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 amount by several fishes [19-24], the exact level of its requirement for successful development of aquaculture. Protein is the most expensive ingredient in comparison to other plant ingredient [1]. The nutritional Blended with Glucosamine on Growth and Body Indices of Asian Catfish (Clarias batrachus) Fingerlings ingredient is regarded as an economical and nutritionally rich food

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

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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 (Boomyx 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 was freshly prepared from in lab from dried trash fishes mainly 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 lab from dried trash fishes mainly *Myristus vitatus*, *Puntius sophore*, etc. Live silkworm pupae were procured from Department of Applied Animal Science, Babasaheb Bhimrao Ambedkar University, Raebareilly Road, Lucknow, cultured upto VI<sup>th</sup> Instar larvae & then de-oiled in the lab by di-ethyl-ether (Merck). The de-oiled pupae was dried in oven at 60°C for an hour and powdered and used for feed preparation. The feeds were prepared by thoroughly mixing of the dry ingredients in a mixer and water was added to make stiff dough. Each feed was cooked in a pressure cooker for 15minutes for the proper gelatinization of the ingredients. Finally cooked moist feeds were stored in plastic zipped polybags in a freezer (-20°C) until used. Freshly hatched *Artemia nauplii* is used as the natural feed.

#### Fish and feeding trial

Newly hatched larvae of catfish, *Clarias batrachus* obtained from a single batch of hatchery bred spawned brood stock were used in the experiment after acclimation for one week. In the wet laboratory the experimental fish, *Clarias batrachus* fingerling (av. wt. 2.2 ± 0.009g to 2.6 ± 0.03g) were subsequently segregated and stocked in separate specially designed plastic pool (capacity 300 L, containing 100 L of tap water with continuous aeration), in a groups of 50 fingerling in each pool. The experimental plastic pools were cleaned manually and through siphoning every alternate day in order to remove fecal matter and unutilized feed. Approximately 30-40% volume of chlorine free bore-well water was used to replace the siphoned water on alternate days. The experiment consisted of three replicates for each feed and continued for 84 days. The experimental feeds were hand-fed at the rate of 10% of the total body weight. Each scheduled daily ration per batch of fish was divided into two equal proportions and distributed to the fish at 11:00 hr and 17:00 hr respectively. Initial and subsequent fortnightly weight gains (g) were recorded on electronic balance (make: Sartorius). At the end of the experiment 6-8 fish from each treatment were sacrificed and analyzed for whole body proximate composition (Total N by Micro-kjeldahl method; Total lipid by Soxhlet apparatus). The water quality parameters were water temp, pH, dissolved oxygen and total alkalinity recorded by using the Kit (Merck, Germany).

### Table 1: Ingredients composition (% of feeds for Clarias batrachus Fingerling)

<table>
<thead>
<tr>
<th>Ingredients Composition (%) of feeds (F:A:P:G)</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein</strong></td>
<td>37.39</td>
</tr>
<tr>
<td><strong>Carbohydrate</strong></td>
<td>45.34</td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td>3.33</td>
</tr>
<tr>
<td><strong>GE/ kg</strong></td>
<td>3845.1</td>
</tr>
<tr>
<td><strong>Kg</strong></td>
<td>16.15</td>
</tr>
</tbody>
</table>
| **P**:A :G = Plant Protein : Animal protein : Glucosamine; CMC= Carboxy – methyl – cellulose. 1HiMedia, Mumbai Lot No: 0000013648; ²HiMedia, Mumbai Lot No: 000016171; ²HiMedia, Mumbai, Lot No: 0000028805; ³HiMedia, Mumbai, Lot No: 0000028340; ⁴HiMedia, Mumbai, Lot No. 0000011421; ⁵HiMedia, Mumbai, Lot No. 0000003862; ⁶Each kg of Vitamin and mineral mixture named ‘ Agrimin Forte’ contain’s 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 5.0mg, S 0.72%, Zn 9600mg, Ca 25.5%, P 12.75% Manufacturer Brindavan Phosphates Pvt. Ltd, 48N, doddballapur Ind. Area, Doddballapura – 561 203, India Batch No. BFA-61

### Table 2: Proximate Composition of Asian catfish (Clarias batrachus) fingerlings

- **Ingredients**
  - Fish Meal
  - Soybean meal
  - Fish meal + Silkworm pupae
  - Silkworm pupae
  - Glucosamine (Chitosamine – HCl)
  - Starch
  - CMC
  - Papain
  - VM + MM
  - Natural - Live food
  - Total
  - **F1**
    - Protein 43.52
    - Carbohydrate 34.70
    - Fat 6.70
    - GE/kg 3990.48
    - K.g 16.76
  - **F2**
    - Protein 43.52
    - Carbohydrate 34.70
    - Fat 6.70
    - GE/kg 3990.48
    - K.g 16.76
  - **F3**
    - Protein 43.52
    - Carbohydrate 34.70
    - Fat 6.70
    - GE/kg 3990.48
    - K.g 16.76
  - **F4**
    - Protein 43.52
    - Carbohydrate 34.70
    - Fat 6.70
    - GE/kg 3990.48
    - K.g 16.76
Protein intake are shown in Table 4. The proximate composition of fish results of percent body weight gain, FCR, SGR, PER, Feed intake and case of control the growth recorded as 13.1 ± 1.8 g in 12 weeks. The plant protein group feeding regime (F4 to F6) as 14.9 ± 0.4g . In as 19.9 ± 1.4g whereas best growth was recorded in fish fed F4 among all the feeding trials (F1 to F7). The best growth was recorded in following methods AOAC 1990 [31]. All samples were analysed in triplicate. The survival ranged between 68 ± 5.2 to 88 ± 3.8% (Initial body weight) / (experimental days) x 100.

Weight Gain (%) = [(Final body weight) – (Initial body weight)]/ (Initial body weight)) x 100 Specific Growth Rate (SGR; % day –1) = [(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 ± 2.7 to 88 ± 2.4 % among all the feeding trials (F1 to F7). The best growth was recorded in fish fed F3 among the animal protein group feeding regime (F1 to F3) as 19.9 ± 1.4g whereas best growth was recorded in fish fed F4 among the plant protein group feeding regime (F4 to F6) as 14.9 ± 0.4 g. In case of control the growth recorded as 13.1 ± 1.8 g in 12 weeks. The results of percent body weight gain, FCR, SGR, PER, Feed intake and Protein intake are shown in Table 4. The proximate composition of fish feeding is shown in Table 5. The synergistic growth on supplementing protein and glucosamine showed significant variation (P<0.05) in case of weight gain, FCR, SGR, PER in all the treatments.

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 in 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 for mullet feeds on the growth & nutritive value of Robu 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 squilla 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 mirgala 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% glucosami-
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 acceptable 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 acceptable 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.

Acknowledgement

Authors are grateful to the Director of the institute for providing the facilities to conduct the experiments.

References


Table 4: Growth performance, nutrient utilization in Clarias batrachus fingerling reared for 12 weeks.

<table>
<thead>
<tr>
<th>Feed</th>
<th>Glucos-amine</th>
<th>Animal : Plant Protein Ratio</th>
<th>In wt (g)</th>
<th>4th week wt. gain %</th>
<th>8th week wt. gain %</th>
<th>12th week wt. gain %</th>
<th>FCR</th>
<th>SGR</th>
<th>PER</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>0.5</td>
<td>2.6 ± 0.02 a</td>
<td>94.6 a</td>
<td>365.4 b</td>
<td>615.4 b</td>
<td>1.61 ± 0.01 b</td>
<td>19.0</td>
<td>0.81 ± 0.006 f</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>5.0</td>
<td>2.3 ± 0.01 a</td>
<td>82.6 a</td>
<td>443.5 b</td>
<td>756.5 b</td>
<td>1.56 ± 0.03 c</td>
<td>20.7</td>
<td>0.77 ± 0.01  e</td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>10.0</td>
<td>2.2 ± 0.01 a</td>
<td>136.4 d</td>
<td>463.6 b</td>
<td>804.5 b</td>
<td>1.59 ± 0.02 d</td>
<td>21.1</td>
<td>0.78 ± 0.03  e</td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>5.0</td>
<td>2.2 ± 0.03 b</td>
<td>95.5</td>
<td>503.6 d</td>
<td>856.8 b</td>
<td>1.73 ± 0.01 c</td>
<td>14.8</td>
<td>0.89 ± 0.04  e</td>
<td></td>
</tr>
<tr>
<td>F5</td>
<td>0.5</td>
<td>2.3 ± 0.02 b</td>
<td>108.7</td>
<td>357.6 d</td>
<td>578.7 b</td>
<td>1.78 ± 0.06 d</td>
<td>15.0</td>
<td>0.90 ± 0.01  e</td>
<td></td>
</tr>
<tr>
<td>F6</td>
<td>10.0</td>
<td>2.4 ± 0.01 a</td>
<td>116.7</td>
<td>408.3 b</td>
<td>479.2 b</td>
<td>1.90 ± 0.04 d</td>
<td>13.7</td>
<td>1.00 ± 0.009 g</td>
<td></td>
</tr>
<tr>
<td>F7</td>
<td>-</td>
<td>2.6 ± 0.02 a</td>
<td>65.4 a</td>
<td>219.2 b</td>
<td>403.8 b</td>
<td>1.65 ± 0.02 d</td>
<td>12.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean Values in same column with different superscript letters are significantly different (P < 0.05). Values are mean ± SE of triplicate determinations (n=3). In = Initial weight of fish before feeding; SGR = Specific Growth Ratio; FCR = Feed Conversion Ratio; PER = Protein Efficiency Ratio.

Table 5: Whole body proximate composition (g.100g-1 DM)* and indices of Clarias batrachus fingerling fed feeds containing different proteins for twelve week

<table>
<thead>
<tr>
<th>Parameters (g.100g-1 DM)*</th>
<th>In</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7(Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (Wet wt. basis)</td>
<td>75.6 ± 1.1 b</td>
<td>72.8 ± 2.4 a</td>
<td>74.5 ± 2.3 a</td>
<td>72.7 ± 2.6 a</td>
<td>74.3 ± 1.3 a</td>
<td>75.2 ± 2.6 a</td>
<td>75.4 ± 2.7 a</td>
<td>72.1 ± 3.9 a</td>
</tr>
<tr>
<td>Crude Fat*</td>
<td>6.6 ± 0.4 a</td>
<td>7.5 ± 0.3 a</td>
<td>7.7 ± 0.2 a</td>
<td>8.1 ± 0.3 a</td>
<td>6.4 ± 0.4 a</td>
<td>7.4 ± 0.2 a</td>
<td>7.5 ± 0.2 a</td>
<td>6.9 ± 0.3 a</td>
</tr>
<tr>
<td>Crude Protein*</td>
<td>56.4 ± 1.4 a</td>
<td>57.3 ± 2.5 a</td>
<td>57.2 ± 1.3 a</td>
<td>58.3 ± 2.7 a</td>
<td>55.2 ± 2.1 a</td>
<td>57.4 ± 1.5 a</td>
<td>57.4 ± 2.1 a</td>
<td>58.8 ± 1.4 a</td>
</tr>
<tr>
<td>Dry Matter*</td>
<td>22.3 ± 1.8 a</td>
<td>24.3 ± 1.2 a</td>
<td>24.2 ± 1.4 a</td>
<td>24.2 ± 1.7 a</td>
<td>24.2 ± 1.3 a</td>
<td>24.6 ± 1.3 a</td>
<td>23.5 ± 1.9 a</td>
<td>26.3 ± 1.7 a</td>
</tr>
<tr>
<td>HSI</td>
<td>0.78 ± 0.02 a</td>
<td>0.78 ± 0.09 a</td>
<td>1.05 ± 0.06 a</td>
<td>1.49 ± 0.04 a</td>
<td>1.94 ± 0.19 a</td>
<td>1.34 ± 0.16 a</td>
<td>0.96 ± 0.18 a</td>
<td>0.67 ± 0.03 a</td>
</tr>
<tr>
<td>VSI</td>
<td>1.92 ± 0.1 a</td>
<td>2.60 ± 0.1 a</td>
<td>3.17 ± 0.2 a</td>
<td>1.90 ± 0.2 a</td>
<td>1.94 ± 0.3 a</td>
<td>2.69 ± 0.09 d</td>
<td>4.82 ± 0.07 b</td>
<td>2.25 ± 0.09 a</td>
</tr>
</tbody>
</table>

Mean Values in same period with different superscript letters are significantly different (P < 0.05). HSI= Hepatosomatic index; VSI= Viscerosomatic index.


