Journal of
Fisheries and Aquatic Science

ISSN 1816-4927

Academic
Journals Inc.

www.academicjournals.com

Journal of Fisheries and Aquatic Science 1 (2): 102-111, 2006
ISSN 1816-4927
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Non-gelatinized Corn Supplemented with Microbial α-amylase at Sub-optimal Protein in the Diet of *Labeo rohita* (Hamilton) Fingerlings Increases Cell Size of Muscle

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Abstract: A 60 days feeding trial was conducted to delineate the effect of both gelatinized (G) and non-gelatinized (NG) corn supplemented with exogenous microbial α-amylase (0, 50, 100 and 150 mg kg^{-1}) at either sub-optimal (28%) or optimal (35%) crude protein (CP) level on muscle protein, muscle protein/DNA ratio and DNA/muscle mass (wet wt.) ratio of *Labeo rohita* fingerlings. Three hundred sixty fingerlings (av. wt. 10±0.15 g) were randomly distributed in 12 treatment groups with each of two replicates. The muscle protein and muscle protein/DNA ratio of NG corn fed groups was significantly higher ($p<0.05$) than the G corn fed groups, whereas reverse trend was found for DNA/muscle mass (wet wt.) ratio. There was no significant effect on muscle protein, muscle protein/DNA ratio and DNA/muscle mass (wet wt.) ratio at both 28% and 35% CP. Supplementation of α-amylase at 50 mg kg^{-1} increased the muscle protein and muscle protein/DNA ratio beyond which no significant changes were observed but DNA/muscle mass (wet wt.) ratio was significantly higher in non α-amylase supplemented group. Hence it concludes that NG corn with 50 mg α-amylase kg^{-1} at 28% CP is optimum in the diet of *L. rohita* fingerling to improve muscle protein, muscle protein/ DNA ratio and DNA/ muscle mass (wet weight) ratio.

Keywords: α-amylase, corn, gelatinization, muscle protein, DNA, *Labeo rohita*

Introduction

Fish in general poorly utilize dietary carbohydrate. Furuichi and Yone (1980) observed depressed growth and feed efficiency in red sea bream, yellowtail and common carp fed high carbohydrate containing diets. A relative inability to metabolize carbohydrates had been reported in several studies in different species of fish (Furuichi and Yone, 1982b; Wilson and Poe, 1987; Gutierrez *et al*., 1991). This inability is reflected as persistent hyperglycemia (Palmer and Ryman, 1972; Furuichi and Yone, 1981; Wilson and Poe, 1987), a lower activity of liver hexokinase (Furuichi and Yone, 1982b), a lack of glucokinase (Nagayama *et al.,* 1980; Cowey *et al*., 1977) and a lower number of muscle insulin receptors (Gutierrez *et al*., 1991).

Indian Major Carps (IMC) and exotic carps are considered to be the major aquaculture species in tropical countries, contributing about 97% of the total freshwater aquaculture production (FAO, 2001). Out of these, 90% of the aquaculture production is contributed only by IMC. *Labeo rohita* is the most preferred species, comprising about 35% of the total IMC production.

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Requirement of dietary carbohydrate has been reported to be 26% for *L. rohita* (Sen *et al.,* 1978) but further study revealed that dietary level of 40% gelatinized carbohydrate improve the growth of the species (Mohapatra *et al.,* 2002). Use of more carbohydrate in the fish diet is required as it significantly reduces the feed cost and hence production cost.

The relative use of dietary carbohydrates by fish varies and appears to be associated with the complexity of carbohydrate. The relative utilization of dietary glucose, dextrin and gelatinized starch has been compared in carp and sea bream. Growth and feed efficiency of carp were highest when fed the gelatinized starch diet, followed by the dextrin and glucose diets in decreasing order, whereas red sea bream did not show any significant difference in growth rates for the various carbohydrate sources (Furuichi and Yone, 1982a). Beside gelatinization, enzyme pretreatment of dietary plant ingredients with carbohydrases (α- amylase, β- glucanases and β- xylanases) may enhance the energy digestibility in fish by releasing previously unavailable glucose, galactose and xylose. Exogenous dietary enzyme supplements, isolated from plants and bacteria, have been used successfully in the pig and poultry feed (Batterham, 1992; Farrell, 1992; Campbell and Bedford, 1992; Chesson, 1993; Bedford, 1996; Dudley-Cash, 1997). Stone *et al.* (2003) had also reported that starch digestibility was significantly affected by different level of Natustarch® (a commercial α- amylase supplement). They found that there was greater effect of Natustarch® on the diet containing raw wheat starch (starch apparent digestibility coefficient increased from 84% to 92%) than on the diets containing gelatinized wheat starch. Hilton *et al.* (1982) and Hilton and Slinger (1983) reported that excess of digestible carbohydrate causes reduction in growth of rainbow trout. Muscle growth in fish, including early myotome expansion, is a plastic process (Weatherley, 1990) that involves a combination of enlargement of the muscle fibres already existing (hypertrophy) and the recruitment of new fibres (hyperplasia) (Koumans and Akster, 1995; Rowlerson and Veggetti, 2001). The balance between these mechanisms determines the particular rate of growth and ultimate size of the species (Weatherley *et al.,* 1988; Zimmermanand Lowery, 1999), but is dependent upon various internal and external factors. This is true for both `white' (fast contracting, glycolytic) and `red' (mainly slow contracting, oxidative) fibre types. DNA content is considered to be an index of cell number (Bulow, 1987), as all cells are assumed to contain the same amount of DNA (the nuclear and mitochondrial genomes). Foster *et al.* (1993) deals that protein/ DNA ratio is an index of cell size and DNA/ muscle mass ratio is an index of cell number. They concluded that muscle cell size increases whereas cell number decrease in growing fish. Relationship of dietary carbohydrate with cell size and cell number in fish is rarely reported.

Rainbow trout fed a carbohydrate deficient diet showed a lower growth rate and reduced size of muscle cell (Peragon *et al.*, 1999). But herbivorous fish may utilize the carbohydrate to a greater extent for growth, which can be measured in term of protein/ DNA ratio and DNA/ muscle mass ratio. However, these types of studies are rarely reported. In all fish, regulation of protein deposition is generally of great importance for control of whole body growth. In fish, other parameters related to tissue growth and protein deposition are content of nucleic acids, RNA and DNA (Buckley, 1984; Bastrop *et al.,* 1992; Foster *et al.,* 1993).

Excess protein in diet leads to utilization of protein for energy source (Phillips, 1972; Prather and Lovell, 1973). Protein sparing effect of carbohydrate is observed at low protein level in the feed (Shiau and Peng, 1993; Erfanullah and Jafri, 1995).

Thus, the aim of this study was to investigate effect of the gelatinized or non-gelatinised corn supplemented with exogenous amylase at sub-optimal (28%) or optimal (35%) protein level on muscle protein deposition, DNA concentration and their ratio in *Labeo rohita* fingerlings.

Materials and Methods

Fish and Experimental Conditions

Labeo rohita (Hamilton) juveniles were brought from the Khopoli Fish Farm (Maharastra, India) to the Fish Nutrition Laboratory, Central Institute of Fisheries Education (Mumbai, India) and acclimatized for 24 days with the control diet (35% crude protein). At the beginning of the study, fish were weighed individually and 15 fish (average wt. 10±0.15 g) were randomly transferred to each of 24 tubs (150 L). Aeration was provided to all the tubs and manual water exchange was carried out every other day. Water quality parameters were checked every week following the standard methods of APHA (1998).

Gelatinization of Corn

The corn was ground to fine powder and made into dough by adding required amount of water followed by cooking in as autoclave at 15 psi for 1 h so as to get maximum gelatinization. The cooked corn was then spread over a tray and dried in an oven at 60^0C. The dried mass was then pulverized in a hammer mill with a 0.5 mm screen and stored in airtight containers until use. The degree of gelatinization of corn was determined as Guraya and Toledo (1993). A known amount (0.2 g) of corn powder was mixed with 15 ml of 0.2 N potassium hydroxide followed by intermittent stirring for 30 min. The pH of the mixture was adjusted to 5.5 using 2N phosphoric acid and the volume was made upto 100 mL with distilled water. Next, 100 μL of aliquot was transferred to a test tube and diluted to 5 mL with distilled water. Then 50 μL of standard iodine solution (4% KI, 1% I_2) was added and the absorbance of the solution was taken at 600 nm (A_1) against the reagent blank. Another aliquot was made by the same procedure by mixing 0.2 g of dried corn powder in 15 mL of 0.6 N potassium hydroxide and the absorbance was taken at 600 nm (A_2) as above. The degree of gelatinization was calculated as follows:

$$\text{Gelatinization \%} = A_1/A_2 \times 100$$

Diet Preparation and Feeding

The composition of the experimental diet is given in Table 1. Fat free casein and gelatin were used as protein source, whereas sunflower oil and cod liver oil were used as lipid source and G or NG corn as carbohydrate source. Ingredients were finely ground and mixed thoroughly with water to make dough. The dough was steam cooked for 5 min in a pressure cooker. Vitamin-mineral premix was mixed after cooling and the dough was passed through a hand pelletizer with a 2 mm die and then dried at 60^0C. The required amount of α-amylase (*Aspergillus* origin, HIMEDIA Laboratories Pvt. Limited, Mumbai, India) was dissolved in 50 mL of distilled water and sprayed over 1 kg of basal diet as described by Robinson *et al*. (2002). Thus, twelve experimental diets with 42.43% of non-gelatinized (NG) or gelatinized (G) corn, two level of crude protein: 35% or 28% and four level of α-amylase: 0, 50, 100 and 150 mg Kg^{-1} feed were prepared viz., T_1 (NG, 35% CP, 0 mg kg^{-1} α-amylase), T_2 (G, 35% CP, 0 mg kg^{-1} α-amylase), T_3 (NG, 28% CP, 50 mg kg^{-1} α-amylase), T_4 (NG, 35% CP, 50 mg kg^{-1} α-amylase), T_5 (G, 28% CP, 50 mg kg^{-1} α-amylase), T_6 (G, 35% CP, 50 mg kg^{-1} α-amylase), T_7 (NG, 28% CP, 100 mg kg^{-1} α-amylase), T_8 (NG, 35% CP, 100 mg kg^{-1} α-amylase), T_9 (G, 28% CP, 100 mg kg^{-1} α-amylase), T_{10} (G, 35% CP, 100 mg kg^{-1} α-amylase), T_{11} (NG, 28% CP, 150 mg kg^{-1} α-amylase) and T_{12} (NG, 35% CP, 150 mg kg^{-1} α-amylase). The feed was stored at 4^0C until use. Each diet was fed twice daily (8.00 and 18.00 h) to satiation for 60 days.

Table 1: Composition of the experimental diets (%DM basis)

	Inclusion (%)	
Ingredients	28% CP	35% CP
Casein [1]	26.57	30.57
Gelatin [2]	4.00	8.00
Corn flour (NG or G) [3]	42.43	42.43
Cellulose [4]	15.00	7.00
Sunflower oil : cod liver oil (2 : 1)	8.00	8.00
Vitamin + Mineral mixture [5]	2.60	2.60
Carboxymethyl cellulose [6]	1.00	1.00
Vitamin C [7]	0.10	0.10
Vitamin B complex [8]	0.10	0.10
Glycine [9]	0.20	0.20
Butylated Hydroxy Toluene [10]	0.02	0.02
Chromic oxide	0.05	0.05

1- Casein fat free: 75%CP (HImedia ltd, India)
2- Gelatin: 96% CP (HImedia ltd, India)
3- Procured from Central poultry farm, Mumbai, India.
4- Sd Fine Chemicals Ltd., India.
5- Composition of vitamin mineral mix (EMIX PLUS) (quantity/2.5 kg)
Vitamin A 55,00,000 IU; Vitamin D_3 11,00,000 IU; Vitamin B_2 2,000 mg; Vitamin E 750 mg; Vitamin K 1,000 mg; Vitamin B_6 1,000 mg; Vitamin B_{12} 6 mcg; Calcium Pantothenate 2,500 mg; Nicotinamide 10 g; Choline Chloride 150 g; Mn 27,000 mg; I 1,000 mg; Fe 7,500 mg; Zn 5,000 mg; Cu 2,000 mg; Co 450 mg; Ca 500 g; P 300 g; L- lysine 10 g; DL- Methionine 10 g; Selenium 50 ppm; Selenium 50 ppm; (Lactobacillus 120 million units and Yeast Culture 3000 crore units).
6- Sd Fine Chemicals Ltd., India.
7- Stay C (Hoffman La Roche, Nutley, NJ, USA) 15% ascorbic acid activity
8- Composition of vitamin B complex (quantity g^{-1})
Thiamine mononitrate 20 mg; Riboflavin 20 mg; Pyridoxine hydrochloride 6 mg; Vitamin B_{12} 30 mcg; Niaciamide 200 mg; Ca pantothenate 100 mg; Folic acid 3 mg; Biotin 200 mcg.
9- Himedia ltd, India.
10- Sd Fine Chemicals Ltd., India.

Table 2: Proximate composition of the different experimental diets (DM basis %)

Treatments	OM[1]	CP[2]	EE[3]	Ash	TC[4]
T_1	96.47±0.85	34.73±0.24	12.53±0.11	3.53±0.02	49.21±0.32
T_2	96.77±0.81	34.78±0.19	10.36±0.09	3.23±0.01	51.63±0.41
T_3	96.81±0.65	26.68±0.25	11.85±0.09	3.19±0.02	58.28±0.36
T_4	96.55±0.74	35.05±0.24	12.45±0.11	3.45±0.02	49.05±0.38
T_5	96.67±0.69	26.68±0.13	11.54±0.08	3.33±0.02	58.45±0.41
T_6	96.69±0.81	33.75±0.31	9.63±0.08	3.31±0.00	53.31±0.32
T_7	96.59±0.92	27.01±0.26	11.48±0.09	3.33±0.01	58.18±0.25
T_8	96.72±0.65	34.82±0.18	12.30±0.10	3.41±0.02	49.47±0.25
T_9	96.73±0.74	27.01±0.24	10.95±0.08	3.27±0.02	58.77±0.35
T_{10}	96.71±0.62	34.82±0.29	9.81±0.08	3.29±0.02	52.08±0.38
T_{11}	96.72±0.65	26.68±0.23	11.20±0.09	3.28±0.01	58.84±0.35
T_{12}	96.61±0.74	34.78±0.21	11.75±0.09	3.39±0.01	50.08±0.28

OM[1] – Organic Matter, CP[2] – Crude Protein, EE[3] – Ether Extract, TC[4] – Total Carbohydrate

Sampling and Analysis of Samples

The proximate composition of all the diets was determined following the standard methods of AOAC (1995), Table 2. In brief, moisture content was determined by drying at 105^{0}C to a constant weight. Nitrogen content was estimated by Kjeltec (2200 Kjeltec Auto distillation, Foss Tecator, Sweden) and CP was estimated by multiplying nitrogen percentage by 6.25. Ether extract (EE) was measured using a Soxtec system (1045 Soxtec extraction unit, Tecator, Sweden) using diethyl ether (boiling point, 40-60°C) as a solvent and ash content was determined by incinerating samples in a muffle furnace at 600°C for 6 h. Total carbohydrate was calculated by difference i.e., total carbohydrate % = 100 - (CP% + EE% + ash %).

Sample Preparation

At the completion of experiment, fishes were anaesthetized with clove oil at 50 μl L^{-1} and killed by giving blow to the head and dissected to collect the muscle for protein and DNA concentration estimation. The muscle was taken from the caudal peduncle region after scraping off the scales. Immediately a 5% homogenate in 250 mM sucrose was prepared for muscle tissues. The homogenate was centrifuged at 5000 rpm for 20 min and the supernatant was collected in a sample vial and kept at -20°C until use.

Protein Estimation

Quantification of protein in the muscle tissues was carried out using Lowry's method (Lowry *et al.*, 1951). Tissue homogenate (0.1 mL) was taken and precipitated using 1 mL of 10% TCA. The protein residue was obtained by discarding the supernatant produced after centrifugation at 5000 rpm for 20 min. The residue was dissolved in 0.5 mL of 0.1 N Na0H and 0.1 mL of the dissolved protein residue was used for further analysis. Alkaline copper sulphate (5 mL) was added and left for 10 min. To this 5 mL Folin's reagent (1N) was added and incubated for 30 min in the dark. Reading was taken at 660 nm against the blank. Bovine serum albumin was used as standard.

Quantification of DNA

Quantitative determination of DNA in tissue was done by pentose analysis (Schnieder, 1945) and was calculated as:

$$\mu g\ DNA/mL = [OD\ value\ at\ 600\ nm / 0.019]$$

Statistical Analysis

The main effect was analyzed by using three-factor ANOVA with starch type (gelatinized and non-gelatinized), levels of protein (35 and 28%) and the amount of enzyme supplemented (0, 50, 100 and 150 mg kg^{-1}) as three fixed factors. Where significant interactions were found between main effects, a one-factor ANOVA was used to compare the simple effects. When results were significant, comparison among the means were made using the Duncan's Multiple Range Test (DMRT). All value was compared at 5% level of significance. Statistical evaluation of the data was carried out using the software SPSS version 11.0.

Results and Discussion

The muscle protein, muscle protein/ DNA ratio and DNA/ muscle mass (wet weight) ratio of *L. rohita* fingerlings fed different test diets are summarized in Table 3 and interaction effect of corn type x protein, protein x α- amylase and corn type x α- amylase are given in Table 4.

In general the muscle protein and muscle protein/ DNA ratio of NG corn fed groups were significantly ($p<0.05$) higher than their G counterpart irrespective of the protein and α- amylase level in the diet, whereas DNA/ muscle mass (wet weight) ratio was significantly ($p<0.05$) higher in G corn fed groups. Peragon *et al.* (1999) observed low growth rate of rainbow trout (*Oncorhynchus mykiss*) which was correlated with increased in relative DNA concentration and decreased total protein content in fish with reduced protein/ DNA ratio fed diet without carbohydrate. Feeding of G carbohydrate to *L. rohita* resulted increased DNA/ muscle mass, which indicate the increase in cell number and not cell size. This suggests that G carbohydrate at level of 42.43% seems to be higher causing metabolic stress

Table 3: Muscle protein, muscle protein/ DNA ratio and DNA/ muscle mass (wet wt.) ratio of *L. rohita* fingerlings fed with different experimental diets

Treatments	Muscle protein (%)	Muscle protein/DNA ratio	DNA/muscle mass (wet wt.) ratio
T_1	44.80^{ab}±1.09	1.74^{b}±0.01	26.17^{bc}±2.97
T_2	40.06^{b}±3.37	1.32^{c}±0.01	32.03^{a}±3.52
T_3	49.65^{a}±3.49	2.09^{a}±0.01	22.53^{bcd}±1.61
T_4	49.92^{a}±2.33	2.07^{ab}±0.11	21.55^{bcd}±1.11
T_5	44.77^{ab}±3.23	2.03^{ab}±0.11	22.05^{b}±0.84
T_6	46.45^{ab}±0.42	1.92^{ab}±0.01	26.36^{b}±1.67
T	49.16^{a}±1.87	2.04^{ab}±0.10	20.52^{cd}±1.26
T_8	49.96^{a}±2.66	2.12^{a}±0.17	21.06^{bcd}±1.32
T_9	46.40^{ab}±2.51	1.95^{ab}±0.01	22.63^{bcd}±1.19
T_{10}	46.75^{ab}±3.68	2.01^{ab}±0.01	21.96^{bcd}±0.74
T_{11}	49.16^{a}±1.71	2.04^{ab}±0.01	21.68^{bcd}±0.95
T_{12}	47.70^{ab}±2.13	2.13^{a}±0.01	19.52^{d}±0.71
		ANOVA	
Corn type	<0.05	<0.05	<0.05
CP	NS	NS	NS
α-Amylase	<0.05	<0.05	<0.05
Corn type x protein	NS	NS	NS
Protein x α-amylase	NS	NS	NS
Corn type x α-amylase	<0.05	<0.05	<0.05

Means with different superscript in a column differ significantly ($p<0.05$). Weight gain% (WG. %) = (final weight-initial weight)/initial weight x 100. NS- non- significant

Table 4: Muscle protein, muscle protein/ DNA ratio and DNA/ muscle mass (wet wt.) ratio of *L. rohita* fingerlings as influenced by starch type (G or NG), amylase, CP level and their interaction

Treatments	Muscle protein (%)	Muscle protein/DNA ratio	DNA/muscle mass (wet wt.) ratio
Corn type			
NG	48.62^{a}±0.96	2.03^{a}±0.04	21.86^{b}±0.65
G	44.89^{b}±1.13	1.85^{b}±0.04	25.01^{a}±0.78
CP			
35%	46.52±0.96	1.90±0.04	24.01±0.66
28%	47.83±1.13	2.03±0.04	21.88±0.78
α-Amylase level			
Amy 0	42.43^{b}±1.78	1.53^{b}±0.07	29.09^{a}±1.23
Amy 50	47.69^{a}±1.26	2.03^{a}±0.05	23.12^{b}±0.87
Amy 100	48.07^{a}±1.26	2.03^{a}±0.05	21.54^{b}±0.87
Amy 150	48.43^{a}±1.78	2.08^{a}±0.07	20.59^{b}±1.23
Corn type x protein			
NGx28%	49.33±1.26	2.05±0.05	21.58±1.00
Gx28%	45.59±1.46	1.99±0.06	22.34±1.23
NGx35%	48.09±1.46	2.02±0.06	22.07±0.87
Gx35%	44.42±1.78	1.75±0.07	26.78±1.00
Protein x α-amylase			
35%x0	42.43±1.78	1.53±0.07	29.09±1.23
28%x50	47.21±1.78	2.06±0.07	22.29±1.23
35%x50	48.18±1.78	1.99±0.07	23.96±1.23
28%x100	47.78±1.78	1.99±0.07	21.58±1.23
35%x100	48.35±1.78	2.07±0.07	21.51±1.23
28%x150	49.16±2.52	2.04±0.09	21.68±1.73
35%x150	47.70±2.52	2.13±0.09	19.52±1.73
Corn type x α-amylase			
NGxAmy 0	44.80^{ab}±2.53	1.75^{b}±0.09	26.17^{b}±1.73
GxAmy 0	40.06^{c}±2.53	1.32^{c}±0.09	32.03^{a}±1.73
NGxAmy 50	49.79^{a}±1.78	2.08^{a}±0.07	22.04^{bc}±1.23
GxAmy 50	45.61^{ab}±1.78	1.98^{a}±0.07	24.20^{bc}±1.23
NGxAmy 100	49.56^{a}±1.78	2.08^{a}±0.07	20.79^{c}±1.23
GxAmy 100	46.57^{a}±1.78	1.98^{a}±0.07	22.29^{bc}±1.23
NGxAmy 150	48.43^{a}±1.78	2.08^{a}±0.07	20.59^{c}±1.23

Value with different superscript within a column differ significantly ($p<0.05$)

as suggested by many authors (Hilton and Slinger, 1983; Kausik and de oliva-Teles, 1985). Muscle protein, muscle protein/ DNA ratio and DNA/ muscle mass (wet weight) ratio of fingerlings fed either 28 or 35% CP were similar, suggesting the protein sparing effect of carbohydrate at sub-optimum protein level (28%). Peragon *et al.* (1999) and Mohapatra *et al.* (2002) found that carbohydrate is used as energy source in fish by their protein sparing effect. Protein sparing effect of carbohydrate may be related to the fact that glucose is the preferred oxidative substrate for nervous tissue and blood cells and carbohydrate present in fish diets can depress gluconeogenic activity thus diverting amino acids away from oxidative pathways (Cowey *et al.*, 1977; Sanchez-Muroz *et al.*, 1996). The absence of carbohydrate in diet increased protein degradation and decrease the absolute protein synthesis rate in the white muscle of rainbow trout (Peragon *et al.*, 1999).

Supplementation of α-amylase at different level in the diet significantly ($p<0.05$) varied the muscle protein, muscle protein/ DNA ratio and DNA/ muscle mass (wet weight) ratio of *L. rohita* fingerlings. Addition of α- amylase significantly improved the muscle protein and muscle protein/ DNA ratio irrespective of the starch type and protein level. However, DNA/ muscle mass (wet weight) ratio was significantly ($p<0.05$) decreased in the α-amylase supplemented groups. Muscle protein, muscle protein/ DNA ratio and DNA/ muscle mass (wet weight) ratio was similar at 50, 100 and 150 mg kg^{-1} α-amylase supplemented groups. Addition of exogenous carbohydrase enzymes to aquafeed has been reported to enhance the utilization of unavailable dietary carbohydrates by Atlantic salmon, *Salmo salar*, larval gilthead seabream, *Sparus aurata* and tiger prawn, *Penaeus monodon* (Kolkovvski *et al.*, 1993; Carter *et al.*, 1994; Buchanan *et al.*, 1997). Thus, in the present study increase in muscle protein and muscle protein/ DNA ratio may be due to more utilization of NG carbohydrate by addition of dietary α-amylase in the diet as reported by Kumar *et al.* (2005).

There was no significant ($p>0.05$) interaction found between corn type x protein and protein x α-amylase on muscle protein, muscle protein/ DNA ratio and DNA/ muscle mass (wet weight) ratio. However, a significant ($p<0.05$) interaction was found between corn type x α-amylase on muscle protein, muscle protein/ DNA ratio and DNA/ muscle mass (wet weight) ratio. From the interaction effect it indicates that lowest muscle protein and muscle protein/ DNA ratio was observed with G corn fed groups without dietary α-amylase but reverse trend was found for DNA/ muscle mass (wet weight) ratio.

Protein/ DNA ratio is an index of cell size and DNA/ muscle mass ratio is an index of cell number (Foster *et al.*, 1993). Thus, in the present study the increased muscle protein/ DNA ratio in NG corn fed groups supplemented exogenous α-amylase was due to increased cell size of the muscle (hypertrophy). Increased cell size was reflected as decrease in cell no. in the form of lower DNA/ muscle mass ratio. This can be correlated with our previous result (Kumar *et al.*, 2005) that higher weight gain % and SGR was observed in NG corn fed groups supplemented with exogenous α-amylase. This is in agreement with Pelletier *et al.* (1995), who observed that protein/ DNA ratio increased with growth rate, whereas DNA/ muscle mass ratio decreased. Similarly, Stickland *et al.* (1988) in Atlantic salmon and Kiessling *et al.* (1991) in trout observed an increase in hypertrophy in muscle fibres of fish, where maximum growth was registered. Foster *et al.* (1993) also reported that muscle cell size increases whereas cell number decrease in growing fish.

From the present experiment, it concludes that supplementation of 50 mg α-amylase kg^{-1} is optimum in the diet of *L. rohita* juveniles containing 42.43% NG corn to improve muscle protein and muscle protein/ DNA ratio. Diet containing either 28 or 35% CP registered similar muscle protein, muscle protein/ DNA ratio and DNA/ muscle mass (wet weight) ratio. Thus a feed containing NG corn with 50 mg α-amylase kg^{-1} with 28 % CP, can be used to improve the muscle protein, muscle protein/ DNA ratio and DNA/ muscle mass (wet weight) ratio in the *L. rohita* fingerlings. This study

also reveals that growth of *L. rohita* fingerling was due to increase muscular hypertrophy, not due to muscular hyperplasia. These data may be helpful to ascertain the effect of dietary carbohydrate on real growth response at short duration.

Acknowledgements

The authors are grateful to the Director, Central Institute of Fisheries Education, Mumbai, for providing facilities for carrying out the work. The first author is grateful to Central Institute of Fisheries Education for awarding the institutional fellowship.

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