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The Use of Black Soldier Fly Larvae Meal *H. illucens* for the Pre Growth of *Oreochromis niloticus* (Linnaeus, 1758) Fingerlings Reared in Floating Cages in Benin

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ABSTRACT

To increase the value of black soldier fly (*Hermetia illucens*) larvae in the diet of Tilapia reared on aquaculture farms in Benin, three diets RR, RT and R55 were tested in triplicate in floating cages for 35 days on *Oreochromis niloticus* fingerlings with a mean initial weight Pmi = 7±0.220 g. The stocking density was 70 fingerlings/m3. RR is an imported commercial feed while RT is a fishmeal diet. R55 is a diet based on Black Soldier Fly Larvae (BSFL) meal with an incorporation rate of 55%. Control fishing was carried out every 7 days and hematological constants were determined. The results show that the feed tested significantly affect zootechnical parameters ($p < 0.05$). The Final Mean weight (FMW) of fingerlings fed the experimental diets ranged from 16.37±1.18 (RT) to 21.53±1.42 (R55).

The best specific growth and economic conversion rates were obtained with R55 (3.21±0.064 %day-1 and 0.95±0.18%), which also had better Hb levels. An incorporation rate of 55% of BSFL meal in the feed of *O. niloticus* fingerlings therefore induced good zootechnical performance and better economic profitability.

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Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), the world's population could exceed 9 billion by 2050. In addition, global demand for food is expected to increase by nearly 70%, leading to a sharp rise in demand for already scarce agricultural resources and ecosystems [1,2]. Aquaculture therefore has a significant role to play in meeting future global food demand, particularly protein requirements, and in contributing to global food security [3].

At the same time, aquaculture production is limited by its dependence on fishmeal, as the main source of proteins in fish feed because of its content of essential fatty acids and amino acids. Given this situation, it is important to find new cheaper protein sources for fish feed formulation. Insects proved to be less expensive and sustainable alternative sources to fishmeal for fish feed [4]. Among those insects, there is the black soldier fly (*Hermetia illucens*) which is considered to be one of the most promising protein source substitutes due to its wide distribution in tropical and subtropical regions [5]. Thus, it should be noted that this species is the subject of several studies that have shown that black soldier fly larvae meal is a nutritious food and an interesting alternative protein source for poultry, pig, and fish feed [6-10]. According to Driemeyer, black soldier fly larvae meal can replace soya, maize, and fish meal that are used today for livestock feed [11]. It would therefore be worth taking advantage of the opportunity offered by black soldier fly larvae meal in fish farming to increase the growth of farmed fish.

In Benin, several studies have been carried out in the field of fish feed to increase fish farming yields while using local raw materials to make the product more accessible on the market and to optimize production costs, thereby reducing the need to import fish feed [12,13]. Nevertheless, fish farmers are still dependent on imported feed. There is therefore an urgent need to find new ways of formulating fish feed while substituting fish meal with black soldier fly larvae. It is in this context that the present study was initiated, to develop black soldier fly larvae (BSFL) meals for the pre-growth of *Oreochromis niloticus*

fry reared in floating cages.

Methodology

Experimental Design and Experimental Feed

This was a randomized design with 3 treatments (diets). Each treatment was repeated three times. Experimental fry was randomly distributed in nine floating cages with a volume equal to 1 m^3, respectively. The stocking density was 70 fingerlings/m^3. A total of 630 fry with an initial mean weight of 7 ± 0.220 g were used. The experiment lasted 35 days.

Three (03) experimental feeds (Table 1) covering the nutritional requirements of *O. niloticus* fingerlings were used during the study. Among the feeds, two (02) were formulated and manufactured with locally available raw materials. These iso-protein, iso-lipid, and iso-energy diets contain, among other ingredients, BSFL flour at increasing rates of incorporation (0% and 55%). The cost per kg of each experimental food was calculated considering the cost of the quantity of each ingredient.

Table 1: Composition of Experimental Diets

Ingredients (%)	Treatments		
	RR	RT	R55
Fish meal	-	46	0
Soya meal		24	14
LMSN meal		0	55
Wheat bran		5	7
Maize flour		18	20
Minero-Vitamin Premixa		1,5	2
Soya oil		2	2
Lysine [a]		1,75	0
Méthionine [a]		1,75	0
Total		100	100
Feed price (Euro/kg)	1,61	0,88	0,77
Nutritional value			
Protein	37	37,04	37,16
Fat	9	8,91	8,98
Brut Energy (KJ/g) [b]	17,01	17,03	17,12
Protein/ Energy [b]	2,18	2,17	2,17

a Drugstore , premix (vitamin – mineral) contains (‰): Vitamin A 4 000 000 U.I; Vitamin D 800 000 U.I; Vitamin E 40 000U.I; Vitamin K3 1600 mg; Vitamin B1 4 000 mg; Vitamin B2 3 000 mg; Vitamin B6 3 800 mg; Vitamin B12 3 mg; Vitamin C 60 000 mg; Biotin 100 mg; Inositol 10 000 mg Pantothenic acid 8 000 mg; Nicotinic acid 18 000 mg; Folic acid 800 mg; Cholin chloride 120 000 mg; Colbat carbonate 150 mg; Ferrous sulphate 8 000 mg; Potassium iodide 400 mg; Manganese oxide 6 000 mg; Cuivre 800 mg; Sodium selenite 40 mcg; Lysine 10 000 mg; Methionin 10 000 mg; Zinc sulfate 8 000 mg; b Calculated from nutrient content: 23.01 Kj/g protein; 38.07 Kj/g lipid and 17.15 Kj/g carbohydrates

A part from BSFL flour, all the ingredients used in the manufacture of the experimental feed were purchased from the local market. The BSFL (*Hermetia illucens*) were produced from a substrate whose centesimal composition is presented in Table 2. Fourteen (14) days after egg incubation, the larvae were harvested, steam-killed, and dried in a dehydrator at 30°C for 36 h. They were then reduced to meal before being incorporated into the experimental feed. They were then ground into a meal before being incorporated into the experimental feed. Table 3 shows the essential amino acid (EAA) profiles of the ingredients used.

Table 2 : Centesimal Composition of LMSN Production Substrate

Ingredients	%
Soya okara	30
Palm kernel meal	25
Brewer's grains	25
Marine fish viscera	20
Total	100

Table 3: EAA Profile of Ingredients Used (g100g-1 dry matter)

Composition in AAE	Fish meal	Soya meal	Wheat bran	LMSN	Maize meal
Threonine	2.31	0.76	0.45	1.94	2.0
Valine	2.77	0.56	0.50	3.09	3.0
Methionine	1.94	0.24	0.20	1.07	0.90
Isoleucine	2.45	0.52	2.50	2.40	2.53
Leucine	3.79	1.72	0.95	3.62	8.82
Phenylalanine	3.74	1.36	1.10	2.11	3.50
Histidine	1.75	0.64	0.70	2.77	2.0
Tryptophane	0.57	0.32	0.40	3.41	1.5
Lysine	4.22	1.20	0.50	3.60	1.80
Arginine	3.43	2.04	2.15	2.55	3.82

During the manufacturing process of the foods to be tested, the raw ingredients are finely ground, weighed, and mixed until a homogeneous product is obtained. Purified amino acids (lysine and methionine), vitamins and minerals, and soya oil are then added. Water was then added at a rate of 50% of the dry matter getting a malleable paste. This dough was used to make spaghetti using a grinder. Extruded feeds were made from these mixtures. The extruded feeds were dried in a dehydrator at 30°C for 36 hours. They were stored in small conditioning boxes which were kept in a cool place (4°C) until the end of the experiment.

Conduct of the Experiment

Before the feeding phase itself, fish were acclimatized to the different experimental diets for one week. So that the experiment properly began. Fingerlings were fed 3 times a day at a ration rate of 10% applied to the biomass. Control fishing was carried out every 7 days where fingerling individual was counted and 20 individuals were randomly selected from each floating cage for weighing in accordance to determine the new biomass and adjust the rations accordingly. Every morning, the physicochemical parameters (temperature, pH, and dissolved oxygen) of the water were measured before the first feeding. A portable multimeter (Calypso ORCHIDIS SN-ODEOA 2138) was used for this purpose.

Biochemical Analyses

Biochemical analyses (proteins, lipids, ash, and dry matter) were carried out in triplicate using the standard methods of the AOAC [14]. These analyses were carried out on ingredients, feed, and experimental fry. The Kjeldahl method (%N x 6.25) was used to determine crude protein. Lipids were measured using the hot Soxhlet method, and a mixture of chloroform and methanol (2 :1 v/v or v: v) was used for lipid extraction [15]. Dry matter was determined by measuring weight loss after drying for 24 hours in an oven at 105°C, whereas ash was determined by incinerating the samples in an oven at 550°C for 16 hours. The brut energy of the experimental diets was calculated according to Azaza [16].

The amino acids of feed ingredients were analyzed with a Waters HPLC system (Waters 474, Waters, Milford, MA, USA) including two pumps (Model 515, Waters), an auto-sampler (Model 717, Waters), a fluorescence detector (Model 474, Waters) and a temperature control module. These amino acid analyses were done following the method previously described by Bosch [17]. Thus, aminobutyric acid was added as an internal standard before hydrolyzation. Amino acids were derivatized with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) and then separated with a C-18 reverse-phase column Waters Acc. Tag (150 mm X 3.9 mm). All of these analyses were conducted twice per sample in the Laboratory of Aquatic Animal Nutrition of Kagoshima University (Japan).

Blood Collection

To assess hematological parameters, blood samples were collected from a sample of five fish randomly choosed per treatment before beginning the experiment. At the end, the same control was done. Fish were not anesthetized. Blood samples were drawn up through the back of the syringe (1 ml). The blood sample was pushed directly into a tube on EDTA anticoagulant and carried to the laboratory.

FE =(Body mass gain (g))/(feed intake (g))

SGR (%)/day = 100 ×((ln[FBW]-ln[IBW])/(Duration of experimentation) ;

Where IBW and FBW are Initial Body Weight and Final Body Weight

PER =(Wet body mass gain)/(Protein intake) ;

LER =(Wet body mass gain)/(Lipid intake) ;

PPV= (Body protein gain)/(protein intake)

LPV= (Body lipid gain)/(Lipid intake)

SR (%) = 100 ×(Final number of fish)/(Initial number of fish)

ECR = Feed cost *FCR

Hematological Parameters

Hematological parameters such as Haemoglobin (Hb), Hematocrit (Hct), Number of Red Cells, and their associated indices such as the Mean Corpuscular Concentration in Hemoglobin, Mean Cell Volume, Mean Corpuscular Haemoglobin are determined as described in the automated system XN330 Sysmes [18].

Data Processing

At the end of the experiment, all the raw data collected were encoded in the Excel 2016 spreadsheet to calculate the zootechnical parameters. For each parameter, the mean and standard deviation were calculated using the same spreadsheet. Graphs were presented and produced using Excel 2016.

For statistical analysis, STATVIEW (version 5.01) was used, with a probability threshold of 5%. Analysis of variance with one classification criterion (ANOVA 1) was used to compare the zootechnical performance of the different treatments. When significant differences were reported between treatments, Fisher's LSD (Least Significant Difference) was used to make paired comparisons of the different means.

Results

Physico Chemical Parameters

The mean values of the water physicochemical parameters in the various treatments were recorded throughout the experiment. For temperature, the mean value was 26.32±0.70°C, and dissolved oxygen was equal to 1.19±0.26 ppm whereas pH was equal to 7.32±0.70 throughout the experiment.

Zootechnical Parameters

The assessed zootechnical parameters were presented in Table 4. It was shown that the diets tested had a significant effect on growth performance, i.e. the specific growth rate (SGR) obtained at the end of the study ($p<0.05$). The highest SGR was observed in fish fed R55. However, the feed conversion ratio (FCR), protein efficiency ratio (PER), lipid efficiency ratio (LER), protein productive value (PPV), and survival rate (SR) did not vary significantly between treatments ($p >0.05$). The LPV value obtained with the RR was twice higher than the other regimes ($p<0.05$). Figure 1 shows the trend of the final mean weight of the fish as a function of the treatments throughout the experiment. The mean final weight of *O. niloticus* fingerlings varied from 16.37±1.18 (RT) to 21.53±1.42 (R55).

Table 4: Zootechnical Parameters of *O. niloticus* Fingerlings in Experiments.

Parameters	RR	RT	R55
IBW (g)	7±0.22 a	7±0.22 a	7±0.22 a
SGR (%/j)	2.50±0.06 a	2.43±0.07 a	3.21±0.06 b
FCR	1.04±0.16 a	1.37±0.16 a	1.23±0.16 a
SR (%)	88.49±0.03 a	87.21±0.07 a	89.44±0.02 a
PER	2.60±0.01 a	2.70±0.03 a	2.69±0.06 a
LER	10.68±0.03 a	8.19±0.01 b	9.05±0.07 ab
PPV	2.03±0.10 a	2.11±0.06 a	1.88±0.02 a
LPV	7.11±0.02 a	3.02±0.01 b	2.80±0.31 b

Mean ± SD values in the same line followed by the same superscript are not significantly different (P<0.05)

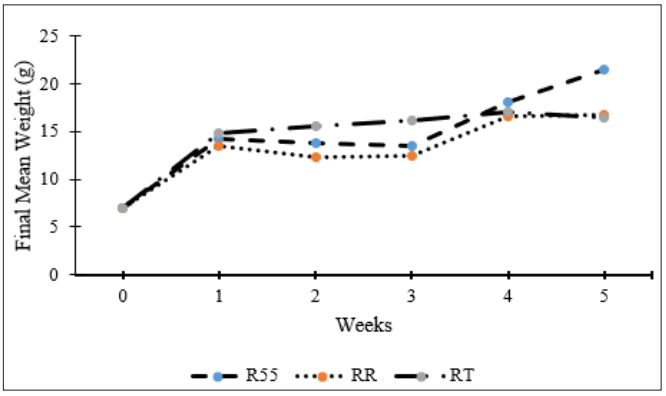

Figure 1: Change in Final Mean Weight Over the Weeks of the Experiment

Bromatological Composition of Fish Carcasses According to Treatments

Table 5 shows that the bromatological composition of the fish carcasses varied according to the treatments. The ash, protein, lipid, and dry matter contents of the carcasses of the final samples were higher than those of the initial fish. The lipid content of fish fed the commercial feed was higher than that of the other two experimental feeds (p<0.05).

Table 5: Bromatological Composition of Fish Carcasses According to Treatments

Parameters (%)	Initial	RR	RT	R55
Ash	4.46±0.11	4.59±0.13^{a}	4.89±0.19^{a}	4.98±0.16^{a}
Protein	13.32±0.31	16.88±0.84^{a}	16.58±1.16^{a}	16.28±0.89^{a}
Lipid	3.28±0.09	6.89±0.32^{a}	5.03±0.29^{b}	5.14±0.66^{b}
Dry matter	89.74±1.21	88.92±1.21^{a}	89.61±1.02^{a}	89.32±0.53^{a}

Economic Parameters

The economic conversion ratio (ECR) varied according to the cost of the experimental feed received by each batch of fish. As it was shown in Figure 2, the ECR of the local feed (RT and R55) was lower than the commercial feed ECR. Moreover, the feed without fish meal (R55) produced a better RCT than the other two tested feeds. The value of the coefficient of determination (R2), which indicates the strength of the correlation between the ECR and the experimental feed (cost per kg of feed), is equal to 1.

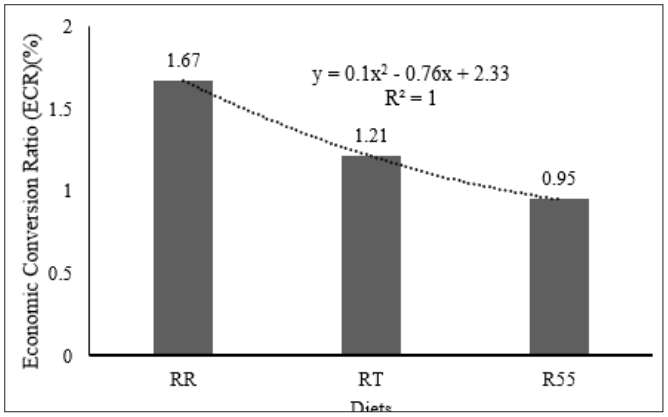

Figure 2: Variation of Economic Conversion Ratio (ECR)

Hematological Parameters of Fish as a Function of Treatments

The variation in blood constant levels from the beginning to the end of the experiment is shown in Table 6. There was a significant variation (p<0.05) in hemoglobin and a high significant variation for RR and RT (p<0.0009). However, no significant difference (p > 0.05) was reported concerning the red blood cells whatever the experimental food.

Table 6: Variation in Blood Constant Levels from the Beginning to the End of the Experiment

Parameters		RR	RT	R55
Hemoglobin (g/dL)	Initial batch	6,7±0,4	6,7±0,4	6,7±0,4
	Experimental	6,4±0,4	12,1±0,3	8,1±0,6
	p-value	>0,05	0,0002	>0,05
Hematocrit (%)	Initial batch	11,1 ± 2,8	11,1 ± 2,8	11,1 ± 2,8
	Experimental	17,9 ± 2,1	16,4 ± 2,9	12,1 ±1,7
	p-value	<0,0001	<0,0009	>0,05
Red blood cells (106/ mm3)	Initial batch	1,5 ± 0,4	1,5 ± 0,4	1,5 ± 0,4
	Experimental	2,3 ± 0,2	2,0 ± 0,5	1,4 ± 0,4
	p-value	>0,05	>0,05	>0,05

Changes in MCHC, MCV and MCH

The general value determined for these three parameters was inferior at the beginning (RR) of the experiment. Like the Mean Corpuscular Haemoglobin Concentration of the diet RR is around the middle of the concentration of other diets (fig. 3). According to Fig 4 and Fig 5, the trend for RT and R55 was the same regarding to MCV and MCH, respectively.

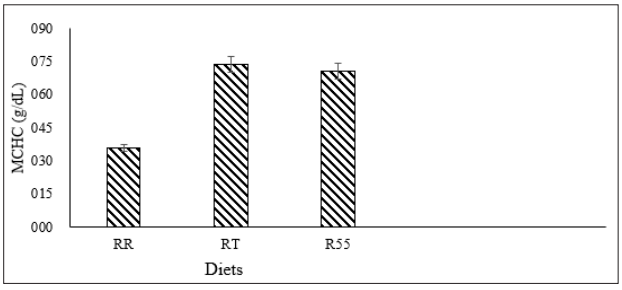

Figure 3: Changes in Mean Corpuscular Hemoglobin Concentration in Fish During the Experiment.

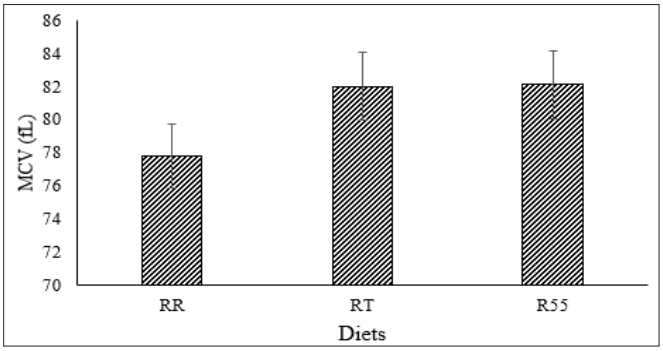

Figure 4: Changes in Mean Cell Volume in Fish

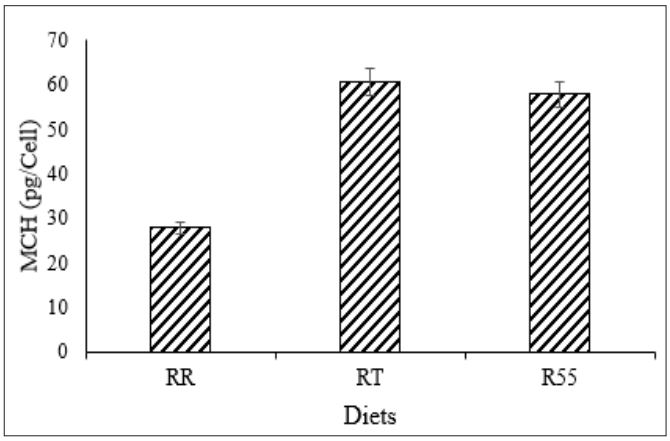

Figure 5: Changes in Mean Corpuscular Hemoglobin Levels in Fish

Discussion

The temperature during the experiment is in line with the recommended standard for tilapia rearing in tropical environments, which should be between 14 and 35°C [19]. The pH values are attested to good water quality and agree with Kanangiré [20] who argued that the best growth performances for this species could recorded with a pH value varying from 6.5 to 9. Furthermore, dissolved oxygen levels in the experimental conditions are very low compared with the required standard (3.1 mg/l) for rearing *O. niloticus* (Cherif and Guechache, 2018). As fish absorb oxygen through direct contact with water, dissolved oxygen is the most important factor in achieving good rearing results. An adequate oxygen concentration continually in the water does not only ensures growth but helps to promote also hygiene, appetite, and fish well-being [19]. Moreover, oxygen helps to reduce the effects of stress on fish, caused by temperature variations. Oxygen appears like a limiting factor and could explain the growth performance obtained with fish subjected to experimental diets. In the case of rearing fish in floating cages in an artificial lake, the problem of dissolved oxygen can quickly arise and affect not only the survival of the fish in the environment but also their growth performance and feed utilization. Our results show that for fish such as tilapia, diets based on black soldier fly larvae meal produce acceptable growth performance during pre-growth. Indeed, black soldier fly (*Hermetia illucens*) larvae are rich in nutrients and represent an extremely interesting alternative feed source for various livestock sectors (chicken, fish, shrimp, etc.) [21]. Furthermore, its production from agri-food waste makes it an excellent potential alternative protein source to fish meal. Its meal can be used as the sole source of high-dose protein, as its methionine and lysine content remain high. They are an appreciable source of animal protein that improves the nutritional value of growing animals [13,22]. In fish farming, Ogundji recommended the use of maggots as a substitute for fishmeal in the diet of *O. niloticus*. A similar recommendation was made by Olaniyi and Salau, who suggested that maggots can replace fishmeal by up to 75% in the diet of African catfish [23]. The growth performance obtained in this experiment with *O. niloticus* fry fed the R55 diet is superior to that obtained by Guedegbe, who completely replaced fishmeal with sun-dried maggot meal [24,25]. This difference can be explained not only by the quality of the rearing water but also by the drying process of the maggots before incorporation into feed formulas for farmed fish Indeed, animal proteins such as fly larvae can be denatured at high temperatures, resulting in reduced growth performance [26,12]. Growth and feed utilization results obtained during the present experiment reveal that *O. niloticus* Fingerlings have adopted all the experimental diets.

Furthermore, in the diet of tilapia (an omnivore with herbivorous tendencies), the ratio of animal proteins to plant proteins plays an important role in growth performance, which increases with the proportion of animal proteins [27]. The difference in performance between the diets RR and RT can be explained, by the species' herbivorous tendency on the one hand, and by the optimum level of incorporation of black soldier fly larvae meal on the other hand. The best results achieved with the R55 diet suggests the optimum level of incorporation of fly larvae into the tilapia diet. Fly larvae give the R55 diet better nutrient utilization than other diets. Furthermore, the results of the economic parameters show that the local feeds (RT and R55) are less expensive and have a good attractive economic conversion rate. These results confirm those obtained by [12,28,29]. In line with the observations of Agbohessou, analyses carried out on the nutritional composition and carcass quality of fish fed with our experimental diets showed that total substitution of fish meal by BSFL meal does not adversely affect the zootechnical performance and carcass nutritional quality of *Oreochromis niloticus* fingerlings [30].

Hematological parameters indicate some variations according to the records. A slight decrease was observed in Hb during the experiment. Although hemoglobin, hematocrit, Number of Red Blood Cells, Mean Corpuscular Hemoglobin Concentration, Mean Cell Volume, and Mean Corpuscular Hemoglobin in fish have not strictly changed as a function of experimental diets, it seems important to admit like that blood parameters variations are affected by several factors including dietary regime, environmental conditions, species and its physiological status, age and size [31,32]. The variations registered in the present study agree with Witeska [33]. According to Well, hemoglobin content and Number of red cells are in perfect correlation in the fish blood [34]. So, when Hemoglobin formation is impaired, the rate of Hemoglobin may significantly decrease (Guyton, 1991). Hemoglobin levels in this experiment ranged from 1.2±0.3 to 6.4±0.4 g/dL. According to Fazio, the hemoglobin level determined in different species is 4.70 to 16.6 g/dL. Literature data early reviewed showed that hemoglobin levels in *C. carpio* range from 34.1 to 1 14.3 g/L [35,36]. In the same way, hematocrit obtained was within the range of values (9.4 to 33.53%). Similar values were recorded for different fish species [37]. In the case of *Cyprinus carpio*, Hematocrit has varied from 14.0 to 44.0% [36]. These showed that the hematocrit value depends on the number and size of red blood cells and can be affected by various factors, such as the physicochemical quality of the water in which the fish are reared, the feed with which they are fed and possibly the presence of certain infectious diseases on the farm. But herein, the deviation

between the number of red cells, Hemoglobin, and hematocrit suggested serious unresolved issues [38]. The very high hematocrit for an almost constant hemoglobin content over the same period could be linked to a loss of water from the blood plasma. However, it could also has assured that this rise in hematocrit is due to the huge increase in the number of red blood cells. This implies that the determination of leucocyte cells and/or blood platelets would have been very useful in assessing fish health. The Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Globular Volume (MGV), and Mean Corpuscular Hemoglobin Rate (MCHR) in fish from all diets obtained during the experiment differed from those collected by Witeska [36] with MCV varying from 130.9 to 367.3 Fl, MHCH from 150 to 446 g/L and MCH from 3 1.8 to 139 pg. Fawole showed that the use of BSFL meal had no negative effect on the growth performance, feed utilization, and health status of C. gariepinus fingerlings.

Conclusion

Incorporating BSFL meal in the diet of farmed fish appears to be non-insignificant approch to improve fish growth and well-being and at a relatively lower cost. An incorporation rate of 55% of BSFL meal in the feed of *O. niloticus* fingerlings therefore induced good zootechnical performance and better economic profitability. It would be more diligent to have an easy technical production sheet for this feed to relieve the pains of fish producers.

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