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Evaluation of Various Leguminous Seeds as Protein Sources for Milkfish, *Chanos chanos* Forsskal, Juveniles

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Abstract

The nutritive value of four leguminous plant seeds: pigeonpea *(Cajanus cajan)*, mungo *(Phaseolus radiatus)*, kidneybean *(Phaseolus vulgaris)* and soybean *(Glycine max)*, were evaluated as protein sources for milkfish *(Chanos chanos)* juveniles in isonitrogenous and isocaloric diets containing 40% protein. The legumes were heat-treated and added at different levels so that their protein contributed 25% of total dietary protein; peruvian fishmeal supplied the remaining portion. A diet with all protein contributed by fishmeal was used as a control. Substitution of 25% of the total 40% animal protein in the control diet by mungo and soybean meal did not adversely affect ($P > 0.05$) fish growth and the efficiency of feed conversion. Diets with 25% of protein contributed by pigeonpea and kidneybean meal resulted in significantly depressed ($P \leq 0.05$) growth and efficiency of feed conversion. Milkfish survival rate was more than 97% throughout the six-week experimental period. We conclude that mungo and soybean meal, contributing at least 25% of dietary protein, can be used to replace fishmeal to reduce the cost of feed without adverse effect on the growth, survival and efficiency of feed conversion of juvenile milkfish.

Introduction

Milkfish, *Chanos chanos,* is the major species cultured in some 200,000 ha of brackishwater ponds in the Philippines. Farmers still rely on detritus and natural food as the food base of milkfish. Few farmers provide milkfish with artificial diets.

In recent years, the profitability of milkfish farming has been steadily declining, and competing land uses have increased the pressure to limit the coastal areas for farming milkfish. These underscore the need to intensify production to increase the yield from the present national average of 870 kg ha^{-1}. Intensification involves feeding milkfish. Thus, studies on nutrient requirement, feed digestibility, digestive physiology and feed formulation and evaluation are needed.

The high biological value of fishmeal for many species has resulted in high costs and dependence on the commodity. Many studies have been conducted to substitute fishmeal either wholely or in part, which often resulted in adverse effects on the growth. Substantial substitution with plant proteins requires proper processing and amino acid supplementation to enhance biological value (Cowey and Sargent 1979; Ketola 1982). Specifically, legumes require heat treatment to remove inhibitors which accentuate digestive losses (Gontzea and Sutzescu 1968).

This study provides baseline information on the potential of legumes as substitutes for fishmeal in order to reduce feed costs. It aims to evaluate the nutritive value of four legumes - pigeonpea *(Cajanus cajan),* mungo *(Phaseolus radiatus),* kidneybean *(Phaseolus vulgaris)* and soybean *(Glycine max)* - for juvenile milkfish based on the proximate analysis of the beans, efficiency of feed conversion and the growth response of milkfish as 25% of the dietary protein is supplied by the beans.

Materials and Methods

Experimental Diets

In order to evaluate the efficiency of utilization of the four locally available leguminous protein sources, different levels of each were added so that each contributed 25% of the dietary protein. Diet 1 served as the control diet, with all of its 40% protein contributed by peruvian fishmeal. Diets 2, 3, 4 and 5 contained 30% protein from peruvian fishmeal and 10% from pigeonpea, mungo, kidneybean and soybean meal, respectively (Table 2). The experimental diets were formulated so that they were approximately isocaloric and isonitrogenous.

Seeds of the four species of legumes were purchased from the local supermarket, rinsed with water and oven-dried for two hours at 110^{o}C. The seeds were ground to pass through a 0.425-mm screen net. Sieved seeds were autoclaved for fifteen minutes and stored in the refrigerator until needed for proximate analysis and diet preparation. Diets were prepared by mixing ingredients one at a time, allowing at least ten minute intervals for mixing. Mixed feeds were extruded using a meat grinder with a 3-mm die. Pelleted feeds were dried in an oven at 40-60^{o}C and stored at 4^{o}C prior to use.

The ingredients and diets were analyzed for proximate composition using standard methods (AOAC 1975). Kjeldahl protein nitrogen was analyzed using the Tecator Kjeltec System.

Analysis of the proximate chemical composition of the leguminous seeds showed that soybean meal contained the highest protein level at 46.8% and the lowest level of nitrogen-free extract (NFE) at 16.11%. Among pigeonpeas, kidneybean and mungo meal, protein ranged from 22.8% to 28.7% while NFE levels were 52.1% to 53.4% (Table 1). Amounts of each legume and of fishmeal in the diets

Table 1. Proximate composition of the protein sources used in the experimental diets.

Component	Peruvian fishmeal (%)	Pigeonpea meal (%)	Mungo meal (%)	Kidneybean meal (%)	Soybean meal (%)
Dry matter	92.17	93.90	95.27	91.20	89.93
Moisture	7.83	6.10	4.73	9.80	10.07
Protein	62.77	28.50	28.67	22.81	46.83
Fat	6.82	2.01	2.04	2.14	14.12
Fiber	2.87	7.79	7.04	6.85	6.49
Ash	15.97	5.49	4.09	5.96	6.38
NFE	3.74	52.11	53.43	53.44	16.11
Calculated ME* (kcal kg^{-1})	3,159	3,028	3,162	2,942	3,590

*Metabolizable energy values (kcal g^{-1}) used were: carbohydrate 3.5, protein 3.9 and fat 8.5.

are shown in Table 2. Corn starch was used as the source of digestible carbohydrate. Carboxymethyl cellulose was used as the filler. The experimental diets had similar proximate composition and calculated metabolizable energy (ME) levels based on the following ME values in kcal g^{-1}: carbohydrate 3.5, protein 3.9 and fat 8.5 (Table 3).

Table 2. Composition of the experimental diets.

Ingredients	Diet 1	Diet 2	Diet 3 (g in 100 g diet)	Diet 4	Diet 5
Peruvian fishmeal	63.72	47.79	47.79	47.79	47.79
Pigeonpea meal		37.74			
Mungo meal			34.86		
Kidneybean meal				43.84	
Soybean meal					21.36
Cod liver oil	2.83	2.99	3.02	2.90	1.86
Vegetable oil	2.83	2.99	3.02	2.90	1.86
Vitamin mix[a]	1.0	1.0	1.0	1.0	1.0
Mineral mix[b]	1.0	1.0	1.0	1.0	1.0
Carboxymethyl cellulose	6.01	2.19	3.96		4.96
Starch	22.62	4.32	5.36	0.57	20.17

[a]Supplied the following in mg kg^{-1}: thiamin.HCL, 25.0; riboflavin, 25.0; pyridoxine. HCL, 20.0; vitamin B_{12}, 0.1; nicotinic acid, 100.0; D-calcium panthothenate, 100.0; L-ascorbic acid, 2,500.0; retinyl palmitate β-carotene, 5.0; cholecalciferol, 5.0; DL-α-tocopherol, 160.0; menadione, 10.0; D-biotin, 2.0; inositol, 1,000.0; folic acid, 7.5; choline chloride, 1,500.0.

[b]Supplied the following in g kg^{-1}, $NaHCO_3$, 2.0; $FeSO_4.7H_2O$, 0.5; $AlK(SO_4)_2.12H_2O$ 0.02; $ZnSO_4.7H_2O$, 0.1; $CuSO_4.5H_2O$, 0.06; $MnSO_4.H_2O$, 0.5; KI, 0.012; $CoCl_2.6H_2O$, 0.02; $NaSeO_3$, 0.012; $MgSO_4.7H_2O$, 2.4; $CaHPO_4.2H_2O$, 4.4.

Table 3. Proximate composition of experimental diets.

Component	Diet 1 Control (%)	2 Pigeonpea meal (%)	3 Mungo meal (%)	4 Kidney bean meal (%)	5 Soybean meal (%)
Dry matter	91.90	92.80	93.00	94.60	93.40
Moisture	8.10	7.10	7.00	5.40	6.60
Protein	35.91	38.13	36.63	37.10	36.75
Fat	9.98	11.75	11.75	11.06	10.83
Fiber	5.64	5.53	4.77	4.89	2.87
Ash	20.51	15.02	16.55	15.77	17.28
NFE	19.86	22.37	23.10	25.78	26.67
Calculated ME* (kcal kg^{-1})	2,944	3,269	3,263	3,289	3,287

*Metabolizable energy values (kcal g^{-1}) used were: carbohydrate 3.5, protein 3.9 and fat 8.5.

Experimental Set-up

Milkfish fingerlings from brackishwater ponds were stocked in 59-l rectangular glass aquaria at 40 fish per tank. The fingerlings were allowed to acclimate in the experimental tanks for one week. During this period, they were fed a practical diet at 10% of body weight daily. Each tank was supplied with aerated recirculating water at 26-29°C at a rate of 1.5 l min^{-1}. The water, maintained at 18-22 ppt salinity, was sand filtered and passed through a 75-cm aeration column. Tanks were cleaned daily by siphoning out particles and feces and replacing the siphoned water.

At the start of the experiment, the fish averaged 2.1 g. They were fed thrice daily at 0800, 1200 and 1600 hours for six weeks. The fish were fed at 8% of body weight during the first four weeks and 6% during the last two weeks. The feeding rate was adjusted every two weeks after determining actual body weight.

Statistical Analysis

The experiment had a completely randomized design with four replicate tanks per treatment. The weight gain, feed conversion and survival rate of fish in the various experimental treatments were tested for significant differences using the analysis of variance (ANOVA).

Results

All experimental diets sustained excellent fish survival of at least 97% throughout the six-week rearing period. Most mortalities were by fish accidentally jumping out of tanks which were not covered during the early phase of the study.

Substitution of 25% of the total animal protein in the control diet (diet 1) by mungo meal (diet 3) and soybean meal (diet 5) did not adversely affect ($P > 0.05$) fish growth and the efficiency of feed conversion (Table 4). Both parameters of measures were significantly

Table 4. Effect of dietary source on the growth, efficiency of feed conversion and survival in milkfish fingerling.

Treatment	Weight gain per fish* (g)	Feed conversion (feed/gain)	Survival (%)
1 Peruvian fishmeal (control diet)	5.41 ± 0.33^{a}	2.12 ± 0.09^{a}	97.5 ± 1.0^{a}
2 Basal diet + pigeonpea meal	4.08 ± 0.31^{b}	2.54 ± 0.12^{b}	100.0 ± 0^{a}
3 Basal diet + mungo meal	5.48 ± 0.07^{a}	2.05 ± 0.01^{a}	97.5 ± 1.8^{a}
4 Basal diet + kidneybean meal	1.73 ± 0.12^{c}	5.31 ± 0.36^{c}	98.1 ± 1.2^{a}
5 Basal diet + soybean meal	5.32 ± 0.15^{a}	1.88 ± 0.10^{a}	98.8 ± 0.7^{a}

*Fish initially average 2.1 g each, and were stocked at a rate of 40 fishes per aquarium.

Note: Values represent the mean ± SEM of four tanks of fish. Values having the same letters in the superscript are not significantly different ($P > 0.05$). Data represent results from a six-week growth period.

depressed ($P \leq 0.05$) when fish were fed diets with 25% of the protein contributed by pigeonpea meal (diet 2) and kidneybean meal (diet 4). Fish fed diet 2 attained 76% of the weight gained by fish fed diets 1,3 and 5, while fish fed diet 4 had the poorest growth.

Discussion

In this study, all the legumes evaluated were subjected to the same heat treatment and were formulated to contribute 25% of the protein in isocaloric diets. The comparable growth resulting from the diets containing mungo and soybean meal with that of the fishmeal control diet suggests the absence of antinutrient effects and the adequacy of nutrients in these diets but not in diets containing pigeonpea and kidneybean meal at the levels tested. Although all the leguminous seeds were heat treated in the same way, the process may not be adequate in removing metabolic inhibitors in pigeonpea and kidneybean meal. There are differences in the degree of metabolic inhibition and the heat treatment required to remove inhibitors among the various legumes (Gontzea and Sutzescu 1968). It is also possible that the concentrations of some nutrients, specifically amino acids like methionine and lysine, are inadequate.

Amino acid supplementation has been shown to improve the nutritive value of soybean meal in trout and carp (Rumsey and Ketola 1975; Ketola 1982; Viola et al. 1982). Complete substitution with soybean meal causes a marked fall in growth rate, but amino acid supplementation can improve growth rate up to more than 80% of that of a fishmeal control (Ketola 1982). Ketola (1982) also showed that a mixture of protein sources with an amino acid profile similar to that of soybean meal alone significantly improved the growth of Atlantic salmon. Milkfish respond positively to amino acid supplementation (Chiu et al. 1986). It would be of great economic interest to determine the maximum rate of inclusion of mungo and soybean meal, through combinations with other protein sources and amino acid supplementation, so that a least-cost formulation that minimizes the use of fishmeal without adverse effects on growth can be attained.

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