Journal of Agriculture and Environment Vol. 12 No. 1, 2016: 89-96
ISSN 1595-465X

EFFECT OF VARYING LEVELS OF FERMENTED SORGHUM (*Sorghum bicolor*) ON GROWTH AND HAEMATOLOGY PROFILES OF *Clarias gariepinus* (BURCHELL, 1822) FINGERLINGS

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ABSTRACT

The availability of suitable and economical feeds is a major constraint to sustainable fish farming in Nigeria and it is a source of discouragement to many prospective fish farmers. Addressing the problem and making fish farming more attractive and sustainable; information should be available especially on feed sources that are less competitive and of low cost value. Feed sources, virtually those of plant origin are found to have anti-nutritional factors which inhibit the growth of fish. However, fermentation has been employed to reduce the effects of anti-nutritional factors in feed ingredients. This study evaluates the growth and haematological performance of *Clarias gariepinus* fingerlings fed fermented sorghum (*Sorghum bicolor*). The sorghum was fermented in solid state form for five days. Four diets; A, B, C, and D were formulated containing 0% (control), 50%, 75%, and 100% fermented sorghum substituted diets respectively. The diets were fed to *C. gariepinus* fingerlings, average weight of 6.79±0.20g for ten weeks. The mean weight gain were significantly different ($p<0.05$) with diet D having the highest (26.54±2.09g), followed by diet B (23.23±4.02g). Diets B and C had 100% survival rate while diet D had the lowest (93.33%), however, with no significant difference ($p>0.05$). The haematology profiles of the experimental fish indicated significant differences in all the parameters examined. The white blood cells and albumin were highest for diet D with 3.57±0.15 and 23.97±1.86g/l and lowest for diet C with 3.20±0.20 and 18.74±2.02g/l respectively. Sorghum (*Sorghum bicolor*) as carbohydrate source in fish diet at 75% fermented combined with 25% unfermented might be a better alternative source of energy in catfish, *C. gariepinus* diet.

Keywords: Haematological profiles; *Clarias gariepinus*; Sorghum; Tannin

INTRODUCTION

Aquaculture development in Sub-Sahara Africa including Nigeria has been reported to be struggling, yet to be properly harnessed compared to the rest of Europe and Asia

(Chungadeya *et al*., 2003), partly due to non-availability of quality feed at cost-effective price. High cost of feed inputs is a major problem facing fish farmers in Nigeria. Fish feed is generally considered as the most expensive item in intensive fish farming. According to NRC (1993) fish feed constitutes about 40-60% of the recurrent cost of most intensive fish farming ventures and can sometimes negate the economic viability of a farm if suitable feed are not used. This problem has become a major source of fear to many prospective fish farmers in Nigeria (Jamiu and Ayinla, 2003). Various studies have been conducted on the use of non-conventional feedstuffs including cereals, root and tubers, and their by-products (Solomon *et al*., 2007; Aderolu *et al*., 2009) with the aim of reducing feed cost and ultimately production cost in fish farming. However, these feedstuffs, virtually those of plant origin are found to have anti-nutritional factors which inhibit growth. They, in most cases form a "shield effect" on nutrients molecules in feeds, especially the protein thereby preventing the protease or similar digestive enzymes from getting them thus causing the molecules to be passed out with the feaces undigested/unabsorbed and hence unavailable to the fish for growth purpose. All plants' feedstuffs such as cereals including sorghum, millet, and wheat; oilseeds including groundnut, soybean and cottonseed contain protease inhibitors, tannins (Sogbesan *et al*., 2006).

Tannins, a toxic component present dominantly in plants including sorghum were in commercial use long before there was any clue about their natural functions. Edwin *et al*. (2001) reported an interesting background regarding the use of tannic acid in leather industry and also demonstrated the toxicity of the tannery effluents to the larvae of mosquito, negative effects of these effluents were also observed in fish. Tannins and their by-products are well known in degrading aquatic habitats, which create problem for fish and inflict mortality in aquatic organisms (Johnson 2001). However, systematic studies are lacking to trace the lethal concentration of tannins in sorghum on fish and identify the solution to the problem. But fermentation has been employed to reduce the effects of anti-nutritional factors such as tannins in feed ingredients (Sogbesan *et al*., 2006). In view of this, the present study aimed at investigating the growth and haematological performances of *Clarias gariepinus* fingerlings fed graded level of fermented sorghum (*Sorghum bicolor*), a cereal usually used as a source of energy in fish diet.

MATERIALS AND METHOD

Study Area

The study was conducted in the Department of Fisheries, Modibbo Adama University of Technology, Yola, Nigeria. Yola, the Adamawa state Capital is on latitude 9° 81'N and 9° 17'N Longitude 12° 25'E.

Feed Preparation

The sorghum (*Sorghum bicolor*) used in the present study were collected from Sabon-Gari market, Girei Local Government Area of Adamawa State, Nigeria. The white sorghum were sorted and solidly fermented according to the methods of Sogbesan *et al*. (2012). The sorghum was fermented in a moist state, wetted at 5% moisture in a plastic container (without any flow of liquid). The contents were covered and allowed to ferment for 5days. At the end of the fifth day, the fermented sorghum were collected, sun-dried for

2 days, grounded, and sieved to produce sorghum meal. Four (4) different diets at 45% crude protein were formulated. The fermented sorghum meal was used to replace unfermented sorghum at 50%, 75%, and 100% in the diets B, C, and D respectively, while the control diet (diet A) contain 0% fermented sorghum meal (Table 1). The diets produced were pelleted, sun-dried to crispy for two days to prevent the growth of moulds. Thereafter, they were packed into waterproofed air tight bag, labeled accordingly and stored at room temperature. The experimental diets produce were subjected to proximate analysis using official methods as recommended by (A.O.A.C, 2001).

Experimental Setup

The experimental fish, *Clarias gariepinus* fingerlings of average weight of 6.79±0.20g were purchased from SB Fish Farm hatchery in Girei, Adamawa State, Nigeria. One hundred and twenty (120) *Clarias gariepinus* fingerlings collected from the hatchery were acclimatized and fed with control diet for four (4) days. Subsequently, they were randomly assigned to the four (4) diet treatments. Each treatment was replicated three times in a completely randomize design having ten (10) fish per replicate in 30litres plastic bowl containing 20litres volume of water in a semi-flow-through system. The fingerlings were fed experimental diets for the period of ten (10) weeks at the rate of 5% body weight per day. Initial mean weight of fish per bowl was taken using electronic top-loading balance (Mettler, E200). The fortnight weights of the experimental fish were monitored for growth determination and the quantity of feed fed were adjusted accordingly. The experimental fish were also monitored for mortality.

Growth Performance and Nutrient Utilization

Weekly weight (experimental fish) and feed were used to assess the growth response to feed in terms of percentage (%) Weight gain, Specific growth rate, Feed conversion rate and Protein efficiency rate.

Blood Collection and Haematological Analysis

Table 1: Composition of the experimental diets (g/100g)

Ingredients	Diet A	Diet B	Diet C	Diet D
Fish meal	25.50	25.50	25.50	25.50
Soybean	30.50	30.50	30.50	30.50
Sorghum (f)	-	19.00	28.50	38.00
Sorghum (uf)	38.00	19.00	9.50	-
Premix	2.00	2.00	2.00	2.00
Methionine	1.00	1.00	1.00	1.00
Lysine	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50
Oil	0.50	0.50	0.50	0.50
Binder	1.00	1.00	1.00	1.00
Total	100	100	100	100

f – Fermented; uf – Unfermented

Blood samples were collected via caudal vein puncture (Kori-Siakpere *et al*., 2005). The samples were used for the determination of packed cell volume (PCV), haemoglobin (Hb) concentrations, leukocyte count. All determinations were done as per the replicates. Blood samples were collected into sample bottle containing ethylene diamine tetra acetic acid (EDTA), as anticoagulant and were thoroughly mixed. Thereafter, the samples were taken for haematological analysis. The haematology parameters including packed cell volume and haemoglobin were analyzed. The white blood cell and differential count (neutrophils, monocytes, and lymphocytes) were analyzed according Davie and Lewis (2001).

Data Analysis

Data generated from the experiment were subject to analysis of variance (ANOVA) using Graphpad Insta window 7. Where significant differences exist Duncan`s Multiple Range Test was used to separate the means.

RESULTS

Proximate Composition

Table 1 presents the composition of the experimental diets while Table 2 shows the proximate values of the four experimental diets; diets A, B, C, and D crude protein were 46.38, 46.25, 47.01 and 48.06% , respectively, with no significant difference (p>0.05).

Table 2: Proximate composition of the experimental diets (g/100g)

Parameters	Diet A	Diet B	Diet C	Diet D
Moisture	9.00	10.50	10.50	11.00
Ash	5.50	5.50	4.50	4.00
Crude fiber	4.50	4.60	4.30	4.00
Crude lipid	5.00	4.50	6.50	7.40
Crude protein	46.38	46.25	47.01	48.06
NFE	30.62	25.65	27.69	25.54

No significant difference (p>0.05) in the values

Water Quality Parameters

Table 3 presents the water quality parameters during the experimental period. The temperature ranged between 34.34 and 36.01°C; dissolved oxygen between 4.20 and 5.00mg/L, while pH ranged between 7.73 and 8.29.

Table 3: Water quality parameters during the experimental period

Parameters	Minimum	Maximum
Temperature (^{0}C)	34.34	36.01
Dissolved oxygen (mg/l)	4.20	5.00
pH	7.73	8.29

Growth Performance

The growth performance, survival rate and feed utilization as presented in Table 4 revealed that *C. gariepinus* fingerlings responded to all the diets, resulting in weight increase. The mean weight gain was significantly different (p<0.05) with diet D having the highest with 26.54±2.09g, followed by diet B with 23.23±4.02g, and the control, diet A had the least, 21.17±4.34g. Diets B and C had 100% survival rate while diet D had the lowest of 93.33%, however, with no significant difference (p>0.05). Diets A and D indicated the least and the highest relative growth rate (%) of 282.27±11.14 and 393.19±10.55 and specific growth rate (%) of 0.83±1.90 and 0.99±1.65 respectively. There were no significant difference (p>0.05) among the protein efficiency ratios, with diet D indicating the highest value of 0.80±0.04 and the lowest value of 0.71±0.05 recorded for the control diet (diet A). Diet A (control) indicated the highest feed conversion ratio of 2.02±0.18 followed by diet C with 1.86±0.10 while diet D had the least value of 1.64±0.14.

Table 4: Growth performances, feed utilization and survival of *C. gariepinus* fingerlings

Parameters	Diet A	Diet B	Diet C	Diet D
Total initial weight (g)	$75.00±2.00^{a}$	$68.87±4.30^{b}$	$67.53±3.50^{b}$	$67.53±2.50^{b}$
Mean initial weight (g)	$7.50±1.20^{a}$	$6.89±0.11^{a}$	$6.75±0.75^{a}$	$6.75±1.06^{a}$
Total final weight (g)	$277.23±10.00^{b}$	$301.20±15.00^{a}$	$292.10±8.69^{a}$	$310.63±10.01^{a}$
Mean final weight (g)	$28.67±5.69^{b}$	$30.12±3.90^{a}$	$29.20±4.22^{ab}$	$33.29±3.05^{a}$
Mean weight gain (g)	$21.17±4.34^{c}$	$23.23±4.02^{b}$	$22.45±3.88^{b}$	$26.54±2.09^{a}$
Percentage weight gain (%)	$282.27±11.14^{c}$	$337.16±17.01^{b}$	$332.59±18.67^{b}$	$393.19±10.55^{a}$
Specific growth rate (%)	$0.83±1.90^{b}$	$0.92±2.00^{ab}$	$0.91±2.05^{ab}$	$0.99±1.65^{a}$
Survival rate (%)	96.70^{a}	100^{a}	100^{a}	93.33^{a}
Feed intake (mg/100g of diet)	$41.60±5.00^{a}$	$43.11±6.99^{a}$	$42.78±4.69^{a}$	$43.60±4.55^{a}$
Protein efficiency ratio	$0.71±0.06^{a}$	$0.77±0.05^{a}$	$0.72±0.08^{a}$	$0.80±0.04^{a}$
Feed conversion ratio	$2.02±0.18^{a}$	$1.84±0.10^{c}$	$1.86±0.10^{c}$	$1.64±0.14^{b}$

Values in the same row with different superscripts are significantly different (p<0.05)

Haematological Profiles

Table 5: Haematology profiles of *C. gariepinus* fed experimental diets

Parameters	Diet A	Diet B	Diet C	Diet D
Packed cell volume (%)	$6.67±0.45^{d}$	$9.00±0.39^{c}$	$21.00±0.67^{a}$	$18.00±0.55^{b}$
Haemoglobin (g/l)	$1.90±0.11^{d}$	$3.46±0.39^{c}$	$6.06±0.45^{a}$	$5.66±0.40^{b}$
Neutrophyles (%)	$7.67±0.30^{d}$	$10.67±0.44^{c}$	$15.50±0.57^{b}$	$16.33±0.50^{a}$
Lymphocytes (%)	$91.00±6.09^{a}$	$83.67±3.07^{c}$	$89.50±5.23^{b}$	$82.33±4.12^{d}$
Monocytes (%)	$4.50±0.68^{a}$	$4.00±0.59^{b}$	$4.50±0.60^{a}$	$4.00±0.55^{b}$
White blood cell (10^4/L)	$3.43±0.14^{c}$	$3.47±0.09^{b}$	$3.20±0.20^{d}$	$3.57±0.15^{a}$
Albumin (g/l)	$22.00±1.68^{c}$	$22.57±1.09^{b}$	$18.74±2.02^{d}$	$23.97±1.86^{a}$
Total bilirubin (µmol/l)	$10.71±0.40^{a}$	$9.85±0.65^{b}$	$1.80±0.02^{d}$	$3.23±0.05^{c}$

Values in the same row with different superscripts are significantly different (p<0.05)

Table 5 documented the haematology profiles of *C. gariepinus* fed the experimental diets. There were significant differences in all the parameters examined. The white blood cells and albumin were highest for diet D with 3.57±0.15 and 23.97±1.86g/l and lowest for diet C with 3.20±0.20 and 18.74±2.02g/l respectively. The packed cell volume and haemoglobin were highest for diets C with 21±0.67% and 6.06±0.45g/l followed by diet D with 18±0.55% and 5.66±0.40g/l respectively.

DISCUSSION

The water quality parameters indicated that the physico-chemical parameters of the culture condition for the experimental fish were within the tolerable limits for African catfish (Olurin *et al*., 2006). The major metabolizable energy source in most compounded fish feed is maize, but it has become eminent that other energy sources including sorghum should be explored (Solomon *et al*., 2007), as maize is being highly demanded for by other livestock and man, and found relatively expensive in the market. However, grains used in fish farming contain variable amounts of protein and carbohydrates depending on the cultivated species.

The increase in weight of the experimental fish fed graded levels of fermented (accompanied by unfermented) white sorghum meal indicated that the diets nutritionally supported the growth of *C. gariepinus*. The growth and nutrient utilization results of the present study were similar to the findings of Aderolu *et al*. (2009) when they fed graded levels of sorghum seed meal to *C. gariepinus*. Although the sorghum meal substitution in their individual dietary treatment was not combined (fermented accompanied by unfermented), their highest graded level, 50% fermented sorghum seed meal dietary treatment gave the best growth performance and nutrient utilization. Contrary to the expectation as reported by Hillestad *et al*. (2001) that starch digestibility decreases with inclusion level, the best growth performance and feed utilization was recorded for 100% fermented white sorghum meal dietary treatment (diet D). This indicated that fermented sorghum meal could be used as a source of metabolizable energy replacement for maize in fish and other animals' diets. The reports of Al-Ogaily and Alli (1996), in which sorghum based diets gave the best growth performance in a study to evaluate effect of feeding different grain sources on the growth performance and body composition of *Oreochromis niloticus*, and that of Aderolu *et al*. (2009), on the substitution effect of sorghum meal for maize meal in the diet of Catfish (*Clarias gariepinus*) juvenile, confirmed the suitability of fermented white sorghum in fish diet; as indicated by the present study. However, Chung *et al*. (1998) in their review stated a different trend where significant decrease in weight gain was observed to occur with sorghum at more than 50% replacement level for maize.

Sorghum in terms of nutrient compositions is similar to maize (Aderolu *et al*., 2009) but the present of anti-nutrition factor like tannin in sorghum could affect its utilization by fish (Soghesan *et al*., 2012). Fermentation has been employed to increase protein content, breakdown complex sugars (carbohydrate) into simpler sugars, and reduce the effects of anti-nutrition factors in feed ingredients (Sogbesan *et al*., 2006). Thus, the better results recorded by the inclusion of the experimental sorghum meal even at up to 100% by weight could be attributed to the processing method (fermentation) adopted. Also, the better crude protein contents, with the highest recorded for diet D with 100% fermented sorghum meal could be attributed to this same fact. Krogdahl *et al*. (2005) reported the possibility of positive effects of combining starch sources. Although, the present study combined fermented with unfermented (differently processed) white sorghum; diets B and C gave better mean weight gain with lower feed conversion ratio in comparison to the diet A (control) with 100% unfermented white sorghum.

The haematology profiles of the experimental fish indicated significant differences in all the parameters examined. The haematology parameters including packed cell volume (PCV), haemoglobin (Hb), neutrophyles, and monocytes were highest in diet C, with 75% fermented while the lowest values were recorded for the control diet with 0% fermented sorghum (100% unfermented sorghum inclusion) followed by diet B with 50% fermented combined with 50% unfermented sorghum. The reduction in value of packed cell volume, haemoglobin concentration, and red blood cells count, as reported by Adeyemo (2005), Osuigwe *et al*. (2005) and Tacon (1992) could be as a result of nutritionally deficient diet or presence of toxic substance in the diet of fish. The above explanation could probably be responsible for these haematological values observed in the present study. Also, as reported by Oyawoye and Ogunkunle (1998), reduction in the value of the PCV in the blood usually suggests the presence of toxic factor which has adverse effect on blood formation. The present of anti-nutrition factor like tannins, a toxic component in sorghum (Edwin *et al*., 2001) could be the source of the toxic substance in the diets.

The white blood cell (WBC) is an important defense cell of the body. The amount has implication in immune responses and the ability of the animal to fight infections (Douglass and Janes, 2010). In this study, the highest WBC recorded for diet D could be indicating that inclusion of sorghum (*Sorghum bicolor*) meal at 100% fermented level in catfish (*Clarias gariepinus*) diet have better stimulatory effects on the fish leucocyte populations as compared to the other inclusions levels (diets). However, the specific reason for the lowest value of white blood cells and albumin indicated by diet C with 75% fermented sorghum meal is unclear.

CONCLUSION

In conclusion, the results obtained from this study indicated that fermented sorghum (*Sorghum bicolor*) meal could be used as carbohydrate source in catfish (*Clarias gariepinus*) diet. The haematology profiles of the experimental fish revealed that fermented sorghum in fish diet could possibly increase the values of fish PCV, Hb and WBC.

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