



## Original Article

# Effects of feeding frequency on growth performance and digestive enzyme activity of sex-reversed Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758)



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## ABSTRACT

The effects were investigated of different feeding frequencies—one meal at 0600 h, two meals (at either 0600 h and 1200 h or 0600 h and 1800 h) and three meals daily (at 0600 h, 1200 h and 1800 h)—on the growth performance, digestive enzyme activity, muscle quality and carcass composition of sex-reversed fingerlings of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758), in a recirculating aquaculture system over a 3 mth period. The experiment involved four replicates following a completely randomized design with 30 fish per replication. The growth performance of the fish fed twice daily at 0600 h and 1800 h and three meals daily were similar ( $p > 0.05$ ) and were higher than for the other treatments. There were no significant differences among the feed conversion ratios of all treatment groups over the 3 mth period. The specific activities of digestive enzyme, amylase, lipase, total proteases, trypsin and the ratios of trypsin to chymotrypsin and amylase to trypsin were similar among feeding frequencies. There was a significant decrease in the chymotrypsin specific activity in fish fed less often compared to the three-meals-daily group. The muscle quality and carcass composition were not affected by feeding frequencies, except for the muscle RNA. These results suggested a superior growth capacity of tilapias fed twice daily (0600 h and 1800 h) which may be of practical use in feeding management for tilapia farming.

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## Introduction

Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1758) is widely cultured in tropical and subtropical areas of the world (Llyuyemi et al., 2010). It constitutes the largest group of freshwater fish production in Thailand (Department of Fisheries, 2014). Despite the high global demand for this fish, its rearing is negatively impacted by fluctuations of feedstuffs, leading to variation in dietary costs, and many farming techniques have been developed to improve the mass production and fillet quality of these species. For example, tilapia fry have been treated with 17- $\alpha$ -methyltestosterone to

induce mono-sex males (Little and Edwards, 2004), providing several desirable characteristics for culture such as fast growth, high fillet quality and good tolerance to a wide range of environmental culture conditions (Little and Edwards, 2004; Getinet and Amrit, 2007). Several studies have been used to increase the feed efficiency and growth in tilapia, such as stocking density for optimal growth (Yi et al., 1996), gastric evacuation time to enhance fish appetite (Riche et al., 2004) and re-feeding after starvation to enhance feed utilization and growth (Chan et al., 2008).

Feeding frequencies or feeding intervals have a direct effect on the water quality, fish welfare and the response of the immune system, as well as on fish growth (Lee et al., 2000; Dwyer et al., 2002; Garcia and Villarroel, 2009). Optimal feeding frequencies may increase growth by improving the food intake due to the return of appetite from gastric evacuation (Riche et al., 2004). The effects of feeding frequencies on growth have been reported in many fish species, such as Korean rockfish, *Sebastes schlegeli* (Lee

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et al., 2000), yellowtail flounder, *Limanda ferruginea* (Dwyer et al., 2002), hybrid sunfish, *Lepomis cyanellus* × *L. macrochirus* (Wang et al., 1998), darkbarbel catfish, *Pelteobagrus vachelli* (Wu et al., 2007), zebrafish, *Danio rerio* (Lawrence et al., 2012) and cobia, *Rachycentron canadum* (Costa-Bomfim et al., 2014).

A feeding frequency of twice daily was optimal for the larvae and juveniles of hybrid tilapia, *Oreochromis niloticus* × *O. aureus* (Jun et al., 2009) while six times daily has also been reported for juveniles alone (Tung and Shiao, 1991). In pure breed Nile tilapia, the mixed sex juveniles acquired optimal growth if fed four times daily under brackish water conditions (Daudpota et al., 2016) while feeding six times daily was optimum for all-male juveniles reared in a freshwater pond (Pouomogne and Ombredane, 2001). These conflicting results are due to the differences in the genetic background of the experimental fish, diet, age and the culture conditions. Optimization of the feeding frequency in farm environments in Thailand is currently lacking and needs to be assessed.

The aim of this study was to investigate the effects of different feeding frequencies on the growth performance, digestive enzyme activity, muscle quality and carcass composition of growing sex-reversed Nile tilapia. The feeding frequencies used were based on practical management for fish farmers in Thailand. The findings from the experiment could be applied to maximize growth using a minimal feed ration.

## Materials and methods

### Fish husbandry and sampling

The GIFT (Genetically Improved Farmed Tilapias) strain of Nile tilapia (14th generation progeny) was used in this study. The sex-reversed fingerlings aged 30 d were obtained from the Khamphaengsaen Fisheries Research Station, Faculty of Fisheries, Kasetsart University, Thailand. The fish were acclimatized indoors in plastic tanks (0.84 m width × 1.97 m length × 0.74 m height) for 1 mth before starting the experiment. Fish with similar weights ( $11.81 \pm 0.23$  g) and lengths ( $8.37 \pm 0.05$  cm) were randomly distributed in 16 aquaria (0.40 m width × 0.76 m length × 0.48 m height) at an initial density of 30 fish per aquarium. The water-recirculating culture system was operated for 3 mth with a regime of 12 h light/12 h dark. The ranges of water quality parameters during the study with the minimum to maximum ranges shown in parentheses were: pH  $7.33 \pm 0.04$  (7.18–7.56), temperature  $29.81 \pm 0.23$  °C (29–31 °C), dissolved oxygen  $4.68 \pm 0.12$  mg/L (4.00–5.15 mg/L), conductivity  $315.61 \pm 9.44$  µs (268.00–352.50 µs), total alkalinity  $42.21 \pm 3.00$  mg/L CaCO<sub>3</sub> (28.39–57.62 mg/L), total ammonia nitrogen  $0.55 \pm 0.03$  mg N/L (0.37–0.67 mg N/L), nitrite  $0.019 \pm 0.01$  mg N/L (0.005–0.041 mg N/L), and nitrate  $10.78 \pm 1.33$  mg N/L (6.08–16.67 mg N/L). Each aquarium was covered with black cloth to avoid changes in appetite and feeding behavior of the fish being fed the dietary treatments. The fish were fed *ad libitum* with a commercial diet for Nile tilapia (containing 18% crude protein) at different daily feeding frequencies—one meal (06:00 h), two meals (06:00 h and 12:00 h or 06:00 h and 18:00 h) and three meals (06:00 h, 12:00 h and 18:00 h)—with four replicates per feeding. Uneaten diets were siphoned off 1 h after feeding, dried at 60 °C until constant weight, and used in calculating the feed conversion ratio (FCR). The body weight and total length of each fish were measured monthly. At the end of the experiment, the fish were starved for 24 h and then were sacrificed by chilling in ice. The body weight and total length were measured before white muscle and digestive tracts were carefully collected. The tissues were then kept at –80 °C until use. Growth and feed utilization parameters of tilapias at harvest were calculated as cumulative values (age 150 d). Both the

FCR and specific growth rate (SGR) were determined at different rearing periods (ages 60–90 d, 90–120 d and 120–150 d) using Equations (1) and (2):

$$\text{FCR} = \text{Dry feed fed/wet weight gain} \quad (1)$$

$$\text{SGR} = 100 [\ln W_t - \ln W_0] / [t - t_0] \quad (2)$$

where  $W_t$  is the mean weight at day  $t$  and  $W_0$  is the mean weight at day  $t_0$  and all weights are in grams.

Additional parameters were determined using Equations (3)–(7)

$$\text{Stomasomatic index (SSI)} = (\text{Wet weight of stomach/Wet body weight}) \times 100 \quad (3)$$

$$\text{Intestinosomatic index (ISI)} = (\text{Wet weight of intestine/Wet body weight}) \times 100 \quad (4)$$

$$\text{Digestosomatic index (DSI)} = (\text{Wet weight of digestive tract/Wet body weight}) \times 100 \quad (5)$$

$$\text{Hepatosomatic index (HSI)} = (\text{Wet weight of hepatopancreas/Wet body weight}) \times 100 \quad (6)$$

$$\text{Condition factor (CF)} = 100 (W/L^3) \quad (7)$$

where  $W$  is the live body weight (grams) and  $L$  is the total body length (centimeters).

### Digestive enzyme studies

#### Enzyme extraction

Frozen intestines of sex-reversed Nile tilapia were extracted in 50 mM Tris-HCl buffer pH 8 containing 200 mM NaCl (1:2 weight per volume) using a micro-homogenizer (THP-220; Omni International; Kennesaw GA, USA). The homogenate was centrifuged at  $10,000 \times g$  for 20 min at 4 °C. The lipid part in the upper layer of crude extracts was then removed. The supernatant was collected and kept at –80 °C in small portions for later determination. The protein concentration in the crude enzyme extract was determined according to Lowry et al. (1951).

#### Digestive enzyme assays

The amylase activity was determined based on Areekijsee et al. (2004) using 5% starch soluble as a substrate. The absorbance at 540 nm was measured and compared to standard maltose. The lipase activity was analyzed based on Winkler and Stuckmann (1979) using 0.01 M *p*-nitrophenyl palmitate as a substrate. The absorbance at 410 nm was measured and compared to standard *p*-nitrophenol. The total proteases activity was assayed using 5% azocasein as a substrate based on Areekijsee et al. (2004). One unit (U) of the enzyme was defined as the amount required to cause an increase of 1.0 absorbance unit at 440 nm under the specified reaction conditions. The activities of trypsin and chymotrypsin were assayed by initial reactions using 1.25 mM benzoyl-*L*-arg-*p*-nitroanilide and 0.10 mM *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide as specific substrates, respectively (Rungruangsak-Torrissen, 2007). The absorbance at 410 nm was measured and compared to the *p*-nitroanilide standard curve.

#### White muscle and carcass analyses

The RNA and protein concentrations from the white muscle and carcass (whole body) were determined using a monophasic

solution of phenol and guanidine isothiocyanate (TRIzol<sup>®</sup> reagent; Invitrogen; Carlsbad CA, USA) for a single-step method, as described in Rungruangsak-Torrissen (2007). The extinction coefficient for RNA is  $E_{260} = 40 \mu\text{g RNA/mL}$ , and for protein is  $E_{280} = 2.1 \text{ mg protein/mL}$ . Lipids were extracted using ethyl acetate, as described by Supannapong et al. (2008). The moisture and ash contents were determined using a moisture analyzer balance (MA 30; Sartorius; Göttingen, Germany) and a muffle furnace (ELF 11/14; Carbolite Limited; Hope Valley, UK), respectively (Thongprajukaew and Kovitvadh, 2013). All muscle and carcass parameters were calculated on a wet weight basis.

### Statistical analysis

Data were expressed as a mean  $\pm$  pooled SE. The percentages were checked for normality after arcsine transformations. Significant ( $p < 0.05$ ) differences between means were tested using Duncan's new multiple range test.

## Results

### Sizes at harvest and digestive organ indices

At harvest (120 d of rearing) there were no differences in the body length of the fish among the two and three feeding times (Table 1). The feeding regimes for two (06:00 h and 1800 h) and three time intervals (06:00 h, 12:00 h and 18:00 h) had similar body and visceral organ weights while feeding only once daily (06:00 h) resulted in an inferior trend in body weight and visceral organ weight, except for the stomach weight. Fish fed twice daily at the shorter interval (06:00 h and 12:00 h) had similar performance relative to the longer interval (06:00 h and 18:00 h) treatment and the three times daily treatment groups, but the digestive tract weight was similar to the fish fed less often. Digestive organ indices in terms of SSI, ISI and DSI did not differ among feeding groups. Only one significant decrease (in HSI) was observed in fish fed less often relative to fish fed twice daily at the longer time interval.

Nile tilapias grew faster under the twice (06:00 h and 18:00 h) and three times daily feeding treatments than the others, as indicated by the cumulative SGR (Table 1). The FCR was not significantly affected by the different feeding frequencies. The CF was

significantly highest in the fish fed three times daily, but there was no significant difference between the two twice-daily regimes.

### Digestive enzyme activities

The specific activities of amylase, lipase, total proteases and trypsin were unaffected by the different feeding frequencies (Table 2). The chymotrypsin specific activity was significantly higher in fish fed three times, followed by those fed twice ( $p > 0.05$ ) and only once ( $p < 0.05$ ), respectively. The activity ratios of trypsin to chymotrypsin (T/C ratio) and amylase to trypsin (A/T ratio) were not statistically different among feeding frequencies.

### Muscle qualities and carcass compositions

Muscle qualities were similar among all feeding treatments (Table 3). The protein concentrations were higher in the muscle than in the carcass, while the lipid contents were lower. Muscle RNA had the highest concentration in fish fed only once or three times daily, followed by the 0600 h and 1800 h group ( $p > 0.05$ ). Ratios of RNA/protein and protein/lipid did not differ among groups. Carcass composition, in terms of moisture, protein, lipid and ash, were also similar among feeding groups.

### Growth performance throughout the rearing period and feed utilization

The progressive body weight of the tilapias showed different patterns during the 3 mth experiment (Fig. 1A). Significant differences in both body weight (Fig. 1A) and total length (Fig. 1B) were clearly observed when fish were aged 150 d. Both these parameters in fish fed one meal daily or two meals daily (06:00 h and 12:00 h) were inferior to the two remaining groups.

The SGR of fish aged 90 d in the three meals per day group was significantly higher than for fish fed twice daily with the shorter interval (06:00 h and 12:00 h) and in those fed only once, respectively (Fig. 2A). No differences were observed between fish fed twice daily with the longer interval (06:00 h and 18:00 h) relative to the highest SGR group. There were no differences in the SGR during 90–120 d. However, at the end of the experiment, fish fed at 0600 h and 1800 h had the highest SGR; this was the only significant difference compared to those fed less often. The FCR of all

**Table 1**  
Body size, parameters related with a digestive system and feed utilization indices measured at harvest (age 150 d) of sex-reversed Nile tilapia reared under different feeding frequencies.

Parameter	Feeding frequencies				Pooled SE	p-Value
	0600 h	0600 h and 1200 h	0600 h and 1800 h	0600 h, 1200 h and 1800 h		
Length (cm)	17.10 <sup>b</sup>	19.70 <sup>a</sup>	21.38 <sup>a</sup>	20.48 <sup>a</sup>	0.39	0.013
Weight (g)						
Body	91.88 <sup>c</sup>	144.68 <sup>ab</sup>	188.57 <sup>a</sup>	180.89 <sup>a</sup>	9.03	0.010
Stomach	0.42	0.39	0.67	0.66	0.06	0.138
Intestine	4.30 <sup>b</sup>	5.57 <sup>ab</sup>	6.96 <sup>ab</sup>	8.48 <sup>a</sup>	0.44	0.034
Digestive tract	4.71 <sup>b</sup>	5.96 <sup>b</sup>	7.53 <sup>ab</sup>	9.00 <sup>a</sup>	0.46	0.029
Hepatopancreas	1.62 <sup>b</sup>	3.28 <sup>ab</sup>	5.25 <sup>a</sup>	4.61 <sup>a</sup>	0.38	0.025
Index (%)						
SSI	0.46	0.35	0.36	0.40	0.03	0.611
ISI	4.56	3.99	4.82	4.68	0.30	0.769
DSI	5.02	4.28	5.18	5.00	0.31	0.747
HSI	1.65 <sup>b</sup>	2.15 <sup>ab</sup>	2.82 <sup>a</sup>	2.55 <sup>ab</sup>	0.16	0.038
SGR (%/d)	2.52 <sup>c</sup>	3.12 <sup>b</sup>	3.65 <sup>a</sup>	3.60 <sup>a</sup>	0.07	0.020
FCR (g feed/g gain)	1.17	1.23	1.61	1.11	0.10	0.567
CF (g/cm <sup>3</sup> )	1.80 <sup>b</sup>	1.85 <sup>ab</sup>	1.91 <sup>ab</sup>	2.09 <sup>a</sup>	0.04	0.040

Values with different lowercase superscripts in each row indicate significant ( $p < 0.05$ ) differences.

SSI, stomasomatic index; ISI, intestosomatic index; DSI, digestosomatic index; HSI, hepatosomatic index; SGR, specific growth rate; FCR, feed conversion ratio; CF, condition factor.

**Table 2**  
Digestive enzyme activities at harvest (age 150 d) of sex-reversed Nile tilapia reared under different feeding frequencies.

Digestive enzyme	Feeding frequencies				Pooled SE	p-Value
	06:00 h	06:00 h and 12:00 h	06:00 h and 18:00 h	06:00 h, 12:00 h and 18:00 h		
Amylase <sup>a</sup>	279.12	384.64	361.92	332.16	26.77	0.568
Lipase <sup>b</sup>	13.53	17.45	14.89	15.39	0.68	0.239
Total proteases <sup>c</sup>	609.46	614.39	706.66	709.80	33.77	0.621
Trypsin <sup>d</sup>	70.07	76.51	92.88	91.30	6.35	0.516
Chymotrypsin <sup>d</sup>	104.75 <sup>b</sup>	121.35 <sup>a,b</sup>	116.88 <sup>a,b</sup>	133.87 <sup>a</sup>	4.09	0.034
Trypsin/Chymotrypsin ratio	0.66	0.63	0.80	0.67	0.04	0.423
Amylase/Trypsin ratio	4.30	5.00	3.82	3.65	0.23	0.262

Values with different lowercase superscripts in each row indicate significant ( $p < 0.05$ ) differences.

<sup>a</sup> Expressed as millimoles of maltose produced/hour/milligram protein.

<sup>b</sup> Expressed as micromoles *p*-nitrophenol produced/hour/milligram protein.

<sup>c</sup> Expressed as milliunits/milligram protein.

<sup>d</sup> Expressed as micromoles *p*-nitroanilide produced/hour/milligram protein.

**Table 3**  
Muscle qualities and carcass proximate compositions at harvest (age 150 d) of sex-reversed Nile tilapia reared under different feeding frequencies.

Parameter	Feeding frequencies				Pooled SE	p-Value
	06:00 h	06:00 h and 12:00 h	06:00 h and 18:00 h	06:00 h, 12:00 h and 18:00 h		
Muscle quality (wet weight)						
RNA ( $\mu\text{g/g}$ )	2,012 <sup>a</sup>	1,555 <sup>b</sup>	1,934 <sup>a,b</sup>	2,065 <sup>a</sup>	64	0.049
Protein (mg/g)	359.07	337.81	364.63	382.02	13.33	0.710
Lipid (mg/g)	13.65	16.54	16.64	19.98	1.49	0.581
RNA/protein ratio ( $\mu\text{g/mg}$ )	5.71	4.67	5.44	5.42	0.26	0.530
Protein/lipid ratio (mg/mg)	27.57	25.58	21.93	21.70	2.58	0.819
Carcass proximate composition (% wet weight)						
Moisture	72.78	69.61	70.88	70.28	0.51	0.208
Protein	14.68	14.80	13.92	15.61	0.56	0.767
Lipid	8.28	9.16	10.62	10.53	0.51	0.345
Ash	4.72	4.88	4.93	5.41	0.40	0.936

Values with different lowercase superscripts in each row indicate significant ( $p < 0.05$ ) differences.

dietary groups was unchanged during 60–90 d and 120–150 d (Fig. 2B). Fish fed twice daily in the period 90–120 d had a significantly lower FCR than fish fed three meals.

## Discussion

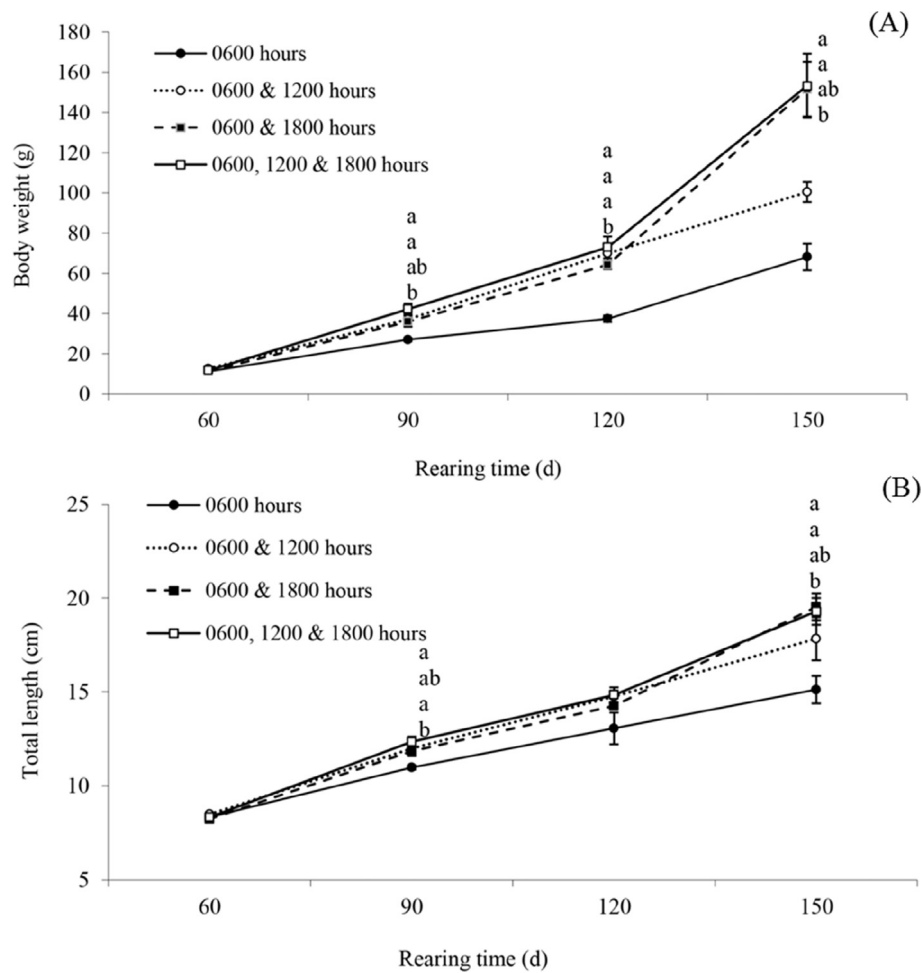
The body weight and length of fish fed twice daily (06:00 h and 18:00 h) or three times daily (06:00 h, 12:00 h and 18:00 h) were similar (Table 1). This trend was found in the weights of the stomach, intestine, hepatopancreas and digestive tract. This might have been due to the fact that the successive growth of fish is driven by the volume of the gastrointestinal tract for the digestion, absorption and utilization of nutrients (Thongprajukaew and Kovitvadhi, 2013). A similar trend in all digestive indices was also observed among dietary groups. This agreed with the unchanged CF and HSI in different feeding frequencies reported by Lee et al. (20:00). There was no significant difference in the FCR among all the feeding frequencies during the experiment. The pattern of daily feeding may explain the constant FCR among feeding intervals. Fish fed two meals daily ingested significantly more at 18:00 h than at 08:00 h, while fish fed three meals daily consumed significantly more at 13:00 h than at 08:00 h or 18:00 h, respectively (Wang et al., 1998). In a similar way, Dwyer et al. (2002) reported behavioral observations for maintaining a similar FCR by fish fed fewer meals daily, as they tended to eat more per meal. Therefore, the effect of feeding frequency on the FCR is usually small (Wang et al., 1998).

More uniform sizes could also arise in fish fed at a higher frequency due to individual fish being less aggressive under such circumstances, or because more food is distributed to locations occupied by subordinates (Başçınar et al., 2001; Aderolu et al., 2010). Behavioral observations were useful for assessing the

feeding response to different meal frequencies. Moreover, aggressive behavior such as fin biting and food seizing were frequently observed in fish fed once a day. This led to significantly lower weight gain of fish in this group compared to the others (Table 1). However, fish fed at the same frequency (twice daily) but with different meal intervals (6 h or 12 h) had differences in the SGR (Table 1). This suggested that the fish in the longer feeding interval group (06:00 h and 18:00 h) have efficiently converted the feed consumed into growth.

The feeding frequency had a significant effect on the growth performances of sex-reversed Nile tilapia. Feeding management with two meals daily (06:00 h and 18:00 h) was appropriate for practical use in this species. This was in agreement with the optimal feeding frequency reported for the larval to juvenile stages of hybrid tilapia (*O. niloticus* × *O. aureus*) by Jun et al. (2009). However, significantly higher weight gain has been reported in hybrid tilapia fed six times compared to those fed twice daily (Tung and Shiau, 1991). With pure breed fish, the optimal frequencies were four times a day relative to two, three or five times daily (Daudpota et al., 2016), or six times daily compared to two or three times (Poumogne and Ombredane, 2001). This information suggests that the genetic background (pure or hybrid), diet, age and the culture conditions play important roles in feed consumption and utilization in tilapia. For other species, optimal feeding frequencies have been also reported, such as three meals daily for both sunfish (Wang et al., 1998) and darkbarbel catfish (Wu et al., 2007), one meal daily for Korean rockfish (Lee et al., 2000), two meals daily for yellowtail flounder (Dwyer et al., 2002) and eight meals daily for juvenile yellow croaker (Xie et al., 2011).

In tilapias, an optimal time between feeding of 4–5 h for *O. niloticus* has been reported (Riche et al., 2004). However, the



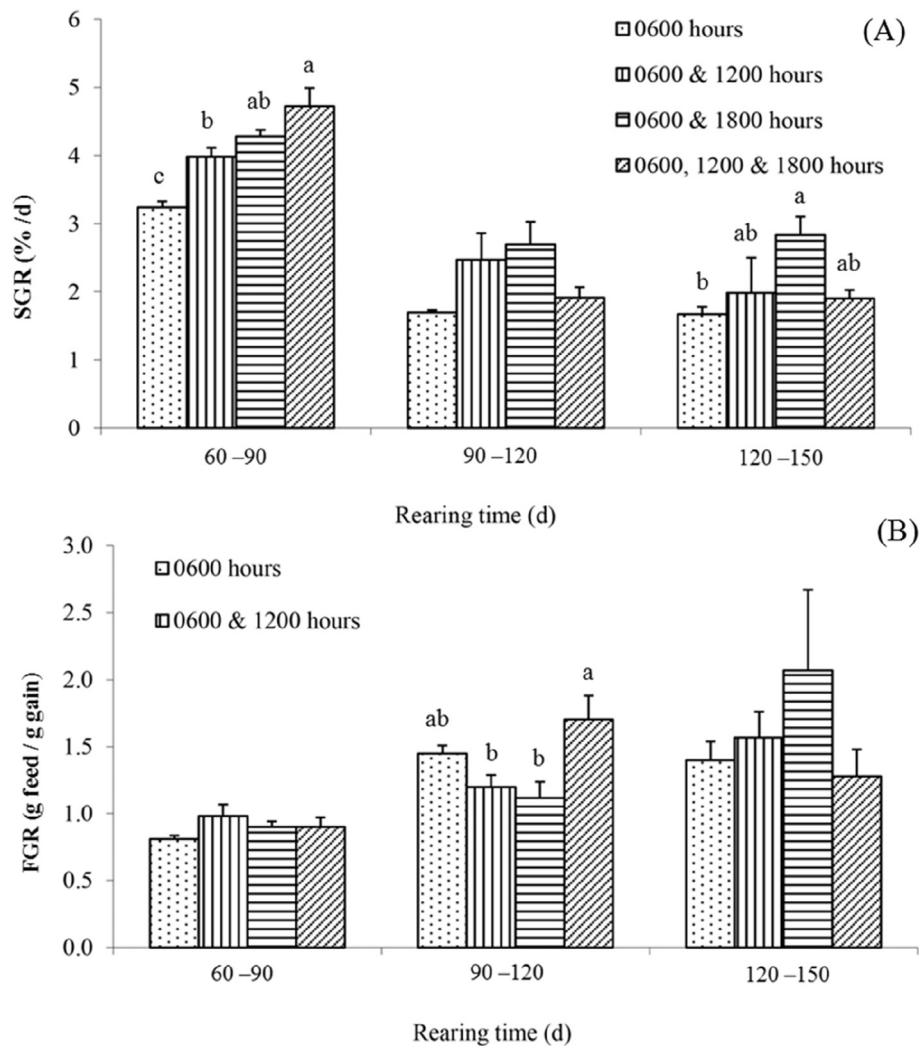
**Fig. 1.** Growth profiles based on total length (A) and body weight (B) of sex-reversed Nile tilapia aged 60–150 d reared under different feeding frequencies. Values are expressed as mean  $\pm$  SE from four replicates. Significant ( $p < 0.05$ ) differences of both parameters among treatments are indicated by different lowercase letters.

feeding interval could not directly explain feed utilization (digestion and absorption). Recently, digestive enzymes have been used for evaluating food utilization, concurrent with growth parameters, in different feeding frequencies (Wu et al., 2007; Jun et al., 2009; Xie et al., 2011). Preliminary studies on the main digestive enzymes during the post-prandial time (0–24 h after feeding) of sex-reversed tilapia showed that the activity profiles were affected by the feeding interval (the present work). Fish fed at 12 h time intervals (06:00 h and 18:00 h) retained greater activity of the intestinal total protease, amylase and lipase, than those with a 6 h time interval (06:00 h and 12:00 h). Thus, the interval might lead to higher feed utilization in the long-term for enzymatic digestion.

Fish fed more than twice a day showed a general increase in the amylase activity compared to the once daily group, though it was not significant. However, no differences were observed in fish fed two and three meals daily. This was in agreement with the unchanged amylase activity reported in juvenile hybrid tilapia (Jun et al., 2009) and large yellow croaker (Xie et al., 2011) after feeding at different frequencies. The specific activity of amylase has been reported to be important for evaluating carbohydrate utilization (Thongprajukaew et al., 2011). In tilapia, the amylase activity showed a positive correlation ( $r = 0.575\text{--}0.744$ ,  $n = 16$ ,  $p < 0.05$ ) with all of the studied enzymes (total proteases, trypsin, chymotrypsin, T/C ratio and A/T ratio). This phenomenon suggests an association between carbohydrate and protein utilization, which is important for improving growth (Kumar et al., 2006;

Thongprajukaew et al., 2011). Regarding the protein-digesting enzymes, fish fed at a proper longer frequency (06:00 h and 18:00 h) had similar activity levels of total proteases, trypsin, chymotrypsin and the T/C ratio compared to those receiving three meals daily. These stable activities indicated unchanged protein digestion in the intestine, suggesting that tilapia appear to have the capacity to adjust their digestive proteases to a range of 2–3 meals daily. For fish fed less often, the significantly decreased chymotrypsin activity might negatively affect the protein utilization in this group.

The muscle quality and carcass composition of all dietary treatments were generally similar, except for the significant reduction in the muscle RNA of fish fed at 06:00 h and 12:00 h (Table 3). This suggested that both parameters did not vary with the feeding frequency. The unchanged muscle or carcass composition in the current study were in partial agreement with meagre (*Argyrosomus regius*) fed five, six or seven days weekly (García-Mesa et al., 2014) but in complete agreement with hybrid sturgeon, *Acipenser schrenckii*  $\times$  *A. baeri*, fed two, four or six times daily (Luo et al., 2015). For Nile tilapia fed two to five times daily, no differences in the carcass moisture, protein and ash were observed while the lipid content increased following feeding four and five times daily (Daudpota et al., 2016). However, changes in the organ or body composition are probably affected by various factors, such as age, feeding frequency, migration, sex, starvation and temperature (Weatherley and Gill, 1987).



**Fig. 2.** Specific growth rate (A) and feed conversion ratio (B) of sex-reversed Nile tilapia reared under different feeding frequencies aged 60–90 d, 90–120 d and 120–150 d (values are expressed as mean + SE from four replicates; significant ( $p < 0.05$ ) differences in both parameters among treatments are indicated by different letters).

In conclusion, different feeding frequencies had a significant effect on the growth performance of sex-reversed Nile tilapia. Superior growth was observed in fish fed twice (with a longer interval) and three times daily compared to those fed at the other frequencies. Both these superior growth feeding groups increased their specific activity of chymotrypsin, relative to fish fed once daily and maintained all observed digestive enzyme levels. These changes had no effects on the muscle quality and carcass composition of the fish. These findings suggest an appropriate daily feeding frequency of 0600 h and 1800 h for higher growth performance but lower variable cost in the feeding management of sex-reversed Nile tilapia.

#### Conflicts of interest

The authors declare that there are no conflicts of interest.

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