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Growth responses on major replacement of animal protein with plant protein and graded levels of dietary supplement amino sugar, glucosamine in threatened Magur (*Clarias batrachus*, Linnaeus, 1758) fry

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ABSTRACT

A 12-week feeding trial was performed to evaluate the use of animal and plant protein, in combination with dietary supplement glucosamine (GlcN) an amino sugar and a prominent precursor in the biochemical synthesis of glycosylated proteins and lipids on threatened Asian catfish fry, *Clarias batrachus* fry (av. wt. 0.23 ± 0.02 to 0.25 ± 0.03g). Six practical feeds (CP 34.27 to 43.52 %, kcal/100g 377.1 to 399, and total fat 1.67 to 6.70%) were formulated on the basis of whole animal or animal-plant combined protein feeds blended with GlcN at 0.5, 5.0 and 10.0%. The six-feeds were prepared by blending with GlcN (F-1, PAG::0:100:0.5; F-2, PAG::0:100:5.0; F-3, PAG::0:100:10.0; F-4, PAG::75:25:0.5; F-5, PAG::75:25:5.0; F-6, PAG::75:25:10.0). The best growth performance was recorded in fish fed F-2 feed among the animal protein group feeding regime (F-1 to F-3) as 0.56±0.04g followed by F-3 (0.46±0.02g) and F-1 (0.38±0.02g). The survival ranged with 60±4.3 to 70±2.4% in animal protein rich diets whereas, the survival range recorded as 50±2.4 to 53±2.4% in plant protein rich diets F-4, F-5 and F-6 in comparison to control feed(F-7) showing 43±1.4% survival. The survival significantly ($p<0.05$) improved in GlcN supplemented feeds (50±2.4 to 70±2.4%) in comparison to control (43±1.4%). The synergistic growth on supplementing proteins and GlcN showed significant variation ($p<0.05$) in case of weight gain, FCR, SGR and PER. Results suggests that animal protein feeds were much acceptable than plant protein diets by *C. batrachus* fry and the dietary supplement amino sugar GlcN showed a significant role in survival and growth of the fish at early stage of life.

1. INTRODUCTION

Feedstuffs of animal origins are in general considered alternative protein sources because their protein context is higher and their supplements of indispensable amino acids is better to those of plant origin [1]. Fishmeal has been substituted by one type of animal protein sources such as maggot meal [2], black soldier fly pupae meal [3], poultry by-product meal [4], poultry viscera meal [5], and feather meal [6]. The major part of the world's animal food protein comes from the fish; hence it is essential to secure fish health in rearing systems [61, 62, 63]. The successful fish culture requires use of efficient feed with optimum composition and output. When fish are raised in closed systems or in high density ponds, they must be provided with a better diet, complete and nutrient balanced, allowing rapid fish growth and good health. Thus, the complete fish feed is essential to provide all essential nutritional components like carbohydrates, lipids, proteins, vitamins and minerals [64, 65]. Substantial research efforts have been made in the recent past towards replacement of fish meal by sustainable alternative plant ingredients sources. The suitability of this nutrient replacement, in terms of survival, and growth performance is highly variable among fish species and rearing conditions. Available knowledge shows that a sensible blending of different plant proteins is needed to balance the indispensable amino acid

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profile in order to minimize the requirement of amino acid supplementation in a balanced diet [49, 50,51,52]. It has been experimented that dietary incorporation of soymeal, groundnut cake and winged bean improved the growth performance, feed intake and feed efficiency of *Clarias gariepinus* [53,54,55,56,57]. The level and balance of amino acids found in soy protein are considered to be the most appropriate plant proteins, although low in methionine level [58,59]. Soybean meal is currently the very commonly used plant source protein source in fish feeds and comprises up to 50% of the aquaculture diet[60] though other sources of cheaper plant protein sources such as cottonseed meal, sunflower meal, linseed, rapeseed meal, sorghum, corn meal, mustard, sesame, copra, leguminous seeds and leucaena have been advocated in view of reducing feed cost[6,7,8,9,10,11,49]. Up to 80% of fishmeal protein was replaced by a combination of processed blood meal and meat meal (4:1) with no bad effect on growth, survival, or efficiency in *Epinephelus coioides* [12]. In general, higher substitutions of plant source protein as a complete replacement for fishmeal protein have resulted in poor growth and feed efficiency in fish particularly those requiring animal protein as a must [13,14,15,16,17,18] reported that incorporation of plant protein by replacing fishmeal did not have a significant effect on the whole body composition of Indian major carps, *L. rohita*, *C. catla* and *C. mrigala*. Similarly, Pongmaneerat & Watanabe[19] reported that soybean meal as the sole protein source did not greatly influence the body protein content of carps. Hence, the dietary requirement of protein is different in various fish species [20,21,22,23,24] reported that partial replacement of FM protein with SBM protein in tilapia diet did not compromise growth of the fish. Substitution of various soy products have shown better growth in many fishes including catfishes [8,25,26,27]. Recently, Sarowar *et al*. [28] have studied the impacts of different diets on survival and growth in *C. batrachus* grow-outs. *C. batrachus*, a native to Asia, is one of the most popular fish of aquaculture and aquarium trade among the Asian species [29, 92]. *Clarias batrachus*, belonging to family Clariidae. It is now called *Clarias magur*, is an air-breathing threatened [30], endangered [31] catfish. It is a promising aquaculture candidate species owing to its good flavour, medicinal importance, growth, hardiness, efficient food conversion efficiency, excellent nutritional profile and high market values [32,33,34]. The objective of the present study was to evaluate and assess the effect of major replacement of animal protein with soybean meal in diet formulations of *C. batrachus* fry in captive rearing conditions with the support blending of glucosaimine (GlcN). The assessment criteria included for the study were the key nutritional parameters such as growth, survival, feed utilization and carcass composition.

2. MATERIALS AND METHODS

2.1. Experimental feeds and preparation

Six feeds were prepared by using plant & animal protein in combination with GlcN source for Asian catfish, *Clarias batrachus*. The feed ingredients and proximate composition of the experimental feeds are given in Table - 1. The natural live feed serves as control. In the experiment, six (34.27 to 43.52 % crude protein, 377.1 to 399 kcal/100g, and crude lipid 1.67 to 6.70%) practical feeds were formulated and their composition is given in Table - 2, Fig. 1 & 2. The animal and plant protein component of the feeds was progressively added with GlcN 0.0, 0.5, 5.0 and 10.0 % with basic ingredients like silkworm pupae, fish meal, casein and soybean meal (F-1, PAG::0:100:0.5; F-2, PAG::0:100:5.0; F-3, PAG::0:100:10.0; F-4, PAG::75:25:0.5; F-5, PAG::75:25:5.0; F-6, PAG::75:25:10.0). Fishmeal was freshly prepared from in lab from dried trash fishes mainly *Puntius sophore*, *Mystus vittatus*, etc. Live silkworm pupae were procured from Department of Applied Animal Science, Babasaheb Bhimrao Ambedkar University, Raebareilly Road, Lucknow, cultured upto VIth 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 mixing of the dry ingredients in a mixer and water was added to make hard dough. Each feed was pressure cooked for 15 minutes for the proper gelatinization of the ingredients. Finally the pressure cooked moist feeds were stored in plastic bags in a deep freezer (-20ºC) until used.

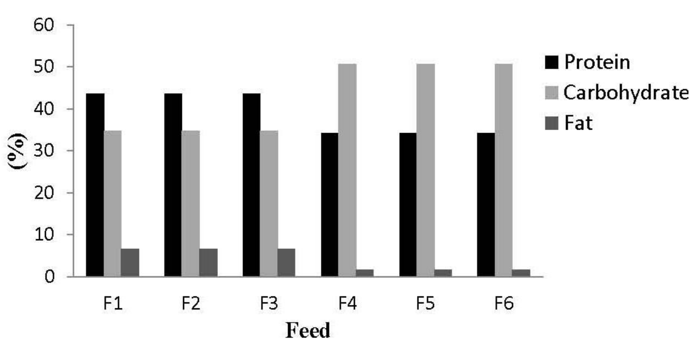

Fig.1: Protein, Carbohydrate and Fat contents of feeds.

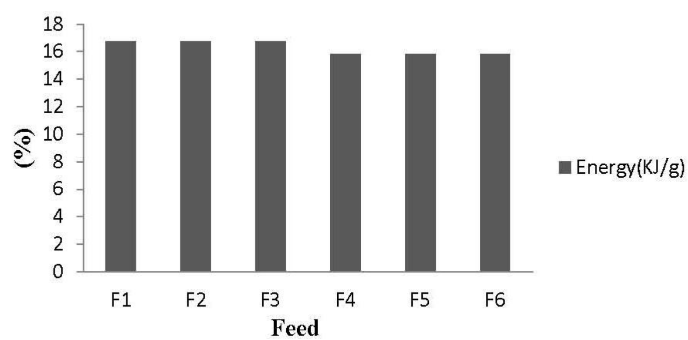

Fig. 2: Gross energy (kJ/g) contents of feeds.

Table 1: Ingredients composition (w/w) of feeds for *Clarias batrachus* Fry.

Feeds Ingredients	F1 PAG 0:100:0.5	F2 PAG 0:100:5.0	F3 PAG 0:100:10.0	F4 PAG 75:25:0.5	F5 PAG 75:25:5.0	F6 PAG 75:25:10.0	Control NATFO
Soybean meal[1]	0.0	0.0	0.0	45.6	45.6	45.6	-
Silkworm Pupae	20.3	20.3	20.3	5.06	5.06	5.06	-
Fish Meal	20.3	20.3	20.3	5.07	5.07	5.07	-
Casein[2]	20.2	20.2	20.2	5.07	5.07	5.07	-
GlcN (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	-	-	-	-	-		100.0
Total	100	100	100	100	100	100	100

P:A:G= Plant Protein : Animal protein : glucosamine (GlcN); 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]Each kg of Vitamin and mineral mixture named 'Agrimin Forte' contains Vit. A 700000 IU, Vit. D_3 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 9600mg, Ca 25.5%, P 12.75% Manufacturer Brindavan Phosphates Pvt. Ltd,

Table 2: Calculated values of Protein, carbohydrate, fat and energy composition of feeds.

	F1	F2	F3	F4	F5	F6
Total Protein	43.52	43.52	43.52	34.27	34.27	34.27
Carbohydrate	34.70	34.70	34.70	50.66	50.66	50.66
Total Fat	6.70	6.70	6.70	1.67	1.67	1.67
GE/ kg (K.Cal)	3990.48	3990.48	3990.48	3771.5	3771.5	3771.5
$KJ.g^{-1}$	16.76	16.76	16.76	15.84	15.84	15.84

2.2. Fish and Feeding Trial

Newly hatched larvae of catfish, *Clarias batrachus* took from a one batch of hatchery bred spawned brooders were used in the experiment after acclimatization for 7 days. In the wet laboratory, the experimental fish, *C. batrachus* fry (av. wt. 0.23 $\pm$ 0.02 to 0.25 $\pm$ 0.03g) were subsequently segregated and stocked in separate specially designed plastic pool (cap. 300 l, containing 50 l of bore water with 24 hour aeration), in a groups of 100 fry in every pool. The experiment consisted of two replications for each feed and continued for 84 days. 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. Initial and subsequent fortnight weight gains (g) were observed and recorded on electronic balance (make: Sartorius). At the end of the experimentation, 6-8 fishes 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.

2.3. Analytical methods & Statistical analyses of data

Proximate compositions of feeds and fish carcasses were analyzed following AOAC methods[35] 1990. 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 growth performance of the feeds, in terms of the weight gain (%), Specific growth rate (SGR), feed conversion ratio (FCR), Protein efficiency ratio (PER) were calculated using the following equations. 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 mean differed significantly (P<0.05) using SPSS version 16.0.

Weight Gain (%) = {(Final body weight) – (Initial body weight)/ (Initial body weight)}x100

Specific Growth Rate (SGR; %day-1)={(Final body weight)-(Initial body weight)/(experimental days)}x100

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).

3. RESULTS

Various water quality parameters: water temperature, pH and dissolved oxygen (DO), total alkalinity were observed and found to be least affected by different feeds. The values of all the parameters of water, i.e. temperature, pH, alkalinity and DO were almost similar for all the feeding treatments during the experimental period and were well within the range. The water quality recorded for water temp, dissolved oxygen, pH and alkalinity as 20 - 24 °C, 6.8 - 7.5, 6.9 - 7.4 ppm and 130 – 138 ppm, respectively (APHA[95].

The survival and average fish weight gain shown graphically in Table-3 & Table-4 and Fig. 3 & 4 respectively. The best growth was recorded in fish fed F2 among the animal protein group feeding regime (F1 to F3) as 0.56$\pm$0.04g followed by F3 0.46$\pm$0.02g and F1 0.38$\pm$0.02g. The survival range 60$\pm$4.3 to 70$\pm$2.4%. The survival range recorded as 50$\pm$2.4 to 53$\pm$2.4% in

F4, F5 and F6. The control feed showing 43 ± 1.4% survival. The growth of the fry was recorded better in animal protein fed fishes but there is no sign of dose-dependent changes on growth performances.

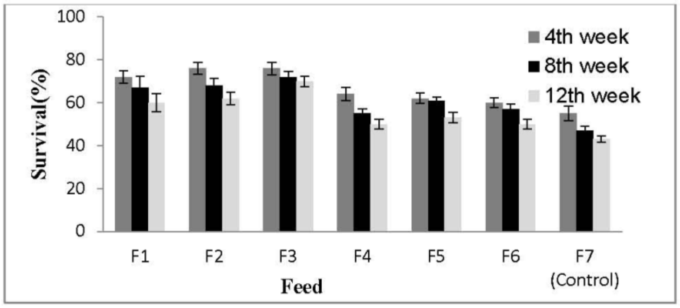

Fig. 3: Survival percentage of fishes till 12th week.

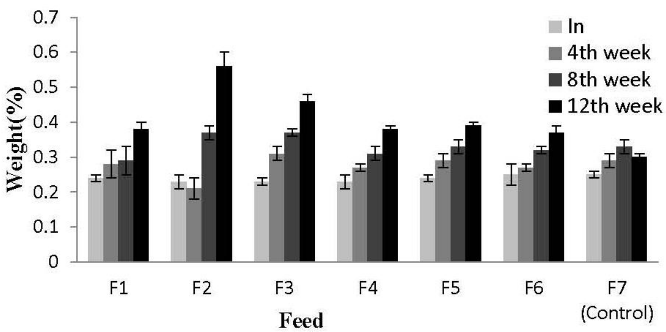

Fig. 4: Weight gain percentage of fishes till 12th week.

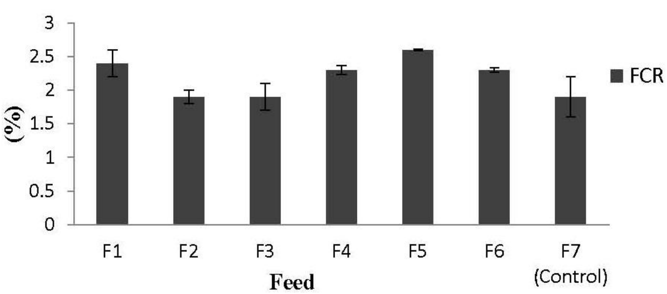

Fig. 5: Feed Conversion Ratio (FCR).

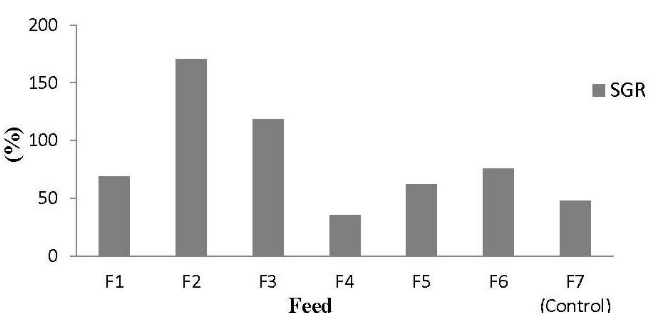

Fig. 6: Specific Growth Ratio (SGR).

The best growth recorded in F2 which contains 5.0% GlcN with 100% animal protein. The plant protein rich diets exhibited poor growth performance in comparison to animal protein rich diets. The control recorded poor growth after 84 day experimental period. The results of FCR, SGR, PER, Feed intake and Protein intake are shown in Table-5, and FCR, SGR, PER in Fig. 5, 6 and 7. The proximate composition is depicted in Table -6, Fig. 08 & 09.

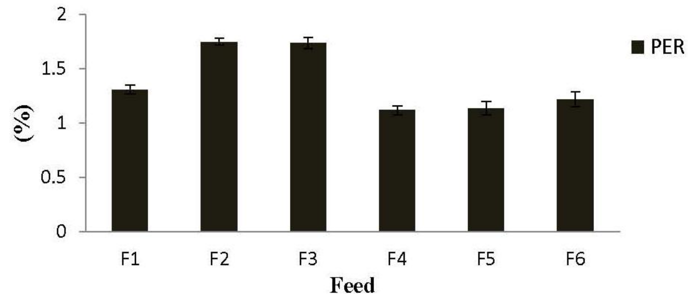

Fig. 7: Protein Efficiency Ratio (PER).

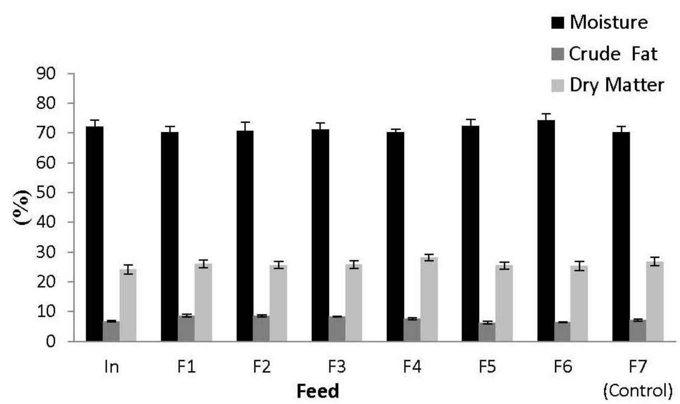

Fig. 8: Moisture, Crude Fat and Dry matter of fish carcass.

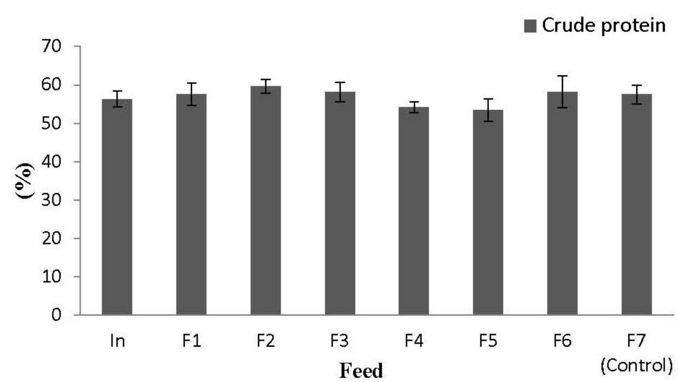

Fig. 9: Crude protein of fish carcass after 12th week.

The synergistic growth on supplementing protein and GlcN 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 3: Survival Percentage of *Clarias batrachus* fry reared for 12 weeks.

Feed	Stocking Nos. (N=100 X 2 replicates)	4th Week	8th Week	12th Week
F-1	200	72± 2.8^{a}	67± 5.2^{a}	60± 4.3^{b}
F-2	200	76 ± 2.7^{a}	68 ± 3.2^{b}	62± 2.9^{c}
F-3	200	76± 2.9^{a}	72 ± 2.7^{b}	70 ± 2.4^{b}
F-4	200	64± 3.0^{a}	55± 2.2^{b}	50± 2.4^{b}
F-5	200	62± 2.4^{a}	61± 1.5^{b}	53± 2.4^{b}
F-6	200	60± 2.4^{a}	57± 2.3^{b}	50± 2.4^{c}
F-7 (control)	200	55 ± 3.3^{a}	47± 2.1^{b}	43± 1.4^{b}

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

Table 4: Growth of *Clarias batrachus* fry reared for 12 weeks.

Feeds	In	4th week	8th week	12th week
F1	0.24±0.01^{a}	0.28±0.04^{a}	0.29±0.04^{a}	0.38±0.02^{c}
F2	0.23±0.02^{a}	0.21±0.03^{b}	0.37±0.02^{b}	0.56±0.04^{c}
F3	0.23±0.01^{a}	0.31±0.02^{a}	0.37±0.01^{b}	0.46±0.02^{d}
F4	0.23±0.02^{a}	0.27±0.01^{a}	0.31±0.02^{a}	0.38±0.01^{c}
F5	0.24±0.01^{a}	0.29±0.02^{a}	0.33±0.02^{b}	0.39±0.01^{c}
F6	0.25±0.03^{a}	0.27±0.01^{a}	0.32±0.01^{c}	0.37±0.02^{d}
F7 (control)	0.25±0.01^{a}	0.29±0.02^{a}	0.33±0.02^{c}	0.30±0.01^{b}

Same alphabet in superscript in a column represents no significant difference in weight gain. *= p< 0.05. The results are of duplicate sets (n =2) of feeding trial. Values = mean± SE

Table 5: Growth performance, nutrient utilization in *Clarias batrachus* fry reared for 12 weeks.

Feed	Dietary Glucosa-mine	Plant: Animal Protein Ratio	In wt (g)	4th week wt. gain % (up to 4 wk)	8th week wt. gain % (up to 8 wk)	12th week wt. gain % (up to12 wk)	FCR	SGR%	PER %	Feed intake (mg)	Protein intake (mg)
F1	0.5	100:0	0.24±0.01^{a}	16.6±1.2^{a}	20.8±1.3^{a}	58.3± 4.7^{a}	2.4±0.2^{a}	69.11	1.31±0.04^{a}	342±20^{a}	106.0±4^{a}
F2	5.0	100:0	0.23±0.02^{a}	8.7±0.9^{b}	60.8±4.5^{b}	143.5± 8.3^{b}	1.9±0.1^{b}	170.5	1.75±0.03^{b}	600±31^{b}	187.6±11^{b}
F3	10.0	100:0	0.23±0.01^{a}	34.78±3.1^{c}	60.6±3.1^{b}	100.1±7.6^{c}	1.9±0.2^{b}	118.8	1.74±0.05^{b}	420±18^{c}	130.9±8^{c}
F4	0.5	75:25	0.23±0.02^{a}	17.4±0.04^{a}	34.8±0.07^{c}	65.2±0.02^{a}	2.3±0.07^{a}	35.29	1.12±0.04^{c}	132±11^{d}	72.1±4^{d}
F5	5.0	75:25	0.24±0.01^{a}	20.8±0.03^{a}	37.5±0.04^{c}	62.5±0.03^{a}	2.6±0.1^{a}	62.34	1.14±0.06^{c}	218±19^{e}	83.0±4^{e}
F6	10.0	75:25	0.25±0.03^{a}	8.0±0.01^{d}	28.0±0.05^{d}	48.0±0.02^{d}	2.3±0.3^{a}	75.52	1.22±0.07^{a,c}	237±14^{e}	101.0±9^{a}
F7	-	-	0.25±0.01^{a}	16.0±0.02^{a}	32.0±0.03^{c}	20.0±0.03^{e}	1.9±0.3^{b}	48.04	-	136±15^{d}	-
	0.5	-	0.23±0.02^{a}	17.4±1.2^{a}	27.4±1.3^{d}		2.3±0.4^{a,b}	51.75	1.20±0.02^{c}	236±18^{e}	88.3±4^{e}
	5.0	-	0.24±0.01^{a}	6.2±0.3^{d}	48.3±2.8^{e}	60.5±4.2^{a}	2.2±0.8^{a}	120.19	1.42±0.08^{d}	423±17^{c}	141.3±4^{c}
	10.0	-	0.24±0.02^{a}	22.3±2.4^{a}	52.2±1.6^{e}		2.2±0.2^{a}	92.42	1.54±0.10^{d}	327±12^{a}	120.7±4^{a,c}
Animal Protein 100%	-	0:100	0.24±0.03^{a}	17.2±1.3^{a}	43.2±3.4^{c}	98.1±4.9^{c}	2.2±0.1^{a}	121.17	1.22±0.12^{a,c}	422±24^{c}	134.8±10^{c}
Plant protein 75%	-	75:25	0.24 ± .04^{a}	15.2±0.5^{a}	33.8±1.3^{c}	55.2±1.7^{a}	2.4±0.7^{a}	60.27	1.15±0.01^{c}	189±15^{f}	81.3±4.7^{e}

Mean Values in same column with different superscript letters are significantly different (P <0.05).
Values are mean± SE of duplicate determinations (n=2). In = Initial weight of fish before feeding.
SGR = Specific Growth Ratio; FCR = Feed Conversion Ratio ;PER = Protein Efficiency Ratio
FI = Feed Intake; PI = Protein Intake

Table 6: Whole body proximate composition (g.kg^{-1} DM) of *Clarias batrachus* fry fed feeds containing different proteins for 12th week.

Parameters (g.kg^{-1})	In W_t	F1	F2	F3	F4	F5	F6	F7 (Control)
Moisture	72.2± 2.1^{a}	70.3± 2.0^{b}	70.8± 2.8^{b}	71.2± 2.2^{a,b}	70.3± 1.1^{b}	72.5± 2.1^{a}	74.3± 2.2^{c}	70.3± 1.9^{b}
Crude Fat	6.8± 0.3^{a}	8.7± 0.4^{b}	8.5± 0.3^{b}	8.3± 0.2^{b}	7.6± 0.3^{c}	6.3± 0.5^{a}	6.4± 0.2^{a}	7.2± 0.3^{c}
Crude Protein	56.3± 2.1^{a}	57.6± 2.9^{a}	59.7± 1.8^{b}	58.1± 2.6^{b}	54.2± 1.4^{c}	53.4± 2.9^{c}	58.2± 4.1^{b}	57.5± 2.4^{a}
Dry Matter	24.2± 1.5^{a}	26.1± 1.3^{b}	25.6± 1.2^{b}	25.8± 1.2^{b}	28.2± 1.0^{c}	25.5± 1.2^{b}	25.3± 1.5^{b}	26.9± 1.4^{b}

Mean Values in same row with different superscript letters are significantly different (P <0.05)

4. DISCUSSION

Yuan and co-workers [36] concluded that fermented Soybean meal is an acceptable alternative plant protein source that can replace up to 35% of fish meal protein in diets in juvenile Chinese sucker, *Myxocyprinus asiaticus* without significant adverse effects on survival, growth, PER FCR, and body composition. Dietary proteins play a dominant role in fish growth [37, 38, 39, 40, 41, 42]. Whether fish will better accept plant or animal protein or combination of both will rest on the type of food habits of the species. In general, herbivorous fishes have a capacity to digest and assimilate plant protein better than the omnivorous or carnivorous fishes whereas, omnivore or carnivore fishes perform better with animal protein.

Yu and co-workers [94] indicated that replacement level up to 40% did not affect the growth, FCR, PER and body composition of juvenile Chinese sucker. However, several of the studies have concluded that even herbivorous fishes when fed with sizable part of animal protein perform better in terms of growth and general health. Rohu, a herbivore fish, when fed separately with plant protein and a combination of plant and animal protein at 35% protein level performed better growth when fed a mixture of both plant and animal protein [43]. Davis & Stickney [44] reported no difference in weight increment of blue tilapia fed diets containing FM or FM-free diets with 74% SBM. Soybean meal is a world- widely available, cheaper protein source with relatively higher digestible protein and energy contents and good amino acid profile[45]. Soybean products as dietary protein source has been reported to affect the growth and feed utilization of many fish species including channel catfish [46,47], tilapia [24,48], rainbow trout [49,66,68,69], red drum [67,70], seabream [71], Asian catfish [27], cobia [72] and cuneate drum [73]. Amongst the animal proteins, fish meal, meat-meal, goat liver, fish and poultry offal's used as fish diet in case of catfishes particularly in *C. batrachus*, fish meal has always been considered to perform better in terms of survival, growth and general well-being [74,75,76,77]. The reason being, fish meal has superior nutritive values over other animal [93] and plant proteins [78], because of its well-balanced amino acid composition and bioavailability [79], which influenced overall growth performance of animal [80]. However, when fish meal is incorporated partly with other animal proteins like goat liver and shrimp-meal, no significant difference was observed in the final body weight, per cent weight gain and net biomass in *C. batrachus* [76]. Similar results were also obtained in the present case as fish fed with mixed animal proteins performed better than the control (F7) and also with feed F4, F5 & F6 prepared by mixing 50% share of both plant and mixed animal proteins. This makes clear that the use of fish meal may be partly supplemented with other animal protein feeds without affecting overall dietary nutritional requirements of *Clarias batrachus.* The dietary protein levels influences the growth, feed efficiency, fish tissue proximate composition, and that the inclusion of dietary protein in the range of 40–43% of the diet is optimum for the growth and efficient feed utilization of protein in *Heteropneustes fossilis.* [49, 69, 81, 82, 83,84]. *C. batrachus* belonging to the same family (siluridae), having similar food and feeding habits performed better when fed protein levels of around 40% [76] and hence the share of protein of 37.39 to 43.52% in the present diets could be considered ideal for this species. Replacement of 75-100% fishmeal finally resulted in down trend growth and feed utilization and also decreased survival percentage. However, the economic viability is a point to be considered as the survival was higher in animal origin protein fed animals (approx. 60 to 70%) than in plant protein fed animals (approx. 50 to 53%). Poor growth of fish has been shown on to the presence of chitinous material in maggot meal and keratin protein in feather meal, which may be more difficult for fish to digest[85]. 5% GlcN in feed with animal protein gave better survival and growth than 0.5 or 10.0 % GlcN in the present study, which shows that 5.0% level of GlcN along with animal protein is good for the health of this fish. However, this was found different in case of plant protein, which showed better performance at 10% GlcN. A good piece of work on the effects of GlcN in case of marine shrimps has been documented but not on fishes. Dietary GlcN has been reported as a growth promoting factor in marine shrimps [86, 87]. The beneficial effects of dietary chitin on the growth and survival of juvenile *Penaeus monodon* has also been documented [88,89]. In what way, the GlcN helps in improving survival and growth in shrimps has not been well understood and, therefore, needs to be reviewed in future studies. Prawn shell waste protein is rich in essential amino acids [90,91] and hence GlcN which is derived from the prawn shell may have some of the essential amino acids which may be prompting higher growth in fish in the instant study. The GlcN has also been shown to reduce free radicals, tissue breakdown, articular cartilage cell death and inflammation in animals and is commonly used as a diet supplement particularly for the treatment of osteoarthritis in human being. It is an amino sugar produced in nature by the body from glucose and glutamine (an amino acid). GlcN is needed to produce glycosamineo-glycans, as they are major compositions of cartilaginous tissues.

5. CONCLUSION

Our results indicate that an animal protein rich feed when blended with GlcN are more acceptable and has direct impact on survival and growth promotion than natural feeds in *C. batrachus*. GlcN being derived from crustacean exoskeleton might also provide natural food flavour to this fish as crustacean's forms major share of diet in *C. batrachus* in natural feeding. The potential of replacing major quantities of animal protein with soybean meal in the feeds of fish needs more evaluation along with synergistic effects/approach of incorporating glucosamine (GlcN) in future research studies.

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