

Growth Performance and Survivability of the Asian Seabass Lates Calcarifer Reared Under Hyper-Saline, Hypo-Saline and Freshwater Environments in a Closed Aquaculture System

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Research Article

Keywords: Lates calcarifer, aquaculture, effects of salinity, growth, survival

DOI: <https://doi.org/10.21203/rs.3.rs-211072/v1>

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Abstract

Salinity is one of the most critical environmental parameters regarding fish physiology, modifying food intake and growth performance in many fish species. The present study has investigated the effects of different salinity levels on growth performance, feeding and survival of Asian seabass *Lates calcarifer* juveniles. Asian seabass juveniles were reared at 0, 5, 22, 36, and 42 ppt salinity. One five hundred ninety-eight fish individuals with an average weight 1.2 ± 0.11 g were randomly distributed (166 fish/Tank) in 5 concrete tanks (30×6×4 feet) each; length × width × depth) and volume 19,122 L, for total forty days. Initially feeding rate of 6% biomass per day with 42% crude protein-containing diets daily. The feeding rate adjusted according to fish biomass every week. The results showed that salinity level had a significant effect on the weight gain, average daily weight gain, specific growth rate, feed conversion ratio, survival rate, total biomass and health indices ($p < 0.05$). The highest WG (39.11 ± 1.49 g), ADWG, (1.00 ± 0.12 g), SGR (8.74 ± 0.03 % d^{-1}) and lowest FCR (0.96 ± 0.20) observed with T3 (22 ppt salinity) treatment, which was significantly higher compared to other treatment groups ($p < 0.05$). Among the health indices, the highest hepatosomatic index and viscerosomatic index found with 22 ppt salinity treatment, which was also significantly higher than the other treatment groups ($p < 0.05$). No significant differences were found among the treatments in terms of survival rate ($p > 0.05$), but the maximum survival rate (98.89 ± 0.0 %) in T3 (22 ppt) and T2 (5 ppt). The maximum level of crude proteins (19.99 ± 1.4 %) was found in the whole-body biochemical composition (% of wet weight) of Asian sea bass juveniles in T3 treatment group reared at 22 ppt salinity. The second-order polynomial regression showed that 20 ppt salinity is optimum for the best growth of Asian sea bass. Thus, our present work would provide valuable information to the fish farmers for culturing the sea bass as well as its management along the inland and coastline of Pakistan.

Introduction

Lates calcarifer (Bloch, 1790) is commonly known as “Barramundi” or “giant sea perch” or “Asian sea bass” throughout the world. It is one of the most suitable species for aquaculture, its tolerance to broad environmental conditions, gregarious (readily school), delicately-flavoured tasty, nutritious meat and fast growth rate that can be reached to 1.5 to 3.0 kg in a year when its environmental conditions are kept optimum, the maximum weight is about 60 kg (Allen et al. 2002), with sustained high demand and market price in both domestic and export markets (Hassan et al. 2020a; Rao et al. 2013). Asian sea bass is a comparatively strong ecological tolerance that tolerates a broad range of salinity from freshwater to complete seawater from 0 to 56 ppt salinity (FAO 2017). Salinity is a critical environmental factor which can highly effect on the physiology of aquatic organisms (Urbina & Glover 2015). Asian seabass has wide physiological tolerances growing in marine, brackish, and freshwater (Anil et al., 2010; Ganzon-Naretetal 2013; Venkatachalam et al. 2018; Yue et al. 2009). Salinity also has an antagonistic effect on growth, survival, fecundity, physiology and osmoregulation (Smith 2003; Smith and Schindler 2009; Anni et al. 2016). Fish at the extreme of their salinity tolerance range often exceed their osmoregulation ability

(Sampaio and Bianchini 2002). The rapid rise in salinity disrupts physiological procedures in fish, leading to greater cortisol concentrations in the blood (Tsuzuki et al. 2007).

Culture of *L. calcarifer* is relatively new in Pakistan (Shah et al. 2020), To initiate advance aquaculture, the development of breeding, larviculture and farming technology is an urgent need to increase and establish Asian sea bass culture industry throughout the country. Specific water parameters and seed production management are required for effective aquaculture practice to avoid the wild fisheries resources declining (Shah et al. 2020) for excellent growth performance and successful aquaculture necessary optimum parameters. Therefore, the objective of this study was to investigate the growth performance, survival, and health status of Asian seabass with a wide range of salinities in a closed aquaculture system.

Materials And Methods

Experimental design

The present study was conducted at the Sindh fish hatchery near Hawkes Bay town from March 2019 to Jan 2020. A total of 598 uniform sized (1.24 ± 1.08 grams) sea bass juveniles were collected from the coastal area of Sindh Sakro and stocked (166 fish/Tank) in five treatments (T1, T2, T3, T4 and T5). Each treatment tank was 30×6×4 feet containing 0, 5, 22, 35 and 42 ppt salinity by following the methods of Azodi *et al.* (2016) with some modifications. Tank water was regulated by blending filtered water from the ocean and freshwater. Water was exchanged for the maintenance of the water quality by up to 50% every 2 days. Fish were fed at 6% of body weight per day ($BW \text{ day}^{-1}$) for 40 days and the feeding rate adjusted every week. The extracted pellet (42.0% crude protein) was used for the manual feeding at two distinct satiation levels twice daily. After 3 hours of feeding, the extra feed was removed. The total consumption of food was reported daily in each treatment. A 42% CP-level diet was formed using various components (Table 1), referred to as the entire diet/ration structure. This diet was offered regularly based on the daily wet bodyweight of the individuals.

Feed formulation and preparation

Feed ingredients were collected from Karachi's local market and formulated as previously methods described by Ghosh et al. (2011). The major source of protein used in diets was fishmeal, and the levels of protein were preserved at 42%. The feed ingredients have been combined to form a dough with fish oil and water. The palletizer was carried through the dough to create the necessary pellet diet. Under dry conditions, the feed was stored in a freezer until used. Proximate dry matter, crude fat, crude protein, crude fibres, and moisture levels were determined in a percentage of the dried feed specimens (Table 1).

Samples collection and Biochemical analysis

The biochemical analysis of feed and fish carcass samples was carried out based on (AOAC 2000). At the end of the experiment, five fish were removed from each tank and then dissected to weigh the liver

and viscera, then HSI and VSI were determined. Techniques (AOAC 2000) were used to analyze crude lipid (CL), moisture and crude protein (CP). Moisture was measured at 105° Celsius in the oven for 24 h (Labostar-LG122 Tabia Espec, Osaka, Japan). Chloroform/methanol (2:1v/v) extraction process predicted for crude lipids. (Folch et al. 1957) The CP analyzed using the automatic processing of Kjeldahl (Buchi430/) Using the Kjeltex method (N 6.25) with the automatic Kjeldahl system (Buchi 430/323) Model 1265, Moline IL, USA). Furthermore, for the determination of ash, the sample was burned at 550 C in a muffle furnace for 3 hours. Gross energy (GE) has been approximated for formulated diets factors 23.62, 39.5 and 17.56 KJ/g for CP, EE and carbohydrate respectively were used (NRC 1993).

Water quality parameters

Water quality parameters such as temperature, salinity, dissolved oxygen (DO), and pH were recorded daily using Celsius glass thermometer, Handheld Refractometer, mobile digital DO-meter (Model: HI9146) and Digital pH meter, respectively. The concentrations of ammonia, alkalinity, nitrate, and nitrite were measured by following the methods of APHA (1995). Details of the water quality parameters recorded during the study period are given in Table 2.

Growth performance analysis

The wet weight gain, average daily weight gain (ADWG), specific growth rate (SGR), feed conversion ratio (FCR), hepatosomatic index (HSI), viscerosomatic index (VSI), Fulton's condition factor (CF), survival rate (SR) and total biomass were calculated with the following formula adopted by Hassan et al. (2020b):

WG=Final weight-initial weight

ADWG= {(Final weight-Initial weight)/Days}

SGR (%) = [(Ln FBW – Ln IBW) / day] × 100

FCR=Food given (g)/Weight gain (g)

Fulton's condition factor (K) = (Weight/Length³) ×100

VSI = {weight of visceral organs and associated fat tissue (g)/wet body weight} ×100

HSI= weight of liver (g)/empty fish weight (g) ×100

SR (%) = (No. of fish survived/ No. of fish released) ×100

Cannibalism (%) = 100 × [(LS – M – LC)/LS]

Where, LS stocked at the beginning of sea bass, LC is the number of sea bass collected at the end of the study and M is natural mortality

Biomass = Average bodyweight × No of fish

Statistical analysis

All data were analyzed using STATISTICA V.13 software (StatSoft Inc., Tulsa, OK, USA). Residuals were tested for normality (Shapiro–Wilk test) and homogeneity of variance (plot of residuals versus predicted values). The percentages data were natural log (LN)-transformed before analysis. The effects of different salinity group on the growth performance and health indices of Asian seabass juveniles at different culture periods were analyzed using a repeated-measures ANOVA model containing culture period (fixed repeated factor) and different salinity groups (fixed factor) main effects as well the culture period × different salinity groups interaction term. Then, the model was decomposed into a series of one-way ANOVA was run to test the effects of different salinity levels on the wet weight gain, average daily weight gain, specific growth rate, hepatosomatic index, viscerosomatic index, Fulton's condition factor, feed conversion ratio, survival rate and biomass for different culture periods. A posteriori analysis was performed using Tukey's multiple comparisons procedures. A significance level of $p < 0.05$ was used for all statistical tests.

Results

Physicochemical parameters

Table 2 shows the minimum and maximum values of water quality parameters in five experimental groups recorded as water pH and the temperature was range from 8.10 to 8.81 and 29.40 to 29.74°C, while dissolved oxygen (DO) was found in the range from 7.16 to 8.16 mg/L, dissolved ammonia concentration was 0.012 to 0.072; whereas, alkalinity was between 126.1 to 162.4 mg/L, nitrates from 0.004 to 2.57 mg/L and nitrites 0.010 to 0.014mg/L respectively.

Growth performance

The saturated repeated-measures ANOVA model revealed a significant effect on the culture period × different salinity groups interaction term ($p < 0.01$). Therefore, the model was decomposed into a series of a lower-order statistical model. Our results showed that salinity level had a significant effect on the weight gain, average daily weight gain, specific growth rate total biomass and health indices ($p < 0.05$) (Table 3). The highest weight gain (39.11 ± 1.49 g), average daily weight gain (1.00 ± 0.12 g), specific growth rate ($8.74 \pm 0.03\% \text{ d}^{-1}$) and production (6654 ± 4.20 g) observed with 22 ppt salinity treatment, which was significantly higher compared to other treatment groups ($p < 0.05$) followed by salinity 42 ppt (T5), 36 ppt salinity (T4), 5 ppt and 0 ppt (T1) respectively. However, the growth curve decreased with increased salinity above 36 to 42 ppt. The second-order polynomial regression showed that 20 ppt salinity is optimum for the best growth of Asian sea bass

Feed utilization parameters

The FCR value significantly variance among the groups (at level $P < 0.05$, Table 3). T3 had the best FCR (0.96 ± 0.20) followed by T1 (1.24 ± 0.10) T2 (1.04 ± 0.00) and T4 (1.23 ± 0.10) while the worst FCR was

achieved by T1 (1.47 ± 0.20).

Morphological indices

Among the health indices, the highest hepatosomatic index and viscerosomatic index found with 22 ppt salinity treatment, which was also significantly higher than the other treatment groups ($p < 0.05$). The better condition factor also found in T3 compared to other treatments.

Cannibalism and survival

However, no significant differences were found among the treatments in terms of survival rate ($p > 0.05$), but the maximum survival rate (99.39 ± 0.0 %) in T3 (22 ppt). The cannibalism noted in T1 and T4 treatments.

Proximate composition of fish carcass

The proximate carcass composition was calculated on basis wet weight and presented in Table 4 carcass content of moisture and protein did not significantly affect by salinity. Conversely,

carcass content of lipid and ash significantly differed among the treatments, where lipid carcass of T1, T2, T3 is lower compared to T4 and T5. The moisture content and protein in carcass did not significantly effect by salinity, were moisture of T1 is higher than the other treatments. There was a positive correlation between the carcass content of crude proteins and increasing of salinity level, The maximum level of crude proteins ($19.99 \pm 1.4\%$) was found in the whole-body biochemical composition (% of wet weight) of Asian seabass juveniles in T3 treatment group reared at 22 ppt salinity (Table 4). Concerning Ash content, there was insignificant among T5, T4, T3 and T2 in Ash content, but Ash content of T1 was 7.82 ± 0.1 and significantly higher than the other treatments.

Discussion

As Asian sea bass *L. calcarifer* has great commercial values among the other marine fish species because of its usage as popular cultured species in most Southeast Asian countries including Thailand, Taiwan, Indonesia, Malaysia, Singapore and Hong Kong near coastal water, brackish and also in their freshwaters resources. Most countries have now used this cultured species for both development and research purposes in pond and cage culture. In this study, the means of physicochemical parameters were recorded in Table 3, did not show and marked variation and fall between the acceptable range for Asian sea bass growth and health According to (Priestly *et al.* 2006; Hassan *et al.* 2020a; Kungvankij *et al.* 1986), who also found water pH ranged from 7.5 to 8.3, temperature 26 to 32°C, dissolved oxygen (DO) from 4.0 to 8.0 mg/L, Dissolved ammonia less than 0.02mg/L and salinity was considered as 10.0 to 31.0 ppt during culturing of fry and fingerling stages of *L. calcarifer* reared in concrete tanks near coastal waters in Thailand. Moreover, the nitrite and nitrate concentrations were comparatively low (> 5 mg/L) in five experimental tanks of this study, because of addition of surface runoff water from rainfall bring pollutants includes domestic sewage wastes, agriculture and industrial wastes in fish ponds along with

water resources used in fish culture and ammonium wastes from fish excreta released in fish ponds during culture of this species as in accordance with Christensen et al. (2012). Nitrate in ponds was higher than in canals because the decomposition of ammonia from fish waste to nitrate. However, nitrate was found in low concentration which meet the surface water quality criteria. Our results of water quality parameters was also found to be optimum for the growth of this species as reported by Jerry (2014). In addition, Abdullah et al. (2018) studied had shown the significant impact of dry and monsoon seasons on the water parameters includes salinity, dissolved oxygen concentration and turbidity of *L. calcarifer* culturing near Sri Tujuh lagoon of north east coast of Peninsular Malaysia.

In this study, the optimum salinity considered best for normal culture of seabass was 22 ppt (T3), which was very close to the results obtained by Ercan et al. (2015). Cheong (1989) had reported that as *L. calcarifer* is a euryhaline species; therefore, it can be easily cultured in both freshwater and brackish water, or even in seawater also. Therefore, this species had been successfully cultured in the freshwater ponds various regions of the world like in Thailand, and also in coastal water ponds like in Tahiti with salinity ranging from 10 to 35 ppt. Hence, most farmers commonly cultured *L. calcarifer* in ponds located along the sea coast with salinities ranging between 10 to 30 ppt, which is considered the optimum range for this species. Euryhaline fish undergo a crucial stage during their acclimatization in a hyperosmotic environment. There is a rapid increase in the gill-ion fluid, followed by a rise in serum electrolytes and osmolality. In the present study, the acclimatization process for the juvenile fish was done to reduce the stress level of the fish during exposure to the different salinity treatments. The juvenile fish were not directly introduced to the various salinity treatments. Indeed, the salinity was decreased or increased slowly to the targeted level using a dropper to avoid fish startling. Thus, the juvenile can survive in a wide range of salinity without affecting its mortality rate (Cotton et al. 2003). In the present study, less mortality of juveniles was found for all the treatment groups (0 to 42 ppt salinity), which indicates that Asian seabass juveniles can resist abrupt hyper/hypoosmotic exposure. When fish have been questioned by medium salinity fluctuations, the ion concentration in body fluids has altered. This is in connection with the higher exchange layer on the skin and gut that lead to higher absorption of water (Shui et al. 2018).

Dendinos & Thorpe (1985) had reported the significant impact of various salinity levels ranged from 0.5 to 33‰ on growth and body composition of European bass *Dicentrarchus labrax* and found that juveniles can survive in salinities between 5 to 33‰. They also observed its maximum growth rate at 30‰, which was decreased with decreasing salinity from 20 to 25‰, and even died in freshwater with 0‰ within few days. However, such changes in salinity levels have no impact on proximate composition of fish body muscles. In the present study, various salinity levels effected on the growth performance and survival rate, where the best growth performance and survival rate were recorded in T3 (22 ppt salinity). The previous study of Bernardino et al. (2016) had also found that salinity is the main factor affecting fecundity, osmoregulation, growth rate, feed consumption and survival of fish. Rearing fish in high salinity conditions has the potential to suppress the appetite of the fish. Li et al. (2008), Partridge et al. (2008) studies suggested that decreasing growth in increased salinity was related to the decrease in food consumption. The hyper-saline environment at 42 ppt salinity negatively effects on the growth rate,

oxygen consumption, histological changes of hepatopancreas and survival as previously reported by Li et al. (2007). *L. calcarifer* at the extreme salinity range often increase their ability for osmoregulation; therefore, Partridge et al. (2008) observed that *L. calcarifer* if reared in salinity levels ranged between 0 to 36 ppt, then its expected specific growth rate (SGR) of its juvenile stages can be obtained during its culture; however, if reared at 37 to 42 ppt salinities, than its may negatively affect its both growth performance and survival rates during culture.

Conclusion

From the above study, it was concluded that the highest growth and survival of Asian seabass *L. calcarifer* were observed at 22 ppt salinity as compared to the other salinity treatments. Therefore, we recommended 20 to 36 ppt salinity for commercial farming of this species under a closed aquaculture system. Sympathetic the physiological capacity of Asian Sea bass to adapt environmental salinity changes reflecting the sustainability of investment possibilities in the aquaculture field. Using a distinct salinity concentration would maximize sea bass production and promote economic growth in aquaculture production.

Declarations

Ethical approval and consent to participate

This study was carried out in strict accordance with the recommendations by the Local Ethical Committee for Experiments on Animals of the University of Karachi, Pakistan

Funding

There was no funding from any funding body or grant agency for conducting this study.

Availability of data and materials

All data analyzed during this study are included in this manuscript.

Consent to Publish

Not applicable. This manuscript did not contain data from any individual person.

Author Contributions

Experimental design: HUH, QMA; Experiment conduct: HUH, QMA; Data analysis: ZM, KG; Manuscript writing: HH, QMA, AR, HR and MAMS Validating and reviewing.

Conflict of Interest

All authors declare no conflicts of interest. All authors listed in the manuscript contributed and attest to the validity and legitimacy of the data and its interpretation and agree to its submission to your journal.

Acknowledgements

The authors are grateful for all the support from the FDB; MNFS&R. We are indebted to the Department of Zoology (MRCC), University of Karachi for laboratory space

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Tables

Table 1
Feed ingredients of the experimental diet and biochemical composition
of the prepared diets used for Asian seabass.

Ingredients (%)	Diet (%)
Fish meal	41.0
Shrimp meal	5.5
Soybean meal	24.5
Squid meal	4.4
Rice bran	7.5
Bread flour	4.4
Cod liver oil	5.4
Mineral and vitamin premix	6.0
Fish protein Hydrolysate	1.3
Total	100
Biochemical composition of diet (%) ¹	
Crude protein ²	41.8 ± 0.5
Crude fiber	9.1 ± 0.5
Crude lipid	8.5 ± 0.06
ASH	9.3 ± 0.5
Moisture	10.8
Nitrogen-free extract ³	31.30 ± 0.5
P/E (mg crude protein KJ ⁻¹)	14.7 ± 0.5
Energy (kJg ⁻¹)	24.5 ± 0.6
Note:	
¹ Dry matter basis (%): mean ± SE, number of determinations = 5	
² Measured as nitrogen × 6.25.	
³ Nitrogen-free extract = 100 – (% protein + % lipid + % fiber + % ash).	

Table 2

Water quality parameters of four experimental tanks at Sindh Fish Hatchery recorded during the experimental period extends from March 2019 to Jan 2020

Water parameter	Treatments				
	T1 (0ppt)	T2 (5ppt)	T3 (22 ppt)	T4(36 ppt)	T5 (42 ppt)
pH	8.10 ± 0.24	8.40 ± 0.22	8.60 ± 0.26	8.80 ± 0.29	8.81 ± 0.28
Temperature (°C)	29.40 ± 1.25	29.60 ± 1.25	29.62 ± 1.10	29.74 ± 1.31	29.74 ± 1.10
D.O (mg/L)	8.16 ± 1.20	7.16 ± 1.20	7.95 ± 0.07	8.03 ± 0.25	7.98 ± 0.08
Ammonia (mg/L)	0.013 ± 0.005	0.012 ± 0.005	0.011 ± 0.005	0.072 ± 0.112	0.013 ± 0.005
Alkalinity (mg/L)	126.1 ± 5.56	143.1 ± 5.56	147.4 ± 5.07	152.6 ± 5.03	162.4 ± 5.07
Nitrite (mg/L)	0.010 ± 0.004	0.012 ± 0.004	0.013 ± 0.005	0.013 ± 0.007	0.014 ± 0.005
Nitrate (mg/L)	0.004 ± 0.001	2.22 ± 0.373	2.32 ± 0.20	2.418 ± 0.48	2.572 ± 0.20

Table 3

The effect of different salinity levels (0, 5,22,36& 42ppt) on growth performance, morphological indices and survival of Asian seabass.

Biotechnical parameters	Salinity level				
	T1 (0 ppt)	T2 (5 ppt)	T3 (22 ppt)	T4(36 ppt)	T5 (42 ppt)
Initial body weight (g)	1.22 ± 0.43	1.22 ± 0.43	1.22 ± 0.35	1.22 ± 0.43	1.23 ± 0.46
Final body weight (g)	37.80 ± 0.45 ^b	38.20 ± 0.42 ^b	40.33 ± 1.84 ^a	37.10 ± 0.40 ^b	36.50 ± 1.06 ^b
Weight gain (g)	36.38 ± 0.02 ^b	37.28 ± 0.01 ^b	39.11 ± 1.49 ^a	36.12 ± 0.03 ^b	34.28 ± 0.39 ^b
Average daily weight gain (g/day)	0.90 ± 0.11 ^b	0.94 ± 0.09 ^b	1.00 ± 0.12 ^a	0.89 ± 0.08 ^c	0.88 ± 0.06 ^c
Specific growth rate (%d ⁻¹)	8.57 ± 0.01 ^b	8.65 ± 0.03 ^b	8.74 ± 0.03 ^a	8.53 ± 0.02 ^b	8.47 ± 0.04 ^c
Hepatosomatic index (HSI)	1.30 ± 0.13 ^b	1.30 ± 0.32 ^b	1.40 ± 0.06 ^a	1.30 ± 0.12 ^b	1.20 ± 0.01 ^c
Viscerosomatic index (VSI)	3.50 ± 0.21 ^c	4.60 ± 0.11 ^b	4.80 ± 0.31 ^a	4.50 ± 0.21 ^b	3.30 ± 0.11 ^c
Condition factor	1.50 ± 0.04 ^a	1.38 ± 0.06 ^b	1.17 ± 0.02 ^c	1.68 ± 0.06 ^a	1.41 ± 0.21 ^b
Feed conversion ratio	1.24 ± 0.10 ^b	1.04 ± 0.00 ^c	0.96 ± 0.20 ^c	1.23 ± 0.10 ^b	1.47 ± 0.20 ^a
Cannibalism (%)	2.40 ± 0.00 ^a	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	1.20 ± 0.00 ^a	0.00 ± 0.00 ^a
Survival rate (%)	97.59 ± 0.0 ^a	98.79 ± 0.0 ^a	99.39 ± 0.0 ^a	97.23 ± 0.0 ^a	96.98 ± 0.0 ^a
Total biomass (g)	5893 ± 4.12 ^c	6113 ± 3.59 ^b	6654 ± 4.20 ^b	6010 ± 22 ^d	5876 ± 43 ^a
The mean ± SE of treatments in the same row with a number of different super-scripts differs significantly between them (P > 0.05).					

Table 4

Whole-body biochemical composition (% of wet weight) of *L. calcarifer* at different salinity level.

Ingredients (%)	Salinity Level				
	T1 (0 ppt)	T2 (5 ppt)	T3 (22 ppt)	T4 (36 ppt)	T5 (42 ppt)
Crude proteins (%)	18.80 ± 1.2 ^b	19.03 ± 1.2 ^{ab}	19.99 ± 1.4 ^a	19.80 ± 1.4 ^b	19.02 ± 1.4 ^{ab}
Crude lipids (%)	8.30 ± 0.60 ^{ab}	8.80 ± 0.60 ^a	8.40 ± 0.8 ^b	8.30 ± 0.77 ^c	8.29 ± 0.77 ^b
Moisture (%)	70.20 ± 1.1 ^a	69.10 ± 2.1 ^a	69.30 ± 1.2 ^a	69.50 ± 2.5 ^a	69.60 ± 2.5 ^a
Ash (%)	7.82 ± 0.1 ^b	7.62 ± 0.2 ^b	7.41 ± 0.3 ^b	6.90 ± 0.8 ^a	6.85 ± 0.6 ^a
Values are the mean ± SE of groups in the same row with different superscripts are significantly different (p > 0.05).					