

Responses of major replacement of dietary proteins, in combination with glucosamine, on the growth performances of *Clarias batrachus* fingerlings

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ABSTRACT

Assessment for the replacement of animal protein by plant protein, blended with glucosamine, feeding trials of 12 weeks was conducted for growth performances in Asian Catfish, *Clarias batrachus* fingerling (av. wt. 2.2 ± 0.01 to 2.5 ± 0.01 g). Six (34.27 to 43.52 % CP; 3771.5 to 3990.48 kcal/100g, GE and crude lipid 1.67 to 6.70%) diets were formulated. The animal or plant protein component of the diets was progressively added with glucosamine 0.5, 5.0 and 10.0 % in basic 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 :: 75:25:0.5; F-5, PAG:: 75:25:5.0; F-6, PAG:: 75:25:10.0). The best growth was recorded in fish fed F1 as 19.4 ± 1.6 g in F1 followed by F2, 18.7 ± 0.6 g and F3, 18.6 ± 0.5 g. Amongst the plant protein fed fishes showed best growth in F4 followed by F6 and F5. The survival was improved in glucosamine supplemented feeds ranging from 73 ± 4.9 to 80 ± 3.8 % whereas, the control showed 69 ± 5.4 %. The FCR, SGR, PER, feed intake, protein intake ranged between 1.9 ± 0.1 to 2.4 ± 0.2 ; 32.88 to 170.5%; 0.99 ± 0.02 to 1.75 ± 0.03 ; 136 ± 15.0 to 600.0 ± 31 mg; 68.5 ± 5 to 187.6 ± 11 mg. The synergistic growth on supplementing proteins and glucosamine showed significance ($p < 0.05$) in case of weight gain, FCR, SGR, PER. 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 when blended with plant origin ingredients.

Key words: Glucosamine, Growth, Animal/plant protein, *Clarias batrachus*, Fingerlings.

INTRODUCTION

As aquaculture production continues to enlarge, the need for high-quality & cost-effective protein sources increases (Mundheim *et al.* 2004). Fish meal and fish oil are main raw materials in the production of fish feeds (Cabellero *et al.* 2002; Bell *et al.* 2010). They are also expensive feed ingredients compared to some alternative plant sources (Mundheim *et al.* 2004). Feed management determines the viability of aquaculture as it accounts for at least 40-60% of the cost of fish production (Jamu & Ayinla 2003). 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 (DeLong *et al.* 1958; Ogino & Satio 1970; Nose & Arai 1972; Anderson *et*

al. 1981; Mazid *et al.* 1979; Wee & Tacon 1982) 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 (Lovell, 1989). The reduced growth may be due to anti-nutritional factors (Ye *et al.* 2011; Francis *et al.* 2001; Leenhouders *et al.* 2006). The histological changes in intestine can also reduce growth performance on feeding plant proteins (Wang *et al.* 2006; Boonyaratpalin *et al.* 1998; Krogdahl *et al.* 2003; Heikkinen *et al.* 2006).

Therefore, many plant protein sources have been used to partially or almost totally replace dietary fish meal in order to reduce cost of feed ingredients (Kaushik *et al.* 1995; Refstie *et al.* 2000). It was found that costly fish meal can be replaced with low in cost but equally effective plant protein sources for the preparation of aquaculture feeds (Eidet *et al.* 2008; Abbas *et al.* 2010; Nazish & Mateen 2011). Soya products have become a widely used protein rich feed ingredient in diets for fish species, which is due to its moderate price, high availability in the market and the relatively well-balanced amino acid profile (Kaushik *et al.* 1995; Davies & Morris 1997; Ustaoglu & Rennert 2002; Romarheim *et al.* 2006; 2008; Bilgüven & Barış, 2011). Due to evermore research data a considerable success has been achieved in supplement of FM with SBM plant proteins in aquatic animals (Dersjant-Li 2002 & Kaushik *et al.* 1995). However, at higher rate of replacement of the fishmeal with SBM encouraged growth retardation may be due to imbalance nutrition in carnivorous fishes (Kaushik *et al.* 1995; Toma's *et al.* 2005; Wang *et al.* 2006; Martinez-Llorens *et al.* 2009; Ye *et al.* 2011) and/or higher ammonia excretion (Ballestrazzi *et al.* 1994, Tantikitti *et al.* 2005). Glucosamine, an amino sugar and a prominent precursor in the biochemical synthesis of glycosylated proteins and lipids synthesizes chitin, is one of the most abundant monosaccharide (Horton & Wander 1980; Roseman 2001; Muzzarelli 1977) 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 (Rao & Kumar 2006). 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*) are a low cost animal protein source, rich in both protein and lipid (Bhuiyan *et al.* 1989).

Different soya products are suitable and result in good growth in catfishes and other fish species (Chowdhary *et al.* 2012; Ustaoglu & Rennert 2002). Sarwar and coworkers (2010) have studied the impacts of different diets on growth and survival of *Clarias batrachus* grow-outs. *Clarias batrachus* is a native to Asia and the most popular in aquaculture and aquarium trade among all the Asian species (Ng & Kottelat 2008). It is a promising aquaculture candidate owing to its good growth, hardiness, efficient food conversion, excellent nutritional profile and high market value. It is one of the most economically important indigenous freshwater in Asia because it is very attractive with good taste hardy rugged medicinally valuable and has tremendous popularity among consumers (Hossain *et al.* 2006; Goswami, 2007; Debnath 2011). A concerted effort needs to be made to enhance the culture performance of *C. batrachus*. The present study was taken up to study the effects of major replacement of dietary animal protein by plant protein, blended with glucosamine, on the growth of *Clarias batrachus* fingerlings.

MATERIALS AND METHODS

Fish and feeding trial

Fingerlings 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 ± 0.01 to 2.5 ± 0.01 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 @ 5-10% 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, Japan). 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.

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 :: 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 *Mystus vittatus*, *Puntius sophore*, etc. Live silkworm pupae were procured from Department of Applied Animal Science, Babasaheb Bhimrao Ambedkar University, Raebareilly Road, Lucknow, reared 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 °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 minutes for the proper gelatinization of the ingredients. Finally cooked moist feeds were stored in plastic zipped polybags in a freezer (-20°C) until used.

Analytical methods & Statistical analyses of data

Proximate composition of feeds and fish carcasses were analyzed following methods AOAC 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 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⁻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 AND DISCUSSION

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, survival and average fish weight gain shown in Table-2, 3 and 4. The results of percent body weight gain, FCR, SGR, PER, Feed intake and Protein intake are shown in Table-5. The proximate compositions of fish fingerling are shown in Table 6. 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 index and Viscerosomatic index are shown in Table –5.

Table -1 Ingredients composition (w/w) of feeds (P:A:: 75:25) for *Clarias batrachus* fingerling

Ingredients	Feeds						Control
	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	
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	-
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. 1HiMedia, Mumbai Lot No: 0000013648; 2 HiMedia, Mumbai Lot No: 0000016171; 3HiMedia, Mumbai, Lot No: 0000028805d ; 4HiMedia, Mumbai, Lot No: 0000028340; 5HiMedia, Mumbai, Lot No. 0000014218; 6HiMedia, Mumbai, Lot No. 0000003862; 7Each kg of Vitamin and mineral mixture named 'Agrimin Forte' contains Vit. A 700000 IU, Vit. D₃ 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, 48N, DoddaballpurInd. Area, Doddaballapur – 561 203, India Batch No. BFA-61

Table -2 Calculated values of Protein, carbohydrate, fat and energy composition of feeds (P:A:: 75:25)

	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	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

Table -3 Survival Percentage of *Clarias batrachus* fingerlings reared for 12 weeks fed with P:A:: 75:25.

Feed	Stocking Nos. (N=50 X 3 replicates)	4 th Week	8 th Week	12 th Week
F-1	150	90 ± 1.8 ^d	85 ± 2.9 ^d	80 ± 3.8 ^c
F-2	150	85 ± 2.8 ^c	82 ± 2.4 ^c	79 ± 3.8 ^c
F-3	150	80 ± 3.2 ^b	78 ± 2.9 ^b	74 ± 2.5 ^b
F-4	150	85 ± 3.3 ^c	80 ± 2.5 ^c	74 ± 5.6 ^b
F-5	150	81 ± 5.8 ^b	80 ± 4.9 ^c	76 ± 6.2 ^{b,c}
F-6	150	82 ± 3.8 ^b	77 ± 5.1 ^b	73 ± 4.9 ^b
F-7 (control)	150	77 ± 4.2 ^a	73 ± 5.3 ^a	69 ± 5.4 ^a

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

Table -4 Growth (g) of *Clarias batrachus* fingerling reared for 12 weeks fed with P:A: 75:25.

Feeds	In	4 th week	8 th week	12 th week
F1	2.4 ± 0.01 ^a	5.29 ± 0.8 ^b	13.34 ± 0.7 ^c	19.4 ± 1.6 ^c
F2	2.2 ± 0.02 ^a	6.44 ± 0.5 ^c	13.30 ± 0.4 ^d	18.7 ± 0.6 ^c
F3	2.5 ± 0.02 ^a	6.75 ± 0.4 ^c	12.42 ± 0.6 ^d	18.6 ± 0.5 ^c
F4	2.3 ± 0.02 ^b	4.29 ± 0.4 ^b	11.31 ± 0.5 ^c	17.5 ± 1.0 ^b
F5	2.4 ± 0.03 ^b	4.24 ± 0.2 ^b	10.24 ± 0.2 ^b	16.4 ± 0.4 ^b
F6	2.2 ± 0.03 ^a	4.27 ± 0.5 ^b	11.53 ± 0.4 ^b	16.5 ± 0.6 ^b
F7 (control)	2.5 ± 0.01 ^a	3.43 ± 0.7 ^a	9.39 ± 0.8 ^a	13.8 ± 1.3 ^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

Neither mortality nor external clinical symptoms was observed in any treatment during the entire period of the experiment. Dietary proteins dietary protein plays a dominant role in fish growth (Cowey *et al.* 1972; Satia, 1974; Cho *et al.* 1976). 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 (Das & Ray, 1991). In the present study, the experimental feeds were formulations with different protein are based on previous reports (Kikuchi, 1999; Kim *et al.* 2002; 2006; Cho *et al.* 2006, Ye *et al.* 2011).

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. 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. 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. These results were in agreement with data obtained by Abdel-Warith *et al.* (2012) reported that plant protein sources such as full fat soybean are unable to substitute 15g/100 protein of a high quality fishmeal protein in the diets of Nile tilapia *O. niloticus*. Growth and feed utilization decreased, which contain 15 or more of the total protein from fishmeal was substituted with the full fat soybean meal these might be the ration of replaced is high also, these diets contain amino acids lower than the minimum requirements of this fish. According to Rao & Kumar (2006), experiment conducted 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 squillameal, which is very much similar to our results. Fish meal has superior nutritive values over other animal proteins (Seenappa & Devraj 1995) and plant proteins (Eyo, 1991), because of its well balanced amino acid compositions and their bioavailability (Moon & Gatlin, 1994), which influenced the

performance of animal (Gaylord & Gatlin 1996). Jahan *et al.* (2012) demonstrated unhindered growth of *L. rohita* fry fed on soybean meal without supplementation of amino acids except fishes under diet, 25% fishmeal protein + 75% soybean meal protein. Although, the inclusion of soybean meal up to 50% in the diet resulted in the growth performance of *L. rohita* fry comparable to that of the control, where 100% fishmeal was used. However, with the replacement of 25% fish protein by soybean protein, the growth performance of *L. rohita* fry was excellent. This result is similar to our results. In accordance to results obtained by (El-Saidy & Gaber 2002) they demonstrated that soybean meal supplementation with 10 g/kg methionine and 0.5 g/kg lysine could replace total FM protein in Nile tilapia diets these due to the high amino acids supplementation.

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 by Mollah & Alam (1990), who obtained value of 15% carbohydrate (as in glucosamine 5.0 and 10.0 % in the present study) in the feed showed retardation of growth. Further, the foregoing results agree and extend the findings of Chakraborty *et al.* (1973) by showing that silkworm pupae, groundnut and wheat bran was better utilized by fry *Labeorohita* and *Cirrhinus mrigala* than that of mustard oilcake and rice bran. Prawn shell waste protein is rich in essential amino acids (Forster 1975; Penafiora 1989). Dietary glucosamine was found to be a growth promoting factor in shrimp (Kitabayashi *et al.* 1971). And the shell (chitin) in shrimp waste growth promoting agents for the prawn *Penaeus indicus* (Vaitheswaran & Ahamad 1986). The effect of dietary chitin on the growth and survival of juvenile *P. monodon* was studied by various workers (Lan & Pan 1993; Sudaryono *et al.* 1996). In the present experiment, conducted to know the effect of animal and/or plant protein incorporated with glucosamine, 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. The results demonstrate 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. In conclusion, Growth performance and feed utilization efficiency of this catfish, fed feeds with animal protein are better than those of plant protein. Furthermore, it is to deduce that, the animal protein rich feeds were better acceptable than alternative plant protein sources for the *Clarias batrachus* fingerlings. The incorporation of soybean meal in the feeds of this fish need more evaluation, however, the synergistic effects of growth promoter like glucosamine exhibits the better impact on the growth performances in asian catfish, *Clarias batrachus*.

Table -5 Growth performance, nutrient utilization in *Clarias batrachus* fingerling reared for 12 weeks

Feed	Glucos-amine	Animal : Plant Protein Ratio	In wt (g)	4 th week wt. gain %	8 th week wt. gain %	12 th week wt. gain %	FCR	SGR%	PER %
F1	0.5	100:0	2.4±0.01 ^a	120.4±10.2 ^c	455.8±22.8 ^{b,c}	708.3±14.3 ^b	2.75±0.3 ^c	82.3	1.24±0.2 ^d
F2	5.0	100:0	2.2±0.02 ^a	192.7±14.4 ^d	504.5±34.7 ^c	750.0±10.2 ^c	2.59±0.1 ^b	76.8	1.53±0.3 ^c
F3	10.0	100:0	2.5±0.02 ^a	170.0±12.5 ^d	396.8±23.4 ^d	644.0±8.7 ^d	2.99±0.2 ^c	110.4	1.56±0.1 ^c
F4	0.5	25:75	2.3±0.02 ^b	86.5±6.4 ^b	391.7±35.7 ^d	660.9±20.8 ^a	2.28±0.2 ^a	78.2	0.92±0.2 ^b
F5	5.0	25:75	2.4±0.03 ^b	76.7±5.7 ^c	326.7±18.5 ^a	583.3±19.8 ^b	2.45±0.1 ^b	65.8	1.11±0.3 ^a
F6	10.0	25:75	2.2±0.03 ^a	94.1±8.4 ^b	424.1±26.7 ^b	650.0±25.2 ^b	2.42±0.3 ^b	72.5	1.12±0.3 ^a
F7	-	-	2.5±0.01 ^a	37.2±7.6 ^a	275.6±24.1 ^a	452.0±24.05 ^a	2.22±0.2 ^a	59.6	-

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

Table -6 Whole body proximate composition (g.100g⁻¹ DM*) and indices of *Clarias batrachus* fingerling fed feeds containing different proteins for twelve week

Parameters (g.100g ⁻¹ DM)*	In W _t	F1	F2	F3	F4	F5	F6	F7 (Control)
Moisture (Wet wt. basis)	74.1 ± 1.8 ^a	75.3 ± 4.5 ^a	76.8 ± 5.2 ^a	72.2 ± 3.9 ^b	76.8 ± 6.4 ^a	76.7 ± 6.2 ^a	77.1 ± 6.5 ^a	77.3 ± 4.5 ^a
Crude Fat*	5.9 ± 0.2 ^a	6.8 ± 0.4 ^b	6.7 ± 0.3 ^b	7.9 ± 0.2 ^d	7.0 ± 0.4 ^c	6.3 ± 0.3 ^a	6.6 ± 0.2 ^b	6.3 ± 0.1 ^a
Crude Protein*	54.1 ± 1.5 ^a	56.2 ± 2.9 ^b	55.6 ± 2.3 ^a	57.3 ± 3.2 ^b	53.8 ± 2.0 ^a	58.4 ± 1.1 ^b	58.8 ± 1.6 ^b	54.2 ± 0.9 ^a
Dry Matter*	24.4 ± 2.3 ^c	23.3 ± 1.7 ^c	22.1 ± 0.9 ^a	26.2 ± 1.1 ^b	21.4 ± 1.5 ^a	22.3 ± 1.0 ^a	22.4 ± 1.4 ^a	21.2 ± 1.3 ^a
HSI	0.77 ± 0.04 ^c	0.96 ± 0.05 ^a	1.20 ± 0.1 ^b	1.49 ± 0.07 ^d	1.44 ± 0.13 ^d	1.34 ± 0.10 ^c	1.23 ± 0.13 ^b	0.90 ± 0.12 ^a
VSI	1.88 ± 0.2 ^c	2.34 ± 0.1 ^a	2.53 ± 0.1 ^d	2.78 ± 0.2 ^b	3.12 ± 0.3 ^c	2.69 ± 0.1 ^b	2.85 ± 0.2 ^b	2.14 ± 0.2 ^a

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

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