Evaluation of Partial Replacement of Dietary Animal Protein from Plant Protein Blended with Glucosamine on Growth and Body Indices of Asian Catfish (Clarias Batrachus) Fingerlings

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Abstract

A 12-week feeding trials was conducted to evaluate the use of animal and plant protein, in combination with Glucosamine source for Asian Catfish, Clarias Batrachus (av. wt. 2.2±0.009 to 2.6±0.03 g). This study was performed to evaluate the effect on fish growth performance by replacing animal protein with vegetable protein sources. In experiment, six (37.40 to 43.52 % crude protein, 16.15 to 16.76 kJ/g energy, and crude lipid 3.33 to 6.69%) practical diets were formulated. The animal and plant protein component of the diets was progressively added with glucosamine 0.5, 5.0 and 10.0% with fish meal, silkworm pupae, soybean meal and casein (F-1, PAG 0:100:0.5; F-2, PAG 0:100:5.0; F-3, PAG 0:100:10.0; F-4, PAG 50:50:0.5; F-5, PAG 50:50:5.0; F-6, PAG 50:50:10.0). The experimental moist diets were fed to triplicate groups of fingerlings at 10% of body weight and results were compared with control feed. Growth performance and feed utilization efficiency of catfish, fed diets with animal protein are better than those of plant protein supplemented feeds. After 12-week study the final weight gain recorded as 18.6g, 19.7g, 19.9g, 14.6g, 14.9g, 13.9g and 13.1g in F1 to F6 and in control fed fishes respectively. The percentage weight gain among the animal protein group (F-1, F2, F-3) were recorded as 615.4%, 756.5% and 804.5%, respectively. And the percentage weight gain in 50% replaced feeds (F-4, PAG 50:50:0.5; F-5, PAG 50:50:5.0; F-6, PAG 50:50:10.0), recorded as 563.6%, 547.8% and 479.2% respectively whereas in control it was 403.8%. The results suggests that the growth is better in total animal protein feeds and the best percentage weight gain (p<0.05) recorded in the feed F3, incorporated with 10% glucosamine (804.5%). The FCR were of the diets along with glucosamine in animal protein content has better growth performances ranged between 1.56 ± 0.03 to 1.90 ± 0.04. The survival was recorded in F1 to F6 as 88 ± 2.4%, 83 ± 2.3%, 76% ± 3.4, 74 ± 4.4%, 71 ± 2.8% and 76 ± 4.7% respectively and in control it is recorded as 68 ± 2.7%. The Hepatosomatic and Viscerosomatic indices ranged between 0.67 ± 0.03 to 1.94 ± 0.19 and 1.90 ± 0.02 to 3.17 ± 0.2 respectively in F1 to F7. The feed efficiency in terms of feed conversion ratio recorded as 1.56 ± 0.03 to 1.90 ± 0.04 among all the feeding trials. The findings shows that feed F3, containing 100% animal protein with 10% glucosamine is performed best. Results indicate that animal protein rich diets with glucosamine were much acceptable than plant protein and/or natural diets (Artemia nauplii) for Asian catfish, Clarias batrachus. And to assess the potential for replacing animal protein with soybean meal in the diets of fish need more evaluation along with synergistic approach of incorporating glucosamine.

Keywords: Growth; Animal protein; Plant protein; Glucosamine; Clarias batrachus

Introduction

Fishmeal as raw material is the first choice in aquaculture production due to high quality protein with balanced amino acid profile [1]. Since last twenty years the production of fishmeal is relatively stable and the increasing requirement could not be matched in present scenario due to increased aquaculture requirement [2]. Moreover the cost of fishmeal is increasing day by day therefore there is an urgent need to evaluate the other ingredients as well find alternative protein source to make up for the shortage of fish meal and fulfill the requirement and secure the supply for commercial feed [3]. In this context soybean meal (SBM) regarded as an economical and nutritionally rich food ingredient in comparison to other plant ingredient [1]. The nutritional evaluation of soybean meal to replace fishmeal has been a long standing priority in fish nutritional research [4]. Due to evermore research data a considerable success has achieved in supplement of FM with SBM plant proteins in aquatic animals [5,6]. However, at higher rate of replacement of the fishmeal with SBM encouraged growth retardation may be due to imbalance nutrition in carnivorous fishes [6-10] and/or higher ammonia excretion [11,12]. The reduced growth may be due to anti-nutritional factors [10,13,14]. The histological changes in intestine can also reduce growth performance on feeding plant proteins [8,15-17].

Air-breeding Catfish, Clarias batrachus (Family: Clariidae), locally known as Magur, is a fish of great demand and attracts the attention of farmers for its high market value. Feed management determines the viability of aquaculture as it accounts for at least 40-60% of the cost of fish production [18]. Reducing the feeding costs could be key factor for successful development of aquaculture. Protein is the most expensive component in fish feeds hence it is known to require in relatively large amount by several fishes [19-24], the exact level of its requirement for...
formulation of well-balanced feed and also the most important factor affecting growth performances of fish and feed cost [25]. Glucosamine a amino sugar and a prominent precursor in the biochemical synthesis of glycosylated proteins and lipids synthesize chitin, is one of the most abundant monosaccharide [26-28] which composes the exoskeletons of crustaceans and other arthropods. It has been well established that animal protein performs better than plant protein in the growth and nutritive value of cultivable fish [29]. Silkworm pupa is one of the unconventional top class animal proteins (65-67%). Recycling of these wastes into an acceptable source of animal protein in the feed of fish is a big challenge in the pursuit of sustained procedure of inexpensive catfish, *Clarias batrachus* feed. Silkworm pupae (Bombyx mori) is a low cost animal protein source, rich in both protein and lipid [30]. This study was taken up as huge mortality is recorded at fingerling stage of this fish in natural condition. Therefore, this experiment was carried out to study the synergistic effects of dietary glucosamine in combinations with plant/animal proteins on the survival and growth performance of *Clarias batrachus* fingerling.

**Materials and Methods**

**Experimental feeds and feed preparation**

Six feeds were prepared by using plant & animal protein in combination with glucosamine source for Asian catfish, *Clarias batrachus*. Ingredients and proximate composition of the experimental feeds are given in Table 1. The animal and plant protein component of the feeds was progressively added with glucosamine 0.0, 0.5, 5.0 and 10.0 % with basic ingredients like fish meal, silkworm pupae, soybean meal and casein (F-1, PAG 0:100:0.5; F-2, PAG 0:100:5.0; F-3, PAG 0:100:10.0; F-4, PAG 50:50:0.5; F-5, PAG 50:50:5.0; F-6, PAG 50:50:10.0). Fishmeal was freshly prepared from in lab from dried trash fishes mainly F-4, PAG 50:50:0.5; F-5, PAG 50:50:5.0; F-6, PAG 50:50:10.0. Fishmeal casein (F-1, PAG 0:100:0.5; F-2, PAG 0:100:5.0; F-3, PAG 0:100:10.0; F-4, PAG 50:50:0.5; F-5, PAG 50:50:5.0; F-6, PAG 50:50:10.0). Fishmeal was freshly prepared from in lab from dried trash fishes mainly F-4, PAG 50:50:0.5; F-5, PAG 50:50:5.0; F-6, PAG 50:50:10.0). Fishmeal casein (F-1, PAG 0:100:0.5; F-2, PAG 0:100:5.0; F-3, PAG 0:100:10.0; F-4, PAG 50:50:0.5; F-5, PAG 50:50:5.0; F-6, PAG 50:50:10.0). Fishmeal was freshly prepared from in lab from dried trash fishes mainly F-4, PAG 50:50:0.5; F-5, PAG 50:50:5.0; F-6, PAG 50:50:10.0).

### Table 1: Ingredients composition (%) of feeds for *Clarias batrachus* fingerling.

| Ingredients                        | F1          | F2          | F3          | F4          | F5          | F6          | Control |
|------------------------------------|-------------|-------------|-------------|-------------|-------------|-------------|---------|
| Soybean meal¹                      | 0.0         | 0.0         | 0.0         | 30.4        | 30.4        | 30.4        | -       |
| Silkworm Pupae                     | 20.3        | 20.3        | 20.3        | 10.1        | 10.1        | 10.1        | -       |
| Fish Meal                          | 20.3        | 20.3        | 20.3        | 10.1        | 10.1        | 10.1        | -       |
| Casein²                           | 20.2        | 20.2        | 20.2        | 10.2        | 10.2        | 10.2        | -       |
| Glucosamine (Chitosamine –HCl)³    | 0.5         | 5.0         | 10.0        | 5.0         | 10.0        | -           | -       |
| Starch⁴                           | 32.0        | 27.5        | 22.5        | 32.0        | 27.5        | 22.5        | -       |
| CMC⁵                              | 2.2         | 2.2         | 2.2         | 2.2         | 2.2         | 2.2         | -       |
| Papain⁷                           | 2.0         | 2.0         | 2.0         | 2.0         | 2.0         | 2.0         | -       |
| VM + MM⁸                          | 2.5         | 2.5         | 2.5         | 2.5         | 2.5         | 2.5         | -       |
| Natural -Live food                 | -           | -           | -           | -           | -           | 100.0      | -       |
| Total                              | 100         | 100         | 100         | 100         | 100         | 100         | 100     |

Composition of Protein, carbohydrate, fat and energy of feeds (P:A :: 50:50).

| F1          | F2          | F3          | F4          | F5          | F6          |
|-------------|-------------|-------------|-------------|-------------|-------------|
| Total Protein| 43.52       | 43.52       | 43.52       | 37.39       | 37.39       |
| Carbohydrate| 34.70       | 34.70       | 34.70       | 45.34       | 45.34       |
| Total Fat   | 6.70        | 6.70        | 6.70        | 3.33        | 3.33        |
| GE/ kg      | 3990.48     | 3990.48     | 3990.48     | 3845.1      | 3845.1      |
| KJ/g⁴       | 16.76       | 16.76       | 16.76       | 16.15       | 16.15       |

### Notes:

P/A = Plant Protein : Animal protein ; CMC = Carboxy – methyl – cellulose.¹HiMedia, Mumbai Lot No: 0000013648; ²HiMedia, Mumbai Lot No: 000016171; ³HiMedia, Mumbai, Lot No: 0000028805; ⁴HiMedia, Mumbai, Lot No: 0000028340; ⁵HiMedia, Mumbai, Lot No: 0000014218; ⁶HiMedia, Mumbai, Lot No. 000003862; ⁷Each kg of Vitamin and mineral mixture named Agrimin Forte contains Vit. A 70000 IU, Vit. D 70000 IU, Vit. E 250mg, Nicotinamide 1000mg, Co 150mg, Cu 1200mg, I 325mg, Fe 1500mg, Mg 6000mg, Mn 1500mg, K 100mg, Se 10mg, Na 5.9mg, S 0.72%, Zn 8600mg, Ca 25.5%, P 12.75% Manufacturer Brindavan Phosphates Pvt. Ltd, 48N, doddballpur Ind. Area, Doddaballapur – 561 203, India Batch No. BFA-61
Analytical methods & Statistical analyses of data

Proximate composition of feeds and fish carcasses were analyzed following methods AOAC 1990 [31]. All samples were analysed in triplicate. Dry matter was estimated after drying in oven at 105°C for 24 hours; crude protein (N x 6.25) by the Kjeldahl method after acid digestion; Crude lipid by di-ethyl ether extraction method using Soxhlet apparatus. The performance of the feeds, in terms of the weight gain (%), Specific growth rate (SGR), feed conversion ratio (FCR), Protein efficiency ratio (PER). The growth in length and weight and the survival data were analysed using Two-way ANOVA. Duncan’s multiple Range test was used to determine which treatment means differed significantly (P<0.05) using SPSS version 16.0.

Weight Gain (%) = (Final body weight) – (Initial body weight)/ (Initial body weight)) x 100

Specific Growth Rate (SGR; % day-) = (Final body weight) - (Initial body weight) / (experimental days) x 100

Survival (%) = 100 x (No. of total fish - No. of dead fish)/Number of total fish

Biomass = Final average weight x Total no. of fish

Feed Conversion ratio (FCR) = Feed given (dry weight) / Body weight gain (wet weight).

Results

Various water quality parameters: water temperature, pH and dissolved oxygen (DO), total alkalinity were observed and found to be least affected by different treatment feeds. The values of all the parameters of ambient water, i.e. temperature, pH, DO and alkalinity were almost similar for all the feeding treatments during the experimental period and were well within the optimal range. The water quality recorded for water temp, pH, dissolved oxygen and total alkalinity as 20 - 24°C, 6.8 - 7.5, 6.9 - 7.4 ppm and 130 – 138 ppm, respectively.

The survival and average fish weight gain shown in Table 2 & Table 3 respectively. The survival ranged between 68 - 7.5, 6.9 - 7.4 ppm and 130 – 138 ppm, respectively. The survival and average fish weight gain shown in Table 2 & Table 3 respectively. The survival ranged between 68 - 7.5, 6.9 - 7.4 ppm and 130 – 138 ppm, respectively.

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Table 3: Growth of Clarias batrachus fingerling reared for 12 weeks.

Discussion

In the present study, the experimental feeds were formulation with different protein are based on previous reports [10,32-35]. In the study, the differences observed in the performance of the dietary animal and plant protein feeds in combination with graded level of glucosamine (0.5, 5.0, 10.0%). The experimental feeds F1, F2 & F3 with animal protein along with glucosamine (0.5, 5.0, 10.0), performed better than the plant proteins based feeds F4, F5 & F6. Dietary proteins play a dominant role in fish growth [36-38]. On the basis of average specific growth rate and % live weight gain, an improvement in growth response was noticed with increase in dietary protein level up to maximum of 35% animal protein (casein) content and thereafter a decrease with further increase in dietary protein concentration [39]. The present study showed that different protein types (plant or animal) significantly affected the growth and feed utilization of Asian catfish, Clarias batrachus. The negative effects of weight gain, FCR, PER in response to dietary plant protein suggesting that dietary plant protein type is poorly suitable than animal protein. Similar reports are recorded in Japanese Flounder [10] by using soybean meal more than 16% and, who found that 43% of fishmeal protein could be replaced by soybean meal (25%) in combination with blood meal (10%) or corn gluten meal (10%) in blue murrels meat (5%) [32]. The data in present study on Clarias batrachus indicated that tolerance to animal protein substitution by plant protein in combination with glucosamine was somewhat low. According to, experiment conducted [29] to know the effect of animal protein incorporated formulated feeds on the growth & nutritive value of Rohu fingerlings, the test feeds containing 35% dietary protein level, showed better performance in growth and fertilization than the control feed having only plant protein and also the test feeds having higher protein levels. This infers that the plant feed (GOC) can be replaced by squid meal (an animal protein), which is very much similar to our results. Fish meal has superior nutritive values over other animal proteins [40] and plant proteins [41], because of its well balanced amino acid compositions and their bioavailability as reported in red drum [42], which influenced the performance of animal [43]. On addition of 0.5 glucosamine with animal protein gives better results than 5.0 or 10% glucosamine with animal protein which shows that 0.5% levels of glucosamine good for the health of fish. Similar results have been reported [44] who obtained value of 15% carbohydrate (glucosamine 5.0, 10.0) in the feed showed retardation of growth. Further, the foregoing results agree and extend the findings [45] by showing that silkworm pupae (animal protein), groundnut and wheat bran was better utilized by fingerling Labeo rohita and Cirrhinus mrigala than that of mustard oilcake and rice bran. Prawn shell waste protein is rich in essential amino acids [46,47]. In the present experiment, conducted to know the effect of animal and/or plant protein incorporated with glucosamine (at graded levels of 0.5, 5.0, 10.0), the test feed F3 (100% animal protein with 10.0% glucosamine) indicated that Clarias batrachus has superior nutritive values over other animal proteins [40] and plant proteins [41], because of its well balanced amino acid compositions and their bioavailability as reported in red drum [42], which influenced the performance of animal [43]. On addition of 0.5 glucosamine with animal protein gives better results than 5.0 or 10% glucosamine with animal protein which shows that 0.5% levels of glucosamine good for the health of fish. Similar results have been reported [44] who obtained value of 15% carbohydrate (glucosamine 5.0, 10.0) in the feed showed retardation of growth. Further, the foregoing results agree and extend the findings [45] by showing that silkworm pupae (animal protein), groundnut and wheat bran was better utilized by fingerling Labeo rohita and Cirrhinus mrigala than that of mustard oilcake and rice bran. Prawn shell waste protein is rich in essential amino acids [46,47].
ne) showed better performance in survival and growth than the other feeds containing plant proteins which have anti-nutritional factors and may have cumulative effects on growth performance in longer days feeding trials.

**Conclusion**

Growth performance and feed utilization efficiency of this catfish, fed with animal protein are better than those of plant protein. Results indicate that animal protein rich feeds were much accepted than alternative plant protein sources for the Asian catfish, Clarias batrachus and the potential for replacing animal protein with soybean meal in the feeds of fish need more evaluation along with synergistic effects of growth promoter like glucosamine. Results indicate that animal protein rich feeds with glucosamine were much accepted than natural feeds for Asian catfish, *Clarias batrachus*. The results suggest that the feeding habit of the fish with small crustaceans is met by the addition of glucosamine therefore, it is confirmed that glucosamine has impact on growth promotion in this fish. And the potential for replacing animal protein with soybean meal in the feeds of fish need more evaluation along with synergistic approach of incorporating glucosamine.

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