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Combined effects of graded levels of glucosamine and total replacement of animal protein from plant protein, on the growth of magur(*Clarias batrachus*) fingerling

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ABSTRACT

A 12-week triplicate feeding trials was conducted to assess the use of variousproteins, in combination with glucosamine by asiancat fish, Clarias batrachus (av. wt. 2.2±0.01 to 2.5±0.02 g) fingerling. Six (31.18 to 43.52 % CP, 15.53 to 16.76 kJ/g energy, and crude lipid 0.0 to 6.70%) practical diets were formulated using plant protein (PP) or animal protein (AP) with glucosamine @ 0.0, 0.5, 5.0 and 10.0 % with basal ingredients(F-1, PAG 0:100:0.5; F-2, PAG 0:100:5.0; F-3, PAG 0:100:10.0; F-4, PAG:: 100:0:0.5; F-5, PAG:: 100:0:5.0; F-6, PAG:: 100:0:10.0). The final weight gain was recorded as 21.2±1.4 g, 19.5±0.8 g, 19.6±0.2 g, 18.4±0.7 g, 17.5±0.6 g, 16.9±0.4 g and 13.24±1.2g in control. It is concluded 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 survival was recorded in between 60±4.1% to 73± 2.8%. Results indicate that animal protein rich diets with glucosamine were much acceptable than plant protein and/or natural diets for C. batrachus, however, the growth performances and FCR with PPwere also improved, from control feeding trials, in combination with glucosamine.

Key words: Glucosamine, growth, animal protein, plant protein, *Clarias batrachus*

INTRODUCTION

Feed management determines the viability of aquaculture as it accounts for at least 40-60% of the cost of fish production [1]. The cost of fishmeal is increasing day by day, therefore, alternative protein source to make up for the shortage of fish meal and fulfill the requirement and secure the supply for commercial feed [2].Reducing the feeding costs could be key factor for successful development of aquaculture. In this way soybean meal (SBM) regarded as an economical and nutritionally rich food ingredient which contain higher protein content in comparison to other plant ingredient [3]. The evaluation of soybean meal to replace fishmeal has been a long standing priority in fish nutritional research[4].Considerable success has achieved in supplement of FM with SBM plant proteins in aquatic animals [5,6]. The 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 nitrogen 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-breathing catfish, *Clarias batrachus*(Family: Clariidae), locally known as Magur, is a fish of great demand and attracts the attention of aquaculturists for its high market value. Protein is expensive component in fish feeds hence it is known to require in relatively large amount by several fishes [18-23] 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 [24]. Therefore, it is important to accurately determine the protein requirements for each species and types of the protein which gives better growth performances. 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 [25-27] 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 [28]. Silkworm pupae (*Bombyxmori*) is a low cost animal protein source, rich in both protein and lipid [29]. 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. This experiment was carried out to study the combined effects of dietary glucosamine in combinations with total replacement of animal protein by plant protein on the growthindices and survival of *Clarias batrachus* fingerling and to evaluate further for reducing the feed cost.

MATERIALS AND METHODS

Fish and feeding trial

Fingerling of catfish, *Clarias batrachus* obtained from a single batch of hatchery bred spawned broodstock were used in the experiment after acclimation for one week. In the wet laboratory the experimental fish, *Clarias batrachus* fingerling (av. wt. 2.2$\pm$0.01 to 2.5g$\pm$0.02g) 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 @ 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 proximate composition of the muscles. The water quality parameters were recorded for water temp, pH, dissolved oxygen and total alkalinity.

Analytical methods & analysis of data

Proximate composition of feeds and fish carcasses were analyzed following methods [30]. 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 micro-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.

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

Experimental feeds and feed preparation

Six feeds were prepared by using plant and 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:: 100:0:0.5; F-5, PAG:: 100:0:5.0; F-6, PAG:: 100:0:10.0. Fishmeal was freshly prepared from in lab from dried trash fishes mainly*Mystusvittatus*, *Puntiussophore*, etc. Live silkworm pupae were procured from Department of Applied Animal Science, Babasaheb Bhimrao Ambedkar University, Raebareilly Road, Lucknow, cultured upto VI[th] Instar larvae & then de-oiled in the lab by di-ethyl-ether (Merck). The de-oiled pupae was dried in oven at 60 ºC 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 (-20ºC) until used.

Table-1 Ingredients composition (w/w) of feeds for *Clarias batrachus* fingerling

Ingredients / Feeds	F1 PAG 0:100:0.5	F2 PAG 0:100:5.0	F3 PAG 0:100:10.0	F4 PAG 100:0:0.5	F5 PAG 100:0:5.0	F6 PAG 100:0:10.0	Control NATFO
Soybean meal[1]	0.0	0.0	0.0	60.8	60.8	60.8	-
Silkworm Pupae	20.3	20.3	20.3	0.0	0.0	0.0	-
Fish Meal	20.3	20.3	20.3	0.0	0.0	0.0	-
Casein[2]	20.2	20.2	20.2	0.0	0.0	0.0	-
Glucosamine(Chitosamine –HCl)[3]	0.5	5.0	10.0	0.5	5.0	10.0	-
Starch[4]	32.0	27.5	22.5	32.0	27.5	22.5	-
CMC[5]	2.2	2.2	2.2	2.2	2.2	2.2	-
Papain[6]	2.0	2.0	2.0	2.0	2.0	2.0	-
VM + MM[7]	2.5	2.5	2.5	2.5	2.5	2.5	-
Natural -Live food (NATFO)	-	-	-	-	-		100.0
Total	100	100	100	100	100	100	100

P:A:G= Plant Protein : Animal protein : Glucosamine; CMC= Carboxy – methyl – cellulose. [1]HiMedia, Mumbai Lot No: 0000013648; [2]HiMedia, Mumbai Lot No: 0000016171; [3]HiMedia, Mumbai, Lot No: 0000028805; [4]HiMedia, Mumbai, Lot No: 0000028340; [5]HiMedia, Mumbai, Lot No. 0000014218; [6]HiMedia, Mumbai, Lot No. 0000003862; [7]Vitamin and mineral mixture 'Agrimin Forte' Manufacturer Brindavan Phosphates Pvt. Ltd

RESULTS

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 proximate composition of feed, survival and average fish weight gain shown in Fig 1 to 4. The survival ranged between 60$\pm$ 4.1to 73 $\pm$ 2.8 % among all the feeding trials (F1 to F7). The best growth was recorded in fish fed F1 among the animal protein group feeding regime (F1 to F3) as 21.2±1.4g whereas best growth was recorded in fish fed F4 among the plant protein group feeding regime (F4 to F6, Table 2) as 18.4±0.7g. In case of control the growth recorded as 13.24±1.2g in 12 weeks. The results of percent body weight gain, FCR, SGR, PER, feed intake and Protein intake are shown in Fig.5 to 8, Table 3. The proximate compositions of fish fingerling are shown in Fig. 9&10, Table 4. 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. The hepatosomatic and viscerosomatic indices ranged between 0.74$\pm$ 0.03 to 1.38$\pm$0.09 and 1.68$\pm$0.01 to 3.42$\pm$ 0.2 respectively in F1 to F7 (Fig. 11&12, Table 4).

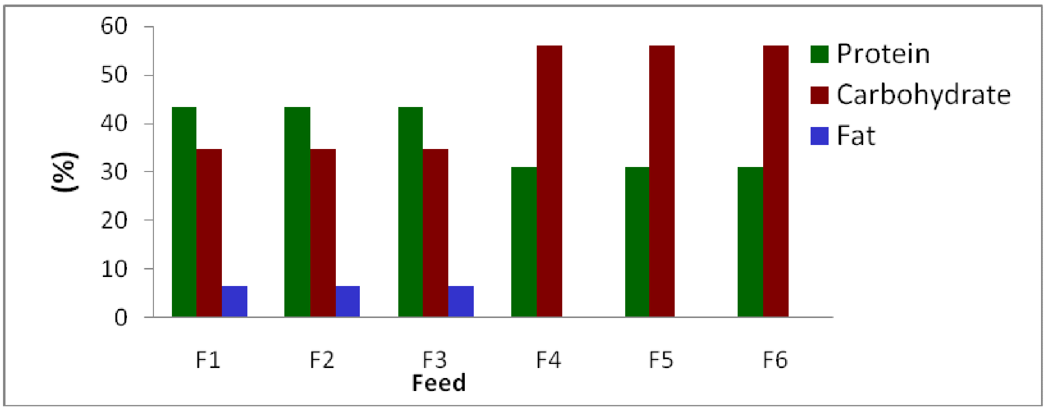


Figure 1

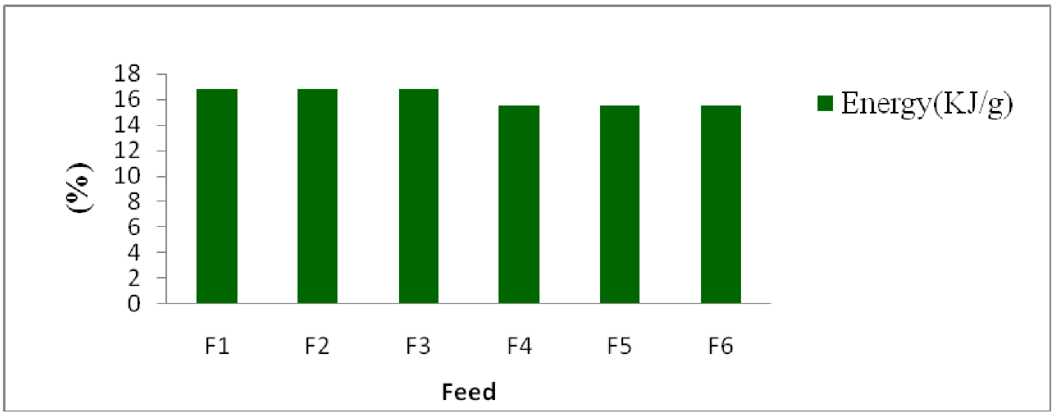
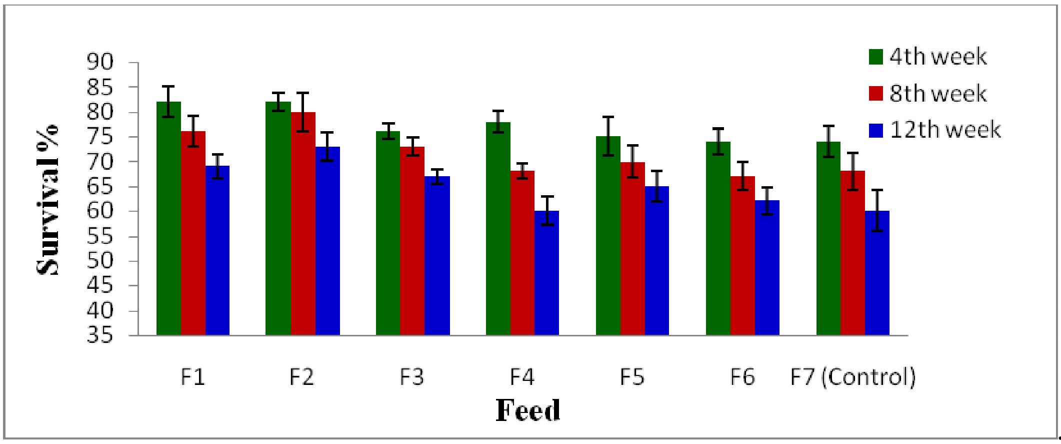


Figure 2



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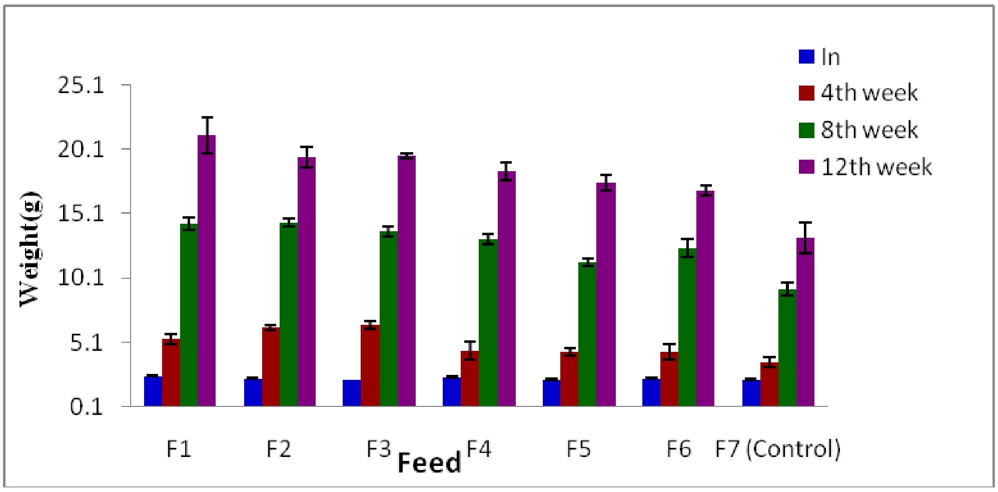

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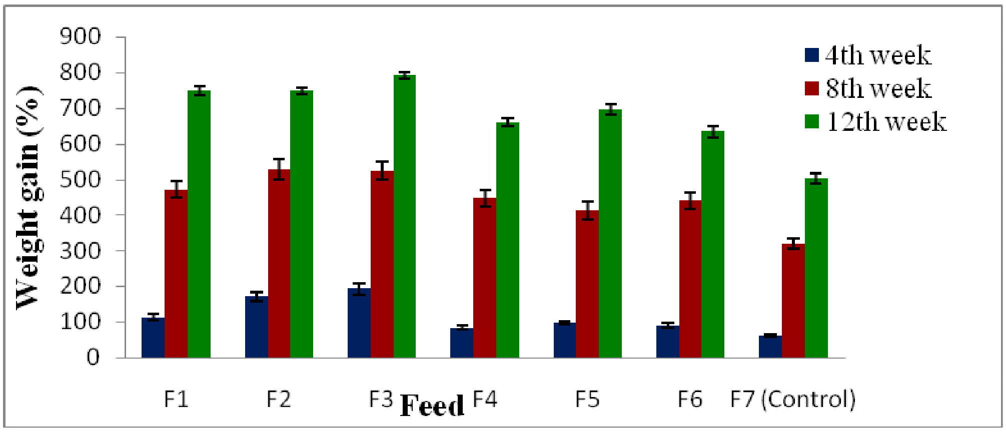

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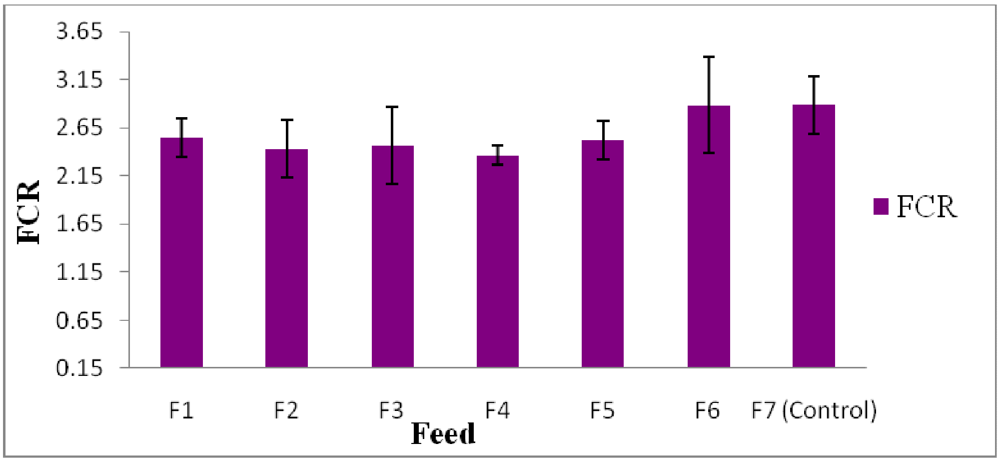

Figure 6

Table – 2 Growth of *Clarias batrachus* fingerling reared for 12 weeks fed with P:A:: 100:0

Feeds	In	4th week	8th week	12th week
F1	2.5±0.02[a]	5.32±0.4[d]	14.28±0.5[d]	21.2±1.4[d]
F2	2.3±0.03[a]	6.23±0.2[c]	14.45±0.3[d]	19.5±0.8[c]
F3	2.2±0.01[a]	6.44±0.3[c]	13.72±0.4[c]	19.6±0.2[c]
F4	2.4±0.03[b]	4.42±0.7[b]	13.12±0.4[c]	18.4±0.7[c]
F5	2.2±0.02[b]	4.34±0.3[b]	11.28±0.3[b]	17.5±0.6[b]
F6	2.3±0.02[a]	4.38±0.6[b]	12.45±0.7[b]	16.9±0.4[b]
F7 (control)	2.2±0.03[a]	3.55±0.4[a]	9.23±0.5[a]	13.24±1.2[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

Table -3 Growth performance, nutrient utilization in *Clarias batrachus* fingerling reared for 12 weeks fed with P:A:: 100:0

Feed	Glucos-amine	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	2.5±0.02[a]	112.8±8.2[d]	471.2±23.4[b]	748.0±12.3[a]	2.55±0.2[b]	78.9	1.20±0.1[a]
F2	5.0	100:0	2.3±0.03[a]	170.9±11.5[c]	528.3±28.9[c]	747.8±9.5[d]	2.43±0.3[b]	75.6	1.49±0.2[c]
F3	10.0	100:0	2.2±0.01[a]	192.7±16.9[c]	523.6±24.8[c]	790.9±7.9[d]	2.46±0.4[c]	102.6	1.47±0.2[c]
F4	0.5	0:100	2.4±0.03[b]	84.2±5.2[b]	446.7±22.1[b]	666.7±10.2[b]	2.36±0.1[c]	76.2	0.96±0.3[b]
F5	5.0	0:100	2.2±0.02[b]	97.3±4.6[b]	412.7±26.4[b]	695.5±13.5[a]	2.52±0.2[b]	64.8	1.14±0.2[a]
F6	10.0	0:100	2.3±0.02[a]	90.4±6.7[b]	441.3±23.4[b]	634.8±16.3[a]	2.88±0.5[a]	70.7	1.17±0.1[a]
F7	-	-	2.2±0.03[a]	61.4±2.9[a]	319.5±15.2[a]	501.8±14.6[a]	2.89±0.3[a]	60.3	-

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

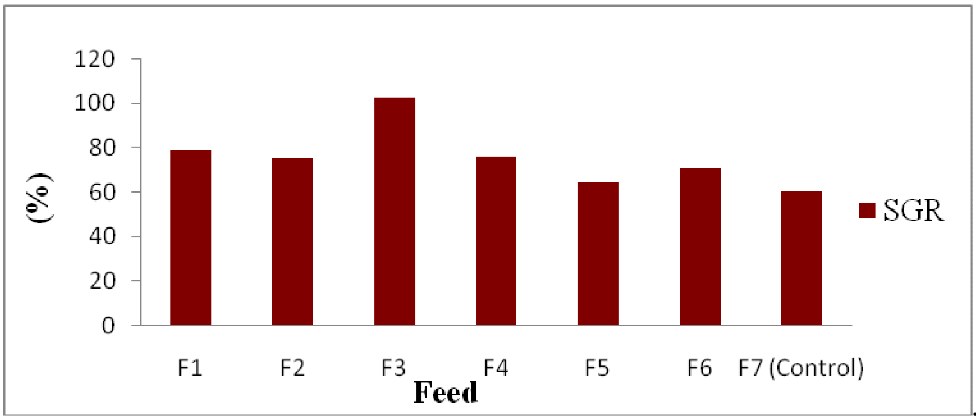

Figure 7

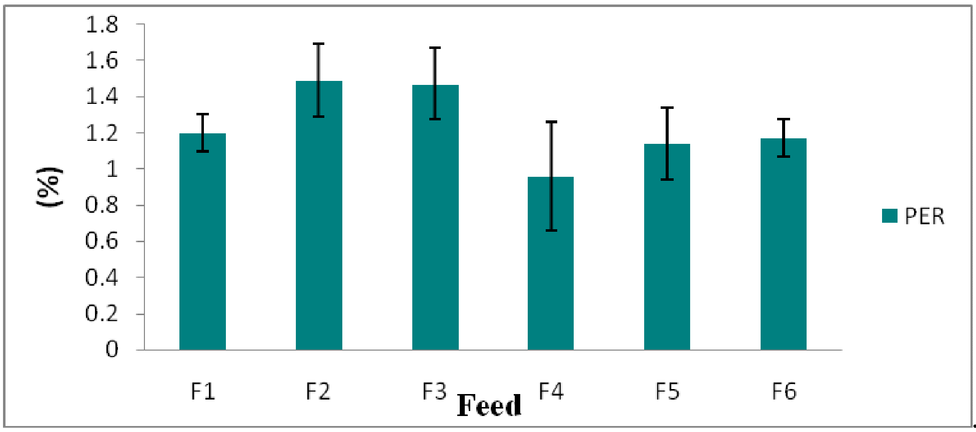

Figure 8

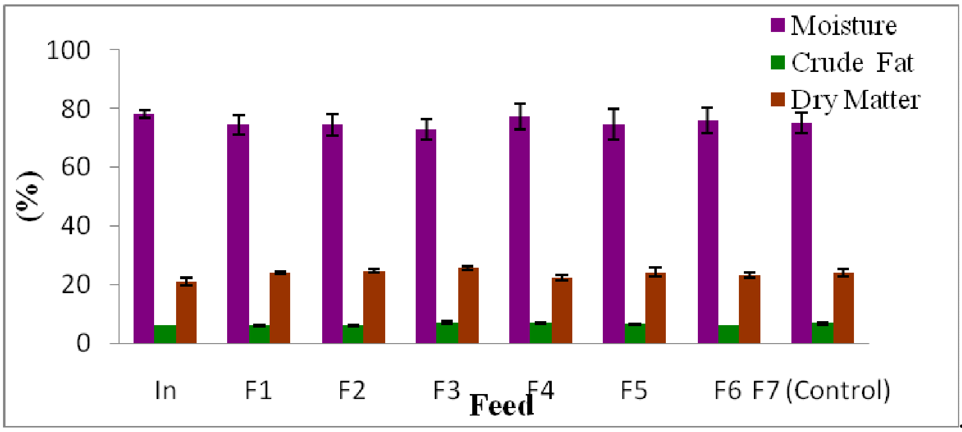

Figure 9

Table -4 Whole body proximate composition (g.100g^{-1} DM*) and indices of *Clarias batrachus* fingerling fed feeds containing different proteins for twelve week fed with P:A:: 100:0

Parameters (g.100g^{-1} DM)*	In W_t	F1	F2	F3	F4	F5	F6	F7(Control)
Moisture (Wet wt. basis)	78.2±1.2^{b}	74.5± 3.1^{a}	74.6± 3.7^{a}	72.9± 3.4^{c}	77.2± 4.3^{b}	74.6± 5.1^{a}	75.9± 4.3^{a}	75.2 ± 3.4^{a}
Crude Fat*	6.2 ±0.1^{a}	5.9 ± 0.3^{b}	6.1 ± 0.2^{b}	7.2 ± 0.4^{c}	6.8 ± 0.2^{a}	6.4 ± 0.2^{a}	5.9 ± 0.1^{b}	6.8 ± 0.4^{a}
Crude Protein*	53.8 ±1.6^{a}	57.6 ± 2.2^{b}	54.9± 2.0^{a}	56.9 ± 2.1^{b}	54.6 ± 1.8^{a}	57.9 ± 1.2^{b}	55.7 ± 1.4^{a}	53.7 ± 1.6^{a}
Dry Matter*	21.2 ±1.2^{b}	24.1 ± 0.5^{a}	24.6± 0.6^{a}	25.7 ± 0.8^{c}	22.3 ± 0.9^{b}	24.2 ± 1.5^{a}	23.2 ± 1.0^{a}	24.1 ± 1.4^{a}
HSI	0.74±0.03^{d}	0.93±0.07^{c}	1.10±0.2^{c}	1.33±0.09^{b}	1.29±0.14^{a}	1.38±0.09^{b}	1.26±0.10^{a}	1.23 ± 0.10^{a}
VSI	1.68 ± 0.1^{d}	2.09 ± 0.2^{a}	2.23± 0.4^{a}	2.37 ± 0.3^{a}	3.42 ± 0.2^{c}	2.56 ± 0.2^{b}	2.42 ± 0.3^{b}	2.27 ± 0.1^{a}

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

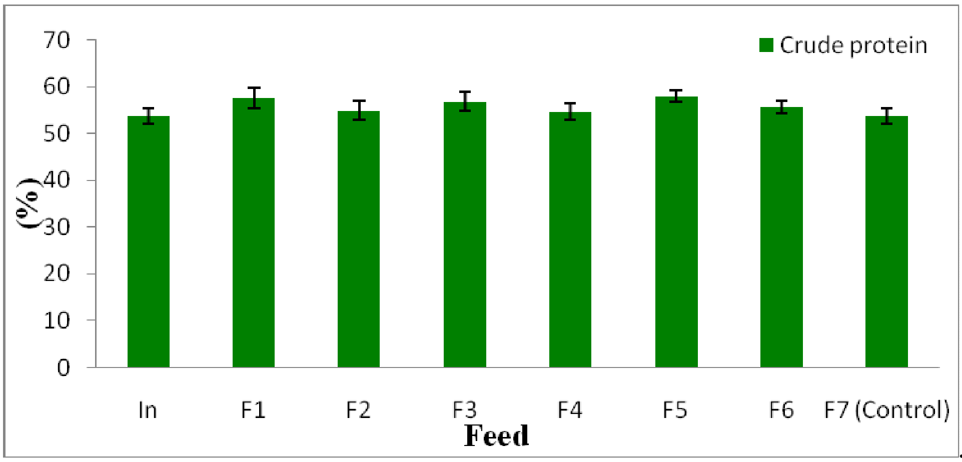

Figure 10

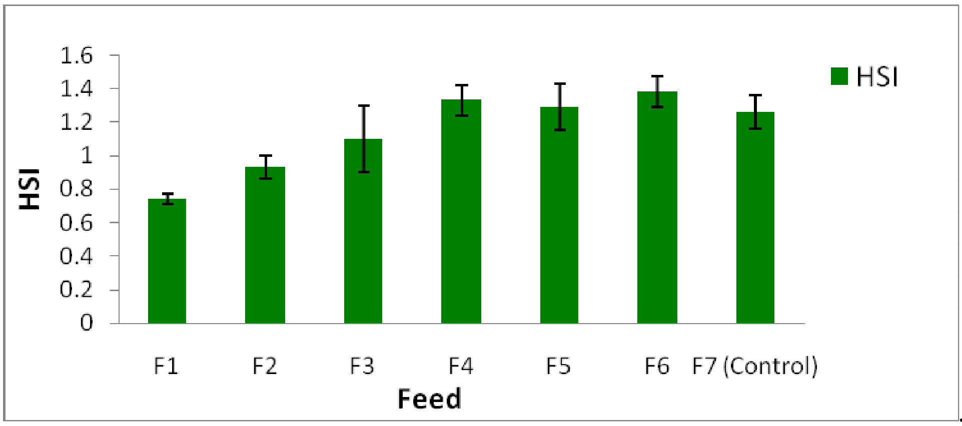

Figure 11

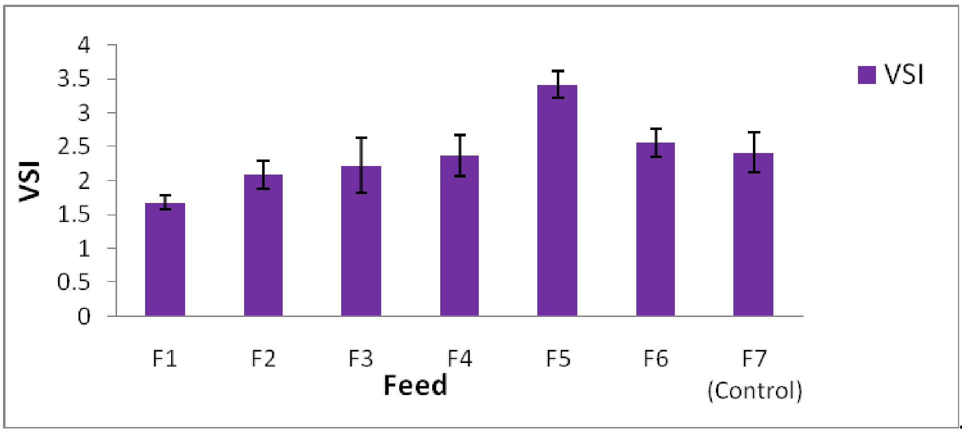

Figure 12

DISCUSSION

In the present study, the experimental feeds were formulated with different proteinswerebased on previous reports [10,31-34]. 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 dietary protein plays a dominant role in fish growth [35-37].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 content and thereafter a decrease with further increase in dietary protein concentration [38]. 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 bloodmealand/ or corn gluten meal 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 [28] experiment conducted to know the effect of animal protein incorporated formulated feeds on the growth andnutritive 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, which is very much similar to our results. Fish meal has superior nutritive values over other animal proteins [39] and plant proteins [40]because of its well-balanced amino acid compositions and their bioavailability in red drum [41], which influenced the performance of animal [42]. 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 [43] 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 [44] by showing that silkworm pupae, groundnut and wheat bran was better utilized by fingerling *L.rohita* and *C.mrigala*than that of mustard oilcake and rice bran. Prawn shell waste protein is rich in essential amino acids [45,46]. Dietary glucosamine was found to be a growth promoting factor in shrimp [47]. And the shell (chitin) in shrimp waste growth promoting agents for the prawn *P.indicus*[48]. The effect of dietary chitin on the growth and survival of juvenile *P. monodon* was studied by various workers [49,50]. In the present experiment, conducted to know the effect of animal and/or plant protein incorporated with different 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. In 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 equallyacceptable than natural feeds for Asian catfish, *Clarias batrachus*.

CONCLUSION

The results suggest that,since the feeding habit of the fish with insects, small crustaceans etc. is met by the addition of glucosamine therefore, it is confirmed that glucosaminehas impact on growth promotion in this fish. However, the potential for replacing animal protein with soybean meal in the feeds of this fish need more evaluation along with synergistic approach of incorporating glucosamine.

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