

Full Length Research Paper

Effects of jackbean seed meal on the intestinal mucosa of juvenile *Heterobranchus longifilis*

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Juvenile *Heterobranchus longifilis* were fed diets that were isonitrogenous (CP 30%) and isocaloric (ME 2900 Kcal/kg) comprising the control diet without jackbean seed meal (JBSM) and those containing raw and 60 min boiled JBSM at different inclusion levels. At the conclusion of the 56 days feeding study, histological examination of the intestinal mucosa of *H. longifilis* indicated that those fed the control diet remained normal. However, progressive damage to the epithelial mucosa of *H. longifilis* intestines was observed with increasing dietary level of JBSM. While fish fed diets with 10% fishmeal substituted by raw JBSM showed minor degeneration of the intestinal mucosa, those fed with 100% fishmeal substituted by raw JBSM showed severe damage. Boiling JBSM however moderated the adverse effect of high dietary JBSM since fish fed diet with 80% fishmeal substituted by boiled JBSM showed only early signs of mucosal degeneration. The findings suggest that boiled JBSM could be used to substantially replace fishmeal in fish diets with no negative effect on fish intestinal mucosa. This development impacts positively on fish production by reducing the cost of fish feed given the comparatively lower cost of JBSM with respect to fishmeal.

Key words: Jackbean, *Canavalia ensiformis*, diets, intestinal mucosa, *Heterobranchus longifilis*.

INTRODUCTION

Fishmeal is the main source of protein used in formulated fish diets. However, production of high quality fishmeal has not met the needs for fish feed production and a future shortage is possible. To forestall this possible situation, investigation of alternative protein sources have been undertaken. Seed of Leguminosae are possible alternatives because of their nutrient composition, availability and low prices (Tacon and Jackson, 1985). One of such seeds is jackbean (*Canavalia ensiformis*) which has been used as animal feed (Udedibie et al., 1996).

Anti-nutritional factors present in legume seeds like protease inhibitors, lectins, saponins and tannins limit

their use as animal feed (Liener, 1979). Raw jackbean seedmeal (JBSM) diets have been reported to cause reduced growth performance in fish while heat treatment of such meals significantly improved growth performance (Osuigwe et al., 2002). However, feeding high dietary levels of properly treated JBSM caused inferior growth in *Heterobranchus longifilis* compared to diets containing fishmeal-based diets (Osuigwe and Obiekezie, 2002). These effects were attributed to the amino-acid imbalance (Bressani et al., 1987) and residual anti-nutritional factors present in heat treated JBSM (D'Mello et al., 1985).

Growth responses alone are not enough to assess the suitability of JBSM as a substitute for fishmeal in fish diets. We earlier reported an inverse relationship between dietary JBSM and fish packed cell volume, red blood cells count and haemoglobin concentration (Osuigwe et al., 2003). The present report describes the

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Table 2. Composition of experimental diets.

Component	Diet No.												
	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	22.00	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	38.89	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	15.20	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	20.07	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 calculated.

Table 1. Chemical composition of JBSM (g/kg DM).

Parameter	Raw	Boiled (60 min)
Protein N x 6.25	282.50	254.00
Ether extract	29.00	28.00
Crude fibre	67.30	62.10
Ash	34.40	29.20
Nitrogen free extract	586.80	626.70
Total Phosphorus	6.20	-
Calcium	0.90	-
Magnesium	0.80	-
Gross energy (Kcal/100 g)	459.32	-

findings on the effect of feeding different levels of JBSM diets for 56 days on the intestinal mucosa of *H. longifilis*.

MATERIALS AND METHODS

Raw and 60 min boiled JBSM (Table 1) processed as described in Osuigwe and Obiekezie (2002) were used to formulate 12 isonitrogenous (30% CP) and isocaloric (ME 2900 Kcal/kg) diets. The control diet contained no JBSM but of the same nutritional regime as the other 12 diets and designated diet 1 (Table 2). Thus 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 while diets 8, 9, 10, 11, 12 and 13 had the fishmeal component replaced by 60 min boiled JBSM at 10, 20, 40, 60, 80 and 100%, respectively. The feedstuffs were thoroughly mixed into mash, hand molded into pellets and dried at 40°C in an oven (Gallenkamp) for 24 h and subsequently stored at -15°C until required for use.

The test diets were assigned randomly using completely

randomized design (CRD) to duplicate groups of 20 fish of average total length 18 cm in 20 litre rectangular plastic aquaria in static water. The fish were fed by hand once daily for 56 days at 3% body weight. Water was replaced completely every 3 days by siphoning. The water quality parameters were monitored daily and mean values were 28.5±1 °C, pH 6.8±0.2 and DO 6.4± 0.5 mg/l

The intestines of 4 fish sacrificed at the commencement and subsequently 2 fish from each aquarium bi-weekly were preserved in Bouins solution for some weeks, processed and infiltrated with paraffin wax. Sections (5 microns thick) were then cut from the processed intestines, stained and examined with a light microscope.

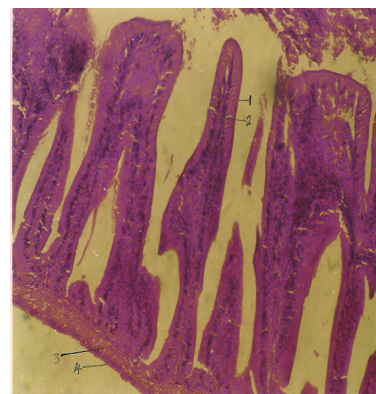


Figure 1. Normal intestinal mucosa at the commencement of the study. 1 Mucosal epithelium, 2 Lamina propria; 3 Muscularis; 4 Serous membrane.

RESULTS AND DISCUSSION

Figure 1 shows the fish intestinal mucosa at the commencement of the study with normal mucosal epithelium, lamina propria, muscularis and serous membrane. After

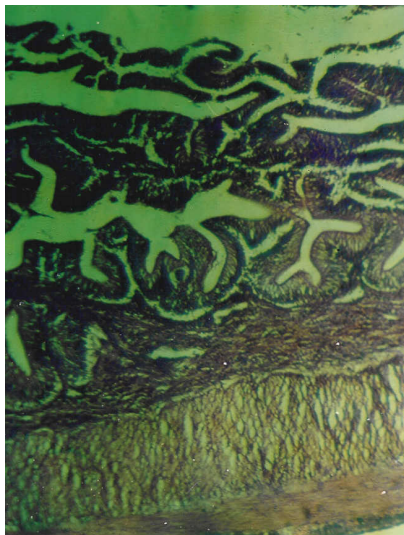


Figure 2. After eight weeks of feeding with control diet, intestinal mucosa remained normal.

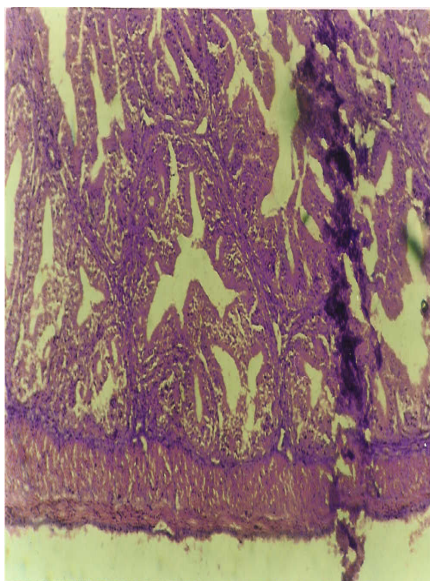


Figure 3. Minor degeneration of intestinal mucosa of fish fed diet 2 (raw JBSM replacing 10% fishmeal).

eight weeks of feeding with the control diet, the intestinal mucosa remained normal (Figure 2). On the other hand, progressive damage of the intestine of fish fed raw JBSM containing diets was observed. The degree of damage was influenced by the dietary level of JBSM. Thus fish fed diet 2 (10% fishmeal substituted by raw JBSM) showed less severe degeneration of the intestinal mucosa (Figure 3) than those fed diets 6 (80% fishmeal substituted by raw JBSM) and 7 (100% fishmeal substituted by raw JBSM) as shown in Figures 4 and 5, respectively.

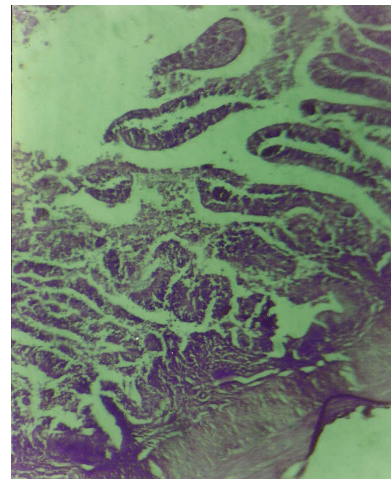


Figure 4. Further degeneration of intestinal mucosa of fish fed diet 6 (raw JBSM replacing 80% fishmeal).

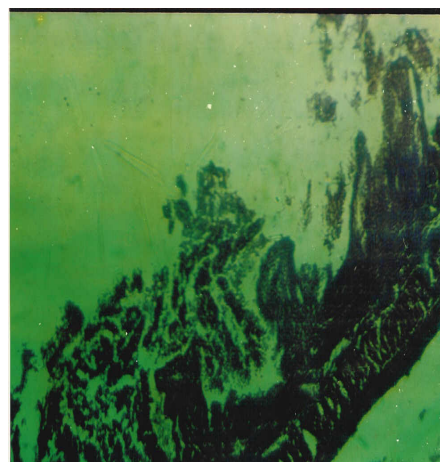


Figure 5. Pronounced degeneration of intestinal mucosa of fish fed diet 7 (raw JBSM replacing 100% fishmeal).

Jackbean seed has been reported to possess several antinutritional factors (Udedibie, 1990) including protease inhibitors, lectins and saponins. Extensive structural and functional disruption of the intestinal microvilli of animals fed JBSM has been reported by Grant (1991). Similarly, D'Mello (1995) reported induction of intestinal abnormalities in animals fed concanavalin A, a lectin present in jackbean seed. Bureau et al. (1998) observed extensive damage of intestinal mucosa of both Chinook salmon and Rainbow trout fed Quillaja bark saponin similar to the condition of fish fed raw soybean meal diet. The damage observed in the intestine of *H. longifilis* fed varying dietary levels of JBSM in this study may be attributed to both lectins and saponins found in jackbean seed meal, since no study yet has linked protease inhibitors to such effects. The damage caused the intestinal mucosa of *H. longifilis* exacerbated with increasing dietary JBSM level. The negative effects of

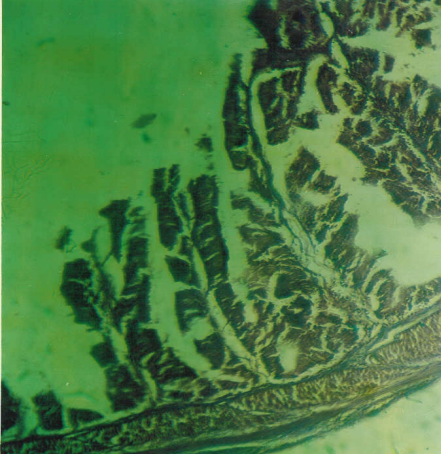


Figure 6. Degeneration of intestinal mucosa of fish fed diet 13 (boiled JBSM replacing 100% fishmeal).

saponins could be caused by the well known effects of these surface-active components on biological membranes (Francis et al., 2001). Lectins and saponins have also been reported to bind the cells of small intestine of chicks altering cellular metabolism thus precipitating hypersecretions of mucus and impairing absorption of nutrients across the intestinal wall (Johnson et al., 1986; Pusztai, 1989). Similar interactions may have been responsible for the poor nutrient utilization in catfish fed JBSM (Osuigwe et al., 2002).

However, it was observed that subjecting JBSM to moist heating (boiling for 60min) substantially reduced its negative effect on *H. longifilis* intestinal mucosa at corresponding dietary levels. Thus, fish fed diet 12 and 13 (80% and 100% fishmeal substituted by boiled JBSM, respectively) only exhibited minimal intestinal damage (Figure 6) after eight weeks. This may be attributed to either considerable reduction or total elimination of the antinutritional factors in JBSM responsible for the damage as a result of moist heating. This assertion is in agreement with the findings of other workers like Norton (1991) who recommended moist heating as a means of reducing protease inhibitors and Areohaore et al. (1998) who reported reduced lectin content in *Jatropha* seed meal after heat treatment. Siddhuraju and Becker (2001) reported 50% reduction in saponin level, 92.5% reduction in trypsin inhibitor, 100% reduction in chymotrypsin inhibitor and 100% reduction in lectin activity after autoclaving.

Martinez-Palacios et al. (1988) reported increased mortality of Tilapia when fed increased dietary JBSM. No such deaths were however, observed in this study with *H. longifilis* even at 100% replacement of fishmeal with JBSM. Udedibie and Carlini (1998) reported that even minute amount of concanavalin A that remain in processed jackbean can constitute a problem to animals in *ad libitum* feeding system. Whether longer period of

feeding with JBSM as processed in this study lead to fatalities remain a further line of investigation.

In conclusion, feeding of high dietary levels of raw JBSM has adverse effects on the intestinal mucosa of juvenile *H. longifilis*. However subjecting JBSM to boiling improves the quality and considerably reduces the adverse effects to the extent that boiled JBSM could substantially replace fishmeal in fish diets. Given the lower cost of JBSM relative to fishmeal, this development will reduce cost of fish feed and impact positively on fish production.

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