Mineral mixture (Alfakema):-Each 1 kg contains:-Vit. A 580000 I.U, Vit. D3 8600 I.U, Vit. E. 720 mg, Vit. K3 142 mg, Vit C 0.1 mg, Vit B1 58 mg, Vit B2 34 mg, Vit. B6 34 mg, Vit.B12 58 mg, Folic acid 86 mg, Pantothenic acid 8 mg, Manganese sulfate 65 mg, Zinc methionine 3000 mg, Iron sulfate 2000 mg, Copper sulfate 3400 mg, Cobalt sulfate 572 mg, Sodium selenite 25 mg, Calcium iodide 25 mg, Calcium carbonate (carrier substance) till 1000 g. **Digestible energy (DE) calculation based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm, NFE 2.5 kcal/gm [30].

Results and Discussion

Growth performance and biochemical analysis

Growth performance of fish fed the experimental diets was presented in Table (2). The result revealed significant increases in total BW, BW gain, BW gain % and SGR in MKM and OE groups. Oat extract had the highest values followed by MKM diets when compared with the control group. These results clearly showed that the OE and MKM stimulated the growth of fish during the entire experimental period. These results confirmed the growth promoting effect (BW gain % and SGR) for diets supplemented with oat extract [11] and MKM [10,23]. Also, Belsare and Singh [24] showed that mango seed kernel at 5% level can be incorporated into the diet of

post-larvae of *M. rosenbergii* for the better growth and survival rate. Also, they proved that proteins and carbohydrates in the mango seed kernel were digestible to the post-larvae. This positive growth promoting effect may be attributed to the actual ingredients that improved feed utilization and had important effects on growth [11]. The current results showed significant (P<0.05) increases in the total protein, albumin and globulin, while significant (P>0.05) decreases in liver enzymes (ALT and AST) in both tested groups (Table 3). These results were comparable with other studies that reported increase in the total protein, albumin and globulin levels of fish supplemented with OE and MKM [10,11,25]. These increases in protein profile might be due to stronger innate response of fishes [26,27]. The liver exerts an important role in lipid metabolism. In this study, the level of liver enzymes significantly decreased in both experimental diets than the control diet. The reduction of liver enzymes reflected to MKM and OE may make cell membrane stabilization and protect liver cells from damages [11].

Table 2: The effect of dietary supplementation with mango kernel meal (MKM) and oat extract (OE) on growth performance of *O. Niloticus*

Parameters	Experimental diets		
	Control	MKM	OE
Initial body weight (g)	14.92±0.10	14.93±0.15	14.93±0.12
Final body weight (g)	29.57±0.19^{c}	31.27±0.29^{b}	34.33±0.09^{a}
Weight gain (g)	14.65±0.13^{c}	16.33±0.28^{b}	19.40±0.12^{a}
Weight gain %	98.21±0.97^{c}	109.40±2.36^{b}	129.93±1.70^{a}
Specific growth rate	49.52±0.35^{c}	53.48±0.81^{b}	60.26±0.53^{a}

^{abc}Means in the same row with different superscripts were significantly different at (P < 0.05).

Table 3: Protein profile and activities of serum enzymes in *O. niloticus* post-treatment with mango kernel meal (MKM) and oat extract (OE)

Parameters	Experimental diets		
	Control	MKM	OE
Total protein (g/100 mL)	3.067 ± 0.088^{c}	4.267 ± 0.145^{a}	3.700 ± 0.104^{b}
Albumin (g/100 mL)	1.323 ± 0.041^{c}	1.837 ± 0.044^{a}	1.553 ± 0.035^{b}
Globulin (g/100 mL)	1.743 ± 0.087^{b}	2.430 ± 0.161^{a}	2.147 ± 0.074^{a}
A/G ratio[1]	0.764 ± 0.050	0.763 ± 0.058	0.724 ± 0.015
ALT (u/L)[2]	13.70 ± 0.19^{a}	10.87 ± 0.04^{b}	11.15 ± 0.03^{b}
AST (u/L)[3]	15.75 ± 0.03^{a}	15.55 ± 0.03^{c}	15.65 ± 0.03^{b}

[1]A/G ratio: albumin globulin ratio, [2]ALT: alanine aminotransferase and [3]AST: Aspartate aminotransferase.
^{abc}Means in the same row with different superscripts were significantly different at (P < 0.05).

Effect on Immune response

Lysozyme activity and immunoglobulin M were significantly (P<0.05) higher in experimental groups when compared with the control group at post-challenge periods (Table 4). Lysozyme activity is the first barrier line in innate immune system [28]. Synthetic and biological immune-stimulant products increased the activity of serum lysozyme. Our study showed a higher positive effect on lysozyme and IgM in *O. niloticus* fed diet supplemented with OE [11] or MKM [10,25]. Improvement in immune status of fish may be attributed to the MKM contains various phenolic compounds which have an antioxidant action [12].

Table 4: The effect of dietary supplementation with mango kernel meal (MKM) and oat extract (OE) on IgM (g/L) and lysozyme (u/mL) in *O. niloticus* after 60 days of feeding.

Experimental diets	Lysozyme (µg/mL)		IgM (g/L)	
	After 2 month	2 weeks post challenge	After 2 month	2 weeks post challenge
Control	352.67±1.45[c]	279.00±2.31[c]	0.423±0.015[c]	0.280±0.012[c]
MKM	540.67±3.48[a]	471.67±7.26[a]	0.760±0.017[b]	0.557±0.020[b]
OE	472.67±1.45[b]	392.67±1.45[b]	0.947±0.023[a]	0.647±0.018[a]

[abc]Means in the same column with different superscripts were significantly different at (P < 0.05).

Challenge with A. Hydrophila.

No obvious clinical symptoms were noticed in both of the experimental groups (MKM and OE), except gentle skin darkness and slight hemorrhagic patches on gut and kidney. The control group after *A. hydrophila* infusion revealed excess mucus and hemorrhage on the skin especially at the base of fins. Ulcers denoted on the gill cover and mildly protruded reddish vent, skin darkness and tail rot were also observed. Petechial hemorrhages were clear in the kidney, submucosa of the intestine and liver and distended hemorrhagic gall bladder were delineated (Figure 1).

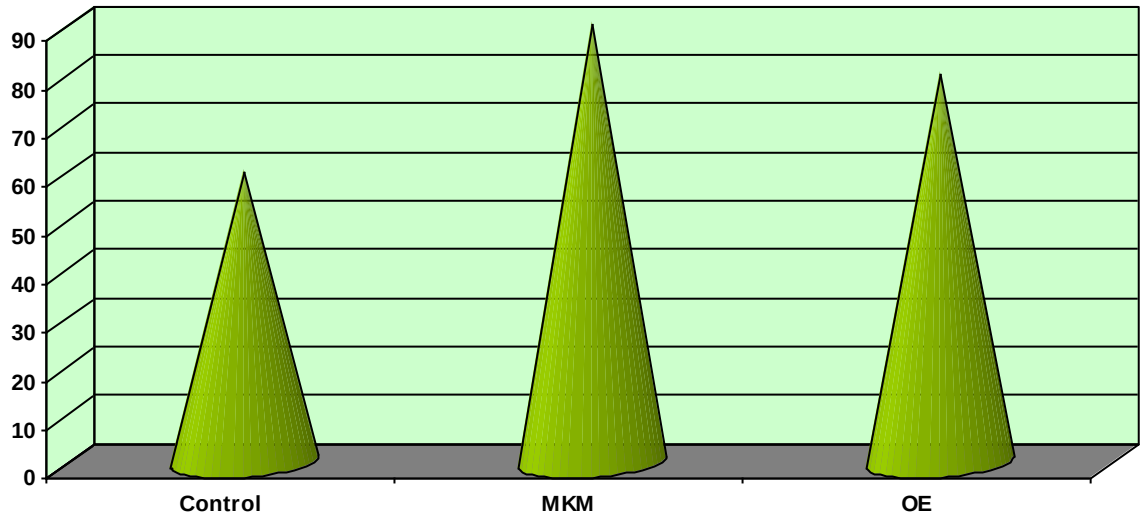

Figure 1: The effect of dietary supplementation with mango kernel meal (MKM) and oat extract (OE) on *survival % of O. niloticus* after challenged with *A. hydrophila*.

Mortalities in the control group appeared after 4 days post-challenge. Survival and mortality percentages were recorded in and the three experimental groups (Figure 1). The obtained results of survival % in fish fed diets supplemented with MKM and OE was 40 and 80%, respectively when compared with the control group (60%). The current results of fish received diets supplemented with oat extract or MKM, showed high survivability rate with no apparent clinical signs and post-mortem lesions [10-12,27]. This may be due to the presence of synergistic effects for active compounds of MKM and OE [29].

Conclusion

In accordance with the obtained results, dietary supplementation of *Oreochromis niloticus* with mango (*Mangifera indica*) kernel meal (MKM) and oat (*Avena sativa*) extract (OE), had a positive effect on growth performance, blood constituents and improved their resistance to *A. Hydrophila* infection. Thus, the use of these natural feed additives at a level of 5 g/kg in *Oreochromis niloticus* diets is recommended as an immune-stimulant and to reduce the amount of more expensive conventional feed additives in order to decrease the feed cost.

Conflict of interest

The authors declare that they have no conflict of interest.

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الملخص العـــــربي

تقييم الإدراج الغذائي لمسحوق نواة المانجو ومستخلص الشوفان على النمو والإستجابة المناعية في البلطي النيلي

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هذه التجربة تمت لتقييم إستخدام مسحوق نواة المانجو ومستخلص الشوفان كإضافات أعلاف طبيعية كمحفز للنمو والمناعة لإصبعيات البلطي النيلي بمتوسط وزن ١٢-١٤جرام , حيث تم إستخدام ١٨٠ سمكة تم تقسيمها إلي ثلاث مجموعات حيث تم تغذيه المجموعة الأولى على عليقه عادية لا تحتوى على أي إضافات بينما أضيف مسحوق نواة المانجو إلى عليقه المجموعة الثانية بمعدل ٥ جرام /كيلو عليقة وكذلك أضيف مستخلص الشوفان إلى عليقة المجموعة الثالثة بمعدل ٥ جرام/كيلو عليقة لمدة شهرين لجميع المجموعات السابقة قبل العدوى. تم فحص معاملات أداء النمو والمناعة بعد ٦٠ يوما من التغذية كما تم إحداث العدوى بميكروب إيروموناس هيدروفيلا تجريبيا لأسماك هذه المجاميع ووضعت المجاميع تحت الملاحظة لمده ١٤ يوما. وسجلت أعلى معدلات للنمو بين الأسماك المعاملة بمستخلص الشوفان وأيضا لوحظ تحسن الحالة المناعية في الأسماك المعاملة بنواة المانجو ومستخلص الشوفان بالمقارنة بالمجموعة التي لم يتم معاملتها بأي إضافات طبيعية. وسجلت المجموعة التي لم يتم لها أي إضافات أعلي نسبة نفوق علي عكس المجموعات التجريبية. وتشير هذه النتائج إلى أن مسحوق نواة المانجو ومستخلص الشوفان يحفزان النمو والمناعة لإصبعيات البلطي النيلي عندما يتم معاملتهم بالطريقة الصحيحة.