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Effects of Substituting Soybean (*Glycine max*) Meal with Bambaranut (*Voandzeia subterranea*) Meal on Growth Performance and Survival of African Catfish (*Clarias gariepinus*) Larvae

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ABSTRACT
Low fingerling production exacerbated by poor nutrition and costly live feed are major setbacks in African catfish *Clarias gariepinus* culture. Larval *C. gariepinus* catfish requires live feed at onset of exogenous feeding after which dry diets can be administered. Dry diets must be nutritious, acceptable and growth supporting. Soybean (*Glycine max*) is the major plant protein component of catfish feed but is costly. There is need for alternative plant proteins. We substituted soybean meal (SBM) with bambaranut (*Voandzeia subterranea*) meal (BNM) in novel diets for larval African catfish, average weight 0.15±0.02 g. The SBM: BNM inclusion levels (%) were, Feed 1(F1), 25:5; feed 2 (F2), 20:10; feed 3 (F3), 15:15; feed 4 (F4), 10:20 and feed 5 (F5), 5:25; the remaining 70% consisted of fish meal (60%); wheat flour (8%) and vitamin mix (2%). Effects of the diets on larvae were evaluated using linear regression analysis. Specific growth rate (SGR) increased significantly while food conversion ratio (FCR) decreased with increasing inclusion of SBM. Survival (c. 30-45% depending on the treatment) of the larval catfish was not attributable to substitution levels. Protein efficiency ratio (PER) and protein retention (PR) increased with increasing inclusion of SBM. Result indicates that BNM can be used to substitute SBM albeit with narrow trade growth tradeoff in terms of FCR; SGR, PER and PR. Equal inclusion of BNM and SBM is beneficial in terms of growth than lower SBM. The catfish grew fast with relatively high mortality.

Keywords: larval diets, SGR, FCR, survival, amino acids.

1. Introduction
Production of African catfish *Clarias gariepinus* is hampered by lack of fingerlings, and further exacerbated by poor quality feeds, predation and cannibalism of larvae [1]. Larval African catfish are voracious feeders and at the first feeding stage they can consume over 50% of their body weight per day [2]. Larval *C. gariepinus* is reported to be more efficient in food conversion and have higher growth rate than many other fishes at the larval stage [3].
African catfish larvae require live food at the onset of exogenous feeding [4, 5], but they can be weaned unto dry diet after four to five days period of live feed [4]. Dry larval diets must provide the required nutrients, amino acids and fatty acids for the developing fish. Due to dwindling supply of fish meal, which normally is the major component of larval feeds, ingredients of plant origin could be an alternative in providing the needed nutrients.
Soybean is the most commonly used plant ingredient in producing fish feed because it is relatively high in protein and amino acids with the exception of lysine and methionine. However, soybean has become costly and the price increase is affecting the feed industry [6]. Conversely, bambaranut (*Voandzeia subterranea*) is a cheap promising tropical legume containing appr. 22-25% protein with good essential amino acid profile [7, 8]. This experiment examined the possibility to use bambaranut meal instead of soybean meal as the plant protein source in larval dry diet. We examined the effects of gradual replacement of soybean meal with bambaranut meal on growth, nutritional performance and survival of African catfish larvae after the first feeding stage.

2. Materials and methods
The larvae were produced by artificial fertilization of African catfish brooders in the laboratory of the University of Jyväskylä, Finland. The hatchlings were reared after yolk sac absorption

with *Artemia* nauplii in 15 L flow through aquaria for 16 days before the start of the experiment. The average water temperature was 30.0±0.2 °C. Water flow rate was maintained at c. 0.4L/min. Photoperiod was maintained at 12D:12L at 60 lux (HD 9221 lux meter Delta OHM, Padua, Italy).

The average dissolved oxygen content of the water was 7.3±0.6 mg/L (YSI oxygen meter model 550A, YSI Inc., Yellow Springs, USA) and pH 6.8±0.3 (Combo pH & EC meter, Hanna Instruments, Arizona, USA), total alkalinity 1.14±0.01 mmol/ L, ammonia 0.20±0.04 mg/ L, and total gas pressure 100% (P4 Tracker total gas pressure meter; Point Four Systems Inc., Mayfield, Canada). Temperature, dissolved oxygen, total gas pressure (TGP) and pH of the water were measured daily.

The average ± SD (n = 20) initial weight of the larvae was 0.15±0.02 g. 350 larvae were distributed into each of the ten aquaria, with two aquaria per feed. Two replicates were considered adequate due to the regression design of the experiment. Two replicates were also used for mixture design trials [9]. Water in the aquaria was constantly aerated through an air stone. The aquaria were cleaned every morning before feeding the fish.

2.1. Experimental feeds

Five experimental diets were produced to vary in percentage inclusion of soybean meal (SBM) and bambaranut meal (BNM) in the following percentage order: Feed 1, 25:5, Feed 2, 20:10, Feed 3, 15:15, Feed 4, 10:20, Feed 5, 5:25 (Table 1). The BNM was made from white cultivar imported from Enugu, Nigeria, while defatted SBM was obtained from a supplier in Finland.

In addition, all feeds contained 60 % of fish meal, 8% of wheat flour and 2% vitamin premix. All ingredients were mixed, some water added, and the dough was steamed and pelleted and dried in an oven at 70 °C. Dried feed was ground to powder and stored in air tight bags and stored at -20 °C till used.

The fish were fed to apparent satiation three times daily (0800 - 0900, 1200 - 1300, 1800 - 1900). The fish were weighed on experimental days 10th and 22nd. Prior to weighing the fish were starved for 16 h. All the fish from each aquarium were weighed (to 0.01 g) in batch and counted.

2.2. Analyses

At the end of the experiment five fish were taken for analyses from each aquarium. The fish were homogenized together and freeze dried. Moisture content was determined gravimetrically after freeze drying. The protein content of the fish and the treatment feeds were determined by Kjeldahl method from freeze dried fish and crude protein was expressed as % N x 6.25. Total lipids of experimental feeds were determined by chloroform–methanol extraction at the ratio of 2:1. This was a modification from [10]. Amino acids of the ingredients were measured after methods of the [11] European Commission (1998). Total peptides (bound and free) were analyzed with Waters Finland Mass Trak UPLC (Water Corporation Milford, USA) and the application was UPLC Amino Acid Analysis Solution®. The amino acid contents of the feeds were calculated based on the results of ingredients and amount of ingredient based on the diet composition table (Table1). The feed formulation (Table 1) ensured complimentary mixtures of essential amino acids from ingredients (Table 2).

Table 1: Formulation of diets (inclusion %) and the proximate composition of larval African catfish feeds (F1 to F5) made to vary in soybean meal (SBM): bambaranut meal (BNM) -ratio.

Ingredients	F1	F2	F3	F4	F5
SBM	25	20	15	10	5
BNM	5	10	15	20	25
Fishmeal	60	60	60	60	60
Wheat	8	8	8	8	8
Vitamin premix	2	2	2	2	2
Proximate composition (%)					
Moisture	3.40	3.14	5.22	8.88	8.35
Lipids	14.78	14.01	11.90	11.50	12.57
Protein	61.34	61.70	60.34	57.55	56.30
Ash	5.67	5.24	7.44	8.25	7.19

[1]Vitamin Prx. The following vitamins were added to supply the following per kg diet: cholecalciferol, 1300 IU; all-race-α-tocopheryl acetate, 140 IU; menadione sodium bisulfite, 12 mg; thiamin HCL, 8 mg; riboflavin, 16 mg; calcium d-pantothenate, 17 mg; biotin, 0.2 mg; folic acid, 5 mg; vitamin B12, 0.02, niacin, 40 mg; pyridoxine HCl, 16 mg; ascorbic acid (Stay C), 80 mg. magnesium phosphate, 5000 mg, potassium carbonate, 400 mg, manganous sulfate, 10; ferrous sulfate, 5 mg; zinc sulfate, 80 mg.

Table 2: Amino acid (AA) contents of ingredients used in formulating diets or larval African catfish (g/ kg feed) as fed

AA	BNM	SBM	FM	Wheat
Arg	16.96	36.70	45.40	6.40
His	6.70	12.20	16.50	3.00
Ile	9.41	21.40	31.30	5.10
Leu	17.24	36.30	51.90	8.90
Lys	13.89	30.80	55.70	3.60
Met	3.11	6.80	20.80	2.10
Cys	2.02	7.50	7.40	2.70
Phe	12.20	24.40	27.10	6.30
Tyr	10.49	17.60	22.01	4.30
Thr	7.27	18.90	29.01	3.70
Val	10.57	25.50	43.00	5.90

Amino acid codes, Arg (arginine), His (histidine), Ile (isoleucine), Leu, (leucine), Lys (lysine), Met (methionine), Cys (cystine), Phe (phenylalanine), Tyr (tyrosine), Thr (threonine), Val (valine).

2.3. Calculations and statistical analyses

Specific growth rate (SGR, %/day) was determined as:

SGR= 100 x (Ln final weight (g) – Ln initial weight (g))/ (period in days)

Feed conversion ratio (FCR) = feed fed (g)/ weight gain (g)

Protein efficiency ratio (PER) = weight gain (g) * protein intake-1 (g)

Relative daily feed given (% of weight) was calculated as, 100 x TFI/IW; where TFI is total feed intake (g/ fish on as fed basis). IW = initial body weight of fish.

Survival (%) = 100 x (final number)/ (initial number)

Protein retention (PR) (%) = 100 x (Protein gain (g))/Protein intake (g)

The data were analyzed using PASW version 18 statistical software (SPSS Inc.). The effect of SBM: BNM-ratio on measured and calculated parameters was analyzed using linear regression analysis, and the average tank value was used as the observational unit, i.e. n=10. The actual SBM and BNM inclusion values (as %) used in the regression analyses for the 5 feeds were 83.3% (F1), 66.6% (F2), 50% (F3), 33.3% (F4) and 16.7% (F5).

3. Results: The catfish larvae readily accepted the experimental dry feeds. Depending on the treatment, catfish average tank wise SGR varied between 13.1%/ day and 14.9%/ day (Table 4). SGR was positively correlated with the amount of SBM in the diet (P=0.01, Fig 1a). Substitution of SBM with up to 50% BNM produced similar growth as F1 which suggests effective substitution and reduction in amount of SBM. The catfish final weight varied between 4.3 g and 2.9 g. Final catfish weight increased much at F3 but drop at F4 and F5.

Food conversion ratio, FCR, was below 0.9 for all diets (Table 4), and it decreased along with the increase of SBM (P = 0.000, Fig. 1b).The relative daily amount of feed fed (% of body weight) was not significantly affected by the SBM: BNM-ratio. Protein efficiency ratio (PER) of the larval catfish was positively related to the SBM content of the diet (P=0.005, Fig 2a). There was also a linear positive relationship between protein retention and the increase of the feed SBM content (P=0.000, Fig 2a).

Survival of the larvae varied between 28.6% and 45.7% (Fig. 2b). There was a tendency of a positive relationship between the larval survival and the SBM inclusion, but however, this correlation was not statistically significant (P=0.28, Fig 2b).

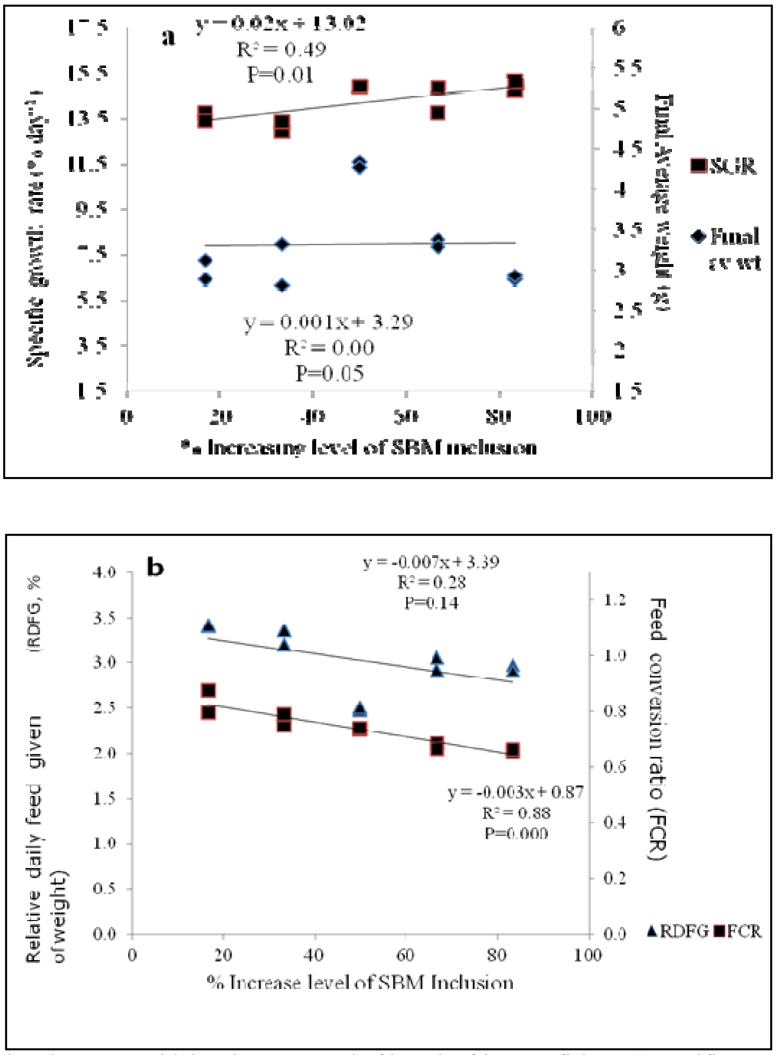
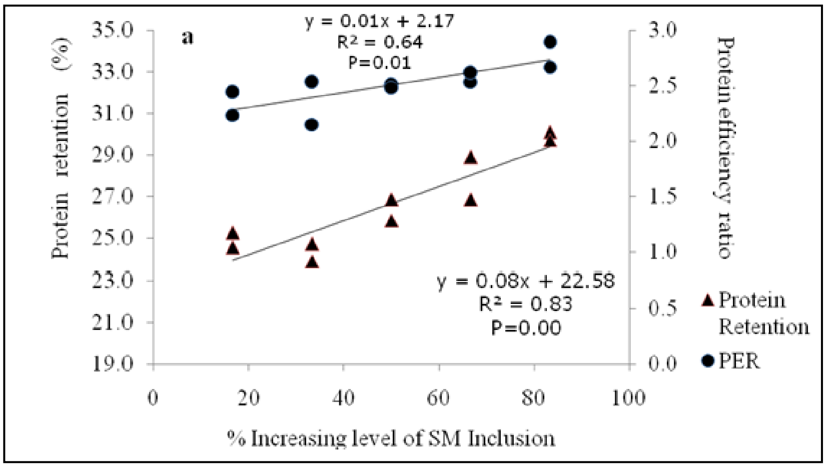


Fig 1: Influence of soybean meal replacement with bambaranut meal of larval African catfish on a) specific growth rate (SGR) and final average weight and b) food conversion ratio (FCR) and relative daily feed intake (RDFI). Values on the x-axis show SBM share of the SBM: BNM-mixture.



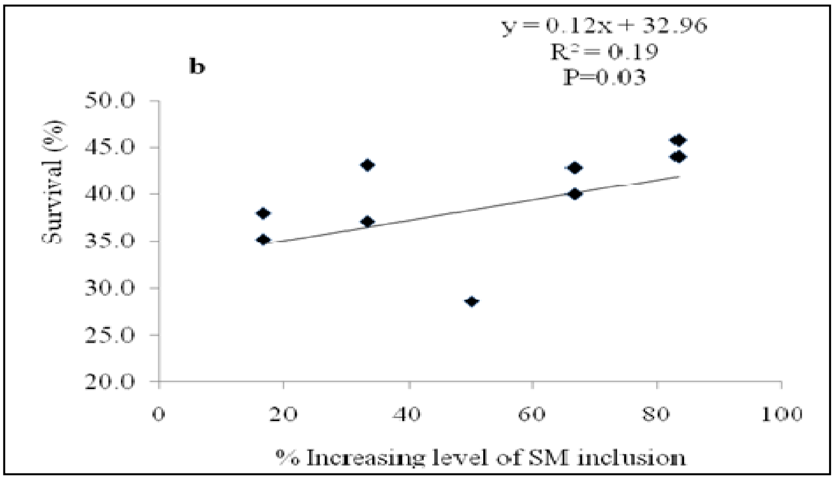


Fig 2: a. Influence of soybean meal replacement with bambaranut meal of larval African catfish on a) Protein retention and protein efficiency. and b) % survival. Values on the x-axis show SBM share of the SBM: BNM-mixture.

Table 3: Amino acid content of larval African catfish diets varying soybean meal with bambaranut meal. Values represent g/ kg feed as fed not dry matter.

AA	F1	F2	F3	F4	F5	Recom.
Arg	37.8	36.8	35.8	31.5	33.8	4.3
His	13.5	13.2	13.0	12.7	12.4	1.5
Ile	25.0	24.4	23.8	23.2	22.6	2.6
Leu	41.8	40.8	39.8	38.9	38.0	3.5
Lys	42.1	41.2	40.4	39.6	38.7	5.1
Met	14.5	14.3	14.1	14.0	13.8	2.3
Cys	6.6	6.4	6.1	5.8	5.5	
Phe	23.4	22.8	22.2	21.6	21.0	5.0
Tyr	18.4	18.1	17.8	17.4	17.0	
Thr	22.8	22.2	21.6	21.0	20.4	2.0
Val	33.2	32.4	31.6	30.9	30.2	3.0

F1-F5 represents diets, where soybean meal was substituted by bambaranut meal at following percentages, 5, 10, 15, 20 and 25. Amino acid codes, Arg (arginine), His (histidine), Ile (isoleucine), Leu, (leucine), Lys (lysine), Met (methionine), Cys (cystine), Phe (phenylalanine), Tyr (tyrosine), Thr (threonine), Val (valine). Recm. Means recommended level for channel catfish (A) g/ kg protein, (B) % of dietary protein) (NRC 1993).

Table 4: Specific growth rate (%/ day), food conversion ratio, final ave. Weight, initial average Weight (g), relative daily feed given (%) of African catfish fed diets varying soybean meal and Bambaranut meal for 22 days

Feeds	SGR	FCR	Final WT	Initial WT
F1	14.93 ± 0.31^{a}	0.66 ± 0.10^{a}	2.90 ± 0.03^{c}	0.12 ± 0.01^{ns}
F2	14.31± 0.81^{a}	0.68 ± 0.01^{a}	3.33 ± 0.06^{b}	0.14 ± 0.03^{ns}
F3	14.89 ± 0.0^{a}	0.74 ± 0.00^{b}	4.30 ± 0.05^{a}	0.16 ± 0.00^{ns}
F4	13.13 ± 0.35^{b}	0.77 ± 0.03^{b}	3.06 ± 0.36^{c}	0.17 ± 0.00^{ns}
F5	13.59 ± 0.2^{b}	0.84 ± 0.06^{d}	3.00 ± 0.17^{c}	0.15 ± 0.00^{ns}

Values not followed by same superscript are statistically significantly different (P<0.05)

4. Discussion

Larval African catfish were able to consume the experimental dry feed as their first dry feed, and also grew fast. African catfish larvae have been noted to growth as fast in previous experiments [2, 3, 12]. The SGR of the African catfish on our experimental dry diets was higher or similar than growth rates of smaller larval catfish in some previous experiments (c. 10 mg initial weight in contrast to our fish of 150 mg), [3, 13, 14] The growth rates of the catfish in this experiment suggest that all tested diets were suitable for rearing catfish larvae from start of exogenous feeding. During the larval stage SGR decreases quickly along with the size of the fish e.g. [3] and thus the growth of this fish in our study can be regarded as fast irrespective of the feed type.

The relationship between SGR and dietary soybean meal inclusion was linear which can be attributed to higher protein and amino acid content and/or availability in soybean than in bambaranut meal. Lysine and methionine are two major essential amino acids lacking in sufficient amounts especially in plant based diets [15, 16]. The experimental feeds contained high amount of these two essential amino acids due to 60% fish meal in all diets (Table 3). Low lysine and methionine levels in catfish feeds negatively affect growth and feed utilization [17]. The lysine content of our feeds varied between 38.7 to 42.1.4 g/kg protein. This was comparable to recommended requirement for closely related channel catfish (*Ictalurus punctatus*) 5.1 % dietary protein (in recalculating 51 g/kg protein) [18]. The low FCR and high SGR achieved by the *C. gariepinus* larvae in our experiment suggest good quality of the diet mixtures. Low dietary FCR has been linked to high amino acids in the culture of channel catfish [19] and Indian *Heteropneustes fossilis* frys [16]. The increased FCR along with the substitution SBM with BNM can be due to the reduced essential amino acid content of the diets with less SBM. The increase of FCR can also be attributed to other non-protein dietary components like anti nutritional factors and carbohydrates and some fiber which may have also affected diet utilization to some extent. At the stage of our research the stomach of African catfish larvae may not have been able to digest such nutrients very well like adult fish. This had been noted previously by [20]. Effects of catfish alimentary canal development on diet digestion and utilization had been reviewed by [4].

However, the larvae should have been able to digest some proteinous and nutritive portions of the dry feeds as they were already over 2 weeks old at the time when the experiment started. According to [4] the larvae could be weaned to dry feeds already four days after hatching. Neither diet nutritional quality alone could have been the reason for low survivability, because the surviving fish grew well and SBM: BNM –ratio did not significantly affect survival. In this experiment we noted some mortality due to stomach rupture which has been noted in African catfish fed dry diets [21]. Ruptures could result from dry diets longer evacuation time when compared to live feed [2], There was also a sudden drop in survival (<30%) with F3 (50% SBM inclusion), but this seems to have been due to the fact that some catfish grew faster than others initiating agonistic behaviors and mortality. Agonistic behavior and mortality had been noted in larval African catfish previously [22] (Kaiser *et al*., 1995).

The catfish larvae protein efficiency ratio (treatment averages ± SD from 2.33±0.36 to 2.77±0.03) and protein retention (30.7±1.83% to 34.1±0.67% to) suggests high dietary protein synthesis of the catfish. This was pointed out previously by [22] and highlighted by [5] and [14] in the *C. gariepinus* larvae fed dry and live diets.

In conclusion BNM is a good substitute of SBM in the diets of larval African catfish. The larval FCR of 0.66 and 0.84 shows good utilization of both SBM and BNM based diets.

Equal inclusion levels of SBM and BNM (F3) produced similar growth rate as F1, indicating beneficial effects of BNM substitution. Although post first feeding African catfish were able to utilize the experimental dry diets and grew fast, it is obvious that use of dry diets induced a relatively high larval mortality.

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