Haematological Effect of Using Jackbean (Canavalia ensiformis (L.) D.C) Seed Meal as An Alternative Protein Source for Clarias gariepinus (Burchell, 1822)

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ABSTRACT

Jackbean seed meal has crude protein and amino acid profile that recommend it for use as a substitute for fish meal in fish feed. It however, has some anti-nutritional factors some of which can be reduced to a large extend by processing (Udedibie, 1990). The study was carried out to evaluate the effect of Jackbean Seed Meal (JBSM) on the haematology of *Clarias gariepinus* when used to replace fishmeal in practical diets of the species. Thirteen isonitrogenous (CP 30) and isocaloric (ME 2900kcal/kg) diets were formulated by substituting fish meal in a control diet with raw and 60 minute-boiled JBSM at 10%, 20%, 40%, 60%, 80% and 100%. The test diets were assigned randomly using completely randomised design (CRD) to duplicate groups of 20 fish of average total length 18cm in 20 litre plastic aquaria. The fish were fed once daily for 8 weeks at 3% body weight. The static water used in rearing was replaced every 3 days. Blood samples were collected from fish tranquilized with MS222 at the commencement and subsequently bi-weekly for determination of some haematological parameters. Results obtained showed that the haematocrit (PCV), red blood cell count, white blood cell count and haemoglobin concentration decreased significantly ($P<0.05$) with increasing dietary levels of JBSM. Though boiling JBSM significantly ($P<0.05$) improved the haematological values of fish fed such diets, the values were still significantly lower than those fed the control diet. The haematological values of fish fed diets with JBSM however, remained within the normal range for C. gariepinus

Keywords: *Canavalia ensiformis* (L.) DC.), *Clarias gariepinus* (Burchell, 1822),Haematology.

INTRODUCTION

In recent times aquaculture has been experiencing rapid growth with a global average rate put at one percent per year between 1984 and 1998 (Misra *et al.,* 2003). This trend is attributed largely to widespread availability and utilization of aquafeeds. Production of aquafeeds with an annual growth rate in excess of 30% per year is one of the fastest expanding agricultural industries in the world (Tacon, 1996).

High quality feed using fish meal is required to meet the expanding aquaculture production system. The fish meal component of aquafeed however contribute substantially to its high cost. To enhance a more economically sustainable aquaculture in the current millennium, many feed ingredient alternatives to fish meal at varying levels are now being sought. Research interest has been directed towards the evaluation and use of unconventional protein sources. Jackbean (*Canavalia ensiformis*) a cheap fast growing legume widely available in the tropics is one of such legumes with a crude protein and amino acid profile that recommend it for use as a substitute for fish meal in fish feed. It however, has some anti-nutritional factors some of which can be reduced substantially by processing (Udedibie, 1990). This study was designed to evaluate 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- 105ºC) or 60 min. Thereafter, the boiled JBSM was spread out and dried in an oven for 24hr at 60ºC.

Determination of the proximate composition of the samples were 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 calorimetric 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 spectrophotometry. The phosphorus content was determined on a spectronic 20 spectrophotometer following development of colour with ammonium molydate. 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 seed meal but of the same nutritional regime as the other twelve diets. Diets 2, 3, 4, 5, 6, and 7 contained 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, 60min. boiled JBSM replaced fishmeal at 10%, 20%, 40%, 60%,80%, and 100% respectively. The feedstuffs were than thoroughly mixed and moistened with water. The diets were than moulded into small pellets and dried in an oven at 40^0c for 24 hours 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 18cm in 20 litre plastic aquaria in static water. The fish were fed once daily for fifty six 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 ± 1^0C; pH 6.8 ± 0.2; DO 6.4 ± 0.5 mgl.

Fish were tranquilized with 150mgl 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 microhaematocrit centrifuge by the Wintrobe and Wester-green method and described by Blaxchall and Daisley (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 Daisley (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

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), polyphenol (Baber *et al.,* 1988), 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. 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). the better performance of *C gariepinus* fed boiled JBSM diets 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

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 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 haematocrit and red blood cell volume. Viola *et al.,* (1983) equally noted that heat treatment causes deficiency imbalance in legumes.

However, it is of importance to note that in spite of the reduction in the levels of haematological 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 observed in the work.

REFERENCES

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.

Baber V.S., Chava J.K. and Kadam S.S (1988). Effects of heat treatments and germination on ttypsin inhibitor activity and polyphenols in jackbean (C. *ensiformis)*. Plant Fd. Hum. Nutr. 38 : 319-324

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

Belmar 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. Journal of Agric. Sci. Cambridge, 123: 407-414.

Belmar R. and Morris T.R. (1994b). Effects of the inclusion of treated jackbeans (*Canavalia ensiformis)* and the amino acid canavanine in chicks diets. Journal of Agric. Sci Cambridge, 123: 393-405

Bressani R. G., Garcia A. and Elias L. G. (1987). Chemical composition, amino acid content and protein quality of C*anavalia* spp seeds J. Sci. Fd Agric. 40 : 17-23.

Cheeke P.R. (1971). Nutritional and physiological implications of saponins: a review. Canadian Journal of Animal Science, 51 : 621-623.

Dick G., Roberts 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 Journal of A:lied Ichthyology, 9 (3-4) : 250-256.

Herman R.L (1970). Effects of gossypol poisoning on rainbow trout (*Salmo gairdneri).* J. Fish Biol. 2 : 293- 304.

Johnson C. M and Ulrich A. (1959). Analytical methods for use in plant analysis. Bull. 766. Califiornia Agr. Exp. Sta., Bekerley.

Klinger R.C., Blazer V.S and Echevarria C. (1996). Effects of dietary lipid on the haematology of channel catfish, *Ictalurus punctatus*. Aquaculture, 147: 225-233.

Liener I.E. (1979). Phytohemagglutinins. In: Herbivores: Their Interaction with Secondary Plant Metabolites (Rosenthal G.A. Ed.). Academic Press, New York, : 567-597.

MERCK (1989). The Merck Index: an Encyclopedia of Chemicals, Drugs and Biological (Budavari, S. O' Neal, M. J., Smith, A., Heckelman, P.E., Eds.), Merck & Co, Rayway, NJ, USA, P 263.

Misra, C. K. Das, B. K. and Mohato, K. N. (2003). Feed management in aquaculture. Fish Farmer International File 17(1): 16-17.

Musa S.O and Omoregie E. (1999). Haematological changes in the mudfish, C*larias gariepinus* (Burchell) exposed to malachite green. Journal of Aquatic Sciences, 14 :37-42.

Nakatsu S., Matsuda M., Sakagami T., Takahashi T. and Yamamoto S. (1996) Decomposition of canavanine in process of germination in the seeds of *Canavalia gladaita.* Seikagaku, 38: 67-71.

Ologhobo A.D., Apata D.F. and Oyejide A.(1993). Utilization of raw Jackbean (*Canavalia ensiformis)* and jackbean fractions in diets for broiler chicks. British Poultry Science, 34 : 323-337.

Rosenthal G.A. (1977). The biological effects and mode of action of L-Canavanine, a structural analogue of L-arginine. Quarterly Review of Biology, 52 : 155-178.

Rosenthal G.A (1992). Non-protein amino acids in the life processes of higher plants. In: B.K.Singh Ed). Biosynthesis and molecular regulation of amino acids in plants. Rockville, MD: American Soc. of Plant Physiologist, : 249-261.

Sandbank E. and Hepher B. (1978). The utilization of microalgae as a feed for fish. Arch. Hydrobiol. Beiheft, 11 : 108-120

Tacon A.G.J. (1992). Nutritional fish pathology: Morphological signs of nutrient deficiency and toxicity in farmed fish FAO Fish Technical Paper No330. Rome, FAO, 75p.

Tacon, A. G. J.,(1996). Global trends in aquaculture and aquafeed production p 90-108. In: International milling directory 1996. Uxbridge, Turret-RAI.

Udedibie A.B.I. (1990). Nutritional evaluation of jackbean (*Canavalia ensiformis)* seed for the Nigeria Poultry Industry. Ambio, 19(8): 361-365.

Udedibie A.B.I and Carlini C.R. (1998). Questions and answers to edibility problem of the *Canavalia ensiformis* seeds: A review. Animal Feed Science and Technology 74 : 95-106

Udedibie A.B.I., Nwaiwu J., Madubuike F. N., Nkwocha C. O. and Imo I.O.(1988). Jackbean (*Canavalia ensiformis):* poison turned to good protein food. Owerri, Nigeria, Federal university of Technology.

Viola S., Mokady S. and Arieli Y.(1983). Effect of soybean processing methods on the growth of carp (*Cyprinus carpio).* Aquaculture, 32: 27-38.

Wagner E. J., Je:sen T. Arndt R., Routledge M. D. and Brddwisch Q. (1997). Effects of rearing density upon cutthroat trout hematology, hatchery performance, fin

erosion and general health and condition. The Progressive Fish-Culturist, 59 : 173-187.

Wedemeyer G. A. and Yasutake W. T. (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 18p.

Table I: 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 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 II: Composition of experimental diets

	Diet No	% Fishmeal substituted by JBSM											
Ingredient	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
Soybean 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	2.50	2.50	2.50
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	29.37	29.67	29.43	29.18

JBSM: diets 2-7 = raw JBSM; 8-13 60min. boiled JBSM, ** Vitamin and mineral premix, * ME Metabolizable Energy calculated**

Table III Effect of replacement of fishmeal in diets by JBSM on the haematocrit (PCV), red blood cell count, white blood cell count and haemoglobin concentration of *C. gariepinus*

% Fishmeal substitution	PCV (%)	RBC count ($X10^6 mm^{-3}$)	WBC count ($X10^3$ mm^{-3})	Hb conc. (g100ml)
0	38.30^{d}	1.58^{f}	23.62^{c}	10.74^{e}
10	34.02^{h}	1.47^{g}	21.92^{e}	$9.69f^{g}$
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^{i}	8.73^{m}
80	31.93^{j}	1.29^{m}	18.32^{k}	8.60^{n}
100	30.26^{I}	1.15^{p}	18.36^{k}	8.43^{p}

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

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 (X 10^6 mm-3)	WBC count (X 10^3 mm^{-3})	Hb conc. %
Raw JBSM	30.27^f	1.27^g	18.48^f	8.24^h
60min boiled JBSM	31.59^e	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 ($p<0.05$)