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GROWTH, HAEMATOLOGY, NUTRIENT RETENTION AND HISTOLOGY OF AFRICAN CATFISH, *Clarias gariepinus* FINGERLINGS FED LARVAE OF *Musca domestica*

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ABSTRACT

A 56-day feeding trial was conducted to evaluate the effect of feeding diets containing Musca domestica larva meal on African catfish (Clarias gariepinus). Five isonitrogenous diets were formulated containing 0, 25, 50, 75 and 100% Musca domestica larva meal replacing fishmeal. Fingerlings (n=225) of African catfish were distributed randomly into triplicate groups of each of the five dietary treatments. Assessment of the effect was done using growth performance, haematology, histology of the liver, as well as kidney and intestine using standard procedures. The second-order polynomial regression model showed the best replacement level that supported optimum weight gain was 80 % with the 0.8477 coefficient of determination that showed a proportion of variation in the dependent variable (weight gain) that was explained by the independent variable (inclusion level). The protein retention by the fish group fed MGM50 was the highest. However, the MGM100-fed fish group significantly had higher lipid and energy retention. Fish fed MGM75 had the highest value of primary and secondary haematological parameters which was not significantly different (p > 0.05) from the MGM50-fed group. There was no significant inflammation and no abnormality seen in fish gut across dietary treatment groups. There were no features of acute and chronic damage in the fish liver and kidney across dietary treatment groups. The liver section of fish fed diets MGM100 showed glycogen accumulation in the hepatocytes. Therefore, feeding Musca domestica larva meal improved growth and impacted no features of acute or chronic injury to the health status of African catfish.

Keywords: Maggot meal, feed utilization, alternative protein, aquaculture, diet formulation

INTRODUCTION

The availability of sufficient protein for the world's growing population is a major challenge (Tripathi *et al.*, 2019). According to Moffitt and Cajas-Cano (2014), aquaculture already provides 50% of all seafood consumed globally and it is developing at a faster rate than any other global food supply sector. Nigeria, with a population of over 200 million people, has the highest demand for fish in Africa and is also the largest producer of African catfish (Adeleke *et al.*, 2020; FAO, 2018). Fishmeal, collected primarily from the marine environment by processing low-value fish and forage fish, was once the primary ingredient in aquaculture feed (Shepherd & Jackson, 2013). The quantity of fishmeal in aquafeed has been significantly reduced in recent years to an optimum level that would support fish physiological performance (Gasco *et al.*, 2018). More so, Tacon *et al.* (2011) observed that fishmeal sustained demand is threatened by the growing aquaculture industry. Hence the need to exert energy and direct research policy on how to

reduce the use of fishmeal in aquaculture feed or search for alternative protein-source feed ingredients that would possess comparable nutrient density in terms of protein content, amino and fatty acid profile to that of fish meal.

Plant protein feed ingredients with good nutrient profiles have been tried as fishmeal substitutes in fish feed (Refstie *et al.*, 2001; Pereira *et al.*, 2002; Espe *et al.*, 2006), but the presence of antinutrients ((Tacon, 1993; Francis *et al.*, 2001; Collins, 2014)) and reduced palatability (Jimoh *et al.*, 2014) have curbed their use. Despite their rich nutrient profile, their negative effect on the enteric or gut (Krogdahl *et al.*, 2003; Merrifield *et al.*, 2011), liver (Jimoh *et al.*, 2015a; Jimoh *et al.*, 2015b), and kidney morphology (Jimoh *et al.*, 2015b) limited their use as fishmeal substitutes. Furthermore, according to Naylor *et al.* (2009), the amount of water and energy needed for the production of these plant-protein sources will restrict their long-term use as viable alternatives to fishmeal. Also. according to Döös (2002), the recently observed ever-increasing human population will restrict the amount of arable land available for the production of these crops.

Since they have a comparable nutrient density to fishmeal, alternative animal-source protein like insect larvae meal may be used to supplement fishmeal in the diets of farmed fishes. For their development, insect larvae need less water, electricity, and arable land (Oonincx & De Boer, 2012; Barroso *et al.*, 2014; Allegretti *et al.*, 2017; van Huis & Oonincx, 2017; van Raamsdonk *et al.*, 2017). Insects are generally high in nutrients, providing high-quality protein comparable to fishmeal and excellent sources of lipid, vitamin B12, zinc, and iron (Finke, 2015; Payne *et al.*, 2016; Koutsos *et al.*, 2019). Insect larvae, such as common housefly (*M. domestica*), house crickets (*Acheta domesticus*), yellow mealworm (*Tenebrio molitor*), black soldier fly (*Hermetia illuscens*) larvae, field cricket (*Gryllus assimilis*), lesser mealworm (*Alphitobius diaperinus*), and banded cricket (*Gryllodes sigillatus*), have the potential to be viable alternatives to conventional protein source such as fishmeal (Van Huis *et al.*, 2013; van Raamsdonk *et al.*, 2017; Gasco *et al.*, 2020). The European Commission approved the inclusion of these insect larvae meals in fish feed through a regulation (Regulation (EU) 2017/893) (Gasco *et al.*, 2020). Among the legally permitted insect larvae meal that can be used in fish and animal feed, Veldkamp and Bosch (2015) found that housefly (*M. domestica*) larvae, black soldier fly (*Hermetia illuscens*) larvae, and yellow mealworm (*Tenebrio molitor*) larvae have the highest potential to be produced in large scale. By 2025, global industrial insect production is expected to reach 1.2 million tonnes (Gasco *et al.*, 2020; Mancuso *et al.*, 2019). The economic outcomes of aquaculture farms are expected to change with the introduction of insect larvae meal in fish feed, according to Arru *et al.* (2019). Bulet *et al.* (1999) and Zhao *et al.* (2010) illustrated the antifungal and antibacterial properties of insect larvae meal, as well as their nutrient stability during storage. In the study by van Huis and Oonincx (2017), the environmental sustainability of insects as fish food was well discussed.

The use of *M. domestica* larva meal had been reported in carp diet (Dong *et al.*, 2013; Yixiang *et al.*, 2013), tilapia diet (Ogunji *et al.*, 2006; Ogunji *et al.*, 2007; Ogunji *et al.*, 2008a; Ogunji *et al.*, 2008c; Ogunji *et al.*, 2008b; Ogunji *et al.*, 2009), and African catfish diet (Idowu *et al.*, 2003; Fasakin *et al.*, 2003; Madu & Ufodike, 2003; Oyelese, 2007; Nsofor *et al.*, 2008; Aniebo *et al.*,

2009). The majority of these catfish studies have only looked at the impact on growth results. There is still a scarcity of information on the impact of feeding *M. domestica* larva meal on *Clarias gariepinus* nutrient retention, haematology and histology of organs involved in food digestion, processing, regulation, and excretion (the gut, liver, and kidney). According to Gasco *et al.* (2018), there is a dearth of knowledge on the dietary impact of insect larvae meal on the histology of fish organs. As a result, the effect of feeding *M. domestica* larva meal on growth performance, haematology, body composition, nutrient retention, and histology of the gut, liver, and kidney was investigated in this study.

MATERIALS AND METHODS

M. domestica larva meal production and processing of feed ingredients

The *M. domestica* larva was raised on a poultry farm in Ilorin, Nigeria, using poultry dung as a substrate following the adapted procedures of Hall *et al.* (2018). After adding a small amount of water to the exposed plastic bowls of poultry dung, signs of larval development were visible in less than 10 hours, and the larvae were left to develop for the next two days. Larvae were collected from dung and extracted using a sieve shaken vigorously in water. The larva was gathered and stored in a polythene bag after being freeze-dried. Other feed ingredients were obtained from a well-known feed mill in Ilorin Metropolis. Soybean, *M. domestica* larva meal, and corn were pulverized separately in a hammer mill and stored at -4^{0}C for later laboratory analysis and diet formulation.

Chemical Analysis

The proximate composition of *M. domestica* larva meal and other basic feed ingredients (fishmeal, soybean meal, and corn) was determined using AOAC (2010) standard procedures. After exposing the samples to 105°C for 24 hours in an oven, the moisture content was determined. The ash content was determined by combusting the samples in a furnace at 600°C for 4 hours (Omegalux LMF-3550). Following acid digestion, crude protein was measured using a Kjedahl protein auto-analyzer. The nitrogen was converted into crude protein using a factor of 6.25. A Soxhlet extraction unit was used to measure crude lipid. A hot extraction fibre analyzer was used to examine the crude fibre. All analyses were measures of triplicate trials (Table 1).

TABLE 1: PROXIMATE COMPOSITION OF SOME FEED INGREDIENTS

	Fishmeal	MM	Soybean	Corn
Dry Matter	92.92	92.80	90.00	89.00
Crude Protein	72.50	64.30	42.30	10.00
Crude Lipid	11.90	11.00	7.30	4.40
Ash	8.52	10.30	5.80	1.90
Crude Fibre		2.20	6.90	2.20
NFE		5.00	27.70	70.50

MM: *M. domestica* larvae meal

Experimental diets

Five iso-nitrogenous feeds (38.5 per cent crude protein) were prepared based on the results of the proximate analysis, in which fishmeal was gradually replaced by MM as a protein source (Table 2). The fishmeal protein part of the control diet was replaced with *Musca domestica* larva meal at a rate of 0% (CTR), 25% (MM25), 50% (MM50), 75% (MM75), and 100% (MM100). The amino acid content of the diets was measured using software established by the (Network of Aquaculture Centre in Asian-Pacific (NACA), 2008). Amino acid requirements of catfish were obtained from the National Research Council (2011). Before adding the fish premix, the ingredients were combined by hand in the laboratory. Following that, fish oil was applied to the dry ingredient and thoroughly mixed. Warm water was applied to the premixed ingredients and mixed until a uniform dough-like paste was created. An improvised manual pelleting machine was used to pelletize the dough (2mm pellet size). The moist pellets were oven-dried at 60°C for 72 hours and placed in airtight containers in a refrigerator at -4°C. Representative samples were taken for proximate analysis.

TABLE 2: GROSS COMPOSITION, PROXIMATE, AND AMINO ACID PROFILES OF THE EXPERIMENTAL DIETS

Ingredient Composition	CTR	MM25	MM50	MM75	MM100	
Fishmeal	26.40	19.76	13.19	6.59	0.00	
Maggot meal	0.00	9.13	18.30	27.40	36.53	
SBM	45.45	45.45	45.45	45.45	45.45	
Corn	10.00	10.00	10.00	10.00	10.00	
*Fish Premix	2.50	2.50	2.50	2.50	2.50	
Fish Oil	1.00	1.00	1.00	1.00	1.00	
Soybean oil	1.00	1.00	1.00	1.00	1.00	
Starch	13.65	11.16	8.56	6.06	3.52	
Proximate composition (g/100g)						
Moisture	8.54	8.37	10.36	10.15	12.08	
Crude Protein	39.4	40.42	41.55	42.62	43.71	
Crude Lipid	7.2	7.39	7.61	7.83	8.05	
Crude Fibre	3.4	3.56	3.76	3.96	4.16	
Ash	5.1	5.45	5.83	6.21	6.59	
NFE	36.36	34.81	30.89	29.23	25.41	
Amino Acid Profile (g/100g diet)						**(** g/kg diet)**
**Arginine	2.95	2.51	1.96	1.58	1.34	10-12
Histidine	0.94	0.79	0.67	0.55	0.42	4-4.2
Isoleucine	1.78	1.56	1.25	1.04	0.89	6-7.3
Leucine	3.00	2.57	2.04	1.68	1.45	9-98
Lysine	2.94	2.30	1.80	1.40	1.07	13-14.3
Methionine	0.86	0.69	0.52	0.38	0.26	6-6.4
M+C	1.36	1.14	0.89	0.69	0.56	
Phenylalanine	1.77	1.52	1.21	1.02	0.92	12-14
P+T	3.06	2.63	2.10	1.76	1.58	
Threonine	1.88	1.49	1.17	0.94	0.77	5-5.6
Tryptophan	0.50	0.40	0.32	0.27	0.23	1.2-1.4
Valine	1.95	1.69	1.33	1.08	0.94	7.1-8.4

*1 kg Aero-mix® fish premix contains Vitamin A 25,000,000 IU, Vitamin D3 2,000,000 IU, Vitamin E 200,000 IU, Vitamin K 8000 mg, Vitamin B2 20,000 mg, Vitamin C 500,000 mg, Niacin 150,000 mg, Pantothenic Acid 50,000 mg, Vitamin B6 12,000 mg, Vitamin B12 10 mg, Folic Acid 4000 mg, Biotin 800 mg, Choline Chloride 600,000 mg, Cobalt 2,000 mg, copper 4,000 mg, Iodine 5,000 mg, iron 40,000 mg, Manganese 50,000 mg, Selenium 200 mg, Zinc 40,000 mg, Antioxidant 100,000 mg, Lysine 100,000 mg, Methionine 100,000 mg manufactured by Aerobic Integrated Concept limited Km 130, Lagos Ibadan Expressway, Hossanah Bus Stop, opposite Islim Filling Station, P. O. Box 22109 UI post Office , Oyo State, Nigeria

** Amino Acid Requirement of Catfish (g/kg diet) (NRC 2011), M+C: Methionine + Cysteine, P+T: Phenylalanine + Tyrosine

Experimental setup

The feeding trial took place at the University of Ilorin's Central Laboratory of the Faculty of Agriculture. *Clarias gariepinus* fingerlings were collected from a reputable hatchery in Ilorin and acclimatized to laboratory conditions in a 1000 litre circular plastic tank for 15 days while being fed a commercial diet (45% crude protein, 1.8mm commercial fish feed). Post acclimation, a triplicate group of fish was bulk weighed (3.50±0.03g) and randomly assigned to each of the five dietary treatments at a rate of fish 15 fish per replicate tank in a completely randomized design. The fifteen rectangular plastic tanks were filled with water up to 60 litres capacity and gently aerated, All the fish were starved for 24 hours prior to the start of the feeding trial to prepare their gastrointestinal tracts for the experimental diet, improve their appetite for the new feed, and minimize stress during weighing and stocking. All of the fish were hand-fed twice a day, at 9:00 a.m. and 5:00 p.m., Bulk weighing was performed at two weeks interval. A combined digital YSI dissolved oxygen meter (YSI Model 57, Yellow Spring Ohio) was used to determine water temperature and dissolved oxygen, while a pH meter (Mettler Toledo – 32, Jenway UK) was used to measure pH weekly. The average water quality parameters were maintained in the culture period; 6.93±0.35 mg/l dissolved oxygen; pH 6.67±0.28; temperature 27.39±0.61^{o}C.

Growth Studies

The following methods in Jimoh *et al.* (2019) were used to measure the growth response and feed utilization indices:

$$\text{Mean weight gain (g)} = \text{Final mean weight (g)} - \text{Initial mean weight(g)}$$

$$\text{Weight gain (\%)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

$$\text{Specific growth rate (\% d}^{-1}\text{)} = \frac{[\ln \text{final weight} - \ln \text{initial weight}]}{\text{time of culture}} \times 100$$

$$\text{Daily Feeding Rate (\% d}^{-1}\text{)} = \frac{\text{Total feed fed}}{[(\text{Initial weight} + \text{Final Weight})/2] \times 56} \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{Total feed fed (g)}}{\text{Weight gain (g)}}$$

$$\text{Protein efficiency ratio} = \frac{\text{Weight gain (g)}}{\text{Total protein fed (g)}}$$

$$\text{Survival (\%)} = \frac{(\text{Total initial number of fish} - \text{mortality})}{\text{The total initial number of fish}} \times 100$$

Body Composition and Nutrient Retention Studies

The initial fish (n=6 fingerlings) before the feeding trial and the final fish (n=5 fish/replicate tank) after the feeding trial were examined for whole-body composition using the procedures of AOAC (2010). The results were used to measure nutrient retention using the procedures described by Jimoh *et al.* (2019) as follows;

$$\text{Protein Retention (\%)} = \frac{\text{Total protein gain (g)}}{\text{Total protein fed (g)}} \times 100$$

$$\text{Lipid Retention (\%)} = \frac{\text{Total lipid gain (g)}}{\text{Total lipid fed (g)}} \times 100$$

$$\text{Energy Retention (\%)} = \frac{\text{Total energy gain (g)}}{\text{Total energy fed (g)}} \times 100$$

Blood Sampling and Haematological Assessment

After the feeding trial, six fish from each experimental tank were removed for blood analysis. The fish was lightly euthanized with 100 mg l^{-1} clove oil prior to this. 2 ml blood was collected per replicate by piercing the caudal vein with a 1ml disposable syringe and a 25G needle and collecting it in an EDTA-treated container for haematological study. The microhaematocrit method, cyanmethaemoglobin method, and total blood cell counts were used to estimate the primary haematological parameters such as packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC), and white blood cell (WBC) counts, respectively (Coles, 1986; Schalm *et al.*, 1975). The standard formula was used to measure secondary haematological parameters such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) (Coles, 1986; Schalm *et al.*, 1975).

Gut, Liver and Kidney Histopathology

Three fish in each treatment replicate were dissected after blood sampling, with the distal gut, liver, and kidney excised and fixed in 10% neutral-buffered formalin, then processed for paraffin parts using a carousel-type automated tissue processor. Following that, the tissue samples were placed in 70% ethanol and processed at different ethanol concentrations. The samples were then cleared in xylene before being impregnated in paraffin wax. All Sections (5 µm) were cut with a rotary microtome and stained with hematoxylin and eosin (H & E) or periodic acid Schiff (liver sections only). The stained slides were photographed using an Olympus BH2 microscope equipped with a photographic attachment (Olympus C35 AD4), a camera (Olympus C40 AB-4) and an automatic light exposure unit (Olympus PM CS5P) at different magnifications (x100 for guts; x400 for kidney and liver) (Olympus PM CS5P). Per replicate slide, a minimum of five (5) photomicrographs were taken. The micrographs were analyzed at the University of Ibadan's Department of Veterinary Anatomy in Nigeria.

Ethics statement

During transportation, accommodation, and termination of the experiment, experimental animals were cared for and used according to standard protocols for animal welfare. In reporting this work, the Animal Research: Reporting of In-Vivo Experiments (ARRIVE) guidelines were followed

Statistical Analysis

Data obtained from the experiment were depicted as mean ± standard error of means (SEM). The statistical analyses were conducted with SPSS version 17.0 using one-way Analysis of variance (ANOVA) while Duncan Multiple Range Test (DMRT) was used to separate the treatment means. Second-order polynomial regression was used to investigate the trend of fish meal replacement level with *M. domestica* larva meal to obtain the optimum level of inclusion of *M. domestica* larva meal.

RESULTS

Growth Performance

Table 3 shows the growth performance of *Clarias gariepinus* fed diets containing graded quantity of *M. domestica* larva meal in place of fishmeal. There were significant

variations ($p < 0.05$) in the various growth performance parameters examined. Fish group fed with *M. domestica* larva meal diets had a better performance than the control-diet fed fish using weight gain, percentage weight gain, and specific growth rate. Fish fed the control diet had the least performance. Among the test dietary treatment groups, the MGM50-fed group of fish had the highest weight gain which was not significantly different ($p>0.05$) from the performance of the fish group fed MGM100. The second-order polynomial regression model (Figure 1) showed the optimum replacement level that best-supported weight gain by *Clarias gariepinus* was 80% with the coefficient of determination of 0.8477 showing the proportion of the variation in the dependent variable (weight gain) that was explained by the independent variable (inclusion level).

TABLE 3: GROWTH PERFORMANCE OF *CLARIAS GARIEPINUS* FED DIETS CONTAINING GRADED LEVELS OF *M. DOMESTICA* LARVA MEAL

Parameter	CTR	MM25	MM50	MM75	MM100
Initial Weight (g)	3.55 ± 0.02^{a}	3.49 ± 0.08^{a}	3.46 ± 0.06^{a}	3.47 ± 0.05^{a}	3.52 ± 0.05^{a}
Final Weight (g)	12.49 ± 0.05^{d}	13.47 ± 0.01^{c}	14.76 ± 0.06^{a}	14.08 ± 0.24^{b}	14.68 ± 0.01^{a}
Weight Gain (g)	8.94 ± 0.04^{d}	9.98 ± 0.08^{c}	11.30 ± 0.11^{a}	10.61 ± 0.27^{b}	11.15 ± 0.05^{a}
%Weight Gain	252.29 ± 1.13^{d}	286.29 ± 8.58^{c}	324.85 ± 8.23^{a}	307.06 ± 12.29^{b}	316.62 ± 6.15^{ab}
SGR (% d^{-1})	1.99 ± 0.01^{e}	2.11 ± 0.00^{d}	2.18 ± 0.03^{bc}	2.15 ± 0.01^{b}	2.25 ± 0.00^{a}
DFR (% d^{-1})	4.32 ± 0.06^{ab}	4.43 ± 0.04^{a}	4.29 ± 0.10^{abc}	4.09 ± 0.08^{bc}	4.07 ± 0.03^{c}
FCR	1.78 ± 0.02^{a}	1.75 ± 0.01^{b}	1.58 ± 0.02^{d}	1.68 ± 0.03^{c}	1.56 ± 0.01^{d}
PER	1.40 ± 0.02^{c}	1.43 ± 0.01^{c}	1.49 ± 0.03^{b}	1.57 ± 0.02^{a}	1.61 ± 0.01^{a}
Survival (%)	86.66 ± 6.67^{a}	82.22 ± 10.18^{a}	82.22 ± 3.85^{a}	80.00 ± 6.67^{a}	82.22 ± 13.87^{a}

Row mean values with different superscripts are significantly different ($p<0.05$) from each other

SGR: Specific Growth Rate
DFR: Daily Feeding Rate
FCR: Feed Conversion Ratio
PER: Protein Efficiency Ratio

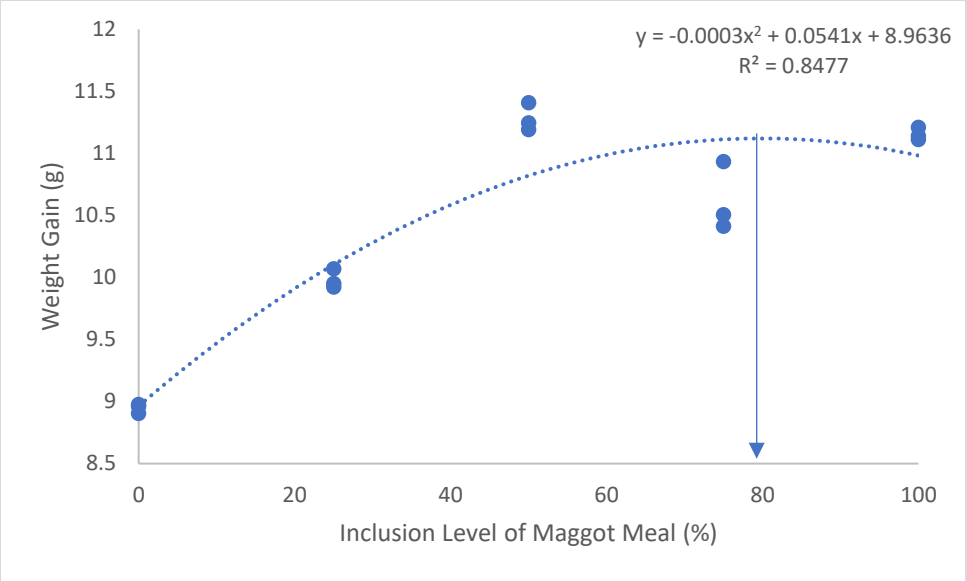


Figure 1: Second-order polynomial regression of inclusion level of *Musca domestica* larva meal against weight gain by *Clarias gariepinus*

Whole-body proximate composition and Nutrient Retention

Table 4 indicates the proximate composition of the entire body and nutrient retention of the fish. Different replacement levels of *M. domestica* larva meal had no effect on whole-body moisture, protein, or ash content ($p > 0.05$). The control group had the lowest body protein content. The lipid and energy contents of the fish fed MM100 were significantly higher than those of the other fish classes ($p < 0.05$). The whole-body lipid content of the fish fed MM75 and MM50 showed no significant differences ($p > 0.05$). Similarly, there were no major differences in whole-body lipid content ($p > 0.05$) between the fish groups fed MM25 and MM50. The fish group that was fed MM50 had the best protein retention. It was significantly higher ($p < 0.05$) than in fish provided other dietary treatments. Protein retention was lowest in MM100 fed classes, but lipid and energy retention were highest in the group.

TABLE 4: WHOLE BODY PROXIMATE COMPOSITION AND NUTRIENT RETENTION OF *CLARIAS GARIEPINUS* FED DIETS CONTAINING GRADED LEVELS OF *M. DOMESTICA* LARVA MEAL

Parameter	Initial	Control	MM25	MM50	MM75	MM100
Moisture (%)	79.84±0.02	77.31±0.83^{a}	76.29±0.59^{a}	75.58±2.43^{a}	75.93±0.56^{a}	73.53±0.88^{a}
Crude Protein (%)	13.12±0.11	13.98±0.52^{a}	14.93±0.49^{a}	14.99±1.58^{a}	14.09±0.01^{a}	15.55±0.64^{a}
Crude Lipid (%)	3.61±0.16	4.19±0.10^{d}	4.60±0.02^{cd}	4.83±0.40^{bc}	5.32±0.30^{b}	6.55±0.05^{a}
Ash (%)	3.43±0.21	4.51±0.20^{a}	4.18±0.12^{a}	4.60±0.44^{a}	4.66±0.26^{a}	4.37±0.29^{a}
Energy (kJ/g)	4.15±0.00	5.29±0.04^{d}	5.45±0.06^{cd}	5.67±0.20^{bc}	5.75±0.12^{b}	6.37±0.04^{a}
Nutrient Retention						
Protein Retention (%)		56.34±7.71^{ab}	58.77±5.53^{ab}	63.16±3.76^{a}	54.23±1.12^{ab}	48.35±10.63^{b}
Lipid Retention (%)		34.03±5.94^{c}	62.45±1.17^{c}	67.14±2.20^{bc}	99.33±1.73^{ab}	125.63±2.32^{a}
Energy Retention (%)		35.66±1.67^{c}	45.00±2.81^{bc}	49.85±8.59^{b}	56.54±5.43^{ab}	62.62±1.24^{a}

Row means with the same superscript are not significantly different (p>0.05) from each other

Haematological Assessment

Table 5 shows the haematological profile of *Clarias gariepinus* fed diets with varying amounts of *M. domestica* larva meal. The primary haematological parameters (PCV, Hb, RBC, excluding WBC) were significantly ($p < 0.05$) higher in the fish fed *M. domestica* larva meal dietary treatments than in the control diet-fed fish. Primary haematological parameters were highest in fish fed MM75 among the test diets. MCH, MCHC, platelet count, white blood cell and its differentials of fish subjected to various dietary treatments did not show any major differences ($p > 0.05$). However, there were significant differences ($p < 0.05$) in the MCV of the fish subjected to various dietary treatments. The highest MCV value was found in fish fed MM75 but was not significantly different ($p > 0.05$) from the MCV found in fish fed control diets. The MCV value of fish fed MM25, MM50, and MM100 showed no significant variation ($p > 0.05$).

TABLE 5: HAEMATOLOGICAL PROFILE OF *CLARIAS GARIEPINUS* FED DIETS CONTAINING GRADED LEVELS OF *M. DOMESTICA* LARVA MEAL

Parameter	Control	MGM25	MGM50	MGM75	MGM100
PCV (%)	25.00 ± 1.41^{c}	26.50 ± 2.12^{bc}	31.00 ± 1.41^{ab}	35.00 ± 1.41^{a}	28.50 ± 1.41^{bc}
Hb (g dL^{-1})	7.70 ± 0.57^{c}	8.20 ± 0.85^{bc}	9.85 ± 0.35^{ab}	10.55 ± 0.35^{a}	8.95 ± 0.92^{abc}
RBC (x10^{12} L^{-1})	2.32 ± 0.09^{c}	2.46 ± 0.21^{bc}	2.83 ± 0.15^{b}	3.20 ± 0.12^{a}	2.64 ± 0.11^{bc}
MCV (Fl)	107.00 ± 2.83^{a}	100.50 ± 0.71^{b}	102.00 ± 1.41^{b}	108.50 ± 0.70^{a}	102.50 ± 0.71^{b}
MCH (ρg)	26.50 ± 0.71^{a}	27.50 ± 0.71^{a}	27.50 ± 2.21^{a}	27.50 ± 0.71^{a}	27.50 ± 0.71^{a}
MCHC (g dL^{-1})	30.50 ± 0.71^{a}	31.50 ± 0.71^{a}	32 ± 0.71^{a}	30.50 ± 0.71^{a}	31.50 ± 0.71^{a}
Platelet Count (x10^{9} L^{-1})	193.50 ± 24.75^{a}	281.00 ± 94.75^{a}	150.00 ± 60.81^{a}	202.00 ± 121.62^{a}	343.50 ± 24.75^{a}
WBC (x10^{9} L^{-1})	8.55 ± 1.91^{a}	12.05 ± 3.89^{a}	7.75 ± 1.20^{a}	10.25 ± 5.59^{a}	10.50 ± 1.13^{a}
WBC Differentials					
Neutrophyl (%)	43.00 ± 9.90^{a}	36.50 ± 12.02^{a}	45.00 ± 15.56^{a}	57.00 ± 26.87^{a}	54.00 ± 11.31^{a}
Lymphocyte (%)	55.50 ± 9.19^{a}	63.50 ± 12.02^{a}	52.50 ± 19.09^{a}	42.00 ± 25.46^{a}	43.50 ± 12.02^{a}
Monocyte (%)	1.50 ± 0.71^{a}	0.00 ± 0.00^{a}	2.50 ± 0.35^{a}	1.00 ± 1.41^{a}	2.50 ± 0.71^{a}

Row means with the same superscript are not significantly different ($p>0.05$) from each other

PCV: Packed Cell Volume
Hb: Haemoglobin content
RBC: Red Blood Cell
MCV: Mean Cell Volume
MCH: Mean Cell Heamoglobin
MCHC: Mean Cell Haemoglobin Concentration
WBC: White Blood Cell

Histology of the intestine of *Clarias gariepinus* juveniles fed a diet containing graded levels of *M. domestica* larva meal.

Figures 4a-e show photomicrographs (x10 magnification) of 5µm sectioned, H&E-stained gut parts of *Clarias gariepinus* fed a diet containing a graded level of *M. domestica* larva meal. Mucosa, submucosa, muscular, and peritoneal layers were visible in all sections examined. In the intestines of fish fed the different dietary treatments, there was no major inflammation or abnormality.

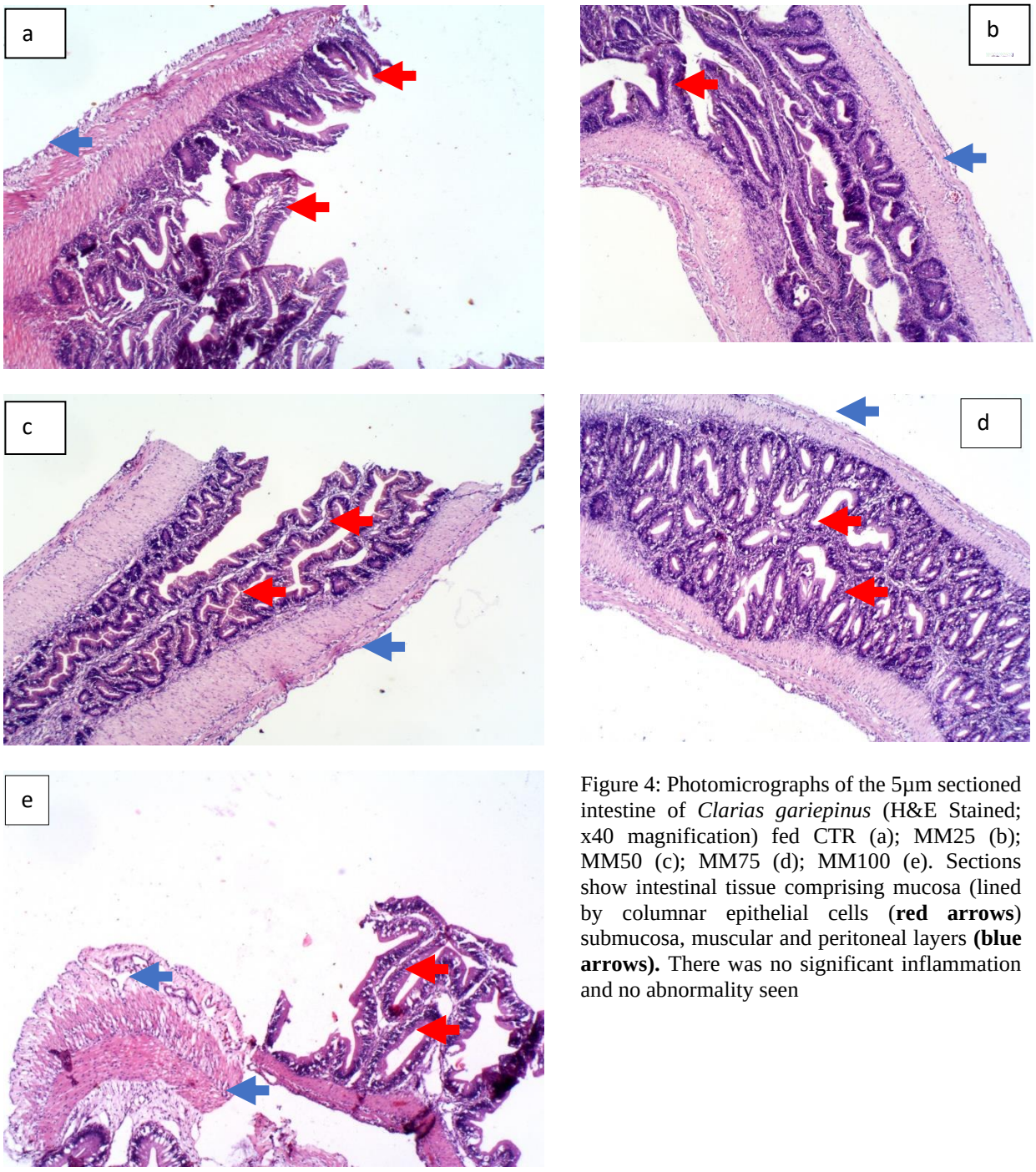
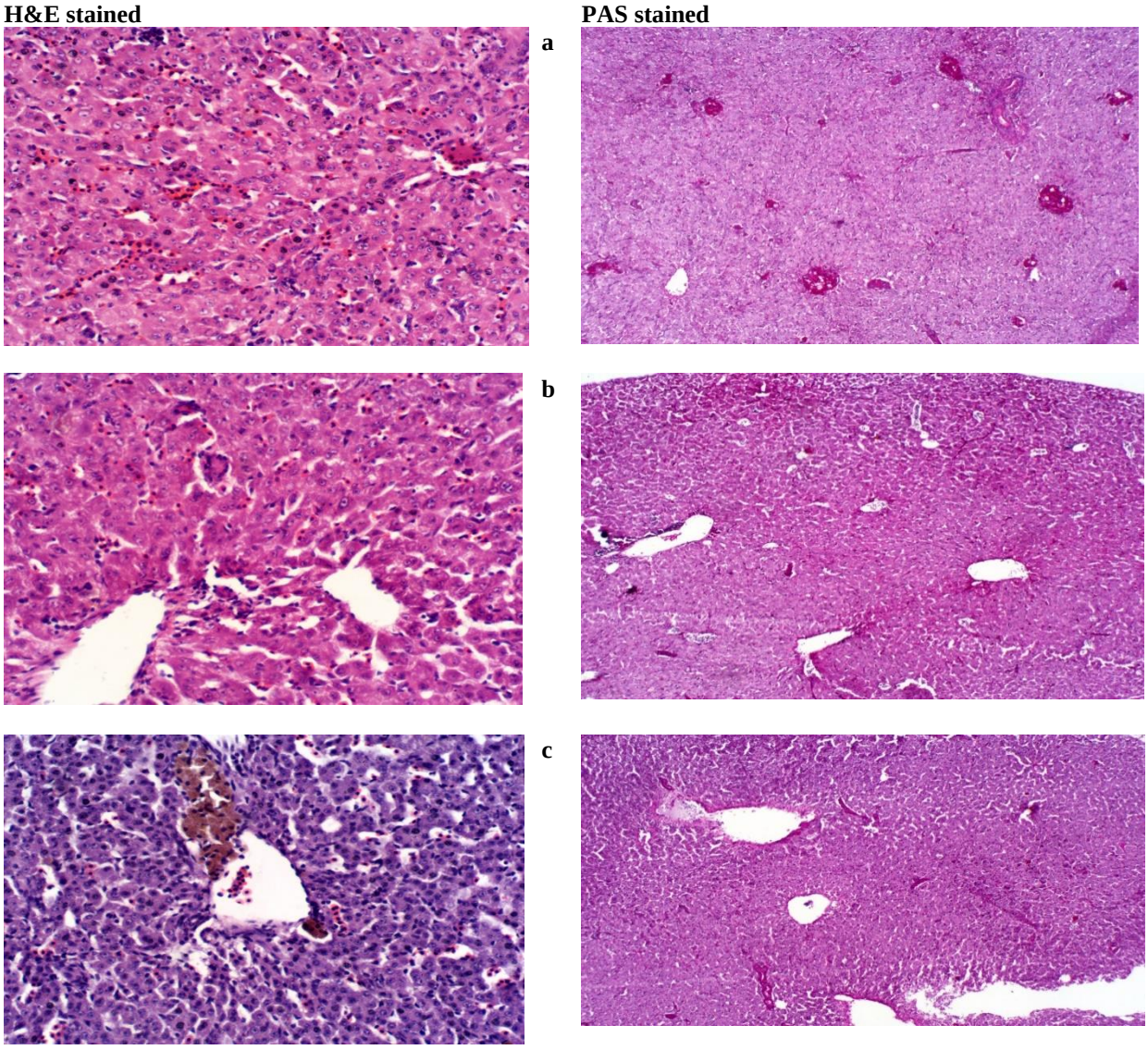


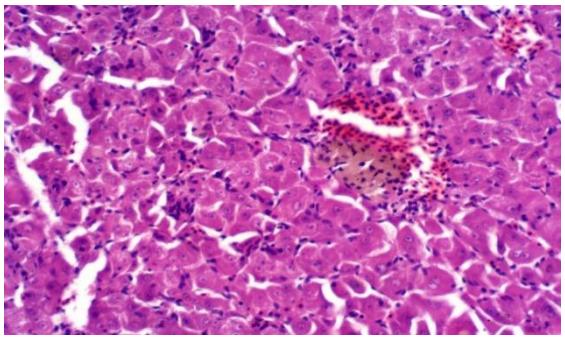
Figure 4: Photomicrographs of the 5µm sectioned intestine of *Clarias gariepinus* (H&E Stained; x40 magnification) fed CTR (a); MM25 (b); MM50 (c); MM75 (d); MM100 (e). Sections show intestinal tissue comprising mucosa (lined by columnar epithelial cells (**red arrows**) submucosa, muscular and peritoneal layers **(blue arrows).** There was no significant inflammation and no abnormality seen

Histological details of haematoxylin and eosin stained and periodic acid Schiff-stained liver of *Clarias gariepinus* fed diet containing a graded level of *M. domestica* larva meal

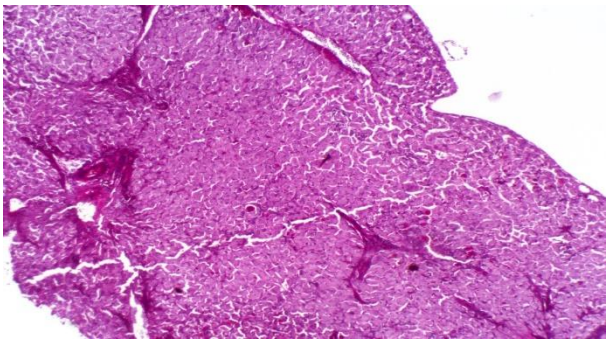
Photomicrographs (x40 magnification) of the 5µm sectioned, H&E and PAS-stained liver of *Clarias gariepinus* fed diets containing graded amounts of *M. domestica* larva meal are shown in Figures 5 and 6. The H&E-stained series revealed liver tissue with

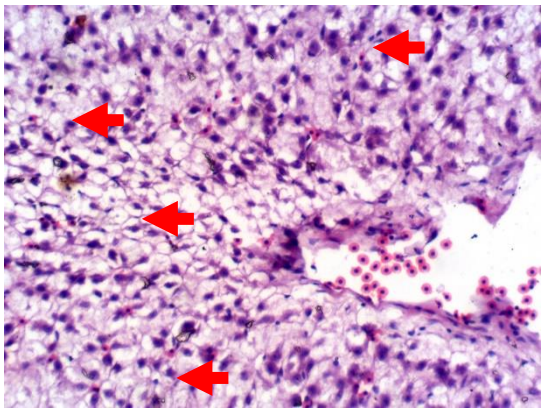
preserved architecture, including hepatocytes, vascular channels, and bile ducts. Up to 75% *M. domestica* larva meal inclusion, there were no signs of acute or chronic injury (Figure 5a-d). The liver tissue of MM100-fed fish (Figure 5e) showed preserved architecture, with hepatocytes with pale staining abundant cytoplasm containing glycogen deposition and vascular channels. Sections from the PAS-stained series (Figures 6a-e) indicated liver tissue with preserved architecture, including hepatocytes, vascular channels, and bile ducts, but no glycogen deposition (Figures 6a-d). Up to 75% *M. domestica* larva meal inclusion, there were no signs of acute or chronic injury. Glycogen accumulation in hepatocytes was seen in the liver sections of fish fed MM100-containing diets (Figure 6e).





d





e

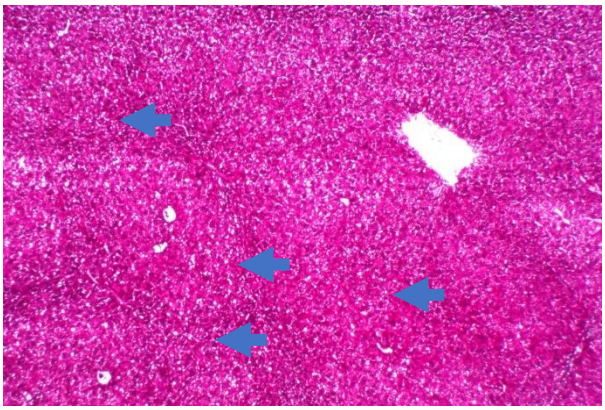


Figure 5: Photomicrographs of the 5µm sectioned liver of *Clarias gariepinus* (H&E Stained; x400 magnification) fed CTR (a); MM25 (b); MM50 (c); MM75 (d); MM100 (e). Sections show liver tissue with preserved architecture comprising hepatocytes, vascular channels and bile ducts. There are no features of acute or chronic injury(a-d). Liver (e) Section shows liver tissue with preserved architecture comprising hepatocytes with pale staining abundant cytoplasm containing glycogen deposition (**red arrows**), and vascular channels. There are no features of acute or chronic injury

Figure 6: Photomicrographs of the 5µm sectioned liver of *Clarias gariepinus* (PAS Stained; x400 magnification) fed CTR (a); MM25 (b); MM50 (c); MM75 (d); MM100 (e). Sections show liver tissue with preserved architecture comprising hepatocytes, vascular channels and bile ducts, which are negative for glycogen deposition. There are no features of acute or chronic injury(a-d). The liver (e) section shows glycogen accumulation in the hepatocytes (**blue arrows)**.

Histological details of the kidney of *Clarias gariepinus* fed diet containing a graded level of *M. domestica* larva meal

Photomicrographs of the 5µm sectioned, H&E-stained kidney of *Clarias gariepinus* fed diets containing graded amounts of *M. domestica* larva meal (x40 magnification) are shown in Figures 7a-e. Renal tissue with preserved architecture and regular glomeruli and tubules was seen in all sections examined. Moderate to thick haemopoietic cells made up of the interstitium were also observed. Across all dietary treatments, there were no signs of acute or chronic damage in the kidneys of the fish.

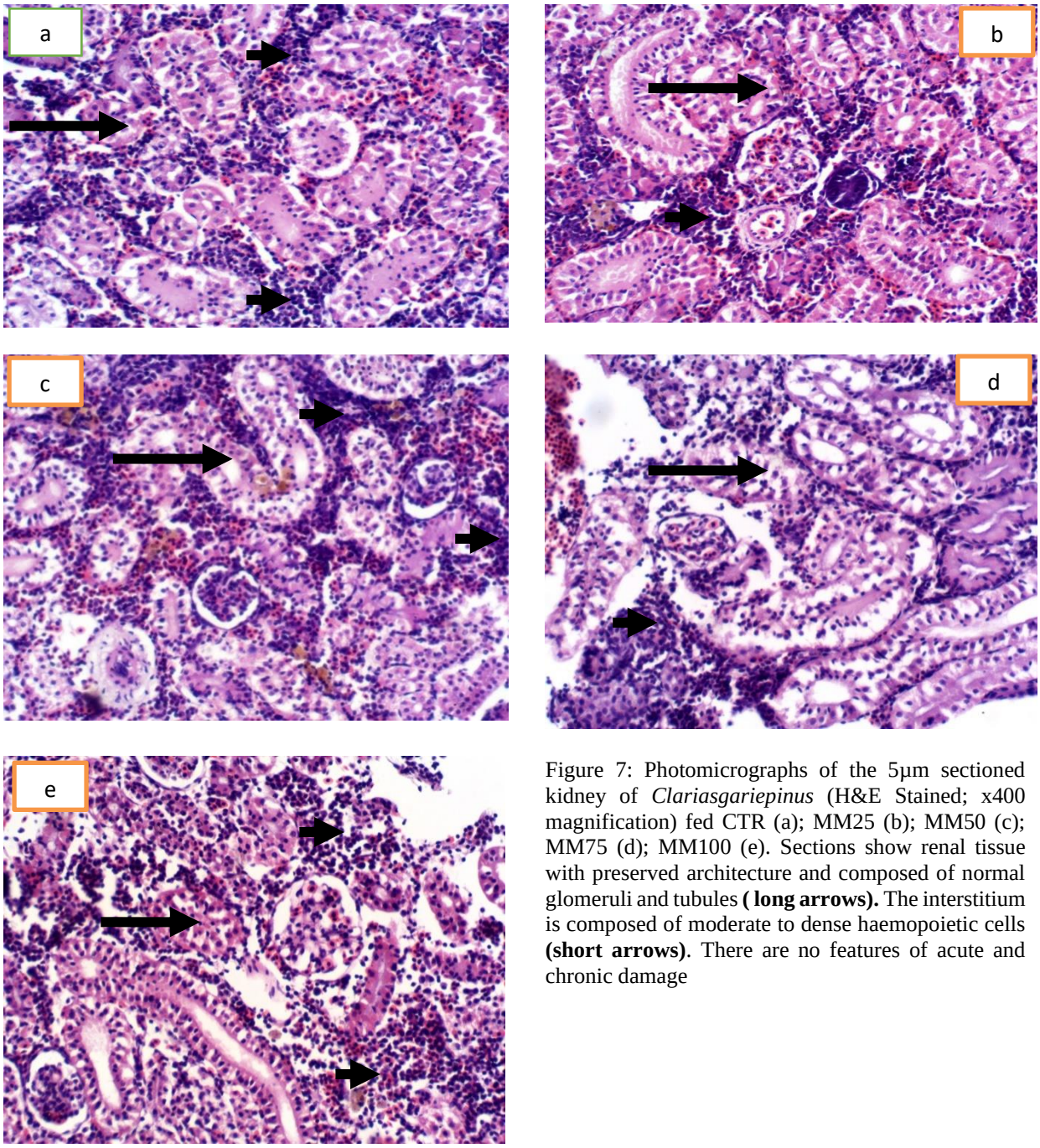


Figure 7: Photomicrographs of the 5µm sectioned kidney of *Clariasgariepinus* (H&E Stained; x400 magnification) fed CTR (a); MM25 (b); MM50 (c); MM75 (d); MM100 (e). Sections show renal tissue with preserved architecture and composed of normal glomeruli and tubules **(long arrows).** The interstitium is composed of moderate to dense haemopoietic cells **(short arrows)**. There are no features of acute and chronic damage

DISCUSSION

Clarias gariepinus fed *M. domestica* larva meal-based diets performed better than the group fed fishmeal-based diets, with 50 per cent replacement of fishmeal by *M. domestica* larva meal resulting in the highest weight gain. This might plausibly be as a result of their high-quality protein and excellent sources of lipid, vitamin B_{12}, zinc, and iron (Finke, 2015; Payne *et al.*, 2016; Koutsos *et al.*, 2019). This is in line with the majority of submissions from researchers who previously worked on the use of *M. domestica* larvae-based diet for fish. Fish fed

M. domestica larva meal performed better in the studies by Fasakin *et al.* (2003), Idowu *et al.* (2003), and Oyelese (2007). The use of *M. domestica* larva meal in place of fishmeal resulted in improved growth and feed utilization in this report. This is consistent with the findings of Ali *et al.* (2015) for Nile tilapia. Although Idowu *et al.* (2003) and Oyelese (2007) revealed a growth reduction in Nile tilapia fed non-defatted *M. domestica* larva meal at higher inclusion levels, the findings from the current study support those of Djissou *et al.* (2016) who found that completely replacing fishmeal with a mixture of other animal protein sources resulted in better growth performance than fishmeal-based diets. Aniebo *et al.* (2009) found that *Clarias gariepinus* fed *M. domestica* larva meal-based diets outgrew fish fed fishmeal-based diets by a non-significant margin.

Haematological parameters are useful resources for assessing fish health in relation to dietary manipulations and tracking physiological and pathological changes in fish as a result of such feed manipulations (Satheeshkumar *et al.*, 2011; Jimoh *et al.*, 2016). Dietary manipulations, malnutrition, and disease conditions can all alter blood composition (Feist & Longshaw, 2000; Jimoh *et al.*, 2020a; Jimoh *et al.*, 2020b). In this study, the superior haematological profile reported in the *M. domestica* larva meal fed group corroborated the growth performance findings. The size of fish and the values of primary haematological parameters have been found to have a strong association (Jawad *et al.*, 2004). Sogbesan *et al.* (2006) attributed the higher biological value of *M. domestica* larva meal in terms of nutrient digestibility and profile to the improved output observed in fish fed the meal. According to Ajani *et al.* (2004), the biological importance of *M. domestica* larva meal is equivalent to that of fishmeal. *M. domestica* larva meal is known for its superior fatty acid profiles, as well as being high in the vitamin B complex and trace elements (Teotia & Miller, 1973; Finke, 2015; Koutsos *et al.*, 2019). The blood parameters of the fish fed the various dietary treatments in terms of RBC, PCV, and white blood cell differential were all within the normal range for fish physiological functioning, as stated by other researchers (Taufek *et al.*, 2016; Fawole *et al.*, 2020). The fish fed *M. domestica* larva meal had significantly higher values of primary haematological parameters than the fish fed control diets, indicating that the fish were able to make greater use of the *M. domestica* larva meal without affecting their physiological functioning or immunity. When fishmeal was replaced with black soldier fly (*Hermetia illucens*) in the diets of African catfish, Fawole *et al.* (2020) recorded similar results, but there were no major differences between the fish fed the different dietary treatments.

Nutrient retention studies, on the other hand, indicated that the higher weight gain found in fish fed 100% *M. domestica* larva meal which was comparable to 50% *M. domestica* larva meal was due to lipid deposition rather than protein accretion. This study demonstrates that the growth observed in catfish fed a 100% *M. domestica* larva meal-based diet was due to lipid deposition rather than protein accretion in the muscle. The fish that were fed MM50 had the best protein retention. This is consistent with the findings of Ajani *et al.* (2004) who found that replacing 50 % of fishmeal with *M. domestica* larva meal improved Nile Tilapia growth, and Nsofor *et al.* (2008) who found that the PER of 50 and 100 percent replacement of fishmeal with *M. domestica* larva meal was superior to that of

fishmeal. The fish fed MM100 had the highest lipid and energy retention, suggesting that the weight gain observed was due to lipid deposition rather than protein accretion. The high glycogen accumulation in the hepatocytes of the H&E and PAS-stained liver section of fish fed MM100 further attest to our claim. This high glycogen accumulation observed in the hepatocytes of the MM100 fed group is a physiological response to high dietary lipid intake by the fish indicating the high lipid contents of the dietary ingredients. Similar findings were made by Jimoh *et al.* (2015a) when *Citrullus lanatus* was fed to Nile tilapia and when *Chrysophylum albidum* was fed to *C. gariepinus* (Jimoh *et al.*, 2015b). This is consistent with other scientists' findings (Martins *et al.*, 2007; Gatta *et al.*, 2011; Valente *et al.*, 2011; Olukunle, 2011).

Except for higher levels of lipid deposition among fish fed diets containing 100 percent *M. domestica* larva meal, feeding *M. domestica* larva meal-based diets did not affect the histology of the gastrointestinal tract, liver, or kidney. According to Ogunji *et al.* (2007), including *M. domestica* larva meal in tilapia diets had no stress on tilapia metabolism because it appears to lack a compound capable of producing reactive oxygen for oxidative stress. Lock *et al.* (2016) and Renna *et al.* (2017) found no difference in the histo-micrographs of fish fed control and insect meal diets. This is consistent with the results of this study.

CONCLUSION

This study revealed that feeding up to 75% *M. domestica* larva meal-based diets to African catfish as fishmeal replacement supported the growth and improved blood profile with no damage to the intestine, liver, or kidneys. Second-order regression optimized the larval meal inclusion level at 80% to support the optimum growth of African catfish. The study also found that the growth reported by catfish fed 100 % *M. domestica* larva meal-based diets was due to lipid deposition rather than protein accretion in the muscle.

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