

Full Length Research Paper

Some haematological changes in hybrid catfish (*Heterobranchus longifilis* x *Clarias gariepinus*) fed different dietary levels of raw and boiled jackbean (*Canavalia ensiformis*) seed meal

D.I. Osuigwe^{1*}, A.I. Obiekezie² and G.C. Onuoha³

¹Department of Fisheries, Michael Okpara University of Agriculture, Umudike, Nigeria.

²Institute of Oceanography, University of Calabar, Calabar, Nigeria.

Accepted 12 August, 2005

Changes were observed in some haematological parameters of juvenile hybrid catfish (*Heterobranchus longifilis* x *Clarias gariepinus*) fed raw and 60 min-boiled jackbean seed meal (JBSM) at different dietary levels for 56 days. The haematocrit (packed cell volume, PCV), red blood cell (RBC) count, white blood cell (WBC) count and haemoglobin (Hb) concentration decreased significantly ($P < 0.05$) with increasing dietary JBSM level. Though the mean values of the blood parameters of fish fed diets containing boiled JBSM (PCV = 29.93%; RBC = $1.2 \times 10^6 \text{ mm}^{-3}$; WBC = $15.91 \times 10^3 \text{ mm}^{-3}$; Hb = 8.31 g/100 ml) showed significant improvement when compared with those fed raw JBSM diets (PCV = 28.68%; RBC = $1.13 \times 10^6 \text{ mm}^{-3}$; WBC = $14.63 \times 10^3 \text{ mm}^{-3}$; Hb = 8.31 g/100 ml) they were, however, lower and significantly ($P < 0.05$) different from those fed the control diet (PCV = 35.50%; RBC = $1.43 \times 10^6 \text{ mm}^{-3}$; WBC = $20.42 \times 10^3 \text{ mm}^{-3}$; Hb = 10.62 g/100 ml). However the observed reduction of the blood parameters did not go below the normal range of values recorded for catfish. Further investigation to improve the quality of boiled JBSM is suggested.

Key words: Hybrid catfish, *Heterobranchus longifilis* x *Clarias gariepinus*, jackbean seed meal, haematology.

INTRODUCTION

Fish is often the cheapest source of animal protein and is, therefore, important in the diets of the lowest income group (Allison, 2001). After a century of rapid development driven by a combination of technological innovation and increasing demand for fish, global landings of wild-caught fish are thought to be close or exceeding their estimated sustainable level. One of the proposed solutions to the crisis in capture fisheries which contributes eighty percent of total fish production (FAO, 1998) has been resort to fish farming.

In the late 20th century, a "blue revolution" (Coull, 1993) has been witnessed with aquaculture regarded as the world's fastest growing food production system (Kureshy

et al., 2000). However, high quality feed using fishmeal is required to meet the expanding aquaculture production system. The use of wild-caught fish as fishmeal in fish diets puts further pressure on stocks (Naylor et al., 2000). For fish culture to successfully divert efforts away from capture fisheries and to substantially contribute to dietary requirement of low-income groups, efforts have to be directed at substantially replacing the fishmeal component of fish feed.

To meet the protein demand in developing countries where animal protein intake is also grossly inadequate and relatively expensive, intensive research effort is geared towards finding alternative sources of protein from underutilized grain legume seeds (Adaparusi, 1994; Fagbenro, 1999; Osuigwe, 1999). Jackbean (*Canavalia ensiformis*) seed is one of such legumes with a crude protein and amino acid profile that recommends it for use as a substitute for fishmeal in fish feed (Osuigwe, et al.,

*Corresponding author. E-mail: diosuk@yahoo.co.uk.

Table 1. Chemical composition of jackbean seed meal (g/kg DM).

Parameter	Raw	Boiled (60 min.)
Protein (NX6.25)	282.5	254.0
Ether Extract	29.0	28.0
Crude Fibre	67.3	62.1
Ash	34.4	29.2
NFE	586.8	626.7
P (total)	6.2	--
Ca	0.9	--
Mg	0.8	--
Gross energy (Kcal/100g)	459.32	--

NFE = Nitrogen free extract; P = phosphorus; Mg = magnesium; Ca = calcium.

Table 2. Composition of experimental diets.

Ingredient	Diet No and amount of fishmeal (%) substituted by JBSM												
	1 control	2 10%	3 20%	4 40%	5 60%	6 80%	7 100%	8 10%	9 20%	10 40%	11 60%	12 80%	13 100%
Fishmeal	22.0	19.80	17.60	13.20	8.80	4.40	0.00	19.80	17.60	13.20	8.80	4.40	0.00
JBSM*	0.00	4.36	8.72	17.44	26.17	38.98	43.61	4.93	9.86	19.71	29.57	39.42	49.28
Maize	35.00	32.84	30.68	26.36	22.03	15.21	12.39	32.27	29.54	24.09	17.63	12.18	6.72
Groundnut meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Soyabean meal	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00	15.00
Wheat bran	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Palm oil	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Bone	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix **	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
% Crude Protein	30.27	30.15	29.93	29.50	29.07	28.39	28.11	30.10	29.83	29.28	28.63	28.09	27.54
ME (Kcal/kg) ***	2986	2975	2964	2943	2920	2903	2933	2973	2916	2937	2967	2943	2918

* JBSM: diets 2-7 = raw JBSM; 8-13 60 min boiled JBSM.

** Vitamin and mineral premix.

*** ME Metabolizable Energy calculated.

2002). It is readily available, cheap and is hardly consumed by man. It however has some antinutritional factors some of which can be reduced to a large extent by processing (Udedibie, 1990). This study was undertaken to establish the effects of feeding raw and boiled JBSM at different dietary levels on some haematological parameters of hybrid catfish (*Heterobranchus longifilis x clarias gariepinus*) since fish haematology can be employed to assess fish health (Klinger et al., 1996).

MATERIALS AND METHODS

Two types of JBSM were obtained by milling the raw seed with hammer mill and subjecting a portion of the milled bean to atmospheric boiling in water (100-105°C) for 60 min. Thereafter, the boiled JBSM was spread out and dried in an oven for 24 h at 60°C. Determinations of the proximate composition of the samples were carried out by AOAC (1990) procedure employing the micro-Kjedahl method for crude protein (CP) and soxhlet method for ether extract

(EE). The gross energy of the sample was assayed using adiabatic oxygen bomb calorimetry technique. The milled raw jackbean seed was also subjected to wet digestion with perchloric acid and nitric acid using the Johnson and Ulrich (1959) method. Following digestion, the calcium and magnesium content were determined by atomic absorption spectrophotometer. The phosphorus content was determined on a spectronic 20 spectrophotometry following development of colour with ammonium molybdate. The results were expressed on the basis of dry matter (Table 1). Thirteen practical isonitrogenous (CP 30) and isocaloric (ME 2900 Kca/kg) diets were formulated (Table 2). Diet 1, which served as the control contained no jackbean seed meal but of the same nutritional regime as the other twelve diets. Diets 2, 3, 4, 5, 6, and 7 had the fishmeal component replaced progressively by raw JBSM at 10, 20, 40, 60, 80 and 100%, respectively. In diets, 8, 9, 10, 11, 12, and 13, 60 min boiled JBSM replaced fishmeal at 10, 20, 40, 60, 80 and 100%, respectively. The feedstuffs were then thoroughly mixed and moistened with water. The diets were then molded into small pellets and dried in an oven at 40°C for 24 h and subsequently stored in a freezer until required for use.

The test diets were assigned randomly using CRD to duplicate groups of 20 fish of average total length 18 cm in 20 litre plastic aquaria in static water. The fish were fed once daily for fifty-six days

Table 3. Effects of replacement of fishmeal diets with JBSM on the haematocrit (PVC), red blood cell (RBC) count, white blood cell (WBC) count and haemoglobin (Hb) concentration of hybrid catfish (*Heterobranchus longifilis* x *Clarias gariepinus*).

%Fishmeal substitution	PCV (%)	RBC count ($\times 10^6 \text{mm}^{-3}$)	WBC count ($\times 10^3 \text{mm}^{-3}$)	Hb concentration (g/100 ml)
0	35.43 ^{fg}	1.43 ^{hi}	20.69 ^g	10.63 ^d
10	33.05 ⁱ	1.38 ⁱ	18.99 ^j	9.41 ⁱ
20	33.04 ⁱ	1.33 ⁱ	18.18 ^k	9.21 ^k
40	31.58 ⁱ	1.27 ⁿ	15.91 ^l	8.59 ⁿ
60	29.99 ^k	1.17 ^o	15.40 ^m	8.51 ^o
80	28.91 ^l	1.13 ^q	15.01 ^{mn}	8.51 ^o
100	27.58 ^m	1.07 ^r	14.73 ⁿ	7.98 ^q

Means on the same column with different superscripts are significantly different ($P < 0.05$).

at 3% body weight. Water was replaced every 3 days by siphoning. The water quality parameters were monitored daily and mean values were temperature $28.5 \pm 1^\circ\text{C}$; pH 6.8 ± 0.2 ; DO 6.4 ± 0.5 mg/l.

Fish were tranquilized with 150 mg/l solution of tricaine methane sulphonate (MS222) (Wagner et al., 1997) for blood collection. Blood samples were collected from 4 fish at the commencement of the feeding trial and bi-weekly subsequently from each aquarium from the caudal artery using 2 ml plastic syringes and needletreated with anti-coagulant and put in sample bottles. Haematocrit (PCV) was determined with microhaematocrit centrifuge by the Wintrobe and Westergreen method as described by Blaxhall and Diasley (1973) with commercially available heparinized capillary tubes of 25 mm. Red Blood Cell (RBC) and White Blood Cell (WBC) counts were determined with a haemocytometer with improved Neubauer counting chamber as described by Blaxhall and Diasley (1973). Haemoglobin (Hb) concentration estimates were determined as described by Wedemeyer and Yasutake (1977).

The data obtained were subjected to analysis of variance and differences between means were determined by Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

The mean values for the haematological parameters of juvenile hybrid catfish studied are shown in Table 3. The haematocrit, red blood cell count, white blood cell count and haemoglobin concentration decreased significantly ($P < 0.05$) with increasing dietary JBSM such that fish fed the control diet had the highest values that were significantly ($P < 0.05$) different from the values obtained from fish fed other diets. Variables such as age, sex, dietary state and stress have been known to alter blood values (Barnhart, 1969; McCarthy et al., 1973). Stress factors due to capture, handling and sampling procedures are factors which can cause intra-species haematological variations (Bouck and Ball, 1966). It has also been shown that haemoglobin concentration and haematocrit of fish blood decreases after the stress of capture and transportation (Hattingh and Van Pletzer, 1974). However, bearing in mind that the fish used in this study were siblings kept under laboratory conditions during the period of feeding with test diets and tranquilized with

MS222 before handling, the effect of stress resulting from handling must have been minimal. Consequently, only the nutritional state of the fish (given the optimal water quality conditions provided) may have significantly affected the haematological parameters.

Jackbean seed has been shown to contain antinutritional factors (Nakatsu et al., 1996). These include concanavalin-A (Con-A), a lectin (MERCK, 1989), canavanine (Rosenthal, 1992), saponins (Belmar and Morris, 1994a,b.), trypsin and chymotrypsin inhibitors (Ologhobo et al., 1993), polyphenols (Baber et al., 1988), cyanogenic glycosides and terpenoids (Udedibie et al., 1988). Some of these antinutritional factors have been known to adversely affect some haematological parameters. Con-A causes agglutination of red blood cells in monogastrics (Liener, 1979) while saponins are known to cause erythrocyte haemolysis and reduction of blood (Cheeke, 1971). Probably the progressive reduction in the values of the haematological parameters of hybrid catfish was caused by the increasing presence of antinutritional factors with increasing dietary JBSM level. This is in agreement with the findings of Dick et al. (1976) that nutritional toxicity is associated with anaemia. Herman (1970) equally observed that gossypol an antinutritional factor found in some legumes severely reduced blood haematocrit and haemoglobin concentration in rainbow trout.

In terms of the processing type, it was observed that juvenile hybrid catfish fed the control diet had haematocrit, red blood cell count, white blood cell count and haemoglobin concentration that were higher and significantly ($P < 0.05$) different from the values of those fed boiled JBSM diets which were in turn higher and significantly different from those fed raw JBSM diets (Table 4). The better performance of fish fed boiled JBSM relative to those fed raw JBSM diets is an indication that boiling significantly improved the quality of some legume seed meals. The improvement may be due to among other factors inactivation of some antinutritional factors present in JBSM as earlier reported by the works of Udedibie and Carlini (1998) and transformation of some

Table 4. Effects of replacement of fishmeal in diets with differently processed JBSM on the haematocrit (PCV), red blood cell (RBC) count, white blood cell (WBC) count and haemoglobin (Hb) concentration of hybrid catfish (*Heterobranchus longifilis* x *Clarias gariepinus*).

Processing type	PCV (%)	RBC count ($\times 10^6 \text{ mm}^{-3}$)	WBC count ($\times 10^3 \text{ mm}^{-3}$)	Hb concentration (g/100 ml)
Raw JBSM	28.68 ^g	1.13 ⁱ	14.63 ^h	8.00 ^e
60 min boiled JBSM	29.93 ^f	1.20 ^h	15.91 ^g	8.31 ^g
Control diet	35.50 ^d	1.43 ^e	20.42 ^d	10.62 ^c

Means on the same column with different superscripts are significantly different ($P < 0.05$).

of the component nutrients to non-toxic more readily digestible absorbable forms (Rosenthal, 1977). The inferior performance of fish fed boiled JBSM diets relative to those fed the control diet may also be attributed to the effect of heat treatment which renders JBSM protein deficient and unbalanced by destroying some amino acids. Bressani et al. (1987) reported that heat treatment not only reduced the level of lysine but also destroyed methionine (both essential amino acids) in jackbean, thus reducing the biological value of JBSM protein. Viola et al. (1983) equally reported that heat treatment causes deficiency and imbalance in the protein quality of legumes. The observed reduction in haematological parameters in hybrid catfish fed boiled JBSM diets in this study therefore conforms with the report of Tacon (1992) that nutritionally deficient diets cause decrease in haemoglobin concentration, reduced haematocrit and red blood cell count. Physiologically, haemoglobin is crucial to the survival of fish being directly related to the oxygen binding capacity of blood. However, the reduction observed in this study (between 7.98-10.63 g/100 ml) may not have had a deleterious effect on hybrid catfish, given that the values are within the normal range recorded for African catfish (Erondu et al., 1993; Musa and Omoregie, 1999). So also were the values obtained for haematocrit (between 27.58 – 35.50%), red blood cell count (between $1.07\text{-}1.43 \times 10^6 \text{ mm}^{-3}$) and white blood cell count (between $14.63\text{-}20.69 \times 10^3 \text{ mm}^{-3}$). The haematocrit values also fall within the normal range of 20-35% and rarely greater than 50% for fish (Clark et al., 1979).

This study therefore highlights the fact that though hybrid catfish could be fed diets containing JBSM for 56 days without apparent deleterious effects on the fish. Also, boiling JBSM before use to a large extent improved the quality of the diet as regards the blood profile. Further investigation to study the effect of incorporating supplementary amino acid observed to be deficient in boiled JBSM on the haematological parameters is advocated.

REFERENCES

- Adeparusi EO (1994). Evaluation of the nutritive potential of cooked pigeon pea (*Cajanus cajan*) meal as a protein source for *Clarias gariepinus* fingerlings. J. Agric. Tech. (1): 48-57.
- Allison EH (2001). Big laws, small catches: global ocean governance and the fisheries crisis. J. Int. Dev. 13: 933-950.
- AOAC (1990). Official methods of analysis of the association of official analytical chemists. 15th edn. AOAC, Arlington Va, USA. p. 550.
- Baber VS, Chava JK, Kadan SS (1988). Effects of heat treatment and germination on trypsin inhibitor activity and polyphenols in jackbean (*C. ensiformis*). Plant Fd. Hum. Nutr. 38: 319-324.
- Barnhart RA (1969). Effects of certain variables on haematological characteristics of rainbow trout, *Salmo gairdneri* (Richardson). Trans. Am. Fish Soc. 98: 411-418.
- Belmar R, Morris TR (1994a). Effects of raw and treated jackbeans (*Canavalia ensiformis*) and of canavanine on the short term feed intake of chicks and pigs. J. Agric. Sci. Cambridge 123: 407-414.
- Belmar R, Morris TR (1994b). Effects of the inclusion of treated jackbeans (*Canavalia ensiformis*) and the amino acid canavanine in chicks diets J. Agric. Sci. Cambridge 123: 393-405.
- Blaxhall PC, Daisley KW (1973). Routine haematological methods for use with fish blood. J. fish Biol. 5: 771-781.
- Bouck RG, Ball RC (1966). Influence of capture methods on blood characteristics and mortality in rainbow trout (*Salmo gairdneri*). Trans. Am. Fish Soc. 95: 167-176.
- Bressani RG, Garcia A, Ellias LG (1987). Chemical composition, amino acid content and protein quality of *Canavalia* spp. Seeds. J. Sci. Fd. Agric. 40: 17-23.
- Cheeke PR (1971). Nutritional and physiological implications of saponins: a review. Can. J. Amin. Sci. 51: 621-623.
- Clark S, Whitmore DH (Jr.), McMahon RF (1979). Consideration of blood parameters of largemouth bass (*Micropterus salmoides*). J. Fish Biol. 14: 147-154.
- Coull JR (1993). Will a blue revolution follow the green revolution? The modern upsurge of aquaculture. Area 25: 350-357.
- Dick R, Roberts RJ, Anderson CD (1976). The hepato-renal syndrome of cultured turbot. Its effect on a 2 year trial of farm reared turbot (*Scophthalmus maximus*). Aquacult 8: 241-249.
- Erondu ES, Nnubia S, Nwudukwe FO (1993). Haematological studies on four catfish species raised in fresh water pond in Nigeria J. Appl. Ichth. 9 (3-4): 250-256.
- Fagbenro OA (1999). Use of fullfat winged bean (*Psophocarpus tetragonolobus*) seed meal as a protein feedstuff in fishmeal-free diets for African catfish (*Clarias gariepinus*). Aquacult. Nutr. 5(3): 99-204.
- FAO (1998). Aquaculture production statistics 1987-1996. Fish Cir. 815 FAO: Rome. p. 96.
- Hattingh J, Van Pletzen AJJ (1974). The influence of capture and transportation on some blood parameter of freshwater fish. Comp. Biochem physiol. 49a: 607-609.
- Herman RL (1970). Effects of gossypol poisoning on rainbow trout (*Salmo gairdneri*). J. Fish Biol. 2: 293-304.
- Johnson CM, Ulrich A (1959). Analytical methods for use in plant analysis. Bull. 766 California Agric. Exp. Sta. Bekerley. p. 15.
- Klinger RC, Blazer VS, Echevarria C (1996). Effects of dietary lipid on the haematology of channel catfish, *Ictalurus punctatus*. Aquacult. 147: 335-233.

- Kureshy N, Davis DA, Arnold CR (2000). Partial replacement of fish–and bone-meal in practical diets for juvenile red drum. *North Am. J. Aquacult.* 62: 266-272.
- McCarthy DH, Stevensom JP, Roberts MS (1973). Some blood parameters of the rainbow trout (*Salmo gairdneri Richardson*). I. The kamloops variety. *J. Fish Biol* 5: 1-8.
- MERCK (1989). The Merck Index: an Encyclopedia of chemicals, drugs and biological (Budavari S, O' Neal MJ, Smith A, Heckelman PE, Eds.) Merck & Co, Rayway, NJ, USA pp. 263.
- Musa SO, Omoregie E (1999). Haematological changes in mudfish, *Clarias gariepinus* (Burchell) exposed to malachite green. *J. Aquat. Sci.* 14:37-42.
- Nakatu S, Matsuda M, Sakagami T, Takahashi T, Yamamoto S (1996). Decomposition of canavanine in the process of germination in the seeds of *Canavalia gladiata*. *Seikagaku* 38: 67-71.
- Naylor RL, Goldberg RJ, Primavera JH (2000). Effect of aquaculture on world fish supplies. *Nature* 405: 1017-1024.
- Ologhobo AD, Apata DF, Oyejide A (1993). Utilization of raw jackbean (*Canavalia ensiformis*) and jackbean fractions in diets for broiler chicks. *British Poult. Sci.* 34: 323-337.
- Osuiigwe DI (1999). Growth responses and nutrient utilization of *Oreochromis niloticus* (L) fed on diets containing *Mucuna* seed meal. *J. Agric. Biotech. Environ.* 1(2):47-52.
- Osuiigwe DI, Obiekezie AI, Ogunji JO (2002). Preliminary evaluation of jackbean (*Canavalia ensiformis*) seed meal as a substitute for fishmeal in diets for *Clarias gariepinus* In: Challenges to organic farming Deutscher Tropentag, Oct. 9-11, 2002 in Kassel-Witzenhause. <http://mars.wiz.unikassel.de/tropentagproceeding/2002>.
- Rosenthal GA (1977). The biological effects and mode of action of L-canavanine, a structural analogue of L-arginine. *Quart. Rev. Biol.* 52: 155-178.
- Tacon AGJ (1992). Nutritional fish pathology: morphological signs of nutrient deficiency and toxicity in farmed fish. *FAO Tech. Paper No. 330*. Rome, FAO, p. 75.
- Udedibie ABI (1990). Nutritional evaluation of jackbean (*Canavalia ensiformis*) seed for the Nigeria poultry industry. *Ambio* 19(8): 361-365.
- Udedibie ABI, Carlini CR (1988). Questions and answers to edibility problem of the *Canavalia ensiformis* seeds: A review. *Anim. Feed Sci. Tech.* 74: 95-106.
- Udedibie ABI, Nwaiwu J, Madubuike FN, Nkwocha Co, Imo 10 (1988). Jackbean (*Canavalia ensiformis*): poison turned to good protein Food Owerri Nigeria, federal University of Technology.
- Viola S, Mokafy S, Arieli Y (1983). Effect of soybean processing methods on the growth of carp (*Cyprinus carpio*) *Aquacult.* 32:27-38.
- Wagner EJ, Jensen T, Arndt R, Routedge MD, Brddwisch Q (1997). Effects of rearing density upon cut throat trout haematology, hatchery performance, fin erosion and general health and condition. *The Prog. Fish-Cult.* 59: 173-187.
- Wedemeyer GA, Yasutake WT (1977). Clinical methods for the assessment of the effects of environmental stress on fish health. Technical papers of the US fish and wildlife service No. 89. Washington, DC, US Dept of the Interior, Fish and wildlife service. p. 180.