Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

Efficacy of green algae and cyanobacteria as feed for juvenile *Labeo gonius*

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Abstract

The present paper deals with efficacy of some dominant microalgae and cyanobacteria in different combinations as fish feed and colour enhancer in the growth of fish *Labeo gonius* (Hamilton) which is commonly cultured fish in pisciculture industry of the region. The survival, specific growth rate, feed conversion ratio (FCR), protein, carbohydrate, lipid and carotenoid content of *Labeo gonius* fed with different algal diets were significantly higher than that fed with normal fish feed. Among the three algal feed, all the parameters measured were maximum in fishes fed with mixed algal feed AF (*Scenedesmus obliguus*; *Chlorella vulgaris; Spirogyra* sp; *Navicula* sp; *Nostoc* sp and *Anabaena* sp) followed by fishes fed with diet containing only green algae AF1 (*Scenedesmus obliguus; Chlorella vulgaris; Spirogyra* sp and were minimum in diet containing mainly two Cyanobacteria and one diatom member AF2 (*Nostoc* sp and *Anabaena* sp and *Navicula* sp).

Key words: algal feed, pisciculture, specific growth rate, feed conversion ratio

Introduction

The use of algae as an additive feed in aquaculture has received a lot of attention due to the positive effect it has on weight gain. Quality of aqua feed is one of the most important criteria for the success of aquaculture. The popularity of microalgae as fish feed is increasing rapidly as suitable alternative source in modern aquaculture industry (Becker, 2004, 2007). Various species of microalgae have been assessed for their nutritional value and incorporated into fish feed formulation for higher fish production and combination of different algal species provided balanced nutrition and improved fish growth better than a diet composed of only one algal species (Sen Roy et al, 2015; Sirakov et al., 2015). Green algal species like *Chlorella, Scenedesmus* containing crude protein(40% to 50%), total carbohydrate content (25%-60%) and a considerable amount of β- carotene content have been reported to be beneficial for the growth of fishes like Korean rockfish, Tilapia (Bai et al., 2001; Tartiel et al., 2008). Use of Cyanobacteria *Spirulina* possessing protein as high as 40–70% is well documented as fish feed. Renaud et al. (1994) reported more protein in diatom species in comparison to Chlorophycean members. In addition, algal feed formed with different microalgae have been used widely to develop efficient colour in ornamental fishes. Microalgae such as *Dunaliella salina* (Dunal) Teodoresco, *Haematococcus pluvialis* (Flotow) and *Spirulina* spp. have been used to enhance the skin coloration in fishes (Gupta et al., 2007).

Fish has long been used as source of protein for human nutrition worldwide. In India, demand for fishes is very high and the rate is going high as the demand is escalating. To fill up the gap between demand and the production, pisciculture industry is gaining attention. Fish meal, the most important source of protein, is an expensive feed ingredient and the supply often could not be met with due to high demand. To meet the demand, use of locally available microalgae as an efficient alternative/additive fish feed is gaining importance in growing pisciculture industries all over the world including India.

In Meghalaya, a state in the North Eastern region of India is very rich in aquatic resources and therefore fish is an important component of food. But there is a huge gap in between fish production and fish supply by the state and therefore the demand is fulfilled by bringing fishes from other neighbouring states. State Government is developing pisciculture industry in large scale to meet the demand of the fast growing population of the region. Therefore, the present work was taken to formulate fish feed using locally available nutritious and coloured microalgae which could develop healthy local fishes and bring down the cost of fishes.

Materials and Methods

Juvenile *Labeo gonius* (Hamilton) is an important fish cultured by the fishery Department of the state. Therefore, in the present study, *Labeo gonius* has been used as experimental fish. Juvenile *Labeo gonius* of same age group were collected from fish breeding farm in Mawpun, Meghalaya.

Collection and culturing of few screened algal species

A mixture of three Chlorophyceae genera *Scenedesmus obliguus* (Turpin) Kützing; *Chlorella vulgaris* Beyerinck (Beijerinck) and *Spirogyra* sp, two cyanobacterial genera *Anabaena* sp and *Nostoc* sp and one Bacillariophyceae genera *Navicula* sp were used as experimental feed. The experimental algal genera were collected from four lakes situated in three different District of Khasi Hills following standard protocols and micro algal cultures were raised for biomass utilization. The three green algal genera were grown using Chu media, diatom was grown using Guillard media supplemented with SIO^4 and for cyanobacteria BG11 media was used. Fishes were acclimatized for 2 weeks in the laboratory condition and were fed with commercial diet for proper rearing. All the fishes were starved for 24h prior to the onset of the feeding trial. A trial for 90 days feeding was carried out in aquarium. Four experimental sets were prepared for each experimental diet with thirty fishes in each set. In set 1, fishes were fed with conventional feed (CF) (Rice Bran and Mustard Oil Cake). This was considered as control feed; in set 2, fishes were fed with the mixture of all the five different algal genera (AF) (*Scenedesmus obliguus*: *Spirogyra* sp*: Chlorella vulgaris: Anabaena* sp: *Nostoc* sp: *Navicula* sp); in set 3, fishes were fed with only green algae (AF1) (*Scenedesmus obliguus*: *Spirogyra* sp: *Chlorella vulgaris*) and in set 4, fishes were fed with a mixture of blue-green algae and one diatom species(AF2) (*Anabaena* sp: *Nostoc* sp: *Navicula* sp).

The pre-weighed dry algae were mixed in a proportion of *Scenedesmus obliguus*: *Spirogyra* sp: *Chlorella vulgaris*: *Anabaena* sp: *Nostoc* sp: *Navicula* sp: 15:35:15:20:10:5 to prepare the composite algal mix for set 2. For set 3 it was mixed in the proportion of *Scenedesmus obliguus: Spirogyra* sp: *Chlorella vulgaris*: 25: 40: 35 and for set 4 *Anabaena* sp: *Nostoc* sp: *Navicula* sp: 40: 30: 30 (Fitzsimmons, 1997). All the fishes were fed with their respective diets to apparent satiation twice a day at 3% of body weight throughout the experimental period and the uneaten food was collected daily. The fishes were weighed and the length was measured every fortnight. Water parameters like temperature, dissolved oxygen and pH were maintained throughout the feeding experiments.

Analysis of growth performances in fish

Growth performance and feed utilization of experimental fishes were evaluated by standard methods (Siddhuraju and Becker, 2003) in terms of final fish weight (g), weight gain (WG, %), specific growth rate (SGR, % day-1) and feed conversion ratio (FCR). The fishes were randomly selected for analysis of different parameters.

The formulae used are as follows:

1. WG (weight gain) = [(Final body weight (g) - Initial body weight (g))/ Initial body weight (g)] x 100

2. SGR (specific growth rate) = [(ln Final body weight (g) - ln Initial body weight (g) / Number of days] x 100

3. FCR (feed conversion ratio) = Dry feed fed (g) / Live body weight gain (g)

Analysis of biochemical parameters in fish

Biochemical parameters like protein, carbohydrate, lipid and carotenoid contents of selected algal species were estimated by the following methods. Protein was estimated following the standard method of Lowry et al. (1951), carbohydrate was estimated by Anthrone method (Roe, 1955), lipid was estimated by Dittmer and Wells (1969) and pigments mainly carotenoid was estimated by Sadasivam and Manickam, (1996) respectively.

The proximal protein, carbohydrate and lipid were analyzed from the tissue of the fishes whereas carotenoid content was analyzed from the skin of *Labeo gonius* following the same method as was done for algal species.

Statistical Analysis

All growth parameter data were subjected to one-way analysis of variance (ANOVA). The significance of difference between means was determined by Duncan's multiple range tests ($P < 0.05$) using Past software.

Results

Growth performance of Labeo gonius with different feeds

The survival of *Labeo gonius* fed with different algal diets was significantly higher than that fed with normal fish feed. Addition of algal biomass to the diet of *Labeo gonius* showed significant increase in the body length and weight of the fishes with time. The maximum increase in mean length and weight was observed after 90 days. Body length ranged from 5.7cm – 9.4cm and weight gain ranged from 3.3g – 7.8 g. (Table.1).

Table 1: Survival rate, body length and body gain of *Labeo gonius* fed with conventional feed and algal feed. AF (*Scenedesmus obliguus*; *Chlorella vulgaris*; *Spirogyra* sp; *Navicula* sp; *Nostoc* sp and *Anabaena* sp), AF1 (*Scenedesmus obliguus*; *Chlorella vulgaris* and *Spirogyra* sp) and AF2 (*Nostoc* sp: *Anabaena* sp: *Navicula* sp).

	Control	AF	AF1	AF2
Survival %				
30 days	83.2±0.51^{a}	85.5±0.36^{b}	84.3±0.3^{c}	83.6±0.55^{c}
60 days	84.8±0.45^{a}	88.8±0.58^{b}	86±0.76^{c}	85.6±0.2^{c}
90 days	85.5±0.25^{a}	89.4±0.46^{b}	86.9±0.15^{c}	85.9±0.37^{d}
Body length (cm)				
30 days	5.3±0.11^{a}	5.7±0.15^{b}	5.5±0.06^{b}	5.5±0.1^{b}
60 days	6.2±0.12^{a}	7.6±0.1^{b}	7.5±0.1^{b}	7.3±0.12^{c}
90 days	7.8±0.06^{a}	9.4±0.15^{b}	8.8±0.14^{c}	8.5±0.12^{d}
Body weight gain (g)				
30 days	2.8±0.3^{a}	3.3±0.1^{b}	3.1±0.5^{b}	3.0±0.15^{b}
60 days	3.7±0.06^{a}	4.9±0.2^{b}	4.6±0.4^{c}	4.4±0.1^{c}
90 days	6.4±0.2^{a}	7.8±0.24^{b}	6.9±0.11^{c}	7.2±0.3^{d}

* Values on the same row with different superscripts are significantly different ($p < 0.05$)

Among the three algal feed, survival rate was maximum in fishes fed with mixed algal feed AF (89.4%), followed by fishes fed with diet containing only green algae (86.9%) and was minimum in diet containing two Cyanobacteria and one diatom (83.6%) (Fig1).

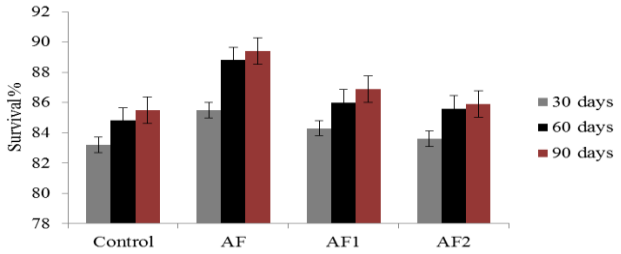

Figure 1: Survival rate of *Labeo gonius* fed with conventional feed and algal feed. AF (*Scenedesmus obliguus*; *Chlorella vulgaris*; *Spirogyra* sp; *Navicula* sp; *Nostoc* sp and *Anabaena* sp), AF1 (*Scenedesmus obliguus*; *Chlorella vulgaris* and *Spirogyra* sp) and AF2 (*Nostoc* sp: *Anabaena* sp: *Navicula* sp).

Body length and weight were maximum when the fishes were fed with mixed algal feed AF: (*Scenedesmus obliguus*; *Chlorella vulgaris; Spirogyra* sp; *Navicula* sp; *Nostoc* sp and *Anabaena* sp). When fed with AF1 (*Scenedesmus obliguus*; *Chlorella vulgaris* and *Spirogyra* sp) the body length and weight gain of the fishes ranged from (5.5cm – 8.8 cm) and (3.1g – 6.9 g) and when fed with AF2: (*Nostoc* sp, *Anabaena* sp and *Navicula* sp) the fishes attained a body length from 5.5cm – 8.5 cm and weight gain was from 3.0g – 7.2 g respectively (Fig. 2).

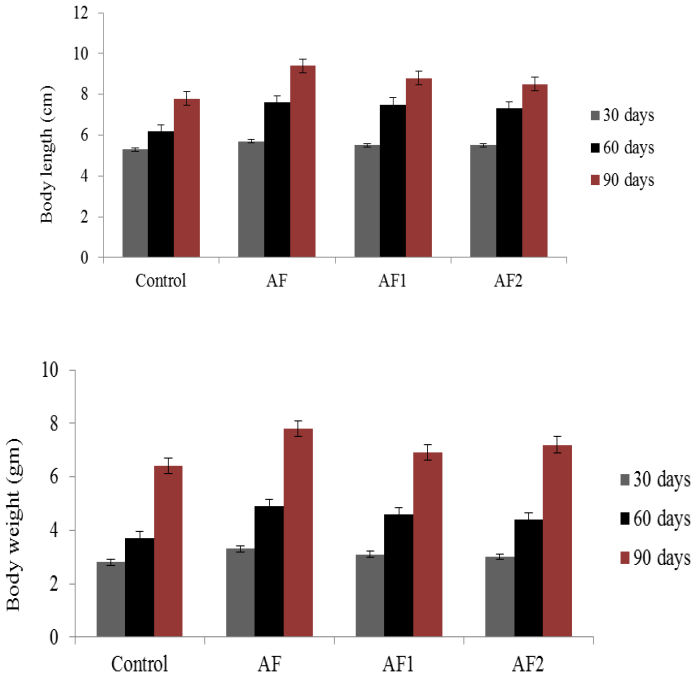

Figure 2: Changes in body length (cm) and weight (g) in *Labeo gonius* fed with conventional feed and algal feed. AF (*Scenedesmus obliguus*; *Chlorella vulgaris*; *Spirogyra* sp; *Navicula* sp; *Nostoc* sp and *Anabaena* sp), AF1 (*Scenedesmus obliguus*; *Chlorella vulgaris* and *Spirogyra* sp) and AF2 (*Nostoc* sp: *Anabaena* sp: *Navicula* sp).

Feed efficiency

Final body weight (FBW), weight gain percentage (WG), specific growth rate (SGR%/day) and feed conversion ratio (FCR) in *Labeo gonius* were significantly high ($p < 0.05$) when the fishes were fed with different algal diets compared to the normal feed (Table. 2). The final body weight gain after feeding conventional feed ranged from (2.8 – 6.4 g) in diet containing mixed algal feed AF, it ranged from (3.3 – 7.8 g), in diet containing only green algae AF1, it ranged from (3.1 – 6.9 g) and after feeding diet containing a mixture of two Cyanobacteria and one diatom AF2, it ranged from (3.0 – 7.2 g) respectively. The weight gain percentage from feeding conventional feed ranged from (27.3 – 190.9 %), feeding diet containing mixed algal feed AF it ranged from (50.0 – 254.5 %), in diet containing only green algae AF1 it ranged from (40.9 – 213.6 %) and in diet containing a mixture of two Cyanobacteria and one diatom AF2, it ranged from (36.4 – 227.2 %) respectively. The specific growth rate after feeding conventional feed ranged from (4.0 – 5.6 % day-1), in diet containing mixed algal feed AF it ranged from (7.3 – 7.9 % day-^{1}), in diet containing only green algae AF1 it ranged from (6.0 – 6.3 % day-1) and in diet containing a mixture of two Cyanobacteria and one diatom AF2 it ranged from (5.3– 6.7 % day-1) respectively. The feed conversion ratio in conventional feed ranged from (0.5 – 0.07), in diet containing mixed algal feed AF it ranged from (0.3 – 0.05), in diet containing only green algae AF1 it ranged from (0.3 – 0.06) and in diet containing a mixture of two Cyanobacteria and one diatom AF2, it ranged from (0.4 – 0.06 %) respectively. Among the three algal diets the final body weight, weight gain percentage and specific growth rate were significantly ($P < 0.05$) higher when the fishes were fed with mixed algal feed (AF) compared to the other two algal diets (only green algae) and (two Cyanobactera and one diatom) indicating the best results among the 3 diets used (Table. 2).

Protein, carbohydrate, lipid and pigment content in selected algal genera

The proximate composition of protein, carbohydrate, lipid and pigment content of the selected algal genera were determined (Table.3). Among the six selected algal genera, protein content was proportionately higher in *Scenedesmus obliguus* (53.3 %) and *Chlorella vulgaris* (48.8 %) and respectively, whereas *Spirogyra* sp had high carbohydrate content (52.1 %) in its nutrient composition. Lipid content was more in *Chlorella vulgaris* (19.3 %) and *Spirogyra* sp (17 %) compared to the other algal genera whereas carotenoid content was maximum in *Anabaena* sp (0.21 %) followed by *Nostoc* sp (0.19 %), *Spirogyra* sp (0.17 %), *Scenedesmus obliguus* (0.13 %), *Chlorella vulgaris* (0.06 %) and was least in *Navicula* sp (0.05 %) respectively (Table.3).

Table 3: Biochemical analysis of the algae used in fish feed

Algae	Protein (%)	Carbohydrate (%)	Lipid (%)	Carotenoid (%)
Scenedesmus obliguus	53.3±0.35	16.1±0.22	12.1±0.78	0.13±0.12
Chlorella vulgaris	48.8±0.27	14.1±0.77	19.3±1.24	0.06±0.01
Spirogyra sp	18.5±0.28	52.1±0.93	17.0±0.52	0.17±0.08
Navicula sp	15.0±0.04	13.8±0.57	2.7±0.37	0.05±0.06
Nostoc sp	31.8±0.36	14.0±0.54	9.5±0.45	0.19±0.09
Anabaena sp	52.1±0.33	29.4±1.11	6.2±0.15	0.21±0.04

Protein, carbohydrate, lipid and pigment content in Labeo gonius

The protein, carbohydrate and lipid contents in *Labeo gonius* increased significantly ($p < 0.05$) when fed with algal feed compared to conventional feed (Table.4). The increase in protein content was maximum when fishes were fed with mixed algal feed (10.6) compared to that algal feed containing only Chlorophyceae members (8.9) or combination of two Cyanobacteria and one diatom species (8.1). The carbohydrate content in *Labeo gonius* was also found to be maximum when fed with mixed algal feed (3.9) followed by diet containing only Chlorophyceae members (3.8) and was minimum when fed with diet containing two Cyanobacteria and one diatom species (3.7). Lipid and carotenoid content in the skin showed the same trend. It was maximum in fishes when fed with mixed algal feed (0.23) and was minimum when fed with diet containing Cyanobacteria and diatom species (0.15) (Table .4).

Table 4: Proximal protein, carbohyrdate, lipids and carotenoids level of *Labeo gonius* fed with conventional feed and algal feed. AF (*Scenedesmus obliguus; Chlorella vulgaris*; *Spirogyra* sp; *Navicula* sp*; Nostoc* sp and *Anabaena* sp), AF1 (*Scenedesmus obliguus; Chlorella vulgaris* and *Spirogyra* sp*)* and AF2 (*Nostoc* sp: *Anabaena* sp*: Navicula* sp*)***.**

Parameters (%)	Initial value	Control	AF	AF1	AF2
Protein	5.8±0.08	6.1±0.05^{a}	10.6±0.07^{b}	8.9±0.02^{c}	8.1±0.11^{d}
Carbohydrate	3.4±0.16	3.5±0.04^{a}	3.9±0.02^{b}	3.8±0.01^{b}	3.7±0.03^{b}
Lipid	1.5±0.04	2.9±0.03^{a}	3.7±0.05^{b}	3.4±0.03^{c}	3.1±0.04^{d}
Carotenoid	0.13±0.02	0.13±0.03^{a}	0.23±0.03^{b}	0.20±0.01^{c}	0.15±0.02^{c}

* Values on the same row with different superscripts are significantly different ($p < 0.05$)

Discussion

Mustafa and Nakagawa (1995) reported enhanced growth, feed utilization, lipid metabolism, body composition, disease resistance and carcass quality of a variety of fishes with supplementation of microalgae meal in the fish feed. In the present study, growth performance of *Labeo gonius* improved significantly ($p < 0.05$) when the fishes were fed with conventional feed mixed with different combinations of algal feed compared to only conventional feed. Among the three different feed, algal diet (AF) comprising six algal species from three different algal groups significantly surpassed the growth performances of fishes subjected to feed containing only Chlorophyceae members (AF1) or feed comprising two Cyanobacteria and one diatom members (AF2) indicating the importance of composite algal mixture supplementation. Singh et al. (2006) reported improved growth performance with algal feed in *Labeo rohita* fingerlings. Badaway et al. (2008) reported better growth performance on partial replacement of fish meal with *Chlorella* and *Scenedesmus* in Nile Tilapia. Sudaporn et al. (2010) reported increased growth performance in *Oreochromis niloticus* and in red sword tail fish by adding *Spirulina* to the diet. Khatoon et al. (2010) reported an increase in body weight as well as specific growth rate percentage in gold fish when fishes were fed with microalgae based feed.

The protein, carbohydrate and lipid contents in *Labeo gonius* increased significantly with addition of algal feed compared to that fishes fed with conventional feed during the experimental tenure. The diet containing mixed algal feed (AF) had a good amount of protein carbohydrate and lipid contents. This could be the reason for the increased growth performances in the fishes fed with algal diet. *Chlorella* sp was considered as a protein source and was used as an energy-rich food source, for fish larvae and rotifers (Tartiel et al., 2008) .The diatom genus *Navicula* had already been reported as a good source of lipid (Sen Roy et al., 2009). But in the present case, lipid content was high in mixed algal feed compared to other two feed used. This could be because biomass of diatom species raised in culture was too less and it was used along with two Cyanobacteria. Many authors while trying in feeding trials with many types of microalgae in fishes reported improved quality of carcass (Belay et al., 1996; Floreto et al., 1996). Addition of *Spirulina* enhanced growth, improved protein digestibility and increased lipid content (Mustafa et al., 1994), stress and disease resistance (Henson, 1990; Olvera-Novoa et al., 1998).

Freshwater green microalgae (*Chlorella, Haematococcus*), cyanobacteria (*Nostoc, Anabaena* and *Spirulina*) have been successfully used to supply certain carotenoids (lutein, violaxanthin, zeaxanthin, astaxanthin, and β-carotene) to ornamental fish, Red Tilapia fish, koi carp, goldfish or cichlid and prawns (Gouveia and Rema, 2005; Kop and Durmaz, 2008; Khatoon et al., 2010). Gouveia et al. (2002) reported the use of *Chlorella vulgaris* biomass in the diets of rainbow trout and gilthead sea bream for better yield and skin pigmentation effects. The present study is in full agreement with the result observed in different fishes by different authors. Carotenoid content in the fish skin of *Labeo gonius* was significantly higher when fed with algal feed compared to normal feed.

Conclusion

The present study confirmed that the locally available rich algal potential could be utilized successfully in pisciculture industry of the region to achieve better growth and enhanced production of fishes at lower rate.

Acknowledgement

Authors are thankful to Fishery Department of Meghalaya Government for providing Juvenile fishes, to UGC for financial help and to North Eastern Hill University for providing all the facilities.

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Table 2: Growth performance of *Labeo gonius* fed with conventional feed and algal feed. AF (*Scenedesmus obliguus*; *Chlorella vulgaris*; *Spirogyra* sp; *Navicula* sp; *Nostoc* sp and *Anabaena* sp), AF1 (*Scenedesmus obliguus*; *Chlorella vulgaris* and *Spirogyra* sp) and AF2 (*Nostoc* sp: *Anabaena* sp: *Navicula* sp).

	After 30 days				After 60 days				After 90 days			
Parameters	Control	AF	AF1	AF2	Control	AF	AF1	AF2	Control	AF	AF1	AF2
Initial Weight (g)	2.2	2.2	2.2	2.2								
Final Weight (g)	2.8±0.15^{a}	3.3±0.25^{b}	3.1±0.06^{c}	3.0±0.05^{c}	3.7±0.11^{a}	4.9±0.2^{b}	4.6±0.1^{c}	4.4±0.16^{c}	6.4±0.35^{a}	7.8±0.32^{b}	6.9±0.15^{b}	7.2±0.21^{b}
WGP (%)	27.3±0.17^{a}	50.0±1.53^{b}	40.9±0.17^{c}	36.4±0.07^{d}	68.2±0.09^{a}	122.7±0.4^{b}	109.0±0.5^{c}	100.0±2.08^{d}	190.9±0.25^{a}	254.5±0.12^{b}	213.6±0.3^{c}	227.2±0.6^{d}
SGR (%day$^{-1)}$)	4.0±0.76^{a}	7.3±0.04^{b}	6.0±0.65^{c}	5.3±0.04^{d}	3.3±0.04^{a}	6.0±0.5^{b}	5.3±0.02^{c}	4.9±0.03^{c}	5.6±0.02^{a}	7.9±0.03^{b}	6.3±0.04^{c}	6.7±0.9^{d}
FCR	0.5±0.21^{a}	0.3±0.03^{b}	0.3±0.03^{c}	0.4±0.04^{d}	0.2±0.15^{a}	0.1±0.2^{b}	0.1±0.01^{c}	0.2±0.02^{d}	0.07±0.01^{a}	0.05±0.01^{a}	0.06±0.02^{a}	0.06±0.1^{a}

*Values on the same row with different superscripts are significantly different ($p < 0.05$). WGP (Weight gain percentage), SGR (specific growth rate), FCR (feed conversion ratio).