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Nutrient Composition of some Tropical Legumes Capable of Substituting Fish Meal in Fish Diets

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Abstract
Sword beans (*Canavalia gladiata*); Jackbean (*Canavalia enciformis* (L)); Mucuna bean (*Mucuna pruriens*); *Mucuna cochiunensis*; Bambara (*Voandzeia subterranea*) and Limabean (*Phaseolus lunatus*) are the tropical legumes considered in this paper. They have been used in the feed of ruminants but very scarcely considered in fish feed. Information about their nutrient composition are also scarce. Results from this study show that the protein contents of the test seeds ranged from 19.94% dry matter (DM), (Bambara) to 36.95% DM (*Mucuna cochiunensis*). Considering the high protein level required by fish for maximum growth and the presence of some ANFs, the seeds may not be able to be used in isolation without supplementing them with other food stuffs having higher protein value. The relatively high content of Nitrogen Free Extract (+ fibre) seem to suggest that the test seeds can be used in a semi-intensive setting to supply carbohydrate in fish diets. The seeds contain considerable amount of linoleic acid (18:2 n-6). The highest occurring in Lima beans. Sword beans and Jack beans are rich in oleic acid (18:1n-9). Palmatic acid (16:0) is high, while stearic acid (18:0) and myristic acid (14:0) are low. The amino acid compositions of the test seeds are not very adequate. Sword beans had a better amino acid profile though it seems deficient in some of the amino acids. The amino acid contents of Jack bean, Mucuna bean, Bambara and Lima bean look inadequate to provide a possible alternative to fish meal on individual basis. If to be used in fish feed formulation, combinating them with other protein sources, possessing higher contents of the limiting amino acids is strongly suggested. The potentials of these seeds in fish feed formulation seem high.

Keywords: Swordbean, Canavalia gladiata, Jackbean, Canavalia enciformis, Mucunabean, Mucuna pruriens, Bambara, Voandzeia subterranea, Limabean, Phaseolus lunatus, fish diet

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1 Introduction

Tropical legume grains represent a potentially important source of protein and energy for farm animals. Many varieties have been identified for use in animal feed because of their rich protein composition, mineral content and widespread distribution in the tropics. Only a few however, have been utilised (ADEPARASI, 1994).

In practical feed formulation wheat has been used as a supplementary feedstuff in European agriculture. The aim is to ensure that essential components of fish feed are not used as energy sources, but only for growth. Legumes have a high content of carbohydrate and can become good energy sources in fish feed just like wheat.

The general poor amino acid profile and deficiencies of some specific essential amino acids in some of these legumes have restricted their exploitation in non ruminant feeding. The occurrence of diverse range of natural compounds capable of precipitating deleterious effects in animals have also been identified in these legumes. These substances, arising principally from secondary metabolism in plants, are commonly referred to as anti nutritional factors (ANF). They reduce food intake and nutrient utilization in animals. They may also be hepatotoxic, neurotoxic and even lethal (D´MELLO, 1991).

The above setbacks not withstanding, nutritionists have continued in their efforts to make these pulses viable for use in animal feed. Supplementation with the limiting amino acids is an obvious method of upgrading the amino acid profile of the legumes and thereby maximize utilization of leguminous seeds. Thermal treatment confers significant nutritional advantages to the legumes, it is also an essential component of a more complex detoxification procedure. A proportion of the anti-nutritional factors present in plant proteins can be destroyed and inactivated by heat treatment process (TACON and JACKSON, 1985).

Sword beans (*Canavalia gladiata*); Jackbean (*Canavalia enciformis* (L)); Mucuna bean (*Mucuna pruriens*); *Mucuna cochiunensis*; Bambara (*Voandzeia subterranea*) and Limabean (*Phaseolus lunatus*) are the tropical legumes considered in this paper. They have been used in the feed of ruminants but very scarcely considered in fish feed. Information about their nutrient composition are also scarce. This paper therefore attempts to provide the basic information about nutrient composition of these tropical legumes while studies on their potentials to substitute fish meal in fish feeds continue.

2 Materials and Methods

Seeds from the test legumes were collected and planted at the Michael Okpara University of Agriculture, Umudike - Umuahia , Nigeria. to obtain enough seeds for proximate, amino and fatty acid analysis. After harvest dried raw seeds were homogenised, freeze dried and used for the respective chemical analysis at the Institute of Freshwater Ecology and Inland Fisheries.

Protein ($N \times 6.25$) was determined by the Kjeltec System (Tecator); crude fat by Soxtec System HT (Tecator) using petroleum ether, and ash by burning in a muffle furnace at 750°C for 4 hours. For the amino acid analysis, 5mg of the freeze dried samples were hydrolysed with 6 $NHCl$ at 110°C for 24 hours. No protecting reagents were added to avoid destruction of sulphur amino acids. Other procedures for the analysis have been reported (OGUNJI and WIRTH, 2001). Fatty acid composition of the samples were analysed using gas-liquid chromatography (with omega-wax capillary column Supelco, USA), as described by WIRTH and STEFFENS (1985). The lipid classes were separated by thin layer chromatography on silica gel G 60 (Merck, Darmstadt), using n-hexane/ethylether/acetic acid (73/25/2/v/v/v) as developing solvent. The fatty acids of phospholipids and triglycerides were transformed with sodium methylate into methylesters.

3 Result and Discussion

Table 1 shows the proximate analysis of the test tropical legumes. The protein contents of the seeds ranged from 19.94% dry matter (DM; Bambara) to 36.95 % DM (*Mucuna cochiunensis*). The values are lower than the recorded content of raw soybean (46.44% DM) but higher than the content of groundnut cake that was used to substitute fish meal in the feed of tiliapia *Oreochromis nitoticus* (OGUNJI and WIRTH, 2001). The protein requirements of cold water fishes and of many warm water fishes are generally high; more than 30% of the diets (WILSON, 1985; NATIONAL RESEARCH COUNCIL (NRC), 1981, 1983). Considering this high protein level required by fish for maximum growth and the presence of some ANFs the test seeds may not be able to be used in isolation without supplementing them with other food stuffs with higher value of protein. OSUIGWE *et al.* (2002) mixed only 9.80% of Jack beans seed meal boiled for 60 minutes with 17.60% fish meal in their diet formulation for *Clarias gariepinus* thus substituting fishmeal by 20%. By boiling, some of the ANFs were inactivated. The relatively high content of Nitrogen Free Extract (+ fibre) seem to suggest that the test seeds can be used in a semi-intensive setting to supply carbohydrate in the diet of fish. This possibility needs to be examined. The seeds are low in fats.

Table 1: Proximate analysis of test tropical legumes.

Sample No.	*Dry Matter*	*Ash**	*Fat**	*Protein**	*NFE [1] **
Swordbean	88.98	4.00 (3.56)	0.89 (0.79)	33.60 (29.90)	61.51 (54.73)
Jackbean-W	85.30	3.00 (2.56)	1.96 (1.67)	32.24 (27.50)	62.80 (53.57)
Jackbean-R	88.22	3.06 (2.70)	1.58 (1.39)	34.59 (30.52)	60.77 (53.61)
Mucuna Bl	89.44	3.58 (3.20)	2.40 (2.15)	33.19 (29.68)	60.83 (54.41)
M. cochi	88.96	3.56 (3.17)	3.39 (3.01)	36.95 (32.87)	56.10 (49.91)
Bambara	91.32	3. 26 (2.98)	5.78 (5.28)	19.94 (18.21)	71.02 (64.85)
Limabean	87.49	4.26 (3.73)	0.97 (0.85)	28.17 (24.64)	66.60 (58.27)

* Values in brackets are presented on wet weight basis.

[1] NFE = Nitrogen Free Extract

Table 2: Amino acid composition (% dry matter) of test tropical legumes.

	Sword Bean	*J-Bean W*	*J-Bean R*	*Mucuna Bl*	*M. cochi*	*Bambara*	*Limabean*
Aspartic acid	1.25	0.86	0.82	0.66	0.62	0.67	0.80
Glutamic acid	2.52	2.01	1.74	1.48	1.57	2.22	1.78
Serine	0.73	0.47	0.43	0.30	0.36	0.43	0.45
Glutamin	-	0.10	0.13	0.08	-	0.14	0.09
Histidine[1]	0.67	0.70	0.77	0.48	0.53	0.84	0.60
Glycine	0.90	-	0.32	-	-	-	-
Threonine[1]	-	0.96	1.01	-	-	-	-
Arginine[1]	2.02	0.76	0.66	0.58	0.63	0.83	0.68
Taurine	0.69	0.57	0.68	0.27	0.33	0.49	0.55
Alanine	0.40	0.16	0.20	0.29	0.23	0.09	0.15
Tryptophan1	0.68	0.35	0.30	0.40	0.27	0.23	0.27
Methionine1	-	-	-	-	-	-	-
Valine1	0.94	0.64	0.73	0.52	0.50	0.60	0.64
Phenylalanine[1]	1.10	0.75	0.62	0.61	0.57	0.74	0.80
Isoleucine[1]	0.90	0.61	0.63	0.51	0.51	0.56	0.65
Leucine[1]	2.15	1.78	1.85	1.16	1.15	1.50	1.63
Ornitine	-	1.08	0.79	0.83	0.83	0.85	1.03
Lysine[1]	1.42	0.87	0.76	0.61	0.62	0.86	0.83

[1] Essential amino acids

The Amino acid composition of the test tropical legumes are shown in Table 2. Among the test seeds, sword beans had a better amino acid profile. It seems deficient in some of the amino acids. However, this is dependent on the amino acid requirement of the fish for which the diet will include sword bean. For instance sword bean can be used in tilapia diet while methionine will be supplemented. Santiago and Lovell (1988) investigated the amino acid requirements for the growth of nile tilapia using purified diet. Reported as percentage dry matter the requirements are: Arginine 1.14, histidine 0.48, isoleucine 0.87, leucine 0.95, lysine 1.43, methionine 0.75, phenylalanine 1.05, threonine 1.05, tryptophan 0.28, and valine 0.78. The digestibility of the feed needs to be put into consideration. The essential amino acid composition of alternative protein sources for fish are not comparable with that of fish meal. The amino acid content of Jack bean, Mucuna bean, Bambara and Lima bean looks inadequate to provide any possible alternative to fish meal on individual basis. When using them in fish feed formulation, combination with other protein sources which may possess different limiting amino acids is strongly suggested.

Legumes similar to oil seeds like sunflower seed and linseed are rich in linoleic and linolenic acid. They may be deficient in long-chain polyunsaturated fatty acids. The fatty acid compositions (%) of phospholipids and tryglycerides from the test tropical legumes are presented in Tables 3 and 4. The test seeds contain a considerable amount

Table 3: Fatty acid composition (%) of phospholipids from the test tropical legumes

Fatty acid	*Sword Bean*	*J-Bean W*	*J-Bean R*	*Mucuna Bl*	*M. cochi*	*Bambara*	*Limabean*
14:0	0,2	0,2	0,1	0,1	0,1	0,1	0,1
15:0	0,5	0,2	0,3	0,1	0,1	trace	0,2
16:0	20,3	17,9	18,2	31,8	30,7	24,4	25,6
16:1 n-9	trace	0,5	trace	trace	trace	0,1	0,1
16:1 n-7	1,0	1,2	0,6	0,2	0,2	0,1	0,1
17:0	0,1	0,2	0,1	0,2	0,1	0,1	0,4
17:01	0,2	trace	0,3	0,1	trace	0,1	0,2
18:0	0,3	0,4	1,2	4,7	7,9	4,3	2,9
18:1 n-9	0,4	0,3	58,3	4,6	4,6	26,5	10,1
18:1 n-7	50,8	62,1	trace	1,3	1,2	0,1	0,1
18:2 n-6	19,8	12,6	16,3	49,5	48,4	37,0	47,8
18:3 n-6	trace	trace	trace	trace	trace	trace	trace
18:3 n-3	5,1	3,4	3,3	3,8	3,1	1,0	10,6
18:4 n-3	trace	trace	trace	trace	trace	trace	trace
20:0	0,1	0,1	0,1	0,3	0,6	trace	trace
20:1 n-11	trace	trace	0,1	trace	trace	0,5	0,2
20:1 n-9	0,2	0,3	0,1	0,1	0,1	0,3	0,1
22.3 n-6	trace	trace	trace	0,3	trace	0,2	0,3
22:4 n-6	0,1	trace	0,1	0,2	0,1	trace	0,1
24:0	0,2	0,2	0,2	0,2	0,3	trace	0,5
24:1 n-9	0,1	trace	trace	trace	0,3	1,5	trace

of linoleic acid (18:2 n-6). Higher concentration was found in the Mucuna species, Bambara and Lima bean both in phospholipids and triglyceride. Sword beans and Jack beans are rich in oleic acid (18:1n-9) while the highest content of linolenic acid (18:3n-3) were found in Lima beans. Palmatic acid (16:0) is high in the test seeds while stearic acid (18:0) and myristic acid (14:0) are low. 14:0 and 16:0. Fatty acids are however, not important for the growth of fish. It should be noted, that 18:2 (n-6) or 18:3 (n-3) as with other vertebrate, cannot be synthesised by fish *de novo*. Hence one or both of these fatty acids must be supplied preformed in the diet depending on the essential fatty acid (EFA) requirements (National Research Council (NRC), 1993). Based on the foregoing, the test seeds can be used to provide the fatty acid source in fish feed provided their inclusion level is not exceeded.

The following aspect of work on these test leguminous seeds are in progress: determination of the dietary inclusion level of these seeds in feed of different fish species; determination of the best processing method to deactivate the ANFs. The potentials of these seed in fish feed formulation seem high.

Table 4: Fatty acid composition (%) of triglycerides from the test tropical legumes

Fatty acid	*Sword Bean*	*J-Bean W*	*J-Bean R*	*Mucuna Bl*	*M. cochi*	*Bambara*	*Limabean*
14:0	0.6	0.3	0.3	0.1	0.2	0.1	0.2
15:0	0.3	0.1	0.2	trace	trace	trace	0.1
16:0	16.9	13.5	14.7	27.3	28.2	17.6	20.3
16:1 n-9	trace	trace	trace	trace	trace	trace	trace
16:1 n-7	2.5	2.6	1.5	0.4	0.3	0.1	0.2
17:0	0.3	0.1	0.1	0.1	0.1	0.2	0.2
17:01	0.1	trace	0.2	trace	trace	trace	0.1
18:0	1.9	1.7	4.2	8.1	7.9	9.0	9.1
18:1 n-9	46.3	52.9	49.8	8.8	8.5	23.0	10.4
18:1 n-7	3.1	3.6	2.4	4.3	3.8	0.1	1.1
18:2 n-6	14.1	10.9	14.3	43.2	45.2	37.3	40.0
18:3 n-6	trace	trace	trace	trace	trace	trace	trace
18:3 n-3	7.8	7.9	6.2	1.6	1.5	2.2	11.8
18:4 n-3	trace	trace	trace	trace	trace	trace	trace
20:0	trace	trace	trace	1.5	1.1	trace	trace
20:1 n-11	0.8	0.7	1.5	0.2	0.2	2.6	0.9
20:1 n-9	1.7	2.7	1.6	0.1	0.1	0.6	0.2
22:3 n-6	0.2	0.1	0.2	0.2	0.1	0.2	0.4
22:4 n-6	0.1	trace	trace	0.1	0.1	trace	0.1
24:0	1.4	1.6	1.7	0.8	0.9	1.7	2.2
24:1 n-9	0.1	0.1	trace	trace	trace	trace	trace

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