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FERMENTED DESERT LOCUST (*Schistocerca gregaria*) BY-PRODUCT MEAL AS A DIETARY PROTEIN SOURCE IN THE DIETS OF OREOCHROMIS NILOTICUS FINGERLINGS (Linnaeus, 1758)

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ABSTRACT

The potentials of Fermented Desert Locust By-product (FDLBP) as a substitute for fishmeal in Oreochromis niloticus diet was evaluated in a 90 day feeding experiment. Four iso-nitrogenous (35.62%) and iso-caloric (16.72 kJ/g) diets were formulated, in which FDLBP was added to supplement fishmeal at three inclusion levels (15, 30 and 45%). Bacillus sustilus was used for the fermentation of the DLBP. Each diet was fed to triplicate groups of 10 fish (8.23±0.24 g) twice daily at 3% body weight per day. An intra-peritoneal challenge with Pseudomonas aeruginosa was carried out on the fish. Proximate composition, chitin content and amino acid profiles of the meals were analyzed. Growth performance in terms of Mean weight gain (MWG), Feed conversion ratio (FCR), Specific growth rate (SGR) and feed utilization were calculated weekly. Blood samples were collected from each group for haematological and blood chemistry analyses. Data obtained were analysed using one way analysis of variance (ANOVA). The SGR (1.10±0.09-1.44±0.14 %) and FCR (1.60±0.10-2.22±0.18) were significantly ($p<0.05$) different among treatments in the group of fish fed control diet (diet 1) relative to others. Fish fed FDLBP 45% (diet 4) had the least overall growth performance. Packed cell volume (24.2± 3.27-34.8±5.36 %), haemoglobin concentration (5.4±0.85-7.9±1.14 g/dl), red blood cells (1.86±0.24-2.80±0.41) and white blood cell (10.74±1.05-11.52±2.09 ×1012/L) were significantly ($p<0.05$) different among the groups. There were no significant difference ($p>0.05$) in the total protein (3.58±0.11-4.42±1.41 g/dl) albumin (2.17±0.15-2.68±0.54 g/dl) and globulin (0.90±0.14-1.84±0.61 g/dl) levels in the blood. This study concluded that FDLBP could be included in the diets of O. niloticus up to 45% without negatively impacting on the fish growth and health status.

Keywords: Desert Locust Waste Meal, Oreochromis niloticus, Proximate composition, chitin content and amino acid

INTRODUCTION

The decline in capture fisheries with increase in the aquaculture industry over the years have resulted to decrease in the availability of fishmeal and fish oil (FAO, 2015). Likewise, the competitive demand for fishmeal and fish oil for livestock and aquaculture feed has resulted to high cost of both fishmeal and fish oil (FAO, 2015). Terrestrial plants, such as soya meal or oil, which are rich in protein and lipids have been used to replace or supplement fishmeal and fish oil (Gatlin et al., 2007). Plant meals are usually limited or deficient of some essential amino acids as a source of protein and are high in fiber, carbohydrates, and other antinutritional factors, which have negative effects on feed intake, digestion, and absorption and result in stunted growth (Krogdahl et al., 2010, Collins et al., 2013).

By 2050, prices for beef, pig, and poultry will have increased by more than 30% from 2000 levels due to the rise in global prices for the most significant agricultural crops (Nelson et al. 2009). The hunt for alternative protein sources, such as cultured meat (Fayaz et al 2011), seaweed (Fleurence 1999), vegetables and fungus (Asgar et al 2010), mini-livestock (Paoletti 2005), and insectmeal (Van Huis, 2013) will be prompted by the rise in food and feed prices in the future. Therefore, there is need for the use of insects as a food source for fish feed since, insects is found to be part of the natural diet of fish species (Howe et al., 2014; Whitley and Bollens, 2014). Although the use of insects in animal feed has drawn a lot of attention, several studies that have typically been created with the use of insects as food have recently been published, attracting the interest of numerous researchers and demonstrating the nutritional potential of insects. These research studies offer intriguing insights for the use of insects for many reasons, such as animal feeding, agriculture, the production of essential oils or biodiesel, as well as the creation of mass raising systems for insects, the current economic crisis, and the rise in food costs (Van Huis, 2013).

The exoskeleton of insects like other arthropods contains a polysaccharide bond by a β (1/4) glycosidic bond, called Chitin. Chitin is a crude fiber which cannot be digested by monogastric animals (Lindsay et al., 1984). Insects possess chitin which varies among different species and in various phases of development. The percentage of chitin in field cricket is 8.7% (Wang et al., 2004) while the larvae of Cirina forda contains 9.4% of chitin content (Akinnawo and Ketiku, 2000). In total, 11.6 and 66.6 mg chitin/kg dry matter in brood of bees and in a silk worms were obtained, respectively (Finke, 2007).

Fish, among other monogastric animals have difficulties in digesting chitin (Rust, 2002). Therefore there is need to reduce the amount of chitin present in animal feed, which could be removed by alkaline extraction from the insect meal (Belluco et al., 2013; Sanchez-Muros et al., 2014). Addition of chitinase or chitinolytic bacteria to insect meal could improve the protein utilization of the chitin-protein complexes (Kroeckel et al., 2012), as demonstrated in tilapia fed diets containing crustaceans shell (Zhang et al., 2014). Alternatively, enzymes or chemicals such as chitosan, acetylglucosamine (GlcNAc) or chito-oligosaccharides that can be used to degrade chitin can be added to fish diets (Lin et al., 2012)

Therefore, this study is necessary to determine the potential of fermented desert locust by-product (discarded parts during pro-

cessing such as head, limbs and wings) as a dietary protein source in the diet of O. niloticus fingerlings.

MATERIALS AND METHODS

Fish and experimental conditions

The feeding trial was conducted at the fish hatchery of Aquaculture and Fisheries Management, Federal University of Agriculture Abeokuta, Nigeria. A total of 120 fingerlings (8.26±0.11 g) of Nile tilapia, sourced from a reputable fish hatchery were randomly stocked in 50-L tanks in batches of 10 fish/tank. The fish were acclimatized for one week and were fed the same diet iso-nitrogenous (35.64%) and iso-caloric (15.45 kJ/g), prepared by grinding equal amounts of each of the four diets, mixed together and then pelletized into 2mm size (Model: CAZL150, Nigeria).

During the period of acclimation, stressed and dead fish were replaced with individuals of similar sizes. The fish were starved for 24 hr prior to the start of the feeding experiment. Each of the four experimental diets (0%, 15%, 30% and 45% FDLBP), was randomly assigned to triplicate groups of fish. The fish were fed at 3% according to Adeoye et al (2016) body weight per day, between 8.00 - 9.00 h and 16.00 – 17.00 h, for a period of 90 days. Feacal materials were removed with the aid of a siphon before the next feeding hour. The fish were fed six days of the week, fish were weighed using Balance Meter Toledo FB602 on the 7th day, the new weight was used to calculate new feeding regimen. For water quality monitoring, temperature and dissolved oxygen were measured daily, total ammonium, nitrite, and pH levels, were measured weekly by standard methods (APHA, 1995).

Bacterial Culture

Bacillus sustilus was collected from the Microbial type culture collection (MTCC), Department of Microbiology, FUNAAB. B. sustilus was grown on nutrient agar.

Diet preparation and formulation

Desert locust by-product (discarded part during processing such as head, limbs and wings) was purchased from a local market, in Dange/Shuni, Sokoto State, Nigeria. Other ingredients were purchased from a reputable livestock feed store in Abeokuta, Ogun State, Nigeria. The insect waste were properly stored in air tight plastic container and placed at room temperature prior to use.

The insect meal was fermented according to Klunder et al. (2012) with slight modifications for the inoculation with starter organisms. One (1) kg of Desert locust by product was milled to powdered form in a blender (3D model) and was transferred into an air tight container as substrate prior to inoculation of starter organisms. 50ml of B. sustilus suspension was carefully added to the substrate and thoroughly mixed with 1000 ml of distilled water. This was left at 32°C in an incubating unit for 3 days. The product was oven dried at 55oC for 24 hours, cooled in air and milled with a food blender (3D model), and sieved to produce fermented desert locus waste meal (FDLBP).

Four iso-nitrogenous (35%) and iso-caloric (16.32 kJ/g) diets were formulated, in which FDLBP was added at three inclusion levels (15%, 30% and 45%), the feeds were labeled, diet 1, 2, 3, and 4 representing 0% FDLBP (Control), 15% FDLBP, 30% FDLBP, and 45% FDLBP respectively (Table 1). The diets were pelleted into 2mm pellets (Model: CAZL150, Nigeria). Thereafter, the pelleted feeds were oven dried at 60oC for 72 hours

and packed in labelled cellophane plastic before stored in a refrigerator.

Chemical analyses

Dry matter and proximate composition of ingredients, feeds and faeces were analysed using the same methods as for diets, which has been briefly described (AOAC, 1990). The chromium oxide content of the feed and faecal samples, in triplicates, was estimated by the method of Furukawa and Tsukahara (1966) using the acid digestion technique (nitric acid and perchloric acid). The protein and dry matter were calculated using the Apparent Digestibility Co-efficient (ADC) formula:

ADC%= 100-[100 x (%Cr_2O_3 in feed/% Cr_2O_3 in faeces) x (%nutrient in faeces / % nutrient in feed)].

Dry matter and proximate composition of ingredients and feeds were analyzed using standard method (AOAC, 2006). The chitin content was estimated according to Finke (2007): chitin (%) = Acid detergent fiber (ADF) – Acid Detergent Lignin (ADF). The amino acid profile of DLBP was determined using methods described by Benitez (1989). The samples were dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer. An integrator was attached to the Analyzer to calculate the peak area proportional to the concentration of each of the amino acids. The chromium oxide content of the feed and faecal samples, was estimated by the method of Furukawa and Tsukahara (1966) using the acid digestion technique (nitric acid and perchloric acid). The ADCs were calculated with the following formula:

ADC%= 100-[100 x (%Cr_2O_3in feed/% Cr_2O_3 in faeces) x (%nutrient in faeces / % nutrient in feed)].

Haematological and Biochemical Analysis

At the end of 90 days feeding experiment three fish from each rearing tank were randomly taken for the analysis

At the end of the experiment, three fish from each tank were randomly taken for blood sampling. Blood (1–2 ml, depending on fish size) was collected from the vertebral blood vessels towards the caudal peduncle of each fish using separate heparinized syringes. The following blood parameters were analysed and recorded: Parked Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC), White blood cell (WBC), Mean Cell Volume, Mean Cell Haemoglobin, Mean Cell Haemoglobin Concentrate, Lymphocyte, Total Protein, Albumin and Globulin as described by Blaxhall and Daisley, (1973); Doumas, (1994); Reinhold, (1988); and Coles, (1998).

Data calculations and statistical analysis

The criteria used to determinate growth and feed conversion ratio were:

Weight gain (g) = final weight - initial weight

Feed Conversion Ratio, FCR = Total dry feed intake / Wet weight gain

Specific Growth Rate, SGR= $\frac{lnW2 - lnW1}{T2 - T1} x\ 100$

Where; ln = Natural logarithm, W2 = final mean weight of fish (g), W1 = initial weight of fish (g) and T2-T1 = Period of days.

Protein Efficiency Ratio, PER= Wet weight gain / crude protein intake

Protein Intake = Feed intake x Crude protein of the feed.

Survival rate (%) = 100 × (final no of fish - initial no of fish)

The feed cost was estimated based on the FDLBP, the cost of feed required to produce 1 kg of biomass was calculated. The economic conversion ratio (ECR) was calculated with the following equation: ECR=Feed cost × Feed Conversion Ratio (FCR). The cost of each feed was determined by multiplying the respective contributions of each feed ingredient by their respective costs per kg and summing the values obtained for all the ingredients in each of the formulated diets.

All values obtained for growth and nutrient utilization such as, feed intake, mean weight gain, feed conversion ratio, apparent protein digestibility, protein productive value (PCV), Hb, WBC, MCHC, feed cost/100kg, estimated investment cost, were subjected to descriptive and inferential statistics (One way ANOVA) and where significant differences were observed, means were separated using Duncan's Multiple Range Test (Sanders, 1990) of the Statistical Package for the Social Sciences (SPSS) version 20. The results were considered as significant when p values were less than 0.05.Values throughout the text are expressed as means ± standard error of means of three replicates (SE|M; n =3).

RESULTS

The crude protein content of fishmeal (70.4%) was higher than that of the fermented DLBP, so also is the crude lipid Table 2. Chitin, acid detergent lignin (ADL) and acid detergent fibre (ADF) content are 23.4±0.1, 6.59±0.09 and 29.99±0.23 % respectively in FDLBP. FDLBP (15.22%) has high ash content than fishmeal (13.6%). Potassium level was high in fishmeal at 11.9 g/kg compared to 10.59 g/kg for FDLBP. Phosphorus level was lower in FDLBP at 5.55 g/kg compared to 19.3 g/kg for fishmeal (Table 2). Calcium level was low 0.95 g/kg in FDLBP relatively to the level 15.4 g/kg in fishmeal. Both essential and non-essential amino acids are low in fishmeal compare to FDLBP except in lysine, tryptophan, alanine, glutamic acid, glycine and aspartic acid (Table 3).

The moisture and protein contents varied slightly among the different groups. The crude protein ranged from 35.64±0.11% to 36.01±0.14% with highest value observed in control and the lowest in diet 3 (30% FDLBP). Crude lipid increased with FDLBP inclusion level and ranged from 4.33±0.44 to 4.88±0.59. Crude fibre increased with FDLBP inclusion levels except in the diet with 30% inclusion. The ash content ranges from 5.79±0.04 - 10.11±0.26, while the Carbohydrate values ranged from 28.30±0.07 - 29.58±0.28 with no significant difference. The chitin content range from 3.4±0.1 (Treatment 1) to 6.71±0.75% (Treatment 2), which increases with the DLBP composition (Table 3).

Growth performance, nutrient utilization and survival after 90 days of feeding trial, there was no significant difference in the initial body weight of O. niloticus fingerlings fed the experimental diets. There was significant difference between growth performance and feed utilization parameters in fish batches fed different experimental diets. Mean weight gain (MWG) and specific growth rate (SGR) ranged, respectively, from 7.63±0.89 - 11.16±1.67 g/fish and 1.10±0.09 - 1.44±0.14%/day with the highest values observed in the Treatment 1 fed with control diet (0% FDLBP) and lowest in those fed with Diet 4 (45% FDLBP). Fish fed with Diet 2, 3 and 4 were not significantly ($p > 0.05$) MWG and SGR. There was no significant difference in MFI which ranged from

Table 1: Gross composition (%) of experimental diets

Ingredients	TREATMENT 1 (Control)	TREATMENT 2 (FDLWM 15)	TREATMENT 3 (FDLWM 30)	TREATMENT 4 (FDLWM 45)
FDLWM	0	2.6	5.15	7.7
Fish meal	10	7.4	4.8	2.3
Groundnut cake	30	30	30	30
Soybean Meal	35	35	35	35
Maize	21	21	21.05	21
Lysine	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
Vit/Min premix*	1	1	1	1
Vegetable oil	1	1	1	1
Chromic Oxide	0.5	0.5	0.5	0.5
Total	100	100	100	100

*Radar vitamin premix supply/100 g diet ^ vitamin A palmitate, 1000 IU; cholecalciferol (D), 1000 IU; a-tocopherol acetate (E), 1.1mg; menadione (K), 0.02 mg; thiamine B1, 0.63 mg; riboavin (B2), 0.5 mg; pantothenic acid, 1.0 mg; pyridoxine (B6), 0.15 mg; cyanocobalamine (B12), 0.001mg; nicotinic acid, 3.0 mg; folic acid, 0.1mg; choline, 31.3 mg; ascorbic acid (C), 0.1mg; ferrous sulphate, 0.05 mg; copper sulphate, 0.25 mg; manganese sulphate, 6.00 mg; cobalt chloride, 0.5 mg; zinc sulphate, 5.0 mg; sodium selenite, 0.02 mg.

Table 2: Proximate composition of Desert locust waste meal (DLWM), Fermented desert locust waste meal (FDLWM), and fishmeal

Parameters	DLWM	FDLWM	Fishmeal
Dry matter	91.7	86.75	92.1
Crude protein	61.65	64.13	70.4
Crude lipid	1.38	0.99	11
Ash	15.93	15.22	13.6
NFE	5.72	4.13	5
GE*(kcal/g)	380.04	391.69	507.80
Calcium (g/kg)	0.90	0.95	15.4
Potassium (g/kg)	9.71	10.59	11.9
Phosphorus (g/kg)	5.37	5.55	19.3
Chitin	24.69±0.18	23.4±0.17	-

Gross energy: calculated after National Research Council (NRC) Committee on Animal Nutrition (1993) as Carbohydrate, 4.11kcal $100g^{-1}$; protein, 5.64 kcal $100g^{-1}$ and lipids, 9.44 kcal $100g^{-1}$; GE = Gross Energy, NFE = Nitrogen Free Extract

Table 3: Amino acids Profile (g/100gprotein) of Desert locust waste meal (DLWM), Fermented desert locust waste meal (DLWM), and fishmeal.

Essential	DLWM	FDLWM	Fishmeal	Dietary requirements for *O. niloticus* (Luquet, 1991)
Arginine	7.74	8.00	5.8	4.20
Histidine	2.56	2.88	2.2	1.70
Isoleucine	5.63	5.93	4.3	3.10
Leucine	7.59	8.20	7.0	3.40
Lysine	4.85	5.15	7.5	5.10
Methionine	2.97	2.99	2.8	2.70
Phenylalanine	4.97	5.15	3.8	3.80
Threonine	4.11	4.55	4.1	3.80
Tryptophan	0.68	0.84	1.1	1.00
Valine	5.38	5.73	4.9	2.80
Non-essential				
Tyrosine	4.13	3.96	2.9	
Proline	6.19	5.28	3.8	
Cystine	2.06	1.94	0.8	
Alanine	4.60	5.31	6.1	
Glutamic acid	9.61	10.45	12.6	
Glycine	5.13	4.89	5.9	
Serine	4.40	5.03	4.0	
Aspartic acid	6.70	7.38	8.7	

Table 4: Proximate composition (%) of experimental diets

Composition (%)	Diet 1	Diet 2	Diet 3	Diet 4
Moisture	8.00±0.03	7.44±0.01	6.71±0.05	8.10±0.01
Crude lipid	4.51±0.66	4.72±0.03	4.88±0.59	4.33±0.44
Ash	5.79±0.04[a]	8.44±0.25	10.11±0.26	5.79±0.32
Crude protein	36.01±0.14	35.78±0.64	35.61±0.28	35.09±.11
Crude fibre	3.31±0.06 [a]	5.98±0.05	9.46±0.06	3.29±0.12
Carbohydrate	29.08±0.78	29.58±1.08	28.30±0.07	29.08±0.56
GE*(MJ/Kg)	15.52±0.14	15.64±0.23	15.44±0.35	15.23±0.17
Chitin (%)	-	3.4±0.15[a]	6.71±0.75 [c]	4.1±0.57 [b]

Means with different superscript on the same row are significantly different ($p<0.05$)
*Gross energy (GE): calculated after National Research Council (NRC) Committee on Animal Nutrition (1993) as Carbohydrate, 4.11kcal $100g^{-1}$; protein, 5.64 kcal $100g^{-1}$ and lipids, 9.44 kcal $100g^{-1}$

16.82±0.76 - 17.79±2.32 (Table 5).

The highest feed conversion ratio (FCR) was observed in FDLBP 45% (2.22±0.18), while the lowest was recorded in Control (1.60±0.10). Protein efficiency ratio (PER) was significantly higher in O. niloticus fingerlings in Control (1.79±0.10), while the lowest was observed in FDLBP 30% (1.15±0.21). There was no significant differ-

ence in survival of fish from all treatments (Table 5).

The PVC values of the fish fed FDLBP 30% and Control had the lowest and highest value of 24.20±3.27 and 27.8±3.24 respectively. Hb value ranged from 5.4±0.85 (FDLBP 30%) – 7.9±1.14 (Control). RBC value ranges from 1.86±0.24 (FDLBP 30%) - 2.80±0.41 (Control). WBC value ranges from 10.74±1.05 (FDLBP 30%) - 11.52±2.09 (Control). There was no significantly different ($p>0.05$) in the values of WBC, Het., Lymphocyte, Mean Cell Volume, and Mean Cell Haemoglobin Concentrate. MCV and MCHC values followed the same pattern. There was increase in the values with increase in inclusion level (Table 6).

The total protein and globulin values increase with increase in inclusion of the FDLBP up to 30% and a slight decrease in fish fed FDLBP 45%. Albumin value ranges from 2.17±0.15 - 2.68±0.54 with no significant difference in among the treatments (Table 7).

The FDLBP group showed higher survival (10%; 87.5%; and 100% respectively) in comparison with control (10%) group (Fig 2). Among treatment groups, FDLBP 45% recorded significantly ($P<0.05$) highest survival compared with other treatments.

Economic parameters of O. niloticus fingerlings fed trial diets (Table 8) showed that feed cost decrease with increase in inclusion level. While the unit feeding cost (UFC) value ranged from N 421.52 – 553.66, Control and FDLBP 45% has the lowest and highest value 451.38±.09 and 588.66±.04 respectively, for Estimate investment cost (EIC). Incidence cost (IC) decreased significantly from

Table 5: Growth performance, nutrient utilization and digestibility of *O. niloticus* fed Experimental diets

PARAMETERS	TREATMENT 1	TREATMENT 2	TREATMENT 3	TREATMENT 4
IMW (g)	8.12±0.00	8.13±0.03	8.14±0.03	8.11±0.08
FMW (g)	19.28±1.65^{b}	16.80±1.70^{a}	16.38±0.71^{a}	15.73±0.91^{a}
MWG (g)	11.16±1.67^{c}	8.68±1.67^{a}	8.24±0.68^{a}	7.63±0.89^{a}
MFI (g)	17.79±2.32	16.99±0.84	16.87±0.15	16.82±0.76
FCR	1.60±0.10^{a}	1.99±0.29^{ab}	2.06±0.16^{b}	2.22±0.18^{c}
PI	6.22±0.81	5.95±0.29	5.91±0.05	5.89±0.27
PER	1.79±0.10^{c}	1.15±0.21^{a}	1.40±0.11^{b}	1.29±0.10^{b}
SGR	1.44±0.14^{b}	1.20±0.17^{a}	1.16±0.07^{a}	1.10±0.09^{a}
DWG (%)	1.86±0.28^{b}	1.45±0.28^{a}	1.37±0.11^{a}	1.27±0.15^{a}
ADMD (%)	77.88±.03^{b}	76.41±.37^{b}	75.47±.66^{a}	76.6±.46^{b}
APD (%)	83.23±.31^{b}	80.35±.46^{b}	78.83±.55^{a}	82.91±.37^{b}
SURVIVAL (%)	90±.0.10	95±.0.50	90±.0.10	95±.0.50

Means on the same row with the different superscript are significantly different $P<0.05$

IMW: initial mean weight; FWM: final mean weight; MWG: Mean weight gain; MFI: Mean Feed intake; FCR: Feed conversion ratio; PPV: protein productivity value; PER: Protein efficiency ratio; APD: Apparent Protein Digestibility; ADMD: Apparent dry matter digestibility; SR: survival rate; SGR: Specific growth rate; FDLWM: Feed Efficiency Ratio.

Table 6: Haematological characteristics of *O. niloticus* fed Experimental diets

PARAME-TERS	TREAT-MENT 1	TREAT-MENT 2	TREAT-MENT 3	TREAT-MENT 4
PCV(%)	34.8± 5.36[c]	30.0±1.73 [b]	24.2± 3.27 [a]	27.8±3.24 [ab]
Hb (g/dl)	7.9±1.14 [c]	6.7±0.46 [bc]	5.4±0.85 [a]	6.3±0.76 [ab]
Rbc ($\times 10^{12/L}$)	2.80±0.41 [c]	2.33±0.12 [b]	1.86±0.24 [a]	2.14±0.25 [ab]
Wbc ($\times 10^{9/L}$)	11.52±2.09	11.27±0.45	10.74±1.05	11.06±1.13
Het. (%)	34.00±6.44	29.67±1.53	30.80±4.32	33.43±3.64
Lym (%)	65.20±6.38	69.67±0.58	67.80±5.12	65.71±3.77
Eos (%)	0.00±0.00	0.00±0.00	0.20±0.45	0.14±0.38
Bas (%)	0.20±0.45	0.33±0.58	0.60±0.89	0.29±0.49
Mono (%)	0.60±0.89 [ab]	0.33±0.58 [a]	0.60±0.89 [ab]	0.43±0.53 [ab]
MCV (fl)	124.14±1.29	128.57±1.10	130.06±1.59	130.00±1.58
MCH (pg)	28.22±0.77 [a]	28.70±0.78 [b]	29.40±0.87 [b]	29.69±0.80 [b]
MCHC (g/dl)	22.74±0.66	22.30±0.53	22.60±0.46	22.83±0.77

Values on the same row with the different superscript are significantly different P<0.05 PVC: Parked Cell Volume, HB: Haemoglobin, RBC: Red Blood Cell, WBC: White blood cell, MCV: Mean Cell Volume, MCH: Mean Cell Haemoglobin, MCHC: Mean Cell Haemoglobin Concentrate, Lym: Lymphocyte.

FDLBP 45% (86.51±.03) to Control (37.31±.01). The highest profit index (PI) and benefit cost ratio (BCR) were obtained with control (1.33±.01) when the lowest were recorded with FDLBP 45% (1.02±.01).

DISCUSSION

Many studies have been carried out on the performance of insects as dietary protein sources in livestock especially in fish; for example Chironomidae larvae (Adams et al. 2008), Trichoptera larvae (Ferriz et al. 2010); Tenebrio molitor (Sánchez-Muros et al. 2016) Hermetia illucens larvae (Reyes et al 2020). However, no studies have been recorded so far on the use of fermented insect byproduct (insect's parts discarded during processing such as head, limbs and wings) as dietary protein source.

Amino acid analysis of the insect waste showed a slight improvement post fermentation, and a reduction in chitin content, increases in all the essential amino acids. This agrees with previous studies (Kook et al, 2014; Mok et al 2020) in which Bacillus has been used in fermentation. There was also a decline in the chitin content post fermentation. Several species of Bacillus are known to degrade chitin, these include B. circulans (Watanabe et al 2003), B. thuringies (Mehmood et al 2015) and B. licheniformis (Songsiriritthigul et al 2010). These are also known to produce along with chitinase, protein degrading enzymes that enhance the improvement in the amino acid profile. Analyzed amino acids profile of the fermented was similar to that of the fish meal used in this study and was within the range recorded for edible insects (Kohler et al, 2019).

Table 7: Total Protein, Albumin and Globulin of the experimental fish

TREATMENTS	TOTAL PROTEIN	Albumin	Globulin
TREATMENT 1	3.58±0.11 [a]	2.68±0.54	0.9±0.14 [a]
TREATMENT 2	3.77±0.11 [ab]	2.17±0.15	1.6±0.31 [ab]
TREATMENT 3	4.42±1.41 [b]	2.58±0.19	1.84±0.61 [b]
TREATMENT 4	4.13±1.61 [ab]	2.4±0.19	1.73±0.61 [b]

Values on the same column with the different superscript are significantly different P<0.05

Table 8: Economic analysis of *Oreochromis niloticus* fed experimental diets

PARAMETERS	TREATMENT 1	TREATMENT 2	TREATMENT 3	TREATMENT 4
FC(₦ /Kg)	32.3	307.1	288.72	271.40
UFC ₦)	416.38	491.36	421.52	553.66
MWF (g)	11.16±1.67[a]	7.73±1.51[b]	8.23±0.92[b]	6.43±0.92[b]
EIC (₦)	451.38±.09[a]	526.36±.12[b]	456.52±.08[a]	588.66±.04[c]
PI	1.44±.42[a]	1.22±.33[ab]	1.42±.02[a]	1.08±.48[b]
IC	37.31±.01[a]	63.57±.03[b]	51.22±.05[b]	86.51±.03[c]
BCR	1.33±.01[b]	1.14±.09[ab]	1.31±.10[ab]	1.02±.01[a]

Values on the same row with the different superscript are significantly different P<0.05 UFC: Unit feeding cost FC: Cost of Feed, MWF: Mean Weight of Fish, EIC: Estimated Investment cost, PI: Profit Index, IC: Incidence of Cost, BCR: Benefit cost ratio.

The chitin content recorded in DLBPM and FDLBPM were significantly higher than those generally observed in insects (Kaya et al 2015). This could be due to the fact that the discarded parts of edible insects contained the highest content of chitin in insects body parts.

In the current study, fish meal was supplemented up to 45% with FDLBPM. Growth and nutrient utilization were significantly different at 25% and above, these were reflected in MWG, SGR, FCR and PER. The observation could have been due to the quality of the protein content being inferior to that of the fish meal used in this study, the crude protein content of the FDLBPM could have also been influenced by chitin which according to Finke (2007) could contribute Nitrogen to crude protein content during analysis. However, the best growth performance was recorded in fish fed control diet, fishmeal-based diet. It was also observed that, the inclusion levels of the FDLWM had a positive effect on the growth and nutrient utilization of the fish. This agrees with Alegbeleye et al., (2012), who reported that addition of 25% Zonocerus variegatus in the diet of C. gariepinus did not affect the growth rate and nutrient utilisation of the fish compared to the control diet (fishmeal based) due to the low chitin content. However, growth was negatively influenced in C. gariepinus fingerlings when the inclusion of Z. variegatus L. exceeded 50%, with a significant decrease in nutritive indices. At 100% inclusion, the growth performance declined significantly (Alegbeleye et al., 2012). Nevertheless, at higher inclusion levels of Z. variegatus L., there was reduced digestibility of proteins and lipids.

The poor growth in the groups fed FDLBP diets can be attributed essentially to decreased digestibility of the feed as a result of the presence of chitin. There was an inverse relationship between inclusion level and the fish performance. Chitin is a major component of the exoskeleton of insects and other arthropod (Lindsay et al., 1984), and in consequence, is not digestible by monogastric animals such as fish. According to Kroeckel et al., (2012) higher chitin in higher inclusion levels of black soldier fly larvae or prepupae was postulated to affect digestibility of diets and the growth of fish. The presence of chitin has been observed to decrease bile acid levels in the pylorus, which is responsible for the activation of lipase, used for the digestibility of lipid (Hansen et al. 2010). ADC of both lipids and protein decreased with increase in FDLBPM; reduction in digestibility coefficient could have been influenced by the level of chitin

The haematocrit values of the different groups of fish fed experimental diets observed in this study is within the range of corresponding values described by Bittencourt et al. (2003) for Tilapia species; Tavares-Dias et al. (2000) for Florida red tilapia and Hrubec et al. (2000) for Tilapia hybrid. The result showed decrease in Haemoglobin concentration with increase in inclusion level of FDLBP. In contrast, Ogunji et al (2007) reported that there was an increase in the haemoglobin concentration with increase in inclusion level up to 55% in maggot meal. It is was observed that, fish group fed with the inclusion of FDLBP 30% showed lowest mean Hb concentration than the other groups.

The RBC count showed significant differences ($P<0.05$) among treatments. The values were within the range of 3.07 to 7.50 x 106/mm3 reported by Fudge (1999) but lower than 5–8 x 106/mm3 reported by Anon (1980). Hackbath et al. (1983) reported that increased RBC values were associated with high quality dietary protein and with disease free animals.

Changes in hemoglobin concentration and hematocrit or red blood cell count after stress may suggest hemoconcentration or hemodilution by osmoregulatory mechanismsn et al., 1996). According to McDonald and Milligan (1997), stress causes hemoconcentration in many fish, which can be observed by higher hematocrit values.White blood cells (also called leukocytes and abbreviated as WBCs) are the cells of the immune system that are involved in protecting the body against both infectious disease and foreign invaders. Decrease in white blood cell as observed in the fish fed FDLBP is attributed to decrease in the production of leucocyte in the haemotopoietic tissue of the kidney and perhaps the spleen, Akinwande et al., (2004) reported that a measurable increase in WBC of fish is a function of immunity response to vulnerable illness and disease. These results indicate that the animals were healthy. Decrease in number of WBC below the normal range is an indication of allergic conditions, anaphylactic shock and certain parasitism, while elevated values (leukocytosis) indicate the existence of a recent infection, usually with bacteria (Ahamefule et al., 2008). Nevertheless, the values in all groups were within the normal range recorded WBC (9300 – 336200 mm3) for teleosts (Adedeji and Adegbile, 2011). The haematological parameters indicated that fish fed with FDLBP had higher health status than those of control fish. Thus it can be concluded that increase in inclusion reduce stress on the health of O. niloticus fed the experimental diets.

Since total proteins, albumin and globulin are generally influenced by total protein intake of the animal (Birth and Schuldt, 1982; Onifade and Tewe, 1993), the values obtained in current study indicate nutritional adequacy of the FDLBP as a dietary proteins. (Not relevant to this paper. Expunge and recast the rest).In this study, total protein, albumin and globulin contents increased in all treatment groups, which indicate the enhancement of innate immunity of the fish. Similar results have been reported in olive flounder (Cha et al., 2008), common carp (Dautremepuits et al., 2004) and E. bruneus (Harikrishnan et al., 2012) after feeding chitosan-supplemented diet. The increase in total blood protein could be attributed to lysozyme activity, bactericidal activity, globulin content and probably some other peptides (Misra et al., 2006). However, Bagni and Archetti (2000) found no significant difference in protein content or in albumin/globulin ratio in seabass (Dicentrarchus labrax) fed with feed containing b-glucan, alpha-tocopherol and ascorbic acid.

The inverse relationship between inclusion level and cost of production of experimental diets is an indication of the cost effectiveness of using insect waste as non conventional feedstuff in fish feed formulation. This could be attributed to the partial replacement of the more expensive fishmeal with FDLBP that has a low cost (Yusuf et al., 2008). This is similar to the report that non-conventional feed resources (NCFRs) are very cheap by-products or wastes from agriculture, farm-made feeds and processing industries (Sogbesan and Ekundayo, 2014). This has resulted in feed cost of diet IV (FDLBP 45%) to be the cheapest (₦0.74/kg) while control diet being the most expensive (₦0.89/kg). The cost of using the four diets is indicated by the incidence of costs (IC), which is defined as the cost of feed to produced a kg of fish (relative cost per unit weight gain) and the lower the value, the more profitable it is using that particular feed (Nwanna, 2004; Abu et al., 2010). In the present study, it was evident that it could be cheaper to raise Nile Tilapia on diet 1 which contained 100% fish meal as the major protein source with the incidence cost of (37.31±.01). However, diet IV which contained highest proportion of FDLBP (45%) as a major protein source had the highest Incidence cost (86.51), indicating that it is more costly to use fermented insect waste in formulating fish rations. Moreover, the less the incidence cost, the higher the profit index (PI) with diet 1 having the highest profit index (1.48), this might be as a result of higher digestibility of fishmeal compare to insect meal. These results disagreed with the experiment done by Aladetohun and Sogbesan (2013) where blood meal was used as protein ingredient in the diet for O, niloticus and the diet with 100% blood meal was cheapest. In this experiment, high profit index in control diet, arose from the fact that fishmeal has no crude fiber or chitin which resulted in better FCR and lowest UFC. The reverse is the case with other diet which could be attributed to the presence of chitin in insect. More so, in this experiment, it is evident that use of a non-conventional protein source of animal origin such as insect meal was not cost effective for tilapia production systems although they are regarded as wastes. The difference in economic performance for the four rations was due to the high FCR and low MWG resulted in diet II to IV. To increase fish farm profits, the cost of feed must be reduced and considerable effort be focused on finding alternatives to fishmeal from both plant and animal protein sources (Fasakin et al., 2003) The result of this find-

ing agrees with Ogunji (2004) who observed that when alternative protein sources are used in tilapia feeds, the rate of fish growth may be reduced leading to increased rearing time. However, this study concluded that FDLWM can replace fish meal in practical diet of O. niloticus fingerlings up to 45% inclusion levels without any adverse effect on the fish growth.

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