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Growth, histomorphometry and whole-body composition of African catfish, *Clarias gariepinus* (Burchell 1822), juveniles fed fishmeal-free diet supplemented with blend of organic acid salts

Agama, J. D.[1], *Olude, O. O.[2] and Lanre-Sobade E. A.[3]

[123]Department of Marine Sciences, University of Lagos, Akoka, Lagos State, Nigeria

*Corresponding Author: oolude@unilag.edu.ng

ABSTRACT

An investigation was conducted to evaluate the outcomes of adding two levels of a blend of organic acid salts (sodium salts of propionic, formic, fumaric and acetic acid) to a fish meal-free diet for *Clarias gariepinus* juveniles. Four isonitrogenous (40% crude protein) and iso-energetic (18.13±0.10 kJ/g gross energy) diets were formulated: a diet containing 33% fish meal (FM_{33}) served as positive control; a diet in which the fish meal component in FM_{33} was replaced by meat and bone meal (MBM) was used as negative control (FM_0); and two other diets in which the cellulose component of the FM_0 was replaced with the blend of organic acid salt (OASB) at 1.0% ($FM_0OAS_{1.0}$) and 1.5% ($FM_0OAS_{1.5}$). The implications of feeding the experimental diets to *C. gariepinus* juveniles (16.25±0.05 g) were assessed based on growth performance, nutrient utilization, carcass proximate composition, intestinal and hepatic morphology and intestinal morphometry in a 60-day trial. Total replacement of FM with MBM resulted in significantly ($p<0.05$) poor growth performance and nutrient utilization of *C. gariepinus* juveniles and the outcome was not substantially ($p>0.05$) improved by the addition of OASB. Carcass crude protein and ash contents were not significantly ($p>0.05$) affected by dietary treatment but crude fat was significantly ($p<0.05$) reduced in the FM_{33}-fed group compared with the other dietary groups. Dietary supplementation of OASB enhanced ($p<0.05$) the length of the villi relative to the control (FM_{33} and FM_0) diets. The intestinal morphology of *C. gariepinus* revealed that the complete replacement of FM with MBM generated an abundance of goblet cells relative to other dietary groups. The results suggest that dietary supplementation of organic acid salt can enhance the gut health of *C. gariepinus* juveniles but cannot substantially improve the utilization of meat and bone meal.

Keywords:

Clarias gariepinus; Growth; Organic acid salt; Tissue histology

Introduction

The aquaculture industry plays a vital role in global food security, sustainability, and economic growth by providing protein-rich food; alleviating pressure on wild fisheries, and meeting the world's growing demand for seafood while supporting livelihoods (Pradeepkiran *et al*., 2019). However, despite its impressive growth and contributions, aquaculture faces sustainability concerns. One primary concern is the industry's

reliance on fishmeal and fish oil, which has significant environmental implications (Ghamkhar and Hicks, 2020).

Traditionally, fishmeal has been the preferred protein source in aquaculture feed, accounting for approximately 68% of global fishmeal production (Tacon and Metian, 2015). The product's popularity stems from its exceptional amino acid profile, palatability, and digestibility, as well as its provision of nucleotides, essential fatty acids, phospholipids, minerals, and water-soluble vitamins (Oliva-Teles *et al*., 2022). Yet, environmental, economic, and local availability are some of the constraints associated with fishmeal inclusion in aquafeed; limiting the further growth of the aquaculture industry (Hardy, 2010; Shepherd *et al*., 2013). Accordingly, there is a need to reduce fishmeal inclusion levels in aquaculture feeds and shift towards alternative and sustainable protein sources (Galkanda-Arachchige *et al*., 2020; Han *et al*., 2018).

To reduce reliance on fishmeal as a protein source, efforts have been made to incorporate plant protein sources in aquaculture. However, these sources have often been linked to reduced growth performance, feed intake, and impaired intestinal health and function among other reported deleterious effects (Xie *et al*., 2021; Olude and Akinduti, 2023). Several animal protein sources have also been explored in developing low-fish meal diets, including processed and rendered animal by-products such as poultry meal and meat and bone meal, due to their high crude protein content (45-65%), amino acid profile, availability, and relatively low cost (Huan *et al*., 2018; Galkanda-Arachchige *et al*., 2020). Nevertheless, in comparison to fishmeal, these products are of only limited utility because they vary in protein quality and digestibility, resulting in considerable variability in fish performance, particularly when used as the sole protein source in the fish diet (Moutinho *et al*., 2017; Galkanda-Arachchige *et al*., 2020).

Many interventions, albeit with varying levels of success, have been made to solve the issues associated with these alternative feedstuffs. One such promising intervention is the use of organic acids and their salts (Ng *et al*., 2009; Ng and Koh, 2017; Huan *et al*., 2018). Organic acid salts have shown great promise as additives in aquaculture diets, particularly when using alternative protein sources (Huan *et al*., 2018; Hossain *et al*., 2024). Organic acids or their salts have also been shown to improve growth, feed efficiency, and disease resistance in a variety of fish species, both temperate and tropical, though these findings have been inconsistent (Asriqah *et al*., 2018; Hussein *et al*., 2023; Hossain *et al*., 2024). Additionally, incorporating organic acids into fish feed has been shown to reduce microbial contamination, decrease toxic byproducts, and enhance nutrient absorption, including absorption of essential minerals like calcium, phosphorus, and magnesium (Zhang *et al*., 2023; Hossain *et al*., 2024).

Given the potential benefits, such as environmental sustainability and cost efficiency, offered by the possibility of a fishmeal-free diet and the significant niche occupied by *C. gariepinus* in Nigeria's aquaculture industry; the present study attempted to determine whether the possible growth compromise usually associated with fishmeal replacement in *C. gariepinus* diet can be compensated for by dietary supplementation of a blend of organic acid salts. Although many attempts (Asriqah *et al*., 2018; Omosowone *et al*., 2018; Saleh *et al*., 2018) have been made to improve the growth and diet efficiency of *C. gariepinus* through dietary supplementation of organic acids or their salts, either singly or in combination,

there is scarcity of investigations focusing on the strategy of offsetting the nutritional imbalances arising from fishmeal replacement in *C. gariepinus diet* through organic acid supplementation. The possibility of such an approach has been successfully demonstrated by Huan *et al*. (2018) and Zhang *et al*. (2023) in diets for tilapia (*Oreochromis niloticus* × *O. aureus*) and hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatu*), respectively. Against this backdrop, this study investigated the effects of a blend of some organic acid salts (sodium salts of propionic, formic, fumaric and acetic acid) on the growth, histomorphometry, and whole-body composition of *Clarias gariepinus* fed fishmeal-free.

Materials and Methods

Experimental system

The study took place at the Aquaculture Unit, Botanical Garden of the University of Lagos, Akoka, Lagos using twelve clean rectangular plastic tanks each measuring 24cm x 30cm x 40cm. The plastics were covered to prevent fish from escaping but multiple holes were bored on the covers to allow for air passage. Water was supplied to the experimental unit from an overhead reservoir which was in turn supplied from the Waterworks Unit of the University of Lagos, Nigeria. The experimental plastics were filled with about 22 litres of water.

Diet formulation

Four diets having similar crude protein (40% crude protein) and gross energy (18.13±0.10 kJ/g) contents were formulated (Table 1). The diets consist of a positive control (FM_{33}) which has a high level of fishmeal (33%) and a negative control (FM_0) in which the fish meal component in positive control was replaced by meat and bone meal (MBM). Sodium salts of propionic, formic, fumaric, and acetic acids were sourced from Uche Scientific, Bariga, Lagos, Nigeria, and thoroughly mixed in the ratio 3:3:1:1 respectively. The ratio was adopted based on the preliminary result of an *in vitro* antimicrobial study (unpublished data). The organic acid salt mixture was added to diet FM_0 by substituting its cellulose content at 1.0% ($FM_0OAS_{1.0}$) and 1.5% ($FM_0OAS_{1.5}$). The major ingredients used in the formulation were obtained from Neuf Link Integration, Fish Farm Estate, Ikorodu, Lagos, Nigeria. The ingredients were combined based on the proportion needed for each ingredient to formulate 1kg of each diet (Table 1). The ingredients were thoroughly mixed after which hot water was added to form a dough. Lastly, lysine, methionine, butylated hydroxytoluene (BHT), and organic acid salt blend were added through soybean oil, mixed thoroughly, and pelleted using a 2 mm die NationalLine meat grinder (NL-800). The pellets were air-dried at room temperature for 48 hours and stored in labeled plastic bags.

Experimental design and feeding

Clarias gariepinus juveniles were purchased from a commercial fish hatchery in Lagos and transported to the experimental site at the University of Lagos, Nigeria using a 50-litre plastic tank half-filled with water. The fish were acclimatized for ten days in a 3000-litre circular plastic tank with a periodic water exchange. Within this period, the fish were fed 2.0 mm Skretting feed. Following this period, the juveniles were randomly distributed into twelve plastic tanks at the rate of 10 fish (average weight, 16.25±0.05g) per tank with each treatment replicated thrice in a completely randomized experimental design. The fish were fed to satiation twice daily at 0900 hours and 1600 hours for 60 days. The amount of feed given to the fish each day was recorded. The water in the tanks was drained, cleaned, and refilled with fresh water every other

day, but as the experiment progressed and the fish grew, the tanks required daily water changes. Dissolved oxygen (6.05±0.05 mg/L) was measured using Oxyguard Handy Polaris (OxyGuard International, Denmark), while pH (6.44 - 6.48), temperature (27.86±0.25 °C), electrical conductivity (2511.17±286.66 µs/cm) and total dissolved solids (1255.33±143.32 ppm) were monitored through multifunction water quality LCD digital meter (Flintronic®-eu). The monitored water quality parameters were found to be within tolerable limits for *C. gariepinus* juveniles (Boyd *et al*., 1998).

Proximate analysis

The proximate composition of the ingredients, diets, and whole-body composition was assessed using the AOAC (1990). Micro-Kjeldahl protocol was followed in the determination of crude protein. The samples were digested using concentrated H_2SO_4 to liberate nitrogen, the digest was distilled over boric acid in the presence of sodium hydroxide after which the trapped nitrogen was estimated by titration using standard hydrochloric acid. Sequential acid-base digestion was used in estimating crude fibre while the Soxhlet extraction method was used to determine crude fat. The ash content was determined after burning up the sample in a muffle furnace at 500ºC while the moisture was calculated following overnight drying of the sample in an oven (GenLab, E8A76752) at 105ºC. Nitrogen-free extract (NFE) was calculated using the relationship:

NFE = 100 - (moisture + ash + crude protein+ ether extract + crude fibre)

Growth performance and diet utilization

The following growth and nutrient utilization parameters were calculated as measures of the effectiveness of each dietary treatment.

Weight gain (WG, g) = $W_f - W_i$

Percentage weight gain (PWG, %) = $(W_f - W_i / W_i) \times 100$

Daily growth index (DGI) = $[(W_f^{1/3} - W_i^{1/3})/t] \times 100$

Specific growth rate (SGR, %/day) = $100 (\ln (W_f) - \ln (W_i) / t$

Feed Conversion Ratio (FCR) = Total dry feed fed (g) / $(W_f - W_i)$

Protein Efficiency Ratio (PER) = $(W_f - W_i)$ / protein fed (g)

Survival = (No. of fish remaining at the end of the experiment / No of fish stocked) × 100

Where W_f and W_i are final and initial live weights over the rearing period t

Intestinal morphometry and tissue histology

At the end of the feeding trial, liver and mid-intestine were excised from representative fish from each treatment and fixed in 10% formal saline. The fixed tissues were subsequently dehydrated in a graded series of ethanol and cleared with xylene. They were infiltrated with paraffin, sectioned (5 µm), and stained with haematoxylin and eosin. Prepared slides were viewed under a bright field compound microscope (Nikon, YS100) enabled with a Nikon camera to observe any aberration that may have arisen due to treatment, and photomicrographs were taken at 400× magnification. Intestinal villi length, villi width, and crypt depth were measured using Image J software.

Data analysis

The data obtained throughout the experiment were subjected to a one-way analysis of variance (ANOVA). Comparisons among treatment means were conducted using the Duncan Multiple Range test (Duncan, 1955) at a 95% confidence level. All calculations were performed using the IBM SPSS statistical package version 27.

Results

Growth performance, nutrient utilization, survival, and carcass composition

The fish were observed to have fed on the different diets presented to them without showing aversion or signs thereof to any of the diets. The assessed growth parameters of the experimental fish fed the various diets varied significantly ($p<0.05$) with better growth performance generally observed in the FM_{33}-fed group while the least performance was observed in the FM_0 group (Table 2). Complete replacement of FM with MBM significantly ($p<0.05$) resulted in growth depression. WG, DGI, and SGR ranged between 77.62±4.65 - 105.96±3.12 g; 3.50±0.13 - 4.25±0.06; 3.12±0.09-3.61±0.03%/day, respectively. Although measurable increases were observed in the measured parameters of growth consequent to the supplementation of the organic acid salts to the MBM-based diet, the observed increases were insignificant ($p>0.05$). The nutrient utilization assessed using FCR and PER also trended in the same manner as growth performance. Experimental fish survival was not influenced significantly ($p>0.05$) by any of the dietary groups and it ranged from 90.00±5.77 to 96.67±3.33%. The results of the carcass composition (Table 3) showed that diet FM_{33} significantly ($p<0.05$) lowered carcass ether extract relative to other groups whereas diet $FM_0OAB_{1.5}$ favoured significantly ($p<0.05$) higher total carbohydrates when compared to other dietary groups. However, dietary treatment did not elicit any significant effect on moisture, crude protein and ash contents of the fish carcass.

Intestinal morphometry and tissue histology

Dietary supplementation of the blend of organic acid salt significantly ($p<0.05$) increased the length of *C. gariepinus* villi (Figure 1). Compared to other dietary groups, this increase was more in the group fed with 1% OASB. The width of the villi of *C. gariepinus* fed the MBM-based diet (without OASB supplementation) was significantly ($p<0.05$) reduced relative to others (Figure 2). The intestinal crypts of the $FM_0OAS_{1.0}$-fed group were also significantly ($p<0.05$) deeper when compared to those that received FM_0 and FM_{33} (Figure 3). The intestinal morphology of *C. gariepinus* revealed that the complete replacement of FM with MBM generated an abundance of goblet cells relative to other dietary groups (Figure 4). Similar to other dietary groups, the intestinal architecture of the group fed FM_0 revealed well-differentiated villi with intact muscularis externa and serosa. The organization of the hepatic tissue in the FM_0- and $FM_0OAS_{1.0}$-fed groups showed a normal structure, well-differentiated hepatocytes, and hepatic cords with more visible sinusoids in the $FM_0OAS_{1.0}$ group (Figure 5). Vacuolation was observed in the well-differentiated hepatocytes of the FM_{33}-fed group. Supplementation of 1.5% OASB to the MBM-based diet caused pyknotic hepatocytes and disarranged hepatic cords, with dilated sinusoids in the experimental fish.

Discussion

Partial or total replacement of fish meal with unconventional plant- or animal-derived proteins has been the primary focus of investigation in modern aquaculture because of issues bordering on sustainability, prohibitive aquafeed pricing, and environmental health. In this study, we investigated the possibility of complete replacement of FM with MBM and also explored the efficacy of enhancing the utilization of MBM with a blend of organic acid salt. Total substitution of FM with MBM resulted in significantly poorer growth performance and feed utilization, indicating that MBM used under the current investigation could not replace FM in the diet for *C. gariepinus*. That outcome was not unexpected as it has been firmly established in previous investigations that MBM cannot completely substitute FM. For instance, Moutinho *et al*. (2017) incorporated MBM at the expense of FM in the diet for gilthead seabream and observed a compromise of growth, feed utilization, and nutrient retention when more than half of FM protein was replaced. Similarly, Goda *et al*. (2007) had earlier reported significant depression in fish performance when MBM completely replaced FM in the diet of *C. gariepinus*.

Reasons previously advanced for the poor utilization of MBM by the various species were usually the nutritional composition of the raw materials transformed to the MBM, especially in terms of high ash content and deficiency of some important amino acids, and the processing technology employed in its production (Rossi Jr and Davis, 2014; Moutinho *et al*., 2017). Despite that knowledge, we ambitiously carried on with the total replacement of FM with MBM and supplemented the diet with two levels of OASB to establish whether there would be any enhancement of growth and feed utilization consequent to the OASB supplementation. Such enhancement had been previously reported by Huan *et al*. (2018) who supplemented hybrid tilapia's diet with a blend of organic acid salt after completely replacing the FM component of the diet with MBM. Zhang *et al*. (2023) also reported growth promotion, immune potency, and improved stomach formation and digestibility when they added a compound acidifier to the diet of hybrid grouper in which fishmeal was largely replaced with cottonseed protein concentrate. Unlike the outcome reported in the investigation of Huan *et al*. (2018), only measurable but not substantial enhancements were observed under the current investigation.

Our results, however, corroborate the outcome of similar research by Ng *et al*. (2009) who supplemented a proprietary six organic acids blend to the diet of red hybrid tilapia and observed no significant increase in growth performance and diet efficiency. Bjerkeng *et al*. (1999) also observed a similar outcome when they supplemented a blend of sodium acetate, sodium propionate, and sodium butyrate to the diet of Atlantic salmon for 175 days. Contrasting growth and nutrient utilization outcomes when assessing the growth-promoting effects of organic acid salts, either singly or in combination, is not unusual and it can be attributed to variations in fish age, species, type, and level of organic acids tested, diet composition, culture conditions and duration of experiment as alluded to by Ng *et al*. (2009) and Rasidi *et al.* (2024). Although a comparative evaluation of the amino acids and anti-nutrients that might be present in the fishmeal and MBM used under the current investigation was not carried out, it is not unlikely that complete fishmeal replacement by MBM in the FM_0 diet caused some nutritional deficiencies and impairments which could not be fully compensated for by the organic acid supplementation.

Notwithstanding the suboptimal growth performance in the treated fish relative to the FM-based control, there were no significant differences in the carcass protein composition, suggesting that dietary protein

across groups was sufficient for protein deposition (Shalata *et al*., 2021). The most noticeable effect of the dietary OASB supplementation in the MBM-based diet is the beneficial improvement of the intestinal health observed in increased villi length of *C. gariepinus*. Increased villi length is usually associated with enhanced capacity for nutrient absorption since longer villi provide increased surface area for nutrient uptake. The present study aligns with the results of previous investigations where different types and combinations of feed additives have been shown to increase fish villi length and width (Shalata *et al*., 2021; Omidi *et al*., 2024).

There was also an observable reduction in the abundance of goblet cells in the groups that received FM-based control and OASB-supplemented diets relative to the MBM-based diet. Goblet cells are unicellular gland that produces and release mucin which creates a barrier of mucosa that shields epithelial cells when triggered by various stressful conditions such as pathogens or infections, environmental stressors, gut inflammation, or dietary factors (Pan *et al*., 2024).

Under the current investigation, we believe that the abundance of goblet cells in the MBM-based diet may be a compensatory response to gut irritation or inflammation perhaps caused by undigested MBM components (Rimoldi *et al*., 2016). Huan *et al*. (2018) had earlier demonstrated that an MBM-based tilapia diet impaired the digestibility of dry matter, protein, calcium, and other essential minerals which were resolved through dietary combined supplementation of organic acid salt blend and protease enzyme. The beneficial effects of the OASB in the present study notwithstanding, it appears that there was an over-supplementation of the OASB at a 1.5% level. This is because pyknosis of the hepatocytes, dilation of sinusoids, and disarrangement of the hepatic cords observed at this level of supplementation suggest disruption of cellular homeostasis and hepatic cell damage which have been previously associated with toxins, nutritional imbalances, or metabolic disturbances (Wolf and Wheeler, 2018). Contrarily, the group that received 1% OASB showed well-maintained hepatic architecture which suggests that this concentration of OASB did not cause metabolic stress and damage to the liver health of *C. gariepinus* juveniles.

Conclusively, these findings demonstrate the potency of the organic acid salts blend supplemented under the current investigation in enhancing the nutrient absorption capacity of the intestine, however, the OASB could not fully mitigate against the growth depression exerted by the complete replacement of fish meal with meat and bone meal in the diets of *C. gariepinus* juveniles. In this context, future studies should seek to optimize the gradual replacement of FM with MBM and OASB supplementation.

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Table 1: Ingredients and the proximate composition (%) of Experimental Diets

INGREDIENTS	FM_0	$FM_0OAS_{1.0}$	$FM_0OAS_{1.5}$	FM_{33}
Groundnut cake¶	10.78	10.78	10.78	10.78
Soybean meal¶	12	12	12	12
Fishmeal¶	0	0	0	33
Meat and bone meal¶	46.2	46.2	46.2	0
Poultry meal¶	8	8	8	8
Wheat meal¶	11	11	11	24.2
Soybean oil	6	6	6	6
Vit-Min premix	2	2	2	2
CMC	1	1	1	1
BHT	0.02	0.02	0.02	0.02
Lysine	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5
Cellulose	2	1	0.5	2
OASB	0	1	1.5	0
Proximate composition (%)				
Dry matter	90.75	90.80	90.80	90.00
Ether extract	5.33	5.35	5.33	6.02
Crude protein	40.23	40.23	40.20	40.70
Crude fibre	6.60	6.60	6.60	6.05
Total ash	9.52	9.51	9.52	7.40
NFE	29.07	29.11	29.15	29.83
Gross energy (kJ/g)*	18.02	18.03	18.02	18.44

Crude protein of Groundnut cake, Soybean meal, Fishmeal, Meat and bone meal, Poultry meal, and Wheat meal used was 45%, 45%, 64%, 50%, 69%, and 15% respectively. Vit-Min – Vitamin-Mineral premix; CMC- Carboxymethylcellulose, BHT-Butylated hydroxytoluene; OASB- Organic acid salt blend; NFE-Nitrogen free extract; Vitamin-Mineral premix (quantity/kg): vitamin A- 12,500,000I.U.; vitamin D3-2,500,000I.U.; vitamin E- 40,000,000mg; vitamin K3 - 2000mg; vitamin B1- 3000mg; vitamin B2- 5500mg; Niacin- 55000mg; calcium pantothenate- 11500mg; vitamin B6- 5000mg; vitamin B12- 25mg; choline chloride- 500,000mg; folic acid- 1000mg; biotin- 80mg; manganese- 120,000mg; iron- 100,000mg; zinc-80,000mg; copper- 8500mg; iodine- 1500mg; cobalt- 300mg; selenium- 120mg; anti-oxidant- 120,000mg. *Calculated based on physiological fuel value of 23.9 kJ/g, 39.8 kJ/g, and 17.6 kJ/g for crude protein, ether extract, and total carbohydrate, respectively.

Table 2: Growth performance and nutrient utilization of *Clarias gariepinus* fed fish meal-free supplemented with varying levels of organic acid salt blend.

PARAMETERS	FM_0	$FM_0OAS_{1.0}$	$FM_0OAS_{1.5}$	FM_{33}
INI(g)	16.33±0.11	16.28±0.11	16.22±0.13	16.12±0.12
FIN(g)	93.95±4.63^{b}	100.80±4.61^{b}	97.25±7.71^{b}	122.08±3.24^{a}
WG(g)	77.62±4.65^{b}	84.53±4.72^{b}	81.03±7.62^{b}	105.96±3.12^{a}
DGI	3.50±0.13^{b}	3.70±0.13^{b}	3.60±0.20^{b}	4.25±0.06^{a}
PWG (%)	475.37±29.37^{b}	519.77±32.63^{b}	499.10±44.09^{b}	657.08±14.35^{a}
SGR(%/day)	3.12±0.09^{b}	3.25±0.09^{b}	3.19±0.13^{b}	3.61±0.03^{a}
FF(g/fish/day)	110.38±5.62^{b}	118.59±3.47^{a}	111.10±5.19^{b}	119.57±5.03^{a}
FCR	1.42±0.01^{a}	1.41±0.04^{a}	1.38±0.07^{a}	1.13±0.01^{b}
PER	1.77±0.02^{b}	1.79±0.05^{b}	1.83±0.09^{b}	2.23±0.03^{a}
SUR(%)	90.00±5.77	90.00±5.77	96.67±3.33	93.33±6.67

Data are expressed as mean ± SE. $^{a-b}$ Mean values in the same row with the superscript were not significantly different ($p > 0.05$). INI - Initial weight; FIN - Final weight; WG - Weight gain; DGI - Daily growth index; PWG- Percentage weight gain; SGR – Specific growth rate; FF - Feed fed; FCR- Feed conversion ratio; PER - Protein efficiency ratio; SUR- Survival

Table 3: Carcass proximate composition of *Clarias gariepinus* fed with fish meal-free diet supplemented with two levels of organic acid salt blend.

PARAMETER	FM_0	$FM_0OAS_{1.0}$	$FM_0OAS_{1.5}$	FM_{33}
Moisture	71.50±0.15	71.05±0.05	69.20±0.2	71.25±0.75
Total ash	1.02±0.02	1.01±0.03	0.98±0.02	0.96±0.02
Crude protein	12.13±0.13	12.25±0.1	12.25±0.15	12.00±0.15
Ether extract	3.20±0.20^{a}	2.80±0.30^{a}	3.20±0.30^{a}	2.40±0.30^{b}
TCHO	11.50±0.50^{b}	12.89±0.48^{b}	14.37±0.67^{a}	13.39±1.22^{ab}

Data are expressed as mean ± SE.$^{a-b}$ Mean values in the same row with the superscript were not significantly different ($p > 0.05$) TCHO- Total carbohydrate

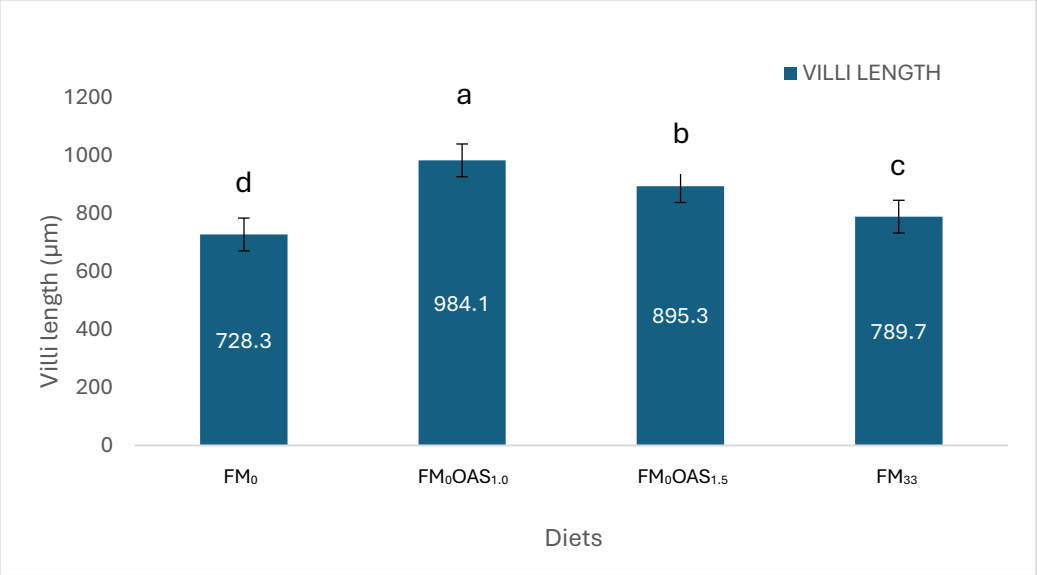

Figure 1: Villi length (µm) of *Clarias gariepinus* fed with fishmeal-free diets supplemented with two levels of organic acid salt blend.

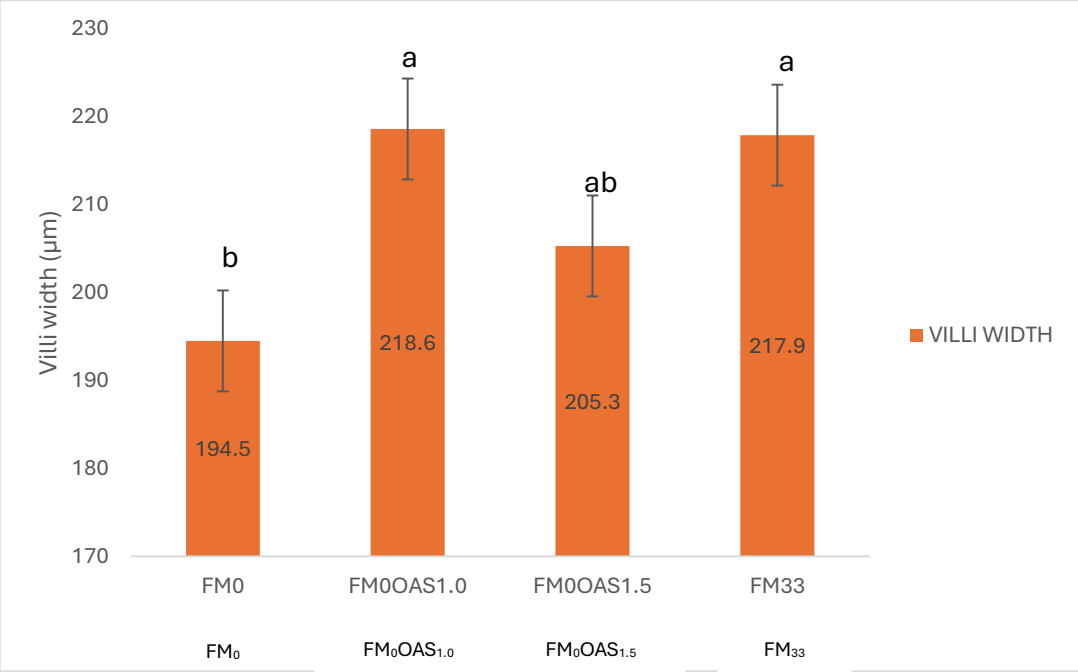

Figure 2: Villi width (µm) of *Clarias gariepinus* fed with fishmeal-free diets supplemented with two levels of organic acid salt blend.

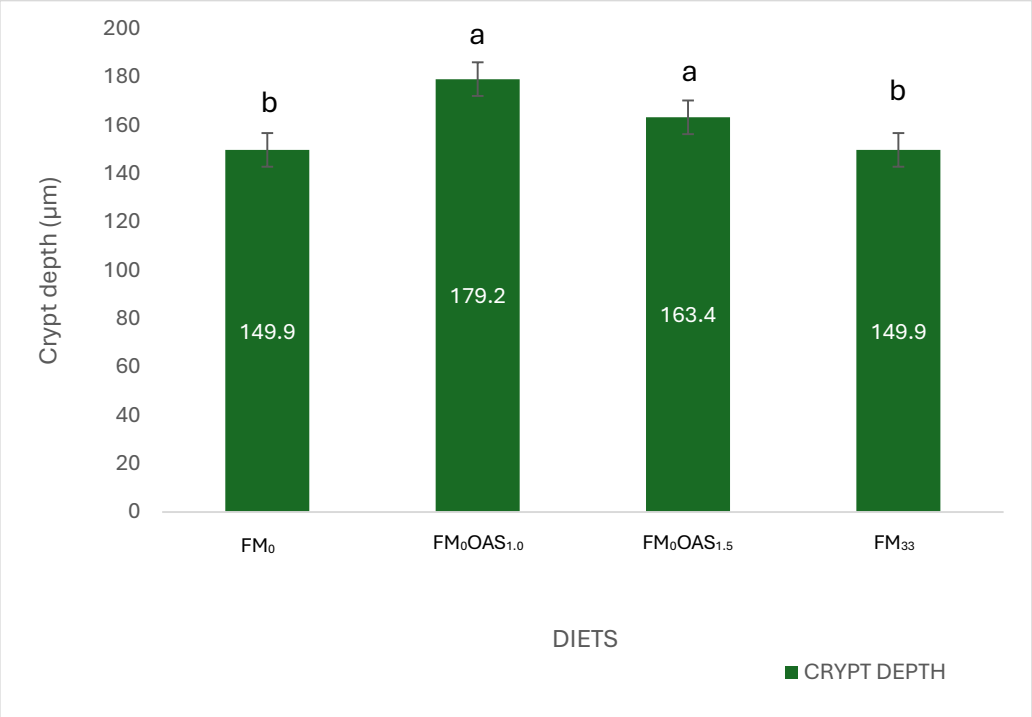

Figure 3: Crypt depth (µm) of *Clarias gariepinus* fed with fishmeal-free diets supplemented with two levels of organic acid salt blend.

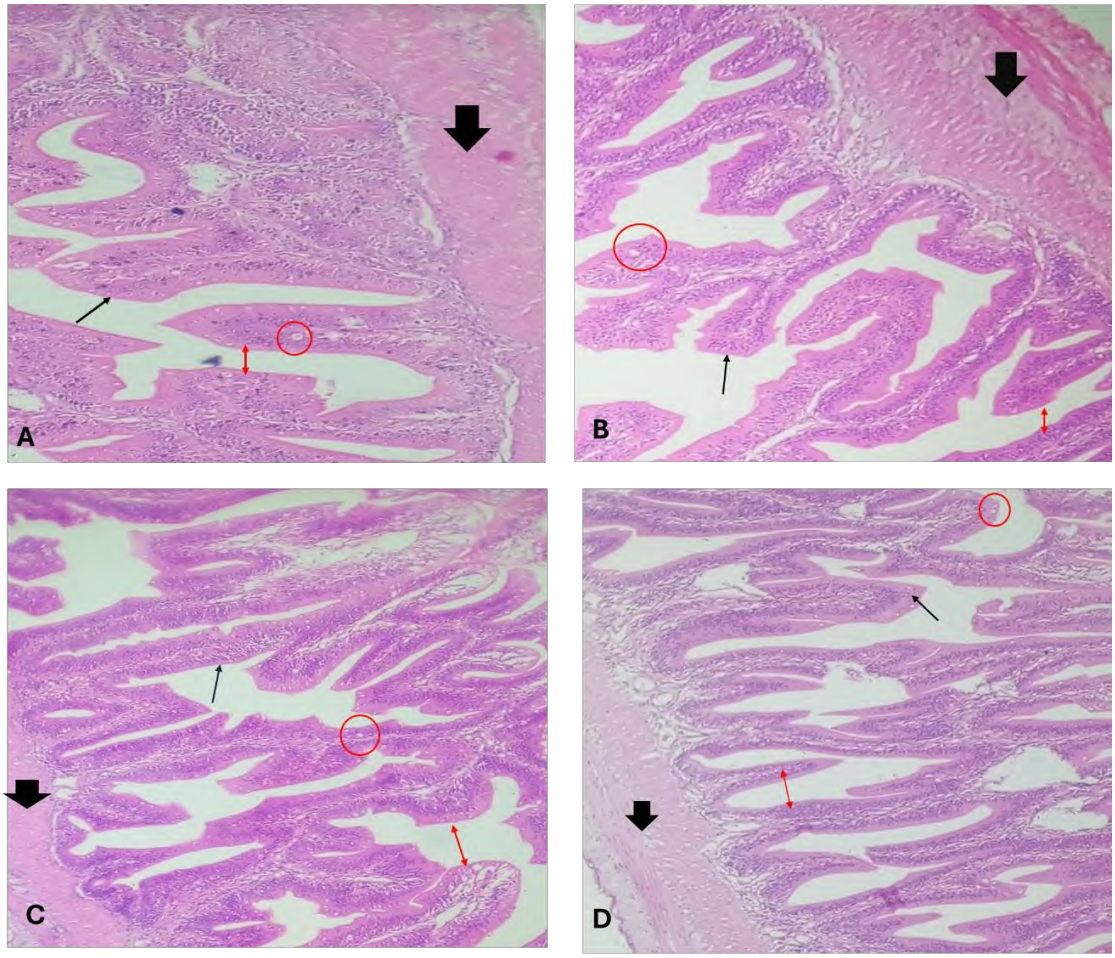

Figure 4: Effect of organic acid salt blend supplementation on intestinal morphology of *Clarias gariepinus* fed fishmeal-free diet (A) FM_0 shows well-differentiated villi (thin black arrow), goblet cells (red circle), muscularis external and serosa (thick black arrow) with duodenal crypts (red double-headed arrow); (B) $FM_0OAS_{1.0}$ shows well-differentiated muscularis layer (thick black arrow), reduced goblet cells (red circle), reduced villi (thin black arrow) and crypts (red double-headed arrow); (C) $FM_0OAS_{1.5}$ shows well-differentiated villi (thin black arrow), reduced goblet cells (red circle), crypts (red double-headed arrow), and muscularis layer (black thick arrow); (D) FM_{33} shows well-differentiated villi (thin black arrow), reduced goblet cells (red circle), muscularis external and serosa (thick black arrow) and crypts (red double-headed arrow). (H/E×400).

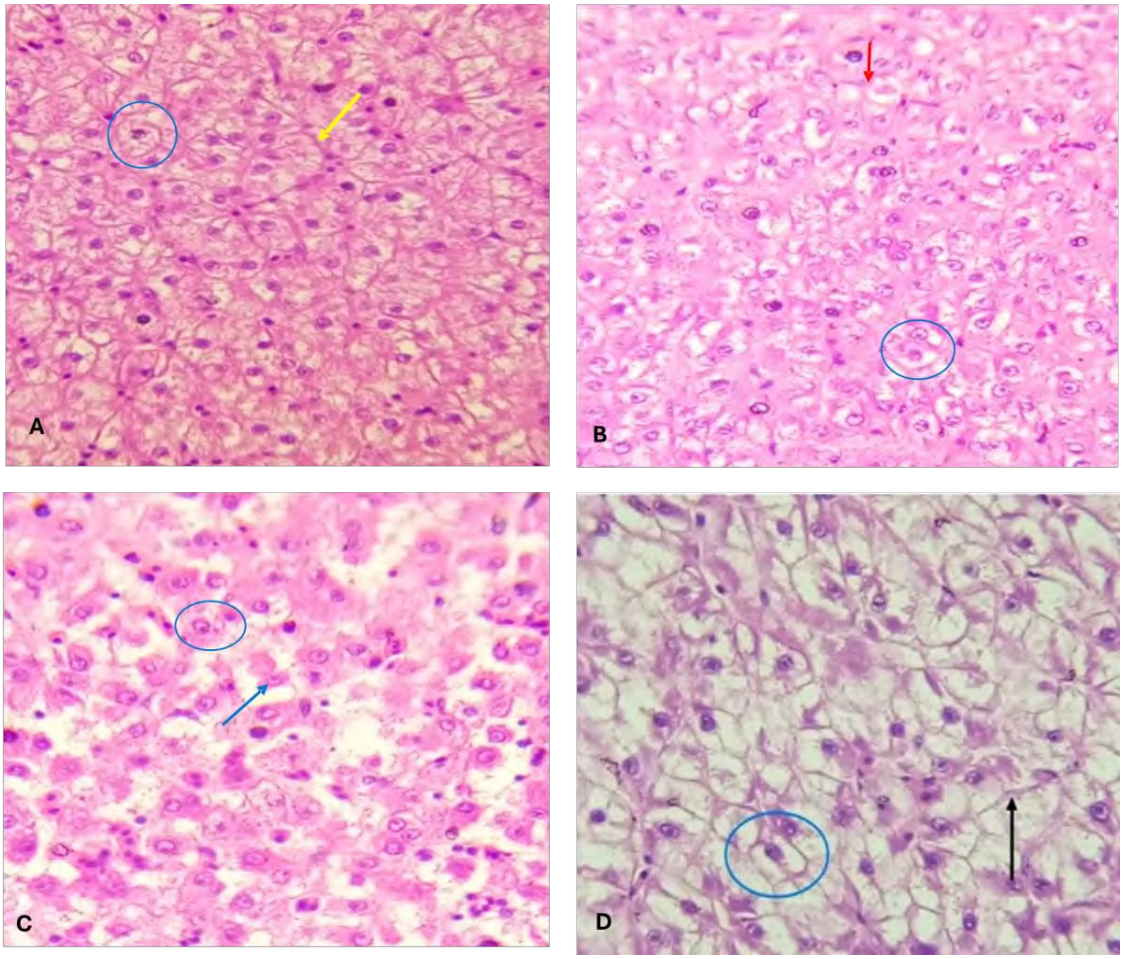

Figure 5: Effect of organic acid salt blend supplementation on the liver morphology of *Clarias gariepinus* fed fishmeal-free diet (A) FM_0 shows well-differentiated hepatocytes (blue circle) and hepatic cords (thin yellow arrow); (B) $FM_0OAS_{1.0}$ shows well-differentiated hepatocytes (blue circle), hepatic cords (thin red arrow) and sinusoids; (C) $FM_0OAS_{1.5}$ shows pyknotic hepatocytes (blue circle), disarrangement of hepatic cords (blue thin arrow) with dilated sinusoids. (D) FM_{33} shows well-differentiated hepatocytes (blue circle) with vacuolation and organized hepatic cords (thin black arrow). (H/E×400).