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RESEARCH ARTICLE

Partial Replacement of Dietary Animal Protein with Vegetable Protein Blend with Different Proportions of Glucosamine on Growth, Feed Efficiency, Body Composition and Survival of Fingerlings of Asian Catfish (*Clarias batrachus*)

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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* (average weight 2.1–2.6 g). This study was performed to evaluate the effect on fish growth performance by replacing animal protein with a blend of vegetable protein sources. In experiment, six (40.45–43.51 % crude protein, 16.45–16.76 kJ/g energy, and crude lipid 5.01–6.69 %) practical diets were formulated. The animal and plant protein component of the diets was progressively added with glucosamine 0.0 %, 0.5 %, 5.0 % and 10.0 % with fish meal, silkworm pupae, soybean meal (SBM) and casein (F1, PAG 0:100:0.5; F2, PAG 0:100:5.0; F3, PAG 0:100:10.0; F4, PAG 25:75:0.5; F5, PAG 25:75:5.0; F6, PAG 25:75:10.0). The experimental diets were fed to triplicate groups of fingerlings ad libitum and results were compared with control feed (natural 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 18.6, 19.7, 19.9, 16.3, 15.2, 15.6 and 13.1 g in control fed fishes. The best growth among the animal protein group (F1–F3) were recorded as 745.5 %, 838.1 % and 765.2 % respectively. The growth percentage in 25 % replaced feeds (F4, PAG 25:75:0.5; F5, PAG 25:75:5.0; F6, PAG 25:75:10.0), recorded as 579.2 %, 484.6 % and 609.1 % respectively whereas in control it was 469.6 %. The results suggests that the growth is better in total animal protein feeds and the best growth ($p < 0.05$) recorded in the feed incorporated with 10 % glucosamine. The data on growth and feed conversion ratio (FCR) were considered animal protein content of the diet along with glucosamine has better growth performances. The survival was recorded in F1–F6 as 88 %, 83 %, 76 %, 78 %, 80 % and 75 % respectively and in control it is recorded as 68 %. The hepatosomatic and viscerosomatic indices ranged between 0.78–1.51 and 1.9–3.03 respectively in F1 to F6. The feed efficiency in terms of FCR recorded as 2.34–2.95 among all the feeding trials. Results indicate that animal protein rich diets with glucosamine were much acceptable than natural diets for Asian catfish, *C. batrachus* and the potential for replacing animal protein with SBM in the diets of fish need more evaluation along with synergistic approach of incorporating glucosamine. Inclusion of plant protein blend affected growth performance and reduced digestibility, but was not compensated by increased feed intake.

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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 20 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 which contain higher protein content in comparison to other plant ingredient [1]. The nutritional evaluation of SBM 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]. So it is important to accurately determine the protein requirements for each species and size of cultured fish. Level of dietary protein is of fundamental important because it is significantly influences growth, survival and yield of fish as well as economics of a farming industry by determining the feed cost which is typically the largest operational cost. 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, *C. 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 *C. batrachus* fingerling.

Materials and Methods

Fish and Feeding Trial

Newly hatched larvae of catfish, *C. batrachus* obtained from a single batch of hatchery bred spawned broodstock were used in the experiment after acclimation for 1 week. In the wet laboratory the experimental fish, *C. batrachus* fingerling (2.1 ± 0.01 to 2.6 ± 0.02 g) 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 experiment consisted of three replicates for each feed and continued for 84 days. The experimental feeds were hand-fed at 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 and 17:00 h 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 proximate composition of the muscles. The water quality parameters were recorded for water temp, pH, dissolved oxygen (DO) and total alkalinity.

Analytical Methods and Analysis of Data

Proximate composition of feeds and fish carcasses were analyzed following methods [31]. All samples were analysed in triplicate. Dry matter was estimated after drying in oven at 105 °C for 24 h; crude protein (N × 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 One-way ANOVA. Duncan's multiple Range test was used to determine which treatment means differed significantly ($p < 0.05$) using SPSS version 16.0

$$\text{Weight gain}(\%) = \{(\text{Final body weight}) - (\text{Initial body weight})/(\text{Initial body weight})\} \times 100$$

$$\text{Specific growth rate}\left(\text{SGR}, \%\,\text{day}^{-1}\right) = \{(\text{Final body weight}) - (\text{Initial body weight})/(\text{experimental days})\} \times 100$$

$$\text{Survival}(\%) = 100 \times (\text{Number of total fish} - \text{Number of dead fish})/\text{Number of total fish}$$

$$\text{Biomass} = \text{Final average weight} \times \text{Total number of fish}$$

Experimental Feeds and Feed Preparation

Six feeds were prepared by using plant and animal protein in combination with glucosamine source for Asian catfish, *C. 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, SBM and casein (F1, PAG 0:100:0.5; F2, PAG 0:100:5.0; F3, PAG 0:100:10.0; F4, PAG 25:75:0.5; F5, PAG 25:75:5.0; F6, PAG 25:75:10.0. Fishmeal was freshly prepared from in lab from dried trash fishes mainly *Mystus vittatus*, *Puntius sophore*, etc. Live silkworm pupae were procured from Department of Applied Animal Science, Babasaheb Bhimrao Ambedkar University, Rae Bareilly Road, Lucknow, cultured up to VIth Instar larvae and 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 15 min for the proper gelatinization of the ingredients. Finally cooked moist feeds were stored in plastic zipped polybags in a freezer (−20 °C) until used.

Results and Discussion

Various water quality parameters: water temperature, pH and DO, total alkalinity were observed and found to be

Table 1 Ingredients composition (w/w) of feeds for *C. batrachus* fingerling

Ingredients	Feeds						
	F1 PAG 0:100:0.5	F2 PAG 0:100:5.0	F3 PAG 0:100:10.0	F4 PAG 25:75:0.5	F5 PAG 25:75:5.0	F6 PAG 25:75:10.0	Control NATFO
SBM[a]	0.0	0.0	0.0	15.2	15.2	15.2	–
Silkworm pupae	20.3	20.3	20.3	15.2	15.2	15.2	–
Fish meal	20.3	20.3	20.3	15.2	15.2	15.2	–
Casein[b]	20.2	20.2	20.2	15.2	15.2	15.2	–
Glucosamine (chitosamine–HCl)[c]	0.5	5.0	10.0	0.5	5.0	10.0	–
Starch[d]	32.0	27.5	22.5	32.0	27.5	22.5	–
CMC[e]	2.2	2.2	2.2	2.2	2.2	2.2	–
Papain[f]	2.0	2.0	2.0	2.0	2.0	2.0	–
VM + MM[g]	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

PAG plant protein:animal protein:glucosamine, *CMC* carboxy-methyl-cellulose

[a] HiMedia, Mumbai Lot no. 0000013648

[b] HiMedia, Mumbai Lot no. 0000016171

[c] HiMedia, Mumbai, Lot no. 0000028805

[d] HiMedia, Mumbai, Lot no. 0000028340

[e] HiMedia, Mumbai, Lot no. 0000014218

[f] HiMedia, Mumbai, Lot no. 0000003862

[g] Each kg of Vitamin and mineral mixture named 'Agrimin Forte' contains Vit. A 700,000 IU, Vit. D_3 70,000 IU, Vit. E 250 mg, nicotinamide 1,000 mg, Co 150 mg, Cu 1,200 mg, I 325 mg, Fe 1,500 mg, Mg 6,000 mg, Mn 1,500 mg, K 100 mg, Se 10 mg, Na 5.9 mg, S 0.72 %, Zn 9,600 mg, Ca 25.5 %, P 12.75 % Manufacturer Brindavan Phosphates Pvt. Ltd, 48N, Doddaballpur Industrial Area, Doddaballapur—561 203, India Batch no. BFA-61

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, DO 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 Tables 2 and 3 respectively. The survival ranged between 75 ± 5.7 to 88 ± 2.2 among all the feeding trials and in control group as 68 ± 3.5. The best growth was recorded in fish fed F3 among the animal protein group feeding regime (F1–F3) as 19.9 ± 1.1 g whereas best growth was recorded in fish fed F4 among the plant protein group feeding regime (F4–F6) as 16.3 ± 1.3 g. In case of control the growth recorded as 13.1 ± 1.1 g in 12 weeks. The results of FCR, SGR, PER, feed intake and protein intake are shown in Table 4. The proximate composition of fish fingerling are 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, However there is no change ($p > 0.05$) in feed intake and protein intake in all the treatments.

Table 2 Survival percentage of *C. batrachus* fingerlings reared for 12 weeks

Feed	Stocking nos. (N = 50 × 3 replicates)	4th Week	8th Week	12th Week
F1	150	94 ± 2.3^{d}	91 ± 3.2^{d}	88 ± 2.2^{d}
F2	150	89 ± 3.4^{c}	85 ± 4.6^{c}	83 ± 2.4^{c}
F3	150	85 ± 2.7^{b}	80 ± 3.8^{b}	76 ± 2.8^{b}
F4	150	87 ± 42^{b}	83 ± 3.3^{c}	78 ± 4.2^{b}
F5	150	85 ± 3.6^{b}	82 ± 4.1^{c}	80 ± 2.9^{c}
F6	150	83 ± 2.6^{a}	78 ± 3.9^{b}	75 ± 5.7^{b}
F7 (control)	150	78 ± 3.1^{a}	72 ± 3.4^{a}	68 ± 3.5^{a}

Same alphabet in superscript in a column represents no significant difference in weight gain $p = <0.05$. The results are of triplicate sets of feeding trial. Values ± SE

Table 3 Growth of *C. batrachus* fingerling reared for 12 weeks

Feeds	In	4th Week	8th Week	12th Week
F1	2.2 ± 0.02^{a}	5.24 ± 0.3^{b}	12.29 ± 0.4^{c}	18.6 ± 1.1^{c}
F2	2.1 ± 0.01^{a}	6.53 ± 0.2^{c}	14.37 ± 0.3^{d}	19.7 ± 0.8^{c}
F3	2.3 ± 0.01^{a}	6.82 ± 0.3^{c}	14.37 ± 0.2^{d}	19.9 ± 1.1^{c}
F4	2.4 ± 0.03^{b}	4.17 ± 0.1^{b}	12.33 ± 0.1^{c}	16.3 ± 1.3^{b}
F5	2.6 ± 0.02^{b}	4.29 ± 0.2^{b}	11.26 ± 0.3^{b}	15.2 ± 0.8^{b}
F6	2.2 ± 0.01^{a}	4.13 ± 0.3^{b}	11.30 ± 0.6^{b}	15.6 ± 0.7^{b}
F7 (control)	2.3 ± 0.02^{a}	3.49 ± 0.2^{a}	10.33 ± 0.5^{a}	13.1 ± 1.1^{a}

Same alphabet in superscript in a column represents no significant difference in weight gain. $p < 0.05$. The results are of triplicate sets of feeding trial. Values = mean ± SE

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 and F3 with animal protein along with glucosamine (0.5, 5.0, 10.0), performed better than the plant proteins based feeds F4, F5 and F6. Dietary proteins dietary protein plays a dominant role in fish growth [36–38]. On the basis of average SGR 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, *C. 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 SBM more than 16 % and, who found that 43 % of fishmeal protein could be replaced by SBM (25 %) in combination with blood meal (10 %) or corn gluten meal (10 %) in blue murrels meat (5 %) [32]. The data in present study on *C. 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 and 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 protein (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 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 10.0 % 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*

Table 4 Growth performance, nutrient utilization in *C. batrachus* fingerling reared for 12 weeks

Feed	Glucosamine	Animal:plant protein ratio	In wt (g)	4th Week (wt gain %)	8th Week (wt gain %)	12th Week (wt gain %)	FCR (%)	SGR (%)	PER (%)
F1	0.5	100:0	2.2 ± 0.02^a	138.2	458.6	745.5	2.72 ± 0.2^a	79.4	1.45 ± 0.1^a
F2	5.0	100:0	2.1 ± 0.01^a	211.0	584.3	838.1	2.61 ± 0.3^b	85.9	1.56 ± 0.08^b
F3	10.0	100:0	2.3 ± 0.01^a	196.5	524.8	765.2	2.34 ± 0.1^b	112.4	1.66 ± 0.09^b
F4	0.5	75:25	2.4 ± 0.03^a	73.8	413.8	579.2	2.73 ± 0.3^a	76.3	0.99 ± 0.07^d
F5	5.0	75:25	2.6 ± 0.02^a	65.0	333.1	484.6	2.95 ± 0.2^c	66.9	1.06 ± 0.07^d
F6	10.0	75:25	2.2 ± 0.01^a	87.7	413.6	609.1	2.85 ± 0.2^c	69.2	1.19 ± 0.4^a
F7	–	–	2.3 ± 0.02^a	51.7	349.1	469.6	2.11 ± 0.1^d	56.3	–

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 100 g^{-1} DM*) and indices of *C. batrachus* fingerling fed feeds containing different proteins for 12 weeks

Parameters (g 100 g^{-1} DM)*	In wt	F1	F2	F3	F4	F5	F6	F7 (control)
Moisture (wet wt basis)	76.1 ± 2.1^b	74.3 ± 2.0^b	73.8 ± 2.8^a	73.2 ± 2.2^a	75.8 ± 1.8^b	74.7 ± 2.7^b	74.1 ± 2.5^b	71.3 ± 1.9^a
Crude fat*	6.3 ± 0.1^a	7.2 ± 0.5^b	7.3 ± 0.4^b	8.4 ± 0.6^c	6.1 ± 0.3^a	6.3 ± 0.2^a	6.2 ± 0.1^a	6.7 ± 0.2^a
Crude protein*	55.2 ± 1.9^b	58.6 ± 2.7^a	56.3 ± 1.6^b	59.4 ± 2.3^a	54.7 ± 2.2^b	59.3 ± 1.9^a	57.0 ± 2.2^a	58.5 ± 1.6^a
Dry matter*	23.1 ± 1.3^c	25.2 ± 1.8^b	25.4 ± 1.1^b	25.3 ± 1.5^b	23.6 ± 1.4^b	24.3 ± 1.2^b	24.2 ± 1.9^b	27.1 ± 1.6^a
HSI	0.78 ± 0.03^d	0.99 ± 0.06^a	1.23 ± 0.2^c	1.51 ± 0.10^b	1.42 ± 0.15^b	1.22 ± 0.21^c	1.40 ± 0.16^b	0.93 ± 0.08^a
VSI	1.90 ± 0.1^a	2.40 ± 0.2^c	2.56 ± 0.3^c	2.87 ± 0.1^b	3.03 ± 0.2^b	2.66 ± 0.3^c	2.96 ± 0.1^b	2.15 ± 0.1^a

Mean values in same period with different superscript letters are significantly different ($p < 0.05$)

HIS hepatosomatic index, *VSI* viscerosomatic index

* Dry Matter basis explained at the top of the table in parameter row

mrigala than that of mustard oilcake and rice bran. Prawn shell waste protein is rich in essential amino acids [46, 47]. Dietary glucosamine was found to be a growth promoting factor in shrimp [48]. And the shell (chitin) in shrimp waste growth promoting agents for the prawn *Penaeus indicus* [49]. The effect of dietary chitin on the growth and survival of juvenile *P. monodon* was studied by various workers [50, 51]. 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 F1 (100 % animal protein with 0.5 % glucosamine) showed better performance in survival and growth than the other feeds containing plant proteins. Growth performance and feed utilization efficiency of this catfish, fed feeds 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, *C. batrachus* and the potential for replacing animal protein with SBM 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, *C. 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 SBM in the feeds of fish need more evaluation along with synergistic approach of incorporating glucosamine.

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