

Digestibility Study and Nutrient Re-evaluation in *Clarias gariepinus* Fed Blood Meal-Rumen Digesta Blend Diet

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Abstract

The nutrient utilization and digestibility of blood meal-bovine rumen digesta blend diet (BMBRD) fed to *Clarias gariepinus burchell* (1822) was assessed using 60 *C. gariepinus* juveniles with a view to confirming the digestibility and suitability of the BMBRD blend inclusion level in the diet of *C. gariepinus* for sustainable clariid production. Two set of three iso-nitrogenous experimental diets containing 35% crude protein were formulated with 0%, 25% and 50% BMBRD inclusion level to substitute the fishmeal component which was the primary protein source. The first set of experimental diet was designed to monitor the growth performance and feed utilization was fed fortnightly in duplicate to *C. gariepinus* juveniles, stocked in glass aquaria (60 cm × 30 cm × 30 cm) at 4% of the body weight in two instalments daily for 10 weeks, while the second experimental diet with 0.5% chromic oxide (Cr₂O₃) inclusion (as a digestibility marker) was prepared and fed to the fish for 7 days to determine to digestibility of the different BMBRD included diets. The obtained results showed that the fish fed 25% BMBRD included diet had significantly higher ($p < 0.05$) growth performance and feed utilization indices than the other experimental diets. Similar to observation in the growth performance and feed utilization indices, the fish fed BMBRD included diets had significantly higher ($p < 0.05$) protein and energy digestibilities than the fish fed 0% BMBRD (control diet) and the study confirmed that fishmeal can be partially replaced up to 25% BMBRD blend in *C. gariepinus* diet for optimum growth performance and feed utilization.

Keywords: blood-meal, *Clarias gariepinus*, diets, digestibility rumen-digesta

Introduction

Fish is a highly nutritious food, containing high amounts of proteins with high biochemical value for humans (Brenden *et al.*, 2003). The importance of fish as a less expensive dietary protein source cannot be over-emphasized especially in developing countries where livestock as sources of dietary protein (meat, egg and milk) are expensive and often beyond the reach of many (Eyo, 2005). Fish is cheaper than meat and also contains essential amino acids such as lysine, methionine and tryptophan, and is also rich in vitamins and minerals (Pearson, 1973). For this reasons, most countries in the tropics have turned their attention to the exploitation, development and sustainable management of their fisheries resources as a means of providing the populace with the needed animal protein from fish products (Eyo, 2005).

Feed is the major input in aquaculture production, however, fish feed technology is one of the least developed sectors of aquaculture particularly in Africa and other developing countries of the world (Akintaro, 2014). High

cost of fish feed was observed as one of the problems hampering aquaculture development in Nigeria (Gabriel *et al.*, 2007) because feed cost accounts for at least 60% of the total cost of production in intensive condition, which to a large extent determines profitability of the fish farming enterprise (Jamiu and Ayinla, 2003). Due to its high palatability, digestibility, excellent supply of essential fatty acids (EFAs), mineral and vitamins as well as balanced amino acids profile (Tacon, 1993), the pisciculture industry has relied heavily upon fish meal as the major dietary protein source of fish feeds (Watanabe *et al.*, 1997). Since fish meal is expensive as a feed ingredient, the use of non-conventional feedstuffs has been reported with good growth and better cost-benefits value (Adewole, 2013). According to Rumsey (1993) and Tacon (1993), cost-effective, practical aquaculture feeds could be produced without the use of fish meal with less apparent loss of fish growth. Blood meal-bovine rumen digesta blend (BMBRD) is a proven cheap, locally available good source of protein supplement in fish feed (Adewumi, 2012; Adewole and Olaleye, 2014). However different inclusion levels have been recommended as being optimum for adequate growth in *C. gariepinus*.

The present study aimed to confirm the appropriate optimum inclusion level of this protein supplement in the *C. gariepinus* diet.

Materials and Methods

Ninety (90) ten (10) weeks old *Clarias gariepinus* juveniles (36.95 ± 1.60 g) were obtained from a reputable Fish Farm in Ile-Ife, Osun State. The fish were collected early in the morning (7:00 am) to reduce stress and transported to the Fish Culture Laboratory, Department of Zoology, Obafemi Awolowo University. The fish were sorted and stocked in 6 glass aquaria (60 cm × 30 cm × 30 cm) at a stocking density of 10 fish per tank. The fish were left to acclimatize for the period of two (2) weeks prior to exposure to dietary treatment. During the period of acclimatization, the fish were fed on Durante feed (2 mm) at the rate of 4% of their body weight per day in two installments to ensure that the experimental fish had uniform nutritional status before being exposed to the experimental diets.

Feedstuffs for experiment diet

The feedstuffs used to compound the experimental diets were wheat offal, yellow maize, fish meal, blood meal-bovine rumen digesta blend, vegetable oil, salt, minerals and vitamin premix. Wheat offal, yellow maize, fish meal, vitamin and mineral premix were obtained at Jeje Feed Mill, Odo-Eran, Abeokuta, Ogun State, while vegetable oil was obtained from Odo-Eran market, Abeokuta. Bovine blood and rumen digesta were collected fresh from Ogo-Oluwa slaughter slab, Opa, Ile-Ife.

Preparation of blood meal-bovine rumen digesta (BMBRD)

The bovine blood and rumen digesta were collected freshly and separately into two different clean plastic buckets. The bovine blood was stirred thoroughly with a paddle to prevent it from coagulation and mixed with rumen digesta obtained from eviscerated cattle in the ratio 1:1 (weight/weight) (Adewole, 2013). The mixture was cooked for 50 minutes with constant stirring until it is almost free of moisture content and was sun dried for five days on a big clean dry polythene sheet. The dried BMBRD was then ground and packed into an air tight container until needed for diet compounding.

Diet formulation and preparation

Based on the proximate composition of the feedstuffs, three approximately isonitrogenous 35% crude protein experimental diets were formulated using fish meal, BMBRD, yellow maize, wheat offal, mineral and vitamin premix (Adewole, 2013) using Pearson's square method (Jhingram and Pullin, 1985). Two (2) sets of experimental diets were formulated for the study. The first set of the experimental diets was formulated to confirm the optimum BMBRD inclusion level in the diet of fish by studying the growth performance and feed utilization by the fish, while the second set of experimental diets was formulated to test the digestibility of the diets by the fish with the inclusion of chromic oxide (Cr_2O_3) as a digestibility marker. In the two

sets of experimental diets, the fishmeal component which is the main source of protein was substituted with the BMBRD at 25% and 50% inclusion levels. The formulated diet without BMBRD and with fish meal as the protein source served as the control diet. To compound the various experimental diets, the various ingredients were weighed according to the calculation and mixed appropriately and were pelletized through a 2 mm die in a G 7682 Model pelleting machine at FUNAAB Fish Farm, Abeokuta, Ogun State. The prepared diets were then sun-dried for 3 days to prevent deterioration. Each of the prepared diets was kept in labelled plastic containers and stored in a cool environment until needed for the feeding trials. The proximate and energy composition of each of the diets were carried out.

Experimental diet feeding

After two (2) weeks of acclimation, 60 healthy juvenile catfish (36.95 ± 1.60 g) were selected and randomly distributed into each of the six labelled tanks (60 cm × 30 cm × 30 cm) at the rate of 10 fish per tank. The fish were stocked in duplicates for each dietary treatment. Total initial weight of the fish per tank was calculated to determine the daily feed allotment. For growth performance evaluation, the fish were fed at 4% of their body weight in two installments between 8:00 - 9:00 am and 5:00 - 6:00 pm for a period of 10 weeks (Adewole, 2013). During the period, feed utilization by the fish was monitored. After each fortnight weighing, the daily feed ration allotted was adjusted accordingly based on the new fish weights. During the feeding trials, the tanks were cleaned thoroughly every other day to remove the uneaten foods and faecal matters and to prevent or remove the hygiene growth of bacteria and fungi in the culture system. After the tenth week, the fish were then fed on 0.5% chromic oxide included diets for 1 week before being sacrificed for digestibility studies.

Digestibility studies

Faeces were collected from each of the experimental dietary group after 7 days of feeding with 0.5% chromic oxide diet as described by Henken *et al.* (1985) and Fagbenro (1996). The fish were sacrificed and faecal matters were removed by rectal dissection (removal of 3.5 cm of the rectum). The faecal matter was removed, pooled for each of the treatments placed in an evaporating dish and oven dried at temperature of 60 °C for 30 mins. Faecal samples were ground and analysed for moisture content, crude protein, crude fat, ash content, as previously described by A.O.A.C. (1990) standard methods while the gross energy content was determined using a bomb calorimetric method. The chromic oxide levels were determined by spectrophotometric method according to standard methods.

Statistical analysis

Data obtained were subjected to One way Analysis of variance (ANOVA) using Post-Hoc Duncan Multiple Test for comparison was used to determine the significant difference between the experimental diets on the growth performance and feed digestibility of the fish specimens at $p < 0.05$ in SPSS software Version 21.

Results

The proximate composition of the blood meal-bovine rumen digesta blend (BMBRD) and other ingredients used to formulate the experimental diet are shown in Table 1. BMBRD which was the alternative protein source had the highest crude protein value of (74.38%), while the lowest crude protein was recorded for yellow maize (10.8%). The fish meal which was used had the highest lipid content of 10.04% and the least lipid content was recorded in the BMBRD blend used. The highest crude fibre content (12.34%) and lowest value of ash content (6.58%) was recorded in the wheat offal used. Table 2 showed the ingredient composition for the formulated diet. The proximate composition of the compounded experimental diets as well as the gross energy and the chromic oxide level is shown in Table 3.

The moisture contents of 0% (8.35%) and 50% BMBRD (8.75%) diets were found not to be significantly different ($p > 0.05$) from each other but the level of moisture content in the two diets were significantly lower ($p < 0.05$) than the moisture content (9.04%) recorded in 25% BMBRD diet. Analyses showed that the crude protein content varied between 37.49% (0% BMBRD diet) and 38.34% (50% BMBRD diet). The observed variation

however, were not significantly different ($p > 0.05$) from each other. Also variations which were recorded in the ash content level in the three diets (9.04% (50% BMBRD diet) and 10.38% (0% BMBRD diet)) were found not to be significantly different ($p > 0.05$). The gross energy and chromic oxide level recorded in the test diets were found to vary significantly ($p < 0.05$) from each other. The 0% BMBRD diet had the least gross energy (3.88 kcal/g) which was found to be close to gross energy value recorded for 25% BMBRD (3.89 kcal/g) and 50% BMBRD diet had (3.92 kcal/g). Variation in the chromic oxide level in the prepared diets followed the same trend as the gross energy content (Table 3).

Fig. 1 showed the various degree of growth of the fish fed the various experimental diets. The fish fed 25% BMBRD diet had the highest weight gained at each fortnight weighing while the fish fed 0% BMBRD diet (the control diet) had the least weight gained. The growth performance indices in the fish fed the three different experimental diets during the period of study showed the fish fed 25% BMBRD had the best and highest growth performance indices, which were significantly higher ($p > 0.05$) than others. During the cultured period, no mortality was recorded in the fish fed 0% and 25% BMBRD diets while the fish fed 50% BMBRD diet had 5% mortality (Table 4).

Table 1. Proximate composition* of the feedstuffs used to formulate the experimental diets

Ingredient	Proximate component (%)					
	Moisture content	Crude protein	Lipid	Ash	Crude fibre	NFE**
BMBRD	10.37	74.33	0.83	7.33	3.17	3.97
Fish meal	7.24	68.50	10.04	11.30	---	2.92
Wheat offal	9.60	16.34	1.69	6.58	12.34	53.45
Yellow maize	11.80	10.80	3.60	8.40	3.50	61.90

*Values are means of 3 determinations

**NFE: Nitrogen Free Extract

Table 2. Ingredient composition (g/100 g) for the formulated experimental diets

Ingredient	0% BMBRD	25% BMBRD	50% BMBRD
BMBRD	-	15.10	30.25
Fish meal	60.50	45.40	30.25
Wheat offal	18.50	18.50	18.50
Yellow maize	18.50	18.50	18.50
Vit/Min premix**	1.00	1.00	1.00
Veg. oil	1.00	1.00	1.00
Salt	0.5	0.5	0.5
Total	100	100	100

*0.5% Cr₂O₃ was included in the experimental diets used for digestibility studies

**Biomix from Bio-organics production were provided per kg of diet: Vitamin A, 12,500 IU; Vitamin D, 2,500 IU; Vitamin E, 40 mg; Vitamin K, 2 mg; Vitamin B1, 3 mg; Vitamin B2, 5.5 mg; Niacin 55 mg; Calcium pantothenate, 11.5 mg; Vitamin B6, 5 mg; Vitamin B12, 0.025 mg; Choline chloride, 500 mg; Folic acid, 1 mg; Biotin, 0.08mg; Manganese, 120 mg; Iron, 100 mg; Zinc, 80 mg; Copper, 8.5 mg; Iodine, 1.5 mg; Cobalt, 0.3 mg; Selenium, 0.12 mg; Anti-oxidant, 120 mg

Table 3. Proximate composition, gross energy and chromic oxide contents of the experimental diets

BMBRD inclusion level	Moisture	Crude protein	Ether extract	Ash	Crude fibre	NFE**	Gross energy (kcal/g)	Cr ₂ O ₃ (g)
0%	8.35 ± 0.16 ^a	37.49±1.27 ^a	8.20 ± 0.10 ^a	10.38 ± 0.43 ^a	7.30 ± 0.10 ^a	28.28	3.88	0.004
25%	9.04 ± 0.06 ^b	37.66±0.23 ^a	7.80 ± 0.15 ^a	10.07 ± 0.03 ^a	7.45 ± 0.15 ^a	27.98	3.89	0.005
50%	8.75 ± 0.06 ^a	38.34±0.44 ^a	7.18 ± 0.08 ^b	9.40 ± 0.10 ^a	5.76 ± 0.16 ^b	30.57	3.92	0.006

*Means within column with different superscripts are significantly different ($p < 0.05$) from each other

**NFE= 100 - (% CP + % LIPID+%ASH+%CF+%MOISTURE)

Table 4. Growth performance of the cultured fish fed different experimental diets

BMBRD inclusion level	Initial mean weight (g)	Final mean weight (g)	Mean weight gained (g)	Percentage body weight gained (%)	Daily weight gained (g)	Specific growth rate	Survival rate (%)
0%	34.18 ± 0.08 ^a	138.10 ± 21.58 ^a	103.92 ± 21.50 ^a	303.88 ± 62.18 ^a	1.49 ± 0.31 ^a	0.86 ± 0.10 ^a	100.0
25%	39.28 ± 1.93 ^a	199.70 ± 10.06 ^b	160.42 ± 11.98 ^b	410.94 ± 50.65 ^b	2.29 ± 0.17 ^b	1.01 ± 0.06 ^b	100.0
50%	37.41 ± 4.61 ^a	150.9 ± 29.35 ^a	113.53 ± 24.74 ^a	299.92 ± 29.22 ^a	1.63 ± 0.36 ^a	0.85 ± 0.05 ^a	95.0

^aMeans within column with different superscripts are significantly different ($p < 0.05$) from each other

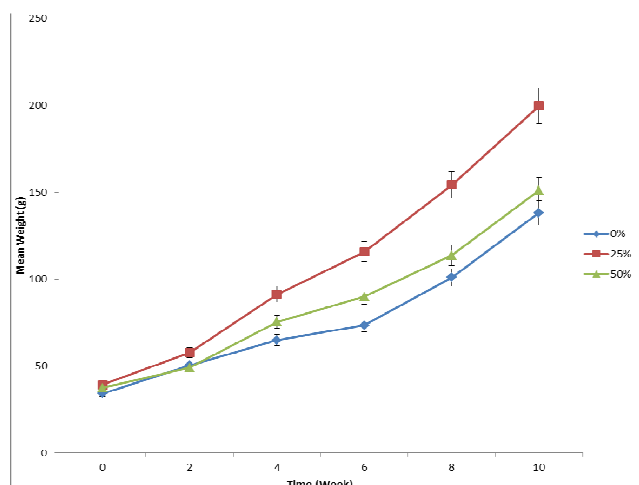


Fig. 1. Weight gained by *Clarias gariepinus* juveniles fed the experimental diets during the period of study

Similar to the observation in the growth performance indices, the fish fed 25% BMBRD had the highest daily feed intake, total feed intake and protein intake with 3.36 ± 0.13 g, 234.84 ± 8.90 g and 117.43 ± 4.38 g values respectively. However, the least daily intake (2.34 ± 0.02 g), total feed intake (163.34 ± 1.27 g) and protein intake (81.73 ± 0.53 g) was recorded in the fish fed 50% BMBRD diet. Analyses showed a significantly higher ($p < 0.05$) difference in the feed utilization of the fish fed 25% BMBRD diet compared to those fed 0% and 50% BMBRD diets which were not significantly different ($p > 0.05$) from each other (Table 5). The food conversion rate (FCR) was also better in the group fed 25% BMBRD diet compared to the other fed the experimental diets (Table 5).

Analyses of the protein and energy digestibilities of the fish fed different experimental diets showed that highest level of chromic oxide was recovered in the fish fed 50% BMBRD diet (45.0%) while the least (35.6%) was recovered in the fish fed 25% BMBRD included diet (Table 6). Analyses also revealed that the fishes fed 50% BMBRD diet voided 51.04% of the crude protein consumed in the faeces compared to 47.32 in 0% BMBRD (control) diet and 39.27% in 25% BMBRD included diets (Table 6). Faeces of the fish fed 50% BMBRD diet also had the highest (44.21%) of the voided energy while the fish fed 25% BMBRD diet had the least (41.93%).

Analyses revealed that the fish fed 25% and 50% BMBRD diets had significantly higher ($p < 0.05$) protein digestibilities than the fish fed 0% BMBRD (control) diet. Despite higher crude protein digestibility value recorded in the fish fed 25% BMBRD diet, statistical analyses however showed the value was not significantly different ($p > 0.05$) from those fed 50% BMBRD diet (Fig. 2). Energy digestibility in *C. gariepinus* juveniles fed the experimental

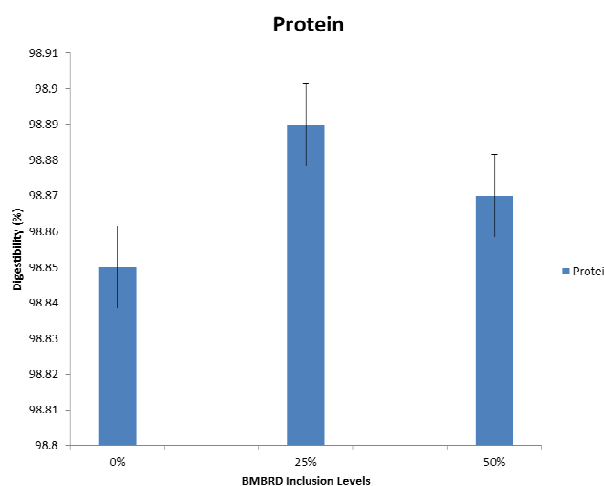


Fig. 2. Protein digestibility in *C. gariepinus* juveniles fed the experimental diets

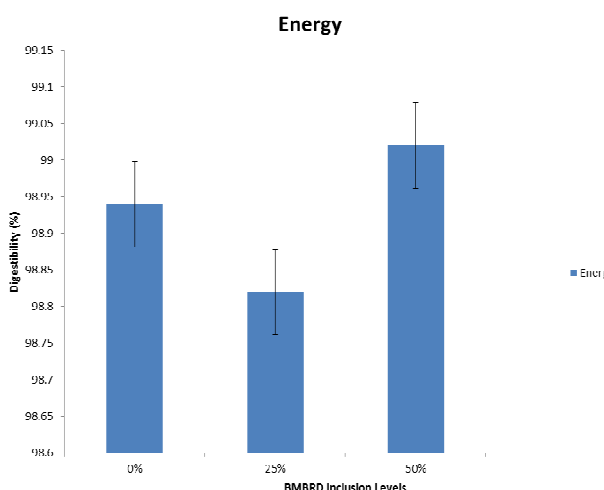


Fig. 3. Energy digestibility in *C. gariepinus* juveniles fed the experimental diets

BMBRD diets and the control (Fig. 3) showed that the fish fed had significantly higher ($p < 0.05$) energy digestibility than those fed 0% and 25% BMBRD diets.

Discussion

The proximate composition of the blood meal - bovine rumen digesta blend (BMBRD) used in this study was found to be higher than what was reported by several authors. In this study, the BMBRD blend was high in (74.38%) crude protein content and 0.83% lipid content. The CP value recorded was significantly higher than 39.80% to 46.10% and the lipid content, lower than 1.15% to 2.13% reported for other BMBRD meals (Odunsi, 2003;

Table 5. Feed utilization in the fish fed the different experimental diets

BMBRD inclusion level	Daily feed intake (g)	Total feed intake (g)	Food conversion ratio (FCR)	Protein intake (g)	Protein efficiency ratio (PER)
0%	2.37 ± 0.26 ^a	165.26 ± 17.94 ^a	1.624 ± 0.164 ^a	82.78 ± 8.93 ^a	1.25 ± 0.13 ^a
25%	3.36 ± 0.13 ^b	234.84 ± 8.90 ^b	1.468 ± 0.054 ^a	117.43 ± 4.38 ^b	1.37 ± 0.06 ^a
50%	2.34 ± 0.02 ^a	163.34 ± 1.27 ^a	1.514 ± 0.34 ^a	81.73 ± 0.53 ^a	1.39 ± 0.31 ^a

*Means within column with different superscripts are significantly different (p<0.05) from each other

Table 6. Feed utilization in the fish fed the different experimental diets

BMBRD Inclusion level	Chromic oxide		Crude protein		Gross energy	
	Level in the faeces	% voided with faeces	Level in the faeces	% of the CP consumed	Level in the faeces (kcal/g)	% of the diet consumed
0%	0.00165 ± 0.00 ^a	41.25	17.74 ± 0.72 ^a	47.32	1.698 ± 0.00 ^a	43.76
25%	0.00178 ± 0.00 ^a	35.6	14.79 ± 0.22 ^b	39.27	1.631 ± 0.00 ^b	41.93
50%	0.0027 ± 0.00 ^b	45.0	19.57 ± 0.01 ^a	51.04	1.733 ± 0.00 ^c	44.21

*Means within column with different superscripts are significantly different (p<0.05)

Dairo *et al.*, 2005; Adeniji and Jimoh, 2007; Adewumi, 2012; Adewole, 2013). The differences in the proximate composition of BMBRD meals used for the experiment with those reported by the other authors were probably due to the differences in chemical composition of the pastures consumed by the cattle on one hand, and also, the period of fasting in the cattle prior to slaughter which probably increased the rumen processing time (Jyotiprabha *et al.*, 2015).

The fish fed 25% BMBRD had the highest feed intake and total feed intake, while the fish fed the 50% BMBRD diet had the least. The result agreed with the results of Adewumi (2012), but was however different from those of Adewole (2013) who reported the highest feed intake was in the fish fed 50% BMBRD diet. The lower feed consumption in the fish fed 50% BMBRD diet could be as a result of a higher combination of blood meal and the rumen contents in the (50% BMBRD) diet, which could probably have a negative influence on the feed palatability resulting in lower consumption by the fish (Odunsi, 2003).

Weight gain is one of the reliable growth indicators for measuring fish responses to experimental diets. During the period of study, the fish juveniles fed the experimental diets and the control responded positively to the diets by showing various degrees of weight increases. *Clarias gariepinus* juveniles fed the 25% BMBRD diet had the highest weight gain, daily weight gain (DWG), percentage body weight gain (PBWG), and specific growth rate (SGR). The higher growth performance recorded in the fish fed 25% BMBRD diet agreed with the result of Adewumi (2012), but was at variance with those of Adewole (2013) who reported better growth performance when the fish was fed 50% BMBRD diet. The better growth in the fish fed the 25% BMBRD included diet could probably be attributed to a better nutrient mix in the test diet.

Determining the efficiency of feedstuffs in promoting fish growth can be evaluated through digestibility studies (Gad and Yehuda, 1999). The determination of digestibility, together with chemical analysis of the diet allowed for the more exact estimation of the natural value of a given protein and carbohydrate source (Plakas and Katamaya, 1981). The result from the study indicated that the digestibility of protein in the fish fed 25% BMBRD and 50% BMBRD diet were relatively high (98.90% and

98.87% respectively) and was different from those reported by Degani *et al.* (1997) in the hybrid tilapia fed diets with protein sources such as fish meal (90%), soybean meal (95%), and poultry meal (92%) but was however similar to the findings of Gad (2004) who reported that protein digestibility in European eel (*Anguilla anguilla*) fed a wheat meal and potato based meal diet was 96.12% and 97.51% respectively. The observed difference could be attributed to the high level of protein in the source (BMBRD), the species of fish, size of fish and probably due to the differences in the method of faecal collection.

The energy digestibility reported in this study, which ranged from 98.82% to 99.02% was also similar to those reported by Gad (2004) (96.66% to 97.11%), although, it was for a different fish which confirmed high dietary energy utilization by the *C. gariepinus* fish juvenile during the period of study. Nwana (2003) reported that low fibre content of any diet was an indication that the meal might be highly digestible which was probably responsible for higher digestibility values recorded in the fish fed 25% BMBRD included diet.

Conclusions

The study revealed that the BMBRD diets were accepted and digested by the experimental fish. The result showed that the fish fed 25% BMBRD diet had the best protein and energy digestibility and this confirmed that BMBRD diet could replace up to 25% fish meal in commercial diet of *C. gariepinus* without any adverse effect on growth performance and feed utilization of fish.

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