Nigerian Veterinary Journal (2003), Vol. 24 (2) 19-26

CHANGES IN THE HAEMATOLOGICAL PARAMETERS OF JUVENILE CLARIAS GARIEPINUS (BURCHELL, 1822) FED DIFFERENT DIETARY LEVELS OF RAW AND BOILED JACK BEAN (CANAVALIA ENSIFORMIS (L) SD.C.) SEED MEAL

OSUIGWE,[1] D. I. AND OBIEKEZIE,[2] A.I.

[1]Department of Fisheries, Michael Okpara University of Agriculture, Umudike, Nigeria;
[2]Institute of Oceanography, University of Calabar, Calabar, Nigeria

SUMMARY

The study evaluated the effect of feeding diets which had the fishmeal sequentially replaced by raw and boiled jackbean seedmeal (JBSM) on some haematological parameters of *Clarias gariepinus*. Thirteen isonitrogenous (CP30) and isocaloric (ME 2900 kcal/kg) diets were formulated by substituting fishmeal in the standard diets with raw and 60 min boiled JBSM at 10, 20%, 40%, 60%, 80% and 100%. The test diets were assigned randomly using CRD to duplicate groups of 20 fish of average total length 18cm in 20 litre plastic aquaria in static water. The fish were fed once daily for 56 days at 3% body weight and water replaced every 3 days. Blood samples were collected from fish tranquilized with MS222 at the commencement of feeding and subsequently bi-weekly for determinatioin of haematocrit (PCV), red blood cell (RBC) count, white blood cell (WBC) count, haemoglobin (Hb) concentration and differential leucocyte count. Analysis of the results obtained showed that the PCV, RBC count, WBC count, Hb concentration and lymphocytes decreased significantly ($p<0.05$) with increasing dietary level of JBSM. Though boiling JBSM, significantly ($p<0.05$) improved the haematological values of the fish fed such diets when compared to those fed raw JBSM, the values were significantly lower than those fed the control diet. The depression in the haematological values observed in fish fed JBSM containing diets however remained within the range reported for *Clarias gariepinus*.

KEYWORDS: *Clarias gariepinus*, feed, fishmeal, Jackbean seed meal, haematology

INTRODUCTION

Aquaculture has been experiencing very rapid growth in recent times. With an annual growth rate of 9.6% since 1984 compared with a growth rate of 3.1% for the terrestrial livestock meat production and 1.6% for capture fisheries production over same period (Tacon and Barg, 1998), aquculture is the world's fastest growing food production system (Kureshy *et al.*, 2000). High quality feed using fishmeal is required to meet the expanding aquaculture production system. Currently, aquaculture feeds use more than 20% of the world's supply of fishmeal put at 6 million metric tons per year (Hardy, 1999). To reduce long-term dependence upon fishery resources for feed production, Tacon and Barf (1998) recommended that effort be placed on use of products arising from terrestrial agricultural production sector.

Successful replacements to fishmeal mostly of animal origin (Meske and Pfeffer, 1978), single cell protein (Attach *et al.*, 1979) and algal meal (Sandbank and Hepher, 1978) have been reported. Unfortunately, most of these materials are scarce and expensive as fishmeal (Viola *et al.*, 1982). The most viable option appears to be the exploitation of neglected novel legumes, which abound in the tropics (Adeparusi, 1994). Jackbean (*Canavalia ensiformis*), is one of such

legumes with a crude protein and amino acid profile that recommend it for use as a substitute for fishmeal in fish feed. It is readily available, cheap and is hardly consumed by man. It however, has some anti-nutritional factors some of which can be reduced to a very large extent by processing (Udedibie, 1990).

This work was designed to study the effect of feeding jackbean seed meal (JBSM) in the raw and boiled forms at different dietary levels on some haematological parameters of *Clarias gariepinus* bearing in mind that 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 mins. Thereafter, the boiled JBMS was spread out and dried in an oven for 24hrs at 60°C.

Determination of proximate composition of the samples was carried out by AOAC (1990) procedure employing the micro-Kjeldahl 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 magnessium contents were determined by atomic absorption spectrophotometry. The phosphorous content was determined on a spectrosonic 20 spectrophotometer following development of colour with ammonium molybdate. The results were expressed on the basis of dry matter (Table I).

Thirteen practical isonitrogenous (CP 30) and isocaloric (ME 2900 Kcal/kg) diets were formulated (Table II). Diet 1, which served as the control contained no jackbean seedmeal 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 JBMS at 10%, 20%, 40%, 60%, 80% and 100% respectively. In diets 8, 9, 10, 11, 12 and 13, 60 mins boiled JBSM replaced fishmeal at 10%, 20%, 40%, 60%, 80% and 100% respectively. The feedstuffs were thoroughly mixed and moistened with water. The diets were then molded into small pellets and dried in an oven at 40°C for 24h 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 of 18cm in 20 litre plastic aquaria in static water. The fish were fed once daily for fifty-six days 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 ± 1°C; pH, 6.8±0.2; DO, 6.4±0.5mg/l.

TABLE 1: Chemical composition of jackbean seed meal (g/kg DM)

	Raw	Boiled (60min.)
Protein (NX6.25)	282.5	254.0
Ether Extract	29.0	28.0
Crude Fiber	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	-

Fish were tranquilized with 150mg/l solution of tricane 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 2ml plastic syringes and needle treated with anti-coagulant and put in sample bottles. Haematocrit (PCV) was determined with microhaematorit centrifuge by the Wintrobe and Westergreen method as described by Blaxhall and Diasley (1973) with commercially available heparinized capillary tubes of 25mm. 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) while differential leucocyte counts were determined by counting stained (Leishman's) blood smear with a light microscope.

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

TABLE II: Composition of experimental diets

Ingredient	Diet No/% Fishmeal substituted by JBSM												
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*
	control	*10%*	*20%*	*40%*	*60%*	*80%*	*100%*	*10%*	*20%*	*40%*	*60%*	*80%*	*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	29.38	28.63	28.09	27.54
ME (Kcal/kg)	2986	29.75	2964	2943	2920	2903	2933	2973	2916	2937	2967	2943	2918

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

** Vitamin and mineral premix

*** ME = Metabolizable Energy calculated

RESULTS AND DISCUSSION

The result obtained showed that the values of the PCV, RBC count, WBC count and HB 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 (Table III). Jackbean seed has been shown to contain anti-nutritional 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), polyhenols (Baber *et al.*, 1998), cyanogenic glycosides and terpenoids (Udedibie *et al.*, 1988). Some of these anti-nutritional factors are known to cause some negative effects on 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 increasing presence of anti-nutritional factors in increasing dietary JBSM caused the inferior haematological parameters observed in *C. gariepinus* fed such diets. This is in line with the findings of Dick *et al.* (1976) that nutritional toxicity is associated with anaemia.

TABLE III: Effect of replacement of fishmeal in diets byJBSM on the haematocit (PCV), red blood cell count, white blood cell count and haemoglobin concentration of *C. gariepinus*

% Fishmeal substitution	PCV (%)	RBC count ($X10^6mm^{-3}$)	WBC count ($x10^3mm^{-3}$)	Hb conc. (g100ml)
0	38.30^{d}	1.58^{f}	23.62^{c}	10.74^{c}
10	34.02^{h}	1.47^{g}	21.92^{c}	9.69^{fg}
20	34.66^{gh}	1.46^{g}	21.12^{f}	9.31^{j}
40	32.12^{j}	1.42^{l}	20.00^{h}	8.89^{l}
60	32.41^{ij}	1.35^{k}	19.50^{l}	8.73^{m}
80	31.93^{j}	1.29^{m}	18.32^{k}	8.60^{n}
100	30.26^{l}	1.15^{p}	18.36^{k}	8.43^{p}

Means on the same column with different superscripts are significantly different (p, 0.05)

Herman (1970) equally observed that gossypol, an anti-nutritional factor found in some legumes, severely reduced blood PCV and Hb concentration in rainbow trout. When viewed from the perspective of diet processing type, it was observed that *C. gariepinus* fed the control diet had PCV, RBC count, WBC count and HB concentration that were higher and significantly ($p<0.05$) different from the values of those fed boiled JBSM diets which were in turn higher than those fed raw JBSM diets (Table IV).

TABLE IV: Effect of differently processed JBSM diets on the haematocrit (PCV), red blood cell (RBC) count, white blood cell (WBC) count and haemoglobin (Hb) concentration of *C. gariepinus*

Processing type	PCV (%)	RBC count ($X10^6mm^{-3}$)	WBC count ($x10^3mm^{-3}$)	Hb conc. (g100ml)
Raw JBSM	30.27^{f}	1.27^{g}	18.48^{f}	8.24^{h}
60min. boiled JBSM	31.59^{he}	1.32^{f}	19.07^{e}	8.61^{f}
Control diet	38.30^{b}	1.58^{c}	23.62^{b}	10.74^{b}

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

The better performance of *C. gariepinus* fed boiled 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 the anti-nutritional factors present in JBSM as earlier reported by the works of Udedibie and Carlini (1998) and transformation of some of the component nutrients to non-toxic, more readily digestible and absorbable forms (Rosenthal, 1977). The inferior performance of fish fed boiled JBSM diets when compared to those fed the control diets may also be attributed to the effect of heat treatment which renders JBSM protein deficient/unbalanced. Bressani *et al.* (1997) reported that heat treatment not only reduced the level of lysine but also destroyed methionine (both of which are essential amino acids) in Jackbean, thus degrading the biological value of JBSM protein. The poor performance of *C. gariepinus* fed boiled JBSM in this work therefore conforms with the report of Tacon (1992) that nutritionally deficient diets cause decrease in haemoglobin concentration, reduced haematrocrit and red blood cell volume. Viola *et al.* (1983) equally noted that heat treatment causes deficiency/imbalance in legumes.

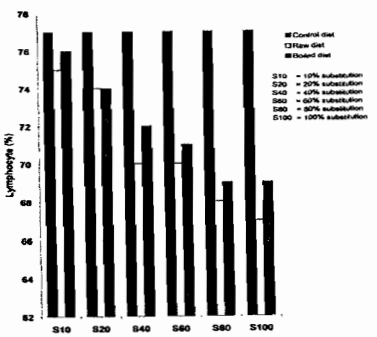

Fig. 1: Effect of substituting fishmeal with raw and processed JBSM on lymphocyte counts of *C. gariepinus*

The decreasing number of lymphocytes with increasing dietary level of JBSM (Figure 1.) may have been caused by the increasing level of anti-nutritional factors in such diets, which causes profound stress on the biological system in the fish. This finding agrees with the report of Baker *et al.* (1994) that stress caused decrease in lymphocyte counts and condition factor of fish. Pickering and Pottinger (1987) also observed a reduction in the circulating lymphocytes and thrombocytes of fish as a result of increased density. However, it is important to note that in spite of the reduction in the levels of haemetological values observed in *C. gariepinus*, they were still within the normal ranges reported for *C. gariepinus* (Erondu *et al.*, 1993; Musa and Omoregie, 1999). This may be responsible for the low mortality (not more than 1.3% for any diet type) observed in the work.

This study thus highlights the fact that though *C. gariepinus* could be fed diets containing JBSM for 56 days without apparent deleterious effects on the haematological parameters (health conditions), boiling JBSM before use in *C. gariepinus* diets to a large extent improves the quality of the diet as regards the blood profile. Further investigation to ascertain the effects of using boiled JBSM diets for longer period on the haematological parameters of *C. gariepinus* is advocated.

REFERENCES

ADEPARUSI E.O. (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.

A.O.A.C. (1990). Official Methods of Analysis of the Association of Official Analytical Chemists. 15th edn. A.O.A.C., Arlington Va, U.S.A.

ATTACK, T.H., JAUNCY, K. and MATTY, A.J. (1979). The use of fishmeal and soyabean meal as protein source in the diet of grass carp fry. *Aquaculture* **18**: 107-114.

BABER, V.S., CHAVA, J.K. and KADAN, S.S. (1988). Effects of heat treatment and germination on trypsin inhibitor activity and polyphenols in jackbean *(C. ensiformis)*. *Plant Fd. Hum. Nutr.* **38**:319-324.

BAKER, D.R., KHAN, R.A., and HOOPER R. (1994). Bioindicators of stress in Winter flounder, *Pleuronectes americanus,* captured adjacent to a pulp and paper mill in St George's Bay, Newfoundland. *Can. J. Fish. and Aquat. Sci.* **51**: 2203-2209.

BLAXHALL, P.C., and DAISLEY, K.W. (1973). Routine haematological methods for use with fish blood. *J. Fish Biol.*, **5**: 771-781.

BELMER, R. and MORRIS T.R. (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. and MORRIS, T.R. (1994b). effects of the inclusion of treated jackbeans (*Canavalia ensiformis*) and the amono acid canavanine in chicks diets. *J. Agric. Sci.* Cambridge, **123**: 393-405.

BRESSANI, R.G., GARCIA A. and ELLIAS, L.G. (1987). Chemical composition, amino acid content and protein quality of *Canavalia* spp seeds *J. Sci. Fd. Agric.*, **40:** 17-23.

CHEEKE, P.R. (1971). Nutritional and physiological implications of saponins: a review. *Can. J. Anim. Sci.* **51**: 621-623.

DICK, G., ROBERT, R.J. and ANDERSON, C.D. (1976). The hepato-renal syndrome of cultured turbot. Its effect on a 2 year trial of farm-reared turbot (*Scophthallmus maximus*) *Aquaculture*, **8**: 241-249.

ERONDU, E.S., NUBIA, S. and NWADUKWE, F.O. (1993). Haematological studies on four catfish species raised in fresh water pond in Nigeria. *J. Appl. Ichthyol.* **9**: 250-256.

HARDY, R.W. (1999). Aquacultures rapid growth: requirement for alternate protein sources. *Feed Management*, **50**:25-28.

HERMAN, R.L. (1970). Effects of gossypol poisoning on rainbow trout (*Salmogairdneri). J. Fish Biol.*, **2**: 293-304.

JOHNSON, C.M. and ULRICH, A. (1959). Analytical methods for use in plant analysis.bull. 766. California Agr. Exp. Sta., Bekerley.