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Effects of Different Processing Methods of Pigeon Pea (*Cajanus cajan*) on the Haematology of African Catfish (*Clarias gariepinus*) Larvae.

Ogunji, Johnny O.[1, 3]; Uwadiegwu, Nicholas[1]; Osuigwe Donald I.[2]; Wirth, Manfred[3]

[1]Ebonyi State University Abakaliki, Nigeria. Department of Animal Production and Fisheries Management.
[2]Michael Okpara University of Agriculture Umudike, Nigeria. Department of Fisheries
[3]Institute of Freshwater Ecology and Inland Fisheries. Müggleseedamm 310 D- 12587 Berlin, Germany.

Contact: Ogunjijo@yahoo.com

Abstract

The need to substitute fishmeal in animal feed has necessitated the use of plant derived feedstuffs. However, problems of anti nutritional factors in tropical legumes have limited their widespread usage and direct incorporation into animal feeds. Different processing methods have been deviced to remove or reduce the concentration of these factors. In this study *Cajanus cajan* was subjected to four different processing methods. These included milling raw, toasting, boiling and soaking. Effect of the different processing methods on the haematology of *Clarias gariepinus* larvae was evaluated. This was aimed at determining the best processing method(s) for optimum utilisation of pigeon pea meal for fish production. Twenty-one test diets were formulated to contain about 39% crude protein. Results obtained showed that Haematocrit (PCV), Haemoglobin concentration, RBC and WBC counts decreased significantly ($P<0.05$) with increasing dietary levels of raw pigeon pea. Fish groups fed diets from other processing methods showed lower values than the control when compared with the initial status. Soaking for 16 hours enhanced the best fish weight gain and haematological values and seems to be the best processing method for *Cajanus cajan*.

Introduction

The need to substitute fishmeal in animal feed has necessitated the use of plant derived feedstuffs. Legume seeds have been highly favourable because of their rich protein composition, energy and, mineral content and widespread distribution in the tropics. However, only few of these plant

proteins have been utilized and investigated (Tacon and Jackson 1985; Webster et al., 1992;, Ogunji and Wirth 2001). The presence of anti nutritional factors in these legumes have limited their widespread usage and direct incorporation into animal feeds.

Pigeon pea (*Cajanus cajan*) seed is one of the tropical legume seeds that has been scarcely used in fish feed production in spite of its crude protein and energy profile. Like other legume seeds, its nutritive value is masked by the occurrence of anti nutritional factors, example trypsin inhibitors haemaglutinin and saponin (Grimaud, 1988; Francis et al., 2001).

Some anti-nutritional factors are known to have negative effects on haematological parameters. Concanavalin A (Con-A) causes agglutination of red blood cells (RBC) in monogastrics (Liener, 1989), while saponins are known to cause erythrocyte haemolysis and reduction of blood (Checke 1971). Anaemia was also reported to be associated with nutritional toxicity (Dick et al. 1976). Gossypol severely reduces blood packed cell volume (PCV) and haemoglobin (Hb) concentration in rainbow trout (Herman 1970).

Haematology has been developed and well utilised in assessing the health of man and livestock. Svobodova et al., (1991) opined that ichthyo-haematology would be useful in the assessment of suitability of feeds and feed mixture, evaluation of fish conditions, determination of toxic effect of substances as well as diagnosis of disease.

The removal of anti-nutritional factors in pigeon pea using different processing methods is important to make it safe for use in fish feed production. Different processing methods have been devised to remove or reduce the concentration of these factors. In this study *C. cajan* was subjected to four different processing methods. These are raw, toasting, boiling and soaking. The effect of these processing methods on the haematology of *Clarias gariepinus* larvae was evaluated.

Methods

Differently processed pigeon pea *(Cajanus cajan)* seed meal (PPSM) were obtained by (a) toasting the seed by frying at a temperature of 120^{O}C, b) subjecting the seeds to atmospheric boiling for 1hr and thereafter sun dried, c) soaking the seeds in water for 16 hours and thereafter sun dried and d) raw seeds. These were then milled using a hammer mill. The differently processed PPSM were used to formulate 20 different isonitrogenous (Crude protein

39%) diets at different dietary levels such that diets A_1 to A_5, B_1 to B_5, C_1 to C_5 and D_1 to D_5 had raw -, toasted -, boiled and soaked – PPSM respectively at different dietary levels. Diet A_0 had no PPSM but of the same nutritional regime as the other and served as control. Table 1 shows a typical dietary inclusion rate used for the formulation of the experimental diets.

Table 1: Typical dietary inclusion rate used for the formulation of the experimental diets

	Experimental Diets A – D					
	A0	**1**	**2**	**3**	**4**	**5**
Fish Meal	55	41	39	37	35	33
Cajanus cajan		45	50	55	60	65
Maize	30	5	2	3	2	-
Palm Kernel Cake	12	6	6	2	-	-
Vit./Min. Premix **	1	1	1	1	1	1
Oil	2	2	2	2	2	1
Total	100	100	100	100	100	100

**Vitamin and trace minerals supplied per Kg of final feed:
Vitamin A 24000 IU, Vitamin D3 2000 IU, Vitamin E 200 mg, Vitamin K3 8 mg, VitaminB1 20 mg, Vitamin B2 30 mg, Vitamin B6 12 mg, Panthonic acid 50 mg, Biotin 0.8mg, Niacin 150 mg, Vitamin B12 0.05mg, Folic acid 4mg, Vitamin C 500mg, Choline chloride 600mg, Inositol 200mg, Batanine 200mg, Cobalt 2mg, Iron 40mg, Iodine 5mg, Manganese 30mg, Copper 4mg, Zinc 40mg, Selenium 0.20mg, Lysine 100mg, Methionine 100mg, Antioxidant 100mg

After 5 days of acclimation and feeding with the control diet each test diet was randomly assigned using CRD (Completely Randomized design) to triplicate lots of 10 liter capacity aquaria each containing seven *C. gariepinus* larvae of average weight 0.46g. The fish were fed 5% of their body weight per day in two portions for 56 days in static water. The aquaria were cleaned and water completely replaced by siphoning every other day to avoid fouling. Water temperature, dissolved oxygen (DO) and PH were monitored and remained relatively stable. Temperature was maintained at 26 ± 0.2^{o}C, dissolved oxygen between 6.5 and 8.0 mg/L and pH between 6 and 8. No critical values were detected in any of the tanks.

Fish were tranquilized with 150mg/L of tricane methane sulponate (ms222) (Wagner et al., 1997) for blood collection. Blood samples were collected from ten fish from the pool at the commencement of the feeding trial. At the end of the experiment 10 fish per feeding group and two fish from control were sampled. Blood was taken from the caudal artery using 2ml plastic syringe and needle treated with anti – coagulant and put in sample bottles. Haematocrit (PCV) was determined with microhaematocrit using heparinized capillary tubes (25mm). Red blood cell

(RBC) and white blood cell (WBC) counts were determined as described by Blaxhall and Diasley (1973). Haemoglobin (Hb) concentration was determined as described by Wedemeyer and Yasutake (1977).

Results and Discussion

The haemoglobin concentration, PCV and RBC decreased significantly with increased dietary PPSM (Table 2). This may have been due to the increasing presence of anti-nutritional factors arising from increasing dietary PPSM in the diets. Pigeon pea seed contains protein inhibitors (Typsin and Chymotrypsin) and amylase inhibitors which affect the activity of digestive enzymes thereby causing digestive losses (Faris and Singh, 1990). These may have contributed to the decreasing haematological parameters observed in this work. Ant-nutritional factors have been reported to have deleterious effects and can interfere with food utilization, health and production of animals (Makkar et al., 1993). Liener (1994[a,b]) recorded that protease inhibitors including trypsin are potential anti-nutrients and are known to decrease growth performance. Tacon (1992) reported that nutritionally deficient diets decrease haemoglobin concentration; reduce haematocrit and red blood cell volume. Dick et al., (1976) found that nutritional toxicity is associated with anaemia in fish. Osuigwe et al., (2003) corroborated these observations by attributing the low values RBC, WBC, PCV count and Hb concentration of *Clarias gariepinus* fed raw Jack bean seed meal to the anti-nutritional factors inherent in the plant ingredients.

Fish fed group D diets recorded Hb concentration, PCV and RBC counts similar to those fed the control diet but significantly different from those fed group A, B and C diets. That is to say that fish fed diets containing soaked PPSM conferred better hematological profile on *C. gariepinus* than those fed diets containing other types of PPSM. This observation agrees with the report that overheating reduces the biological value of legumes (McDonald et al., 1987). Heating solubilizes and reduces nitrogenous compounds in legume seeds (Udedibie and Nwaiwu, 1987; Osuigwe et al., 2003). This seems to be responsible for the inferior performance of fish fed diets containing toasted and boiled PPSM. However, the WBC counts for fish fed heated PPSM generally were similar to those fed the control diet than those fed diet containing soaked PPSM. This may be attributed to wider fluctuations in values usually associated with WBC counts.

It is therefore concluded from this study that soaking for 16 hours is the best processing method for PPSM used in *C. gariepinus* diets.

Table 2: Haematological parameters (mean) and weight of *Clarias gariepinus* larvae fed four differently processed diets of pigeon Pea.

Experimental Diets	Final Fish weight (g)	Haemoglobin (Hb) (g/dl)	PCV (%)	WBC×10^3 (mm^{-3})	RBC×10^3 (mm^{-3})
Initial Status	–	33.3^{n}	70.00^{i}	8.20^{g}	22.00^{i}
A0 (Control)	1.30^{bcd}	33.1^{n}	69.50^{hi}	7.37^{d}	22.05^{i}
A1	0.98^{abc}	25.15^{e}	51.50^{e}	5.83^{c}	18.00^{d}
A2	0.93^{abc}	23.25^{d}	49.50^{d}	4.75^{b}	16.50^{c}
A3	0.70^{ab}	20.65^{c}	47.75^{c}	4.40^{ab}	15.50^{b}
A4	0.65^{a}	18.20^{b}	47.00^{b}	4.23^{a}	15.00^{ab}
A5	0.62^{a}	17.58^{a}	45.50^{a}	7.60^{def}	14.50^{a}
B1	1.12^{abce}	31.00^{hi}	69.00^{gh}	7.90^{efg}	20.50^{ef}
B2	1.03^{abcd}	30.50^{gh}	69.50^{hi}	7.85^{defg}	21.25^{ghi}
B3	1.03^{abcd}	29.50^{f}	68.00^{f}	8.00^{efg}	20.67^{fg}
B4	1.3b^{cde}	31.00^{hi}	70.00^{i}	7.75^{defg}	21.00^{fgh}
B5	1.58^{def}	30.00^{fg}	69.50^{hi}	7.54^{de}	20.00e
C1	1.4^{cde}	31.17^{i}	68.50^{fg}	7.73^{defg}	21.00^{fgh}
C2	1.54^{cde}	32.00^{jk}	69.00^{gh}	7.90^{efg}	20.50^{ef}
C3	1.24^{bcde}	31.37^{i}	68.50^{fg}	7.85^{defg}	20.50^{ef}
C4	1.35^{cde}	32.00^{jk}	69.50^{hi}	7.90^{efg}	20.50^{ef}
C5	1.44^{cde}	31.62l^{j}	68.50^{fg}	8.05^{efg}	20.00^{e}
D1	1.51^{cde}	32.12^{jkl}	69.50^{hi}	8.20^{g}	21.00^{fgh}
D2	2.39^{f}	32.67l^{mn}	69.17^{gh}	8.07^{efg}	21.75^{ij}
D3	2.13^{fgh}	32.78^{mn}	70.00^{i}	8.07^{efg}	21.25^{ghi}
D4	2.13^{fgh}	33.23^{n}	68.50^{fg}	8.10^{fg}	20.00^{e}
D5	2.21^{gh}	32.37^{klm}	69.50^{hi}	8.12^{fg}	21.50^{hij}

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