



EFFECT OF DIETARY BLENDED ORGANIC ACID ON GROWTH, DIGESTIBILITY AND IMMUNITY OF AFRICAN CATFISH (*Clarias gariepinus*)

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ABSTRACT

The present experiment was conducted to evaluate the effect of acidifiers on growth performance and immunity of the African catfish *C. gariepinus*. This experiment was carried out in the wet lab of Fish Production Branch, Department of Animal Production, Faculty of Agriculture, Ain Shams University. A total of 120 fish were selected as healthy catfish which had a uniform size and average weight of 94.93 ± 0.89 g. A thirty of catfish were used in each treatment divided into three replicates as (10 fish/ quadrate tank). Four experimental diets were formulated at 35 % crude protein with different levels of organic acid blend (OAB); diet was supplemented with 0, 2, 4 and 6 ml of organic acid per kilogram diet. Growth parameters, apparent digestibility of crude protein and ether extract were measured. The chemical proximate analysis for whole-body of fish was conducted at the end of the experimental period. Hematological parameters of fish and liver function enzymes were also measured. The fish group fed on 0.6 % organic acids blend showed the highest significance ($P < 0.05$) increase in total weight gain (438 ± 14.24 g), specific growth rate, best feed conversion ratio, feed efficiency ratio and highest protein efficiency than the rest of experimental groups. The highest apparent digestibility for crude protein were achieved in the third fish group which fed diet supplemented with acidifiers at 4 ml / kg diet and have significant difference ($P < 0.05$) with other groups. Data obtained in hepatosomatic index % showed no significant difference among different treatment refers to the health status of liver. However, ALAT

and ASAT recorded high values in the higher group of OAB-treated fish indicating that liver could be affected. Hematological parameters were in normal range except for PCV, Hb, RBCs, and WBCs count which were decreased in the high dose of treated fish. Finally, it could be concluded that using of acidifiers as feed additives at lower doses can enhance growth, productivity and immunity of the African catfish (*C. gariepinus*).

Keywords: Catfish, Organic acid, Growth, Survival rate, Nutrients digestibility, Immunity

INTRODUCTION

Feed additives play an important role in the future of aquaculture diets. However, the utilization of organic acids to improve growth performance is widely used in farm livestock, the research conducted on organic acids in aquaculture is very limited. Most of the studies done on rainbow trout (Hernández et al 2012), tilapia (Li et al 2009), red sea bream (Hossain et al 2007) and white shrimp (Su et al 2014). A broad range of organic acids, salts or admixtures are used to improve growth, feed utilization and disease resistance in fish. Routine use of antibiotics as growth promoters is a matter of debate in the animal farming industry. In the field of aquaculture, the inclusion of antibiotics in fish diets (Ahmad and Matty, 1989) can promote growth and feed conversion. But the use of low levels of these antibiotics in fish feeds possesses the possibility to transfer bacterial immunity to pathogenic species in animals and humans (Liem, 2004). Recently research directed into the

utilization of acidifiers as growth enhancer in fish feeds (Silva et al 2013; Chuchird et al 2015; Ng and Koh, 2017). Acidifiers are functioning as conserving agent by reducing the pH of the feed, thereby inhibiting microbial growth, thus lower the possibility of uptake pathogenic organisms and their toxic metabolites by the farmed animals. Due to the specific spectrum of each of the organic acids, organic acids blend is widely used in animal feed. The organic acids blend has a broader spectrum effect on the pathogenic bacteria than each of the organic acid alone. Also, the use of organic acids blend led to reduction of the dose used in animal feeds, which consequence reduce the costs (Dibner and Buttin, 2002).

The aim of the present experiment was to investigate the effect of acidifier on growth performance, feed utilization, digestibility and the immunity of the African catfish (*Clarias gariepinus*).

MATERIALS AND METHODS

1. Location of the experiment

The study was carried out in the wet lab of Fish Production Branch, Department of Animal Production, Faculty of Agriculture, Ain Shams University, in a recirculating system. The study was started from 1/12/2015 to 30/5/2016 included 21 days as adaptation period.

2. Description of the rearing system

Fish were kept in quadrate fiberglass tanks (60 × 60 × 40 cm) supplied with dechlorinated water in a recirculating system. Each quadrate fiberglass tank had a central stand pipe to remove any of the accumulated excreta; tanks were weekly cleaned to remove any of the residual's adhesive in the tank. Water in each tank was continuously aerated by air stones connected to air blower. Three tanks were used as replicates for each treatment.

3. Water quality

Water temperature and dissolved oxygen were measured by Mettler Toledo, model 128, s/no 1242, the average water temperature was 24.30 ± 1.1 °C and dissolved oxygen was above 5.4 ± 0.5 mg/l. Water pH and ammonia were measured every two days by pH meter (Orion model 720A, s/no 13062) and ammonia by Hanna ammonia meter, the average level of ammonia was 0.012 ± 0.02 mg/l and pH was 7.7 ± 0.7 throughout the experi-

mental period. All water quality parameters were within the accepted levels for African catfish (Boyd, 1984).

4. Fish

Juveniles *Clarias gariepinus* were obtained from the private fish farm, EL-Manzala, Port Said, Egypt. Fish were acclimated in indoor tanks for 21 days, after that a total of 120 fish were selected as healthy catfish which had a uniform size with an average weight of 94.93 ± 0.89 g. Fish in each tank were weighed every two weeks to avoid handling stress.

5. Diet formulation and feeding regime

Four experimental diets were formulated with different levels of organic acids (Table 1). Fish feed raw ingredients were bought from the local market. Feed pellets were prepared by using electrical meat mincer, then sun dried, packed in bags and frozen until use. Diet was supplemented with 0, 2, 4 and 6 ml of organic acid blend per kilogram diet. Fish were daily fed three times at 7:00 am, 12:00 pm and 4:00 pm at a rate of 3% of its body mass. The proximate chemical analysis of the diet composition (%) on dry matter basis is summarized in (Table 2).

6. Acidifiers

Dietary acidifier used in the present experiment was a blend of organic acids which was formulated according to OAB blend. The OAB was prepared by adding 55.4 ml formic acid (85%) and 27 ml phosphoric acid (85%) and 10.1 ml of acetic acid was added to the previous solution, then 5.8 g of citric acid powder plus 1.1 g of copper sulfate were dissolved in 7.5 ml distilled water to obtain 100 ml of a final organic acid blend (OAB) solution. Each 100 ml of this solution were used in three treatments as 2, 4, and 6 ml per kilogram diet.

7. Design of the experiment

One hundred and thirty catfish were used in the present experiment, where 10 fish were sacrificed as a zero group for body composition analysis. The rest (120 fish) were divided randomly into 4 fish groups, where thirty fish were used in each treatment divided into three replicates as (10 fish/ tank). Groups 1, 2, 3, 4 were fed one of the formulated diets, where the ratios of acidifiers included in the diets were 0%, 0.2%, 0.4% and 0.6%, respectively.

Table 1. Diet formulation

Ingredients	T1 Without OAB	T2 2ml OAB/ kg diet	T3 4ml OAB/ kg diet	T4 6ml OAB/ kg diet
Yellow corn	31.7	31.5	31.3	31.1
Residues of poultry slaughter	25	25	25	25
Fish oil	1	1	1	1
Soy oil	1	1	1	1
Rice bran	7	7	7	7
Soy 48%	20	20	20	20
Fishmeal 65%	10.3	10.3	10.3	10.3
Salt	2	2	2	2
Vit. Premix	2	2	2	2
Organic acid blend (OAB)	0	0.2	0.4	0.6

Table 2. The proximate chemical analysis of diet composition (% on DM basis)

Moisture %	8.7
Crude protein %	35
Crude fiber %	5.31
Ether extract %	10.35
Ash %	7.1
Total carbohydrates	42.24
Gross energy (Kcal/100g)	490.5 Kcal/100g

Parameters of growth performance

• **Total weight gain**

TG (g) = $W_1 - W_0$
 as W_1 = the final weight (g), W_0 = the initial weight (g).

• **Specific growth rate**

SGR % = $100 (\ln \text{ final weight} - \ln \text{ initial weight}) / \text{period (days)}$.

• **Average daily gain**

ADG = $(W_1 - W_0) / \text{days of feeding in the experimental period}$.

• **Relative growth rate**

RGR = $\text{wt. gain by fish (g)} / \text{Initial body wt. (g)} \times 100$.

Feed utilization parameters

• **Feed conversion ratio**

FCR = $\text{Feed intake (g)} / \text{Weight gain (g)}$.

• **Feed efficiency ratio**

FER = $\text{Weight gain (g)} / \text{Feed intake (g)}$.

Protein utilization parameters

• **Protein efficiency ratio**

PER = $\text{Total weight gain} / \text{Protein intake}$.

• **Protein productive value (%)**

PPV % = $\text{Protein gain (g)} / \text{Protein intake (g)} \times 100$.

Survival rate

SR % = $(\text{No. fish at the final} / \text{No. fish at the start}) \times 100$.

Digestibility trial

By the end of the growing period, a group of 4 fish in each treatment were fed for 21 days the same feeding regime and feces were collected by siphon and by the central standpipe, then the total fecal was obtained by the basic pipe outlet and placed in a fine mesh net, fecal samples were removed and collected immediately on filter paper. Fecal materials of each treatment were blended together and dried at 60 °C in the oven and kept at -20 °C. Apparent digestibility coefficient of crude protein and ether extract were measured according to the direct methods (Bureau et al 1999; Zhou et al 2004).

[100x (protein in diet – protein in feces)/ protein in diet].

[100x (E.E. in diet – E.E. in feces) / E.E. in diet].

Proximate analysis of whole-body composition

The chemical proximate analysis for whole body of fish was conducted according to Association of Official Analytical Chemists (AOAC, 1995). At the end of experimental period, from each tank a random 3 fish were selected, pooled, ground and frozen in poly ethylene bags for determination of body crude protein, lipid, ash and moisture.

Blood sampling and biochemical analysis

At the end of the experimental period, blood samples were collected from the caudal vein of each fish using syringes transferred to heparinized Eppendorf tubes (10 units/ ml). Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) enzymes were measured according to (Reitman and Frankel, 1957) to examine the status of liver function.

Hematological parameters

Hematocrit (packed cell volume, PCV %) and hemoglobin (Hb) values (g/dl) were determined according to (Dacie and Lewis, 1975). Red blood cells (RBCs) and white blood cell (WBCs) were counted by a Neubauer hemocytometer. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated by using the formula according to (Dacie and Lewis, 1975).

$MCV = (PCV \% \times 10 / RBCs) \text{ as } \mu\text{m}^3$.

$MCH = (Hb \text{ g/dl} / RBCs) \times 10 \text{ as } (\text{pg})$.

$MCHC = (Hb \text{ g/dl} / PCV \%) \times 100 \text{ as } \%$.

Hepato-somatic index (HSI) were calculated by:

$HSI = \text{Liver weight (g)} / \text{Fish body weight (g)} \times 100$

Statistical Analysis

The obtained data were analyzed statistically by submitting one-way analysis of variance (ANOVA) using the general linear model procedure of the SPSS statistical software package version 18.0 (SPSS Inc., 2009). The test of significance between means was carried out by using Tukey's post-hoc test considering the significance level of ($P < 0.05$).

RESULTS AND DISCUSSION

1. Growth Performance

The effect of acidifier's supplementation on growth performance and feed utilization of *Clarias gariepinus* are summarized in Table 3. Fish groups fed on 0.6 % blended organic acids (group 4) showed the highest significance ($P < 0.05$) increase in total weight gain ($438 \pm 14.24 \text{ g/ fish}$), average daily gain (ADG) (2.6 g/ day) and specific growth rate (SGR) ($1.04 \% / \text{ day}$). The total weight gain (TWG) improved significantly ($P < 0.05$) by increasing the levels of dietary acidifiers 0.2 % and 0.4 % comparing with the control diet (no acidifier). Total weight gain (TWG) and specific growth rate (SGR) of *Clarias gariepinus* fed dietary acidifiers at 0.4 % was not differ significantly ($P > 0.05$) from that fed 0.2 %, however, SGR of the group 2 was not differ significantly with the control group (Table 3). Average daily gain (ADG) was significantly higher at level 0.6 % and 0.4 % of dietary organic acids recording the highest value of 2.61 ± 0.08 , $2.03 \pm 0.04 \text{ g}$, respectively, compared with the basal diet and 0.2 % of dietary organic acids. The highest significant value of relative growth rate (RGR) $474.56 \pm 13.01 \%$ was recorded in the fourth treatment at level 0.6 % of organic acid blend, whereas the lowest value ($298.76 \pm 17.44 \%$) was recorded in the control diet. In the present study, African catfish fed blended organic acidifier in diets tended to increase final body weights and specific growth rates (SGR) compared to fish fed on the control diet. The same results were found by (Romano et al 2015; Sherif and Doaa, 2013) who tested OAB blend at different levels ranged from 1 % to 4 %; or 0.1 % to 0.3 % OAB tested by Ng et al (2009). On the contrary, Anuta et al (2011) found no significant differences ($P > 0.05$) in growth performance parameters of white leg juvenile shrimp when fed diets containing calcium sulfate acidifier (vitoxal). Also, Romano et al (2016) found no significant response in growth weight parameters, when they fed tilapia fish diets included different levels (1 to 4%) of sodium citrate acidifier. These differences in the previous results which using acidifiers in diets may be contributed to some variations among fish species, size, strain, age, nutrient content of experimental diet and level of acidifiers and their compositions, in addition to the rearing culture system, water quality and feeding management.

Table 3. Effect of dietary acidifiers on growth performance and feed utilization of *Clarias gariepinus*

Parameter	IW	FW	ADG	TWG	SGR	RGR	FCR	FER
T1	96.83 ± 0.79	385.96 ± 15.00 ^b	1.72 ± 0.09 ^b	289.12 ± 15.42 ^c	0.82 ± 0.026 ^c	298.76 ± 17.44 ^c	1.82 ± 0.09 ^a	0.55 ± 0.03 ^b
T2	95.50 ± 2.42	415.37 ± 16.20 ^b	1.90 ± 0.08 ^b	319.87 ± 14.09 ^b	0.87 ± 0.01 ^{bc}	334.74 ± 8.80 ^{bc}	1.68 ± 0.06 ^b	0.60 ± 0.01 ^b
T3	95.00 ± 1.64	436.25 ± 7.53 ^b	2.03 ± 0.04 ^a	341.25 ± 6.59 ^b	0.91 ± 0.01 ^b	359.30 ± 6.67 ^b	1.64 ± 0.08 ^{bc}	0.61 ± 0.02 ^b
T4	92.40 ± 1.81	530.88 ± 15.33 ^a	2.61 ± 0.08 ^a	438.48 ± 14.24 ^a	1.04 ± 0.01 ^a	474.56 ± 13.01 ^a	1.40 ± 0.08 ^c	0.72 ± 0.03 ^a

Values (means ± SE) within the same row have different superscript letters indicate significant differences (P < 0.05).

2. Feed utilization efficiency of *C. gariepinus*

At the end of the trial, fish showed improvement in feed utilization represented as feed conversion ratio (FCR) and feed efficiency ratio (FER). The acidifiers supplementation in diets had significantly increased (P < 0.01) the feed efficiency ratio and achieved the best feed conversion values. The fourth group of fish, which was fed on 0.6 % acidifiers supplemented diet showed the best highly significant (P < 0.01) feed conversion ratio and feed efficiency ratio compared to the other groups (Table 3). Our findings agree with those obtained by Sherif and Doaa (2013) who tested cultured *Oreochromis niloticus* on different doses of OAB and reported a significant improvement in FCR, SGR and survival rates by increasing the level of acidifiers comparing to the control group. Abu Elala and Ragaa (2014) also observed that graded levels of KDF (potassium diformate) 0.2 and 3 % significantly improved FCR. In despite of the previous results, a study showed that there was no significant difference at (P > 0.05) among different levels of OAB (0.1 %, 0.2 %, and 0.3 % organic acid blend) and 0.2 % KDF in red hybrid tilapia (Ng et al, 2009).

The protein efficiency ratio (PER) was significantly affected by the experimental diets, where

the best significant (P < 0.05) protein efficiency ratio was found by fish group fed the diet containing the highest level of the acidifier (0.6 %). Whereas no significant effects were recorded between the other experimental treatments (Table 4). The protein productive values (PPV %) showed that with increasing the acidifier percentage in the experimental diets, protein deposition in fish body was significantly (P < 0.05) decreased. Fish group fed diet contained 0.6 % organic acids had the highest significant (P < 0.05) protein efficiency ratio (PER) than the rest of experimental groups. It was 30.5 % higher than the control diet. However, the same group (T4) showed the lowest significant (P < 0.05) protein productive value (PPV %). This is may be due to the higher moisture content and lower protein deposition in fish body found in this group (Table 4). In the present study, the protein efficiency ratio was also increased gradually in T2 and T3 as (8.4 and 11.7%, respectively) more than that of the control diet. Similar findings were recorded by (Hassan et al., 2014; Sherif and Doaa, 2013; Abu-Elala and Ragaa, 2014). The later result of PER is disagreeing with that found by (Ng et al., 2009) who reported that protein efficiency ratio was not significantly different (P > 0.05) among different levels of OAB (0.1 %, 0.2 % and 0.3 %).

Table 4. The mean (± SE) of protein utilization efficiency of African catfish (*Clarias gariepinus*) fed diets containing different levels of blended acidifiers

Items	T1	T2	T3	T4
PER	1.75 ± 0.08 ^b	1.89 ± 0.04 ^b	1.93 ± 0.05 ^b	2.28 ± 0.08 ^a
PPV %	33.17 ± 0.05 ^{bc}	44.47 ± 1.90 ^a	35.76 ± 0.71 ^b	30.06 ± 1.20 ^c

Values (means ± SE) within the same row have different superscript letters indicate significant differences (P < 0.05).

Supplementation of the control diet with lower doses of acidifiers significantly improved PPV. The diet supplemented with 0.2 % organic acid had the best protein productive value which increased significantly ($P < 0.05$) as 34.05% over the control diet. In the present work, the protein productive value decreased gradually in T4 compared to the basal diet. However, the data indicating no signifi-

cances difference between T 3, or T4 and the control diet (**Fig. 1**). This result is similar to that obtained by **Hassan et al (2014)** for *Oreochromis niloticus* fed on diet supplemented with 1 % calcium lactate as organic acid salt, they recorded significant increase in protein productive value as (35.04 %) over the other treatments.

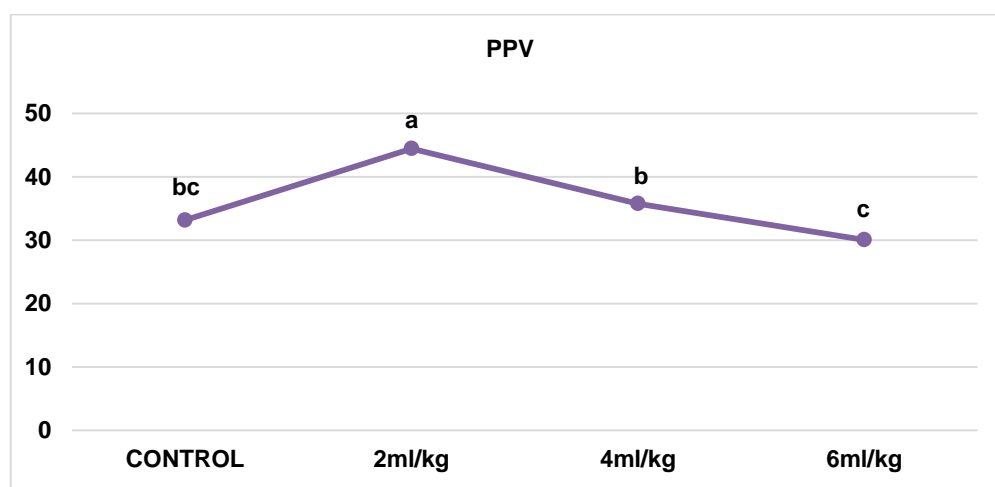


Fig. 1. Protein productive value of *Clarias gariepinus* at different level of organic acid blend

Several hypotheses have been suggested about the effect of acidifiers on improvements of nutrient's utilization, by lowering pH in the gut leading to increase pepsin enzyme activation, pancreatic enzyme (trypsin, lipase), intestinal enzyme activities (leucin, amino peptidase and phosphorus) which may increase mineral solubilization as chelating agents binding up various cations along the intestine, leading to increase mineral absorp-

tion and decreasing intestinal microbial activity (**Castillo et al 2014; De Wet, 2005**).

3. Survival rates

There were no significance differences ($P < 0.05$) on the survival rates (**Fig. 2**) at various levels of organic acidifier.

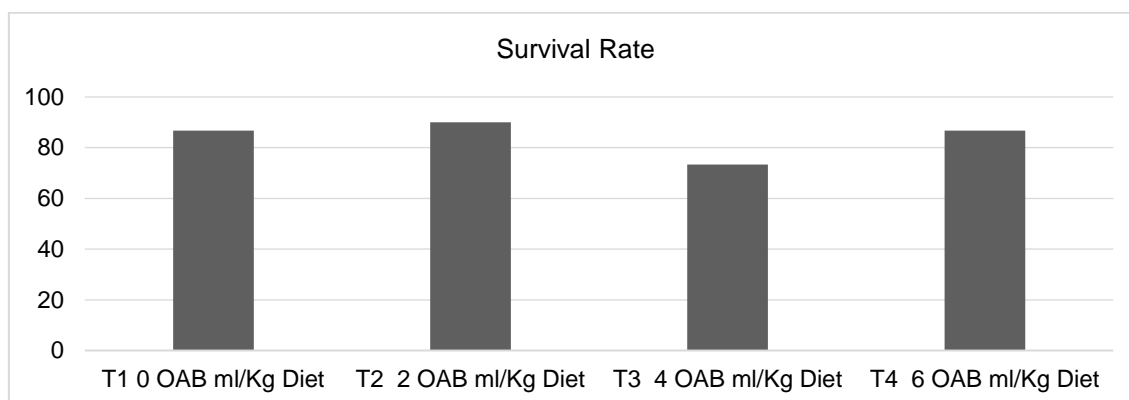


Fig. 2. Survival rates of *Clarias gariepinus* at different levels of organic acid blend.

4. Apparent digestibility coefficient

Apparent nutrients digestibility among different dietary acidifiers groups are presented in **Table 5**. There are no significant differences ($P > 0.05$) in the dry matter digestibility between varied levels of organic acids blend, similar finding to this result with a slight enhancement in the dry matter digestibility was demonstrated by (**Silva et al 2013**), who fed marine shrimp *Litopenaeus vannamei* diets supplemented with 2 % salts of organic acid (acetate, propionate and butyrate) and their results

showed no significant differences in the apparent dry matter and crude protein digestibility.

In the present study the highest apparent digestibility of crude protein was achieved in the third fish group fed diet supplemented with acidifiers at 4 ml/ kg diet which was significantly different ($P < 0.05$) with other groups. This result could be attributed to some factors such as dietary acidification and solubilization which reduce intestinal pH and could be also due to binding various cations along the intestine (**Khajepour and Hosseini, 2012**).

Table 5. Apparent nutrient digestibility coefficient % of *Clarias gariepinus* fed acidifiers supplemented diets (data are means \pm SE of three replicates of fecal samples)

Parameter	T1	T2	T3	T4
Dry matter	68.32 \pm 3.91	69.81 \pm 3.77	69.68 \pm 1.24	65.51 \pm 3.80
Crude protein	55.34 \pm 3.70 ^{ab}	40.93 \pm 3.20 ^c	58.56 \pm 0.45 ^a	49.70 \pm 1.33 ^b
Lipid	54.80 \pm 1.33 ^a	5.58 \pm 2.53 ^c	18.75 \pm 0.91 ^b	62.45 \pm 5.87 ^a

Values within the same row have different superscript letters indicate significant differences ($P < 0.05$).

These results agreed with that found by (**Ng et al 2015**) who fed tiger shrimp *P. mondon* 1, 2 and 4 % organic acid blend diet and found that group fed 2 % organic acid blend had higher significant ($P < 0.05$) growth performance and higher apparent crude protein digestibility than the other groups. Similar findings were detected by (**Hassan et al 2014**) on *Oreochromis niloticus* fed on diet supplemented by calcium lactate and calcium propionate at different levels 0.5 %, 1 % and 1.5 %, the result tends to no significant difference in the dry matter digestibility but showed highly significant difference in the apparent digestibility of crude protein and crude lipids. On the contrary, (**Romano et al 2015**) tested the microencapsulated organic

acids blend on white shrimp *Litopenaeus vannamei* at levels of 1 %, 2 % and 4 % OAB and demonstrated that the highest dry matter digestibility was detected from shrimp fed on 2 % OAB diet.

In previous study (**Ng et al 2009**) on red hybrid tilapia fed diets supplemented with different levels of 0.1 %, 0.2 % and 0.3 % OAB compared to potassium diformate (KDF) at 0.2 % concentration, their results showed that no significant differences in the apparent dry matter, crude protein and crude lipid digestibility among the various treatments. These results could be attributed to the type of acidifiers and species used as experimental aquatic animal.

Table 6. Proximate analysis of whole-body composition of African catfish fed acidifiers supplemented diets

Parameter	T1	T2	T3	T4
Dry matter	28.51 \pm 3.52	27.18 \pm 3.39	27.28 \pm 2.11	31.04 \pm 3.42
Crude protein	65.87 \pm 1.13 ^c	74.39 \pm 1.17 ^{ab}	78.93 \pm 1.15 ^a	72.21 \pm 1.94 ^b
Crude lipid	9.25 \pm 0.56	9.46 \pm 2.29	7.32 \pm 0.68	6.81 \pm 0.65
Ash	0.71 \pm 0.02 ^b	0.67 \pm 0.05 ^b	0.78 \pm 0.01 ^{ab}	0.97 \pm 0.06 ^a

Values (means \pm SE) within the same row have different superscript letters indicate significant differences ($P < 0.05$).

5. Whole-body composition of *C. gariepinus*

The effect of acidifiers supplemented diets on the proximate analysis of whole-body composition of *C. gariepinus* are represented in **Table 6**. The highest value of crude protein and ash were recorded at 4 ml/ kg and 6 ml/ kg dietary supplemented with OAB, respectively. In this field (**Hassan et al 2014**) found that the highest values of crude protein and ash were detected at 1 and 1.5 % Ca lactate compared with the basal diet or other tested diets for *O. niloticus*. In the present study body moisture was mostly not significantly affected by different levels of acidifiers, this result is in agreement with (**Ng et al 2009**) who tested different levels of dietary KDF and organic acids blend on red hybrid tilapia, they did not find negative effects on fish body composition and any hazard effects or toxicity on all treated fish which appeared to be in healthy status as that of the control. Similar findings were observed by (**Romano et al 2015**) who found the highest muscle crude protein levels in shrimp fed on 2 % OAB diet, although there are no significant differences from that of the control diet.

6. Hematological parameters

Hematological parameters for *C. gariepinus* fed on acidifiers supplemented diets are represented in **Table 7**. All fish groups fed on the different doses of organic acids blend showed significant differences ($P < 0.05$) in red blood cells and white blood cells count, the third treatment recorded the highest values of RBCs and WBCs, while in T2 (0.2 %) and T4 (0.6 %) WBCs were decreased recorded the lowest counts, respectively. On the contrary (**Sherif and Doaa, 2013**) demonstrated that the highest record of WBCs was achieved with in-

creasing the concentration of acidifier in the diet. Hematological parameters including hemoglobin (Hb), hematocrit (PCV), MCV, MCH, and MCHC are very close in the different levels of acidifiers treated fish and, in the control, except for RBCs, WBCs, Hb and PCV values which were decreased in (T4) (**Table 7**). **Hastuti and Subandiyono (2018)** reported different values of $1.6 - 1.7 \times 10^6 \mu\text{l}^{-1}$, $175 - 206.3 \times 10^3 \mu\text{l}^{-1}$, $6.4 - 7.5 \text{ gdl}^{-1}$ and $21.7 - 26.2 \%$ for RBCs, WBCs, Hb and PCV, respectively, in the North African farmed catfish, *C. gariepinus*. However, **Erhunmwunse and Ainerua (2013)** reported almost similar values of blood parameters in juvenile African catfish, where RBCs ($\times 10^6 \mu\text{l}^{-1}$) 2.88 ± 0.7 , WBC ($\times 10^3 \mu\text{l}^{-1}$) 41.1 ± 11.05 and PCV (%) 36.0 ± 9.01 .

Blood profiles can be used to evaluate physiological responses of fish (**Hastuti and Subandiyono, 2018**). Under stress conditions fish will decrease the number of red blood cells, hematocrit values and hemoglobin levels, while number of white blood cell count tends to be increased (**Hastuti, Subandiyono, 2018**). The results of this study indicate that WBCs shows the opposite response, i.e. the WBCs value was decreased in the T4 (**Table 7**), and the values of RBCs, Hb, and hematocrit were also decreased. Similar trend of hematological parameters were observed in the African catfish reared under stress of high stocking density (**Hastuti and Subandiyono, 2018**). Decreasing of the WBCs value in 6 ml^{-1} OAB-treated fish along with number of RBCs, hematocrit and hemoglobin indicates that the immune system of the fish is weak, so its body will be vulnerable to the threat of infections. Moreover, hematology test could be a good indicator for the quality and safety of the diet.

Table 7. Hematological parameters for *Clarias gariepinus* fed acidifiers supplemented diets

Parameter	T1	T2	T3	T4
Hematocrit (PCV %)	30.47 ± 3.79	31.33 ± 2.85	32.33 ± 2.85	24.00 ± 2.00
Hemoglobin (g/dl)	10.27 ± 1.19	10.57 ± .93	10.37 ± 1.19	8.23 ± 0.38
RBC ($\times 10^6 \mu\text{l}$)	3.80 ± 0.50 ^{ab}	3.50 ± 0.21 ^b	4.27 ± 0.09 ^a	2.83 ± 0.12 ^c
WBC ($\times 10^3 \mu\text{l}$)	20.31 ± 2.67 ^b	18.69 ± 1.09 ^c	22.80 ± 0.47 ^a	15.14 ± 0.64 ^d
MCV (μm^3)	84.23 ± 3.99	88.17 ± 3.41	79.33 ± 2.96	82.33 ± 2.90
MCH (pg)	27.33 ± 3.17	29.33 ± 1.33	25.00 ± 2.64	27.33 ± 1.66
MCHC (%)	33.00 ± 1.00	33.00 ± 0.57	31.67 ± 1.45	31.67 ± 0.72

Values (means ± SE) within the same row sharing the same superscripts are not significantly different ($P > 0.05$).

7. Biochemical analysis of liver enzymes

Table 8. HSI and liver enzymes of *C. gariepinus* fed acidifiers supplemented diets

Parameter	T1	T2	T3	T4
HSI %	1.4 ± 0.01	1.4 ± 0.13	2.03 ± 0.4	1.2 ± 0.15
ALAT (UI ⁻¹)	48.3 ± 2.9 ^b	61.0 ± 14.4 ^b	75.6 ± 6.5 ^{ab}	113.0 ± 15.3 ^a
ASAT (UI ⁻¹)	311.6 ± 18.8	303.3 ± 20.3	335.0 ± 29.3	374.3 ± 21.3

Values (means ± SE) within same row sharing the same superscripts are not significantly different (P > 0.05).

The results showed a slight increase in hepatosomatic index (HSI %) in T3 compared with other treated and non-treated fish groups. Liver appeared normal with no hemorrhages or tissues damage indicating no hazard effects of dietary supplementation of acidifiers. Similar result was obtained by (Ng et al 2015; Sherif and Doaa 2013; Ng et al 2009; Abu Elala and Ragaa 2014). However, fish fed dietary supplemented with 6 % acidifiers had higher values of ALAT and ASAT enzymes compared with the other treatments (Table 8). ALAT and ASAT enzymes are important indicators for the status of liver function, when large amounts of ALAT and ASAT released into blood will refer to liver cell damage (Suharjo and Cahyono, 2009; Jafarpour and Nekuie 2016). The present data showed increase values of ALAT and ASAT with increasing OAB especially in T4, revealing the possibility of liver cell damage. Hastuti and Subandiyono (2018) reported lower concentrations of ASAT (200 – 232 UI⁻¹) than the values of the present study and almost similar or higher values for ALAT (104 – 108 UI⁻¹) in catfish. The authors mentioned that the recorded higher values of ALAT and ASAT in *C. gariepinus* cultured using biofloc technology is suspected to be due to that fish liver cells work hard to face of stress conditions caused by the high density of 1,000 fish m⁻³, these values were lower 185.3 ± 5.0 UI⁻¹ and 63.7 ± 3.2 UI⁻¹ for ASAT and ALAT, respectively in *C. gariepinus* cultured at lower density of 200 fish m⁻³ (Hastuti and Subandiyono, 2013). Jafarpour and Nekuie (2016) reported lower values of (44.5 ± 6.1 UI⁻¹) for ALAT and (38.5 ± 8.9 UI⁻¹) for ASAT in *Oncorhynchus mykiss* which are very close with those of the control group. On other hand (Romano et al 2016) demonstrated that the

liver from all red hybrid tilapia fish showed some necrosis and hemorrhaging became apparent in the 2 % sodium citrate treated fish, although the authors reported no significant differences were observed with sodium citrate treatments at different levels.

The acidification of the diet markedly reduces the microbial load by reducing gut pH and due to the diffusion of the organic acids into the bacterial cells which in turn reducing cytoplasmic pH of bacterial cells, eventually cause cell death (Booth and Stratford, 2003). Finally, there are many factors that may affect the utilization of acidifiers such as species, type of fish culture, physiological status, age, levels of organic acids, in addition to the type of acidifiers used as growth promoters.

CONCLUSION

The results of this study demonstrated that diet supplemented with acidifiers will improve growth performance, nutrients utilization and immunity for catfish. However, using higher concentrations of OAB as feed additives for prolonged period may affect liver function and cause liver damage.

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تأثير استخدام مخلوط الأحماض العضوية على النمو والهضم والمناعة لأسماك القراميط (القط الأفريقي)

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الموجز

البروتين مقارنة ببقية المجموعات التجريبية. تم تقدير معامل الهضم الظاهري للبروتين الخام والدهن الخام والذي حقق أفضل القيم في المجموعة الثالثة (4 مل/كجم عليقة) مقارنة بباقي المجموعات الأخرى. لم تظهر البيانات أي فروق معنوية في دليل الكبد بين المعاملات المختلفة مما يشير إلى الحالة الصحية الجيدة ظاهريا للكبد. ولكن ، سجلت أنزيمات وظائف الكبد ASAT ، ALAT قيماً عالية في المجموعة الرابعة (6 مل / كجم عليقة) مما يشير إلى تأثير الكبد بالتركيزات العالية من مخلوط الأحماض العضوية. أشارت قراءات الدم كذلك إلى انخفاض في عدد كرات الدم الحمراء والبيضاء وتركيز الهيموجلوبين ونسبة الهيماتوكريت في المجموعة الرابعة مقارنة بالمجموعة الكنترول أو المجموعات التجريبية الأخرى. يتضح من الدراسة أن استخدام مخلوط الأحماض العضوية بتركيزات منخفضة كإضافات في علائق أسماك القرموط يؤدي إلى تحسين في أداء النمو وزيادة قدرة الأسماك على الاستفادة من العليقة وتحسين معاملات الهضم ورفع المناعة ولكن يجب تجنب الإفراط في استخدامها بمستويات عالية لما قد تسببه من أضرار صحية خاصة في الكبد وصورة الدم.

الكلمات الدالة: أسماك القراميط، الأحماض العضوية، أداء النمو، معاملات الهضم ، المناعة.

أجريت تلك الدراسة لتقييم تأثير إضافة مخلوط الأحماض العضوية للعليقة على أداء النمو ومناعة أسماك القرموط الأفريقي. تم إجراء هذه التجربة في المعمل الرطب بشعبة إنتاج الأسماك، قسم الإنتاج الحيواني ، كلية الزراعة ، جامعة عين شمس. تم اختيار 120 سمكة قرموط بمتوسط وزن 94,93 ± 0,90 جم وزعت على 4 معاملات لكل معاملة 3 مكررات بمعدل 10 أسماك لكل حوض تجريبى. استخدمت في الدراسة أربعة علائق تجريبية تحتوى على 35% بروتين خام مضاف إليها تركيزات مختلفة من مخلوط الأحماض العضوية (OAB) بمعدل 0 ، 2، 4 ، 6 مل / كجم عليقة. تمت تغذية الأسماك بالعليقة التجريبية بمعدل 3 % من الوزن الحى مقسمة على 3 وجبات /يوم. كان يتم وزن الأسماك كل 15 يوم لضبط العليقة. تم حساب مقاييس أداء النمو وكفاءة التحويل ومعدلات الاستفادة من الغذاء ومعدلات الإعاشة ، كذلك تم التحليل الكيمياءى لجسم الأسماك في نهاية الفترة التجريبية. أظهرت مجموعات الأسماك التي غذيت على مخلوط من الأحماض العضوية بنسبة 0,6 % أعلى قيمة للزيادة في الوزن الكلي (438 ± 14,24 جم) ، تحسن في معدل النمو النوعى ، أفضل معامل تحويل غذائى ، وأعلى كفاءة في استخدام

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