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Studies on the Effect of Acidifier on Cultured *Oreochromis niloticus* Fish

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ABSTRACT

This work was conducted to study the effect of diets supplemented with different levels of acidifiers (0.0, 1, 2, 3 and 4 ml/kilogram) on growth performance, feed utilization and some blood parameters of Nile tilapia (*Oreochromis niloticus* fingerlings) and eventually resistance to *Vibrio anguillarum (V. Angullirum)* infection. Experimental diets were isonitrogenous and isocaloric (CP 30.5 % and 4.35kcal/GE/g respectively). A total number of 150 Nile tilapia fingerlings with average initial body weight (BW) of 20 ± 1.6 g/fish were randomly distributed into five treatments. Fish in the five experimental treatments were fed at a rate of 3% of the body weight BW daily for 60 days (experimental period). Results indicate that, significant differences ($P<0.05$) were detected in feed utilization criteria between fish groups fed diets supplemented with acidifier. Results showed that, liver enzymes and kidneys test were not significantly different ($P>0.05$) affected by of acidifier supplementation while serum total protein were increased significantly. Mortality rate had decreased in all groups fed diet supplemented with acidifier when experimentally challenged with *V. anguillarum* 10^5 CFU per day over a period of 20 days. So it could be concluded that, the diets supplemented with acidifier improve growth performance of Nile tilapia and enhance the resistance against *V. anguillarium.*

Keywords: acidifier, *Oreochromis niloticus*, growth performance, blood parameters and *Vibrio anguillarium*).

INTRODUCTION

A potential concern of fish culturists is reduced resistance to bacterial and viral infections which could be caused by inadequate rearing conditions and/or malnutrition. The preventative effects of supplements on disease resistance have been investigated by examining growth performance, mortality and blood properties (**Nakagawa *et al.*, 1981**and **1986**). Routine use of antibiotics as growth promoters is a matter of debate in the animal farming industry, in the field of aquaculture it is well established so far that the inclusion of antibiotics into the diets of fish **(Ahmad and Matty, 1989)** can promote growth and feed conversion. But The use of low levels of these antibiotics in animal feeds possesses the possibility to transfer bacterial immunity to species pathogenic in animals and humans (**Liem 2004**). ***Vibrio*** species are gram negative bacteria affected all type of fish of either marine or freshwater

fish allover the world in the different areas of Asia, America ,Australia ,Africa and Europe (**Austin and Austin 1999**). In animal nutrition, acidifiers exert their effects on performance via three different ways (**Freitag**

2007): (a) in the feed; (b) in the gastro-intestinal tract of the animal; and (c) due to effects on the animal's metabolism.**Luckstadt (2008)** peculated that dietary potassium diformate could stimulate a beneficial bacterial colonization of the intestine. Acidifiers function as conserving agent by reducing the pH of the feed, and thereby inhibiting microbial growth and thus lower the uptake of possibly pathogenic organisms and their toxic metabolites by the farmed animals. Tilapia (***O. niloticus***) fed different organic acids had enhanced feeding behaviour and growth performance **(Xie *et al.*, 2003 and Petkam *et al.*, 2008)**. So this study was conducted to evaluate the effect of acidifier on growth performance and resistance to ***V. anguillarium***.

MATERIALS AND METHODS

Experimental fish

A total of 150 apparently healthy ***O. niloticus*** fish were collected from private fish farms at Tolompate 7, Kafr El-Sheikh Governorate. They seem to be likely had a uniform size and with average weight 20 ± 1.6 grams. The experimental fish were acclimated to the aquaria conditions in indoor tanks for 2 weeks. Aquarium (70 x 30 x 40 cm) containing about 70 liters of dechlorinated water and water temperature was adjusted at 25±1.5 ^{o}C as well as continuous oxygen supply.

Experimental diets formulation and feeding system design

In this experiment tilapia fingerlings were allotted into 5 treatments, of which the first was the control group **(C)** supplied with free acidifier diet, the second **(T1)**, Third (**T2),** forth **(T3)** and fifth **(T4)** 1, 2. 3 and 4 ml/kg respectively, each treatment had 3 replicates that distributed in 3 aquaria (10 fish / aquarium). The feeding duration longed for a period of 60 days. Every seven days, the fish in each aquarium were weighed and the amount of feed ***O. niloticus*** was corrected according to the new fish biomass as 3 % of live body weight.

Acidifiers used in the study

Dietary supplementation acidifiers trade name Acilux: a blend of organic acids plus copper sulfate (formic acid 47.1%, phosphoric acid 23.0%, citric acid 5.8%, acetic acid 10.1% and copper sulfate 1.1%) Produced by Jodoco Belgium.

Calculations of feed utilization parameters

Average daily gain (ADG): ADG = (W1-W0)/T

Where, W0 and W1 were the initial and final body weight per gram, and T is the number of days in the feeding experimental period.

Total weight gain: TG (g) = Wt_1-Wt_0

Where wt1 is the final body weight (g) and wt0 is the initial body weight (g)

Survival rate: SR (%) = (No. of fish at end / No. of fish at the start) ×100

Feed conversion ratio: FCR = Feed intake (g)/ weight gain (g)

Where, the weight gain is (the biomass of fish at the start + the biomass of the dead fish- the biomass of the fish at the end)

Specific growth rate: SGR (%/day) = 100(Ln final weight–Ln initial weight)/experimental period (d).

Protein efficiency ratio: PER (%) = total weight gain (g) / protein intake (g)

Blood sample collection and heamatogram

Blood samples were prepared according to the method described by Lucky (1977). Differential leukocytic count was calculated according to Schalm (1986). Hemoglobin concentration Hb was calculated according to the formula mentioned by Dacie and lewis (1975). Red blood cell (RBCs) and White blood cell (WBCs) counts were counted by haemocytometer according to Stoskopf (1993).

In addition, M.C.V. Mean Corpuscular Volume, M.C.H. Mean Corpuscular hemoglobin and M.C.H.C. Mean Corpuscular hemoglobin concentration were calculated according to the formula mentioned by Dacie and lewis (1975).

M.C.V. = (PCV / RBCs) x 10 as m/mm^3.

M.C.H. = (HB content gm/100ml/ RBCs) x 10 as m/mm^3.

M.C.H.C. = (HB content gm/100ml / PCV) x100 as %.

The concentration of total protein (TP) Weichsellbaum (1946). The activity of the liver enzymes, Aspartate Amino Transaminase (AST) and Alanine Amino Transaminase (ALT) was determined according to (Reitman and Frankel, 1957).

Serum urea was determined according to Patton and Crouch (1977) Serum creatinine was determined according to Henry (1974).

Challenge test

After 60 days of feeding, a total number of 150 fish (10 fish from each treatment) were fed diet infected with the pathogenic *V. anguillarium* at 10^5 CFU per day over a period of 20 days according to (Ramli *et al.*, 2005), *O. niloticus* were kept under observation for 14 day to record the mortality rate (MR).

MR % = No. of death in specific period / Total population during that period x 100

Statistical analysis was performed using the analysis of variance (ANOVA). Duncan's Multiple Range Duncan (1955) was used to determine differences among treatments mean at significance level of 0.05. All statistics were run on the computer using the SAS program (SAS, 1998).

RESULTS AND DISCUSSION

Growth performance and survival rate

Results represented in Table (1) showed that, FCR, FW, SGR, FI and survival rate had enhanced significantly (P>0.05) by increasing the addition of acidifier 1, 2, 3 and 4 ml/kg comparing with control group. The highest significantly (P>0.05) DWG, TWG and final weight had achieved by treatment received 4 g/kg acidifiers, 0.53and 53.3g respectively. The highest survival rate recorded by treatments supplemented with acidifier. Our findings agreed with those obtained by Petkam *et al.* (2008) who determined the effects of an acid blend, containing calcium formate, calcium propionate, calcium lactate, calcium phosphate and citric acid at levels of 1.5% the diet of *O. niloticus* resulted in a numerical (11%) increase in body weight. Also similar results were obtained by Zhou *et al.*(2008) who tested hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*) fingerlings (2.7 g initial weight) in a dose response study with potassium diformate (0%, 0.3%, 0.6%, 0.9% and 1.2%), while also comparing the results with an antibiotic growth promoter (8 mg/kg Flavomycin). Also Ajiboye *et al.* (2012) stated that acidifiers (consisting of organic acids and their salts) as a potential replacement for antibiotic growth promoters in order to improve growth, feed utilization. The 2 kg ton^{-1} inclusion of the potassium salt of the formic acid lead to an improvement in weight gain and feed conversion ratio in Tilapia by 18.6% and 8.2% respectively (Ramli *et al.*, 2005). However different results obtained Owen *et al.* (2006) tested the sodium salt of butyric acid as a feed additive in the omnivorous tropical catfish *Clarias gariepinus* at 2 kg / t in a fish meal based diet and in a defatted soya concentrates diet. No significant differences were found while supplying sodium butyrate if compared with the negative control. Concerning data survival rate our findings agreed with Luckstadt (2008) who claimed that acidifiers

Table (1): Feed utilization of ***O. niloticus*** fed diet containing acidifiers.

Item	IW	FW	DWG	TWG	SGR	PER	FCR	FI	SR
C	20.8 ^{a}	33^{c}	0.2^{c}	12.2^{c}	0.76^{c}	1.17^{c}	2.82^{a}	34.24^{c}	80^{b}
	±1.0	±1.9	±0.06	±1.5	±0.06	±0.08	±0.2	±2.3	±10
T1	20.8^{a}	34.5^{c}	0.23^{c}	13.8^{c}	0.84^{c}	1.44^{b}	2.27^{b}	31.2^{c}	83.^{3ab}
	±0.3	±1.78	±0.01	±1.8	±0.09	±0.1	±0.15	±3.1	±11.5
T2	20.57 ^{a}	38.77^{b}	0.3^{b}	18.2^{b}	1.06^{b}	1.5^{b}	2.12^{b}	38.7^{b}c	86.7^{ab}
	±0.3	±0.8	±0.06	±0.9	±0.04	±0.12	±0.16	±5	±5.7
T3	20.77 ^{a}	42^{b}	0.35^{b}	21.2^{b}	1.17^{b}	1.5^{b}	2.19^{b}	46.^{3ab}	86.7^{ab}
	±0.49	±0.45	±0.08	±0.9	±0.06	±0.15	±0.2	±6.4	±5.7
T4	20.7 ^{a}	53.3^{a}	0.53^{a}	32.6^{a}	1.57^{a}	1.96^{a}	1.67^{c}	54.5^{a}	96.7^{a}
	±0.44	±3.2	±0.07	±2.9	±0.08	±0.07	±0.02	±3.6	±5.7

Group with different letter within the same column are significantly different at P< 0.05. T= Treatment, IW= Intial Weight, FW= Final Weight, TG= Total weight Gain, DWG= Daily Weight Gain, FCR= Food Conversion Ratio SGR= Specific Growth Rate, FI= Feed Intake, PER Protein Efficiency Ratio and SR=Survival rate.

can mitigate the impact of bacterial infections, thereby preventing diseases and thus affording higher survival rate.

Blood parameters and biochemical analysis of O. niloticus serum

Table (2) showed that, heamatogram criteria showed enhanced nutritional and health status of ***O. niloticus***. The significantly highest RBCs, Hb, MCV, MCH and MCHC were achieved by treatments received supplementation with acidifier 4g/kg. A different results obtained by **Lim *et al.* (2010)** who mentioned that there were no significant differences among hematological parameters

Table (2) Heamogram, activities of serum enzymes and total protein in ***O.niloticus***.

Item	C	T1	T2	T3	T4
RBC X 10^{3}	1.51^{b} ± 0.2	1.55^{ab} ± 0.3	1.56^{ab} ± 0.24	1.58^{ab} ± 0.3	1.64^{a} ± 0.26
WBC X 10^{3}	55.4^{d} ± 0.15	57.8^{c} ± 1.7	58.1^{bc} ± 1.75	60.1^{ab} ± 0.8	61.5^{a} ± 0.56
Hb (g/dl)	6.2^{b} ± 0.7	6.63^{ab} ± 0.13	6.7^{ab} ± 0.15	7.17^{ab} ± 0.55	8.15^{a} ± 0.17
PCV %	18.6^{d} ± 1	22.2^{c} ± 0.4	22^{bc} ± 0.76	24.37^{b} ± 0.76	27.3^{a} ± 0.7
MCV m/mm^{3}	123.4^{b} ± 8.5	136.5^{b} ± 3.8	138.9^{b} ± 3.8	156.9^{a} ± 3.3	166^{a} ± 6.9
MCH m/mm^{3}	41.2^{b} ± 4.4	41.5^{b} ± 0.11	42.6^{b} ± 1.23	46.1^{ab} ± 1.26	49.6^{a} ± 2.4
MCHC (g/dl)	33.3^{a} ± 4.9	31.15^{b} ± 1.1	29.8^{c} ± 0.9	29.4^{c} ± 2	29.8^{c} ± 1.18
ALT (IU/l)	21.8 ^{ab} ± 2	26^{a} ± 2	24.33^{a} ± 0.31	21.7 ^{ab} ± 3.2	17.5 ^{b} ± 1.5
AST (IU/l)	80^{a} ± 2	81.3^{a} ± 3.8	77^{a} ± 6.5	77.5^{a} ± 3.2	77^{a} ± 3
Urea (mg/dl)	2.8^{a} ± 0.07	2.77^{a} ± 0.19	2.89^{a} ± 0.06	2.63^{a} ± 0.1	2.6^{a} ± 0.2
Creat (mg/dl)	0.83^{a} ± 0.04	1.05^{a} ± 0.3	1.05^{a} ± 0.18	0.98^{a} ± 0.1	0.93^{a} ± 0.26
TP (g/dl)	4.1^{c} ± 0.1	4.4^{bc} ± 0.5	4.5^{bc} ± 0.44	4.9^{ab} ± 0.07	5.3^{a} ± 0.26

Group with different letter within the same column are significantly different at P<0.05. T= Treatment, RBC= Red Blood Cell, WBC= White Blood Cell, MCV= Mean Corpuscular Volume, MCH= Mean Corpuscular Haemoglobin, MCHC= Mean Corpuscular Haemoglobin Concentration, Hb = Hemoglobin, PCV=Packed Cell Volume, AST= Aspartate transaminase and Alt= Alanine amino transaminase, Creat= Creatinine and TP= Total Protein.

(total, red and white blood cell counts, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) of Nile tilapia fed diets with different levels of acidifiers.

Moreover, the measurement of AST, ALT, Creatinine and Urea in plasma showed no significant differences among treatments indicating no adverse or hazourd effect of acidifiers addition on liver or kidneys of ***O. niloticus***.

Concerning serum total protein in ***O.niloticus*** fed on different levels of acidifier showed that (T4) 4ml/kg and (T3) 3 ml/kg had improved significantly 5.3 and 4.9 respectively compared with other control and other treatment. These results maybe explained that feed utilization concerning PER had improved. Different results obtained by **Lim *et al.* (2010)** did not differ among Nile tilapia fed diets with different levels of acidifiers. This difference in results concerning incorporation of acidifiers in tilapia diets may be due to variations among fish species, strain, size or age, levels of inclusion, composition and nutrient content of experimental diets, buffering capacity of dietary ingredients, culture and feeding management, and water quality.

Experimental infection of O.niloticus with V. angullirum

Data in Table (3) concerning *O.niloticus* mortalities after infected with *V. anguillarium* showed that treatment supplemented with acidifiers had decreased as percentages acidifier increased. Addition of acidifier 1ml/kg diet had lower mortality rate (40%) compared with control group (50%) while addition 4 ml/kg diet had the lowest mortality rate (20%). These results could be explained by acidifier raise the pH of the diet thus lower the uptake of possibly pathogenic organisms and their toxic metabolites thus higher dosages had lower mortality rate. These findings agreed with those of Luckstadt (2008) who stated that the application of potassium diformate at 0.2% is an efficient tool to control bacterial infections in tropical tilapia culture and added that survival rates of fish after the challenge with *V. anguillarium* on day 10 were also significantly higher compared to the negative control and the effect was dose dependent ($P<0.01$). Ajiboye *et al.* (2012) acidifiers play an important role disease resistance in fish. Similar findings obtained by Ramli *et al.* (2005) who observed decrease in mortality 15 days post challenge with *V. anguillarium* in Nile tilapia fed potassium diformate containing diets but significantly improvement was obtained with the 0.5% potassium diformate diet compared with control group 11% and 33% respectively. Ng *et al.* (2009) also reported that dietary supplementation of organic acid blend or potassium diformate significantly reduced mortality in red hybrid tilapia 15 days post challenge with *V. anguillarium*. Also our findings agreed with those obtained by Da Silva *et al. (*2013) mentioned that salts of organic acids possess inhibitory activity against pathogenic vibrio species in marine shrimp, exhibiting the highest inhibitory capacity ($p < 0.0001$). Furthermore, decreased the vibrio species concentration in the intestinal microbiota of marine shrimp ($p \leq 0.05$), *Litopenaeus vannamei*. A different results were observed by Lim *et al.* (2010) who mentioned that supplementation of potassium diformate had no effect on mortality 15 days post challenge with *Streptococcus iniae* and post-challenge antibody titers.

CONCLUSION

From this study we can concluded that the addition of acidifier at levels 3and 4 gram/kg feed to the diet of Nile tilapia fish working to improve their health and their ability to resist bacterial infection with ***V. Anguillarium*** and also raise Tilapia's to take advantage of the feed.

Table (3): ***Mortality rate of O. niloticus fed with diet containing acidifiers challenged with V. anguillarium.***

Item	Total no.	Dead no.	Sur %	Mor %
C	10	5	50	50
T1	10	4	60	40
T2	10	4	60	40
T3	10	3	70	30
T4	10	2	80	20

T= Treatment, Total no. = Total number of fish, Dead no. =Dead number, Sur %= Survival rate and Mor %= Mortality rate.

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بعض الدراسات عن احد الاحماض العضوية علي أسماك البلطى النيلى المستزرعة

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تهدف الدراسة إلى تقيم الاحماض العضوية كأضافة غذائية لأعلاف إصبعيات البلطي النيلي المستزرع. فقد تم تغذية إصبعيات الاسماك لمدة 60 يوما علي4 مستويات (1 و 2 و 3 و 4 مللي / كيلوجرام علف) بالاضافة إلى المجموعة الضابطة (علف خالي من الاحماض العضوية). وكانت أهم النتائج المتحصل عليها هي تحسن معنوي في اوزان الاسماك ومعدلات اكتساب الوزن اليومي وكذلك زيادة قدرتها علي الاستفادة من بروتين العلف. ولم يكن لإضافة الاحماض العضوية أي تأثير علي انزيمات الكبد أو كفاءة الكلي. ولقد تم تسجيل ارتفاع في البروتين الكلي بسيرم الاسماك التي تم تغذيتها على الأعلاف المحتوية على الأحماض العضوية مما كان له دلالة علي تحسن الاستفادة من العلف وتحسن الحالة الصحية للأسماك. كما تم إجراء اختبار تحدي علي عدد10 أسماك من كل مجموعة من خلال ختبار التحدي بميكروب الفيبروانجيوليرم (10^5 خلية بكتيرية/سمكة) لمدة 20 أيام ولقد اتضح إرتفاع مقاومة المجموعات المغذاة علي الاحماض العضوية للأصابة بالعدوي. وبالتالي يمكن أن نستخلص من هذه الدراسة أن إضافة الاحماض العضوية إلي أعلاف أسماك البلطي النيلي بمستويات 1 و2 و3 و4 مللي / كيلوجرام علف تعمل علي تحسين صحة الأسماك وقدرتها علي مقاومة بكتريا الفبريو انجيوليرم وكذلك رفع قدرتها علي الاستفادة من الاعلاف.