

## Combined effects of temperature and salinity on energy budget of red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*)

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**Abstract.** Research on the energy budget (or energy partitioning) of red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*) juveniles (mean weight of 10.8 g) was conducted with a 2 x 2 factorial design consisting of two environmental culture conditions: temperature (28°C and 34°C) and salinity (0 ppt and 12 ppt). The results showed that there was no synergistic effect of experimental temperature and salinity on the energy partitioning of red tilapia. Feed intake, growth, ingested energy (C), production energy (P), ingested nitrogen, and production nitrogen of experimental fish were significantly reduced at high salinity (12 ppt) while only fecal loss nitrogen was substantially decreased at high temperature (34°C). The lowest values of feed intake, growth, C, P, F (fecal loss energy), U (excretory loss energy), and R (heat loss energy) were found at 28°C-12ppt. The energy budget of red tilapia at 34°C-12 ppt is as follows:  $100 C = 26.7 P + 19.6 F + 3.65 U + 50.0 R$ , which indicates that this red tilapia can develop and grow well at 34°C-12 ppt.

**Key Words:** climate change, energy budget, ingested energy, partitioning, tilapia.

**Introduction.** The global tilapia culture has rapidly increased over the past ten years, from approximately 2.7 million tonnes in 2010 to 4.5 million tonnes in 2018 (FAO 2020), and reaching 6.5 million tonnes in 2019 (Roderick 2020). Similarly, red tilapia (*Oreochromis* sp.) production also showed a fast growth, from around 0.5 to 1.0 million tonnes in 2010 and 2018, respectively (FAO 2020). This species has become popular, especially in southeast Asia (Jayaprasad et al 2011). In Vietnam, red tilapia is one of the common freshwater culture species in the Mekong Delta (MD) due to its fast growth, high tolerance capacity against changes of environmental conditions, e.g., salinity and temperature (Watanabe et al 1993; Pradeep et al 2014), especially in the context of climate change.

Change in temperature and salinity intrusion into freshwater is getting common in MD (Tuan & Williams 2007). A projected change indicates that the current average water temperature 27°C (Gasparrini et al 2017) can be as high as 33°C at the end of the 21st century (IPCC 2018). Nhan et al (2012) observed around 1.3 million ha of the freshwater area extending 40-50 km inland from estuaries was affected by saline water (above 5 ppt) during the dry season and this can worsen in the coming years. This current and expected future change in temperature challenges the sustainability of red tilapia (*Oreochromis mossambicus* × *O. niloticus*) farming in the MD. The salinity of 12 ppt was known as the isotonic point of tilapia (unpublished data). Information about the effects of temperature and salinity or their combined effect on red tilapia is not well studied but essential for the continued growth of their farming.

Water temperature is one of the major factors determining the metabolic rate and energy expenditure of poikilothermic animals, such as teleost and crustacean (NRC 2011). Many studies reported the effects of temperature on the growth, digestibility, and feed utilization of different fish species, i.e. Nile tilapia (*Oreochromis niloticus*) (Iqbal et al 2012), *Oreochromis aureus* Küçük et al 2013), white leg shrimp (*Litopenaeus vannamei*)

(Wang et al 2015). The performance and feed utilization of Nile tilapia (Likongwe et al 1996) or GIFT tilapia (Qiang et al 2013) increased with the rise of temperature. Also, many researchers studied the effects of salinity on fish growth (Rodriguez-Montes de Oca et al 2015), and feed utilization (Hassanen et al 2014). The blue tilapia (*Oreochromis aureus*) showed the best growth and metabolic rates at the salinities lower than 12 ppt (Küçük et al 2013). The red tilapia responded to salinity and temperature in different levels (Watanabe et al 1993; Pongthana et al 2010; Hassanen et al 2014; Barreto-Curiel et al 2015).

Energy budget is known as the standard energy partitioning of ingested energy from feed into fecal loss energy F, excretory loss energy U (including urinary and branchial energy), heat loss energy R, and production energy (growth) P (Brett & Groves 1979). Each part of the energy budget depends on species, size, environmental conditions, especially water temperature (NRC 2011). Such environmental conditions affect metabolic processes in the fish body, and thus influence digestibility and fish growth. These effects will be reflected in the portions for which the fish spend ingested energy.

Many studies reported the energy budget of different fish species cultured in different temperatures, i.e., Nile tilapia (Xie et al 1997, 2011), bull trout (*Salvelinus confluentus*) (Selong et al 2001), cobia (*Rachycentron canadum*) (Sun et al 2006), striped catfish fed different diets (Tran-Tu 2010), yellow catfish (Zhang et al 2017), snakehead (*Channa striata*) cultured in different salinity and temperature conditions (Lan et al 2019). Moreover, different tilapia species are exposed to different effects on salinities (Likongwe et al 1996; Küçük et al 2013; Hassanen et al 2014; Barreto-Curiel et al 2015).

There is almost no study about the combined effects of temperature and salinity on the energy budget of tilapia. As in MD both these environmental factors are changing, it is important to generate knowledge on the combined effect of salinity and temperature on the energy budget of red tilapia (*Oreochromis mossambicus* × *O. niloticus*). The aim of this study was to see how the particular red tilapia species respond to the single or combined effect of salinity and temperature using energy budget as an indicator of the performance of nutritional physiology.

**Material and Method.** The experiment was conducted from January 2019 to December 2020 at the College of Aquaculture and Fisheries, Can Tho University, Vietnam.

**Experimental materials.** Red tilapia was derived from artificial reproduction hatcheries in Can Tho city. Fish were acclimatized in tanks (0.5 m<sup>3</sup>) with continuous aeration for two weeks. Fish were fed experimental feed twice daily during the acclimation period. After recovering time from transportation, the fish were then acclimated to the experimental salinity and temperature before setting up of the experiment. The salinity in the fish tank was raised by 2 ppt per day until it reached the target level. The desired temperature was controlled by using heaters (2 heaters per tank) to increase 1°C daily (Hassanen et al 2014).

The tap water with continuous aeration was used in the experiment as the freshwater source. Saline water of 70-80 ppt was treated with chlorine at 30 ppm. It was then strongly aerated for 24 hours before use. The salinity was adjusted by diluting saline water with freshwater. The experimental feed was a commercial feed containing 36% crude protein, 5.6% crude fat, 10.9% crude ash, gross energy 19.1 KJ g<sup>-1</sup>, and a pellet size of 1.2 mm.

**Experimental design and sampling.** The study consisted of two experiments. The experimental treatments were conducted 2 x 2 factorial design with two factors: (i) temperatures (room- and high temperature), and (ii) salinity levels (freshwater and brackish water). The experiments were conducted with four cultured conditions (treatments) coded as: MT1 (28°C-0 ppt)-positive control with room temperature (approximately 28°C) and freshwater (0 ppt); MT2 (34°C-0 ppt)-high temperature and freshwater; MT3 (28°C-12 ppt)-room temperature and high salinity; and MT4 (34°C-12

ppt)-combined high temperature (34°C) and high salinity (12 ppt). Each treatment was triplicated.

*Experiment 1 - Growth trial.* Red tilapias with an initial mean weight of 10.8 g were selected randomly to stock into twelve 200-L composite tanks, thirty fish per tank. During the experimental period, the fish were fed apparent visual satiation twice a day at 8 am and 4 pm. The experiment lasted for 59 days. All the fish in each tank were counted and weighed at the beginning and the end of the experiment. The amount of eaten feed from each tank was recorded daily. Temperature, pH, and dissolved oxygen were measured twice a day (9 am and 5 pm) by Hanna pH/temperature tester. Total ammonia nitrogen (TAN) and nitrite (N-NO<sub>2</sub>) were measured once a week by Sera test kits (Germany). Ten (10) initial fish before the experiment and 10 final fish from each tank after the experiment were killed by putting them into ice water for 30 minutes, then stored at -20°C for chemical composition analysis: dry matter, ash, fat, and protein content.

*Experiment 2 - Digestibility trial.* After finishing experiment 1, the remaining fish were kept in the same tanks. Fish were fed the same feed as the first experiment with 1% Cr<sub>2</sub>O<sub>3</sub> (marker) supplementation followed by these steps: ground, marker added, water added, pelleted 1.2 mm, dried, and stored at -20°C until feeding. Feces samples were collected after continuously feeding the experimental feed for 7 days. On the feces sampling days, the abundant feed from each tank was siphoned out after 1 hour of feeding. Right after, feces samples were collected by siphoning with a net; washed through freshwater, then washed through distilled water; and stored at -20°C for chemical composition analysis: dry matter, Cr<sub>2</sub>O<sub>3</sub>, ash, fat, and protein content. Feces samples were collected for about 21 days until getting an approximately 10-15 g dried weight per sample.

*Sample analysis.* The chemical compositions were analyzed by following AOAC (2016). Identical analyses were applied for feed samples, feces samples, and body homogenates. Primary moisture (for fish body composition): samples were dried at 60°C for 24-48 hours. Dry moisture: samples were dried at 105°C for 24 hours. Crude protein was determined by the Kjeldahl method. Crude fat was determined by extracting samples in petroleum ether in the Soxhlet system. Total ash was determined by burning the sample in a furnace at 560°C for 8 hours. Only Cr<sub>2</sub>O<sub>3</sub> was determined following Furukawa & Tsukahara (1966).

### **Data calculation**

Survival rate (SR, %) = final number of fish / initial number of stocked fish \* 100

Energy (KJ/g) = [(protein \* 23.64) + (lipid \* 39.54) + (CHO \* 17.57)]/100

Apparent digestibility coefficient of feed ADC (%) = 100 - (100 x A/B)

Apparent digestibility coefficient of nutrients in the feed

ADC<sub>Nutrient</sub> (%) = 100 - [100 x (A/B) x (B'/A')] where: A = % marker in feed; B = % marker in feces; A' = % nutrient in feed; B' = % nutrient in feces

Daily weight gain (g fish<sup>-1</sup> day<sup>-1</sup>) (mean final fish weight - mean initial fish weight)/59 days

Total energy budget: C = P + F + U + R

Ingested energy C (MJ kg<sup>-1</sup> day<sup>-1</sup>) = feed intake g kg<sup>-1</sup> day<sup>-1</sup> \* energy in feed

Production-retained energy for growth P

P (MJ kg<sup>-1</sup> day<sup>-1</sup>) = [final fish energy - initial fish energy]/59days

Fecal loss energy F (MJ kg<sup>-1</sup> day<sup>-1</sup>) = [(100 - ADC)/100] \* C

Excretory products U (mg nitrogen kg<sup>-1</sup> day<sup>-1</sup>) U<sub>N</sub> = C<sub>N</sub> - F<sub>N</sub> - P<sub>N</sub>

Excretory loss energy (Brafield & Llewellyn 1982)

U (MJ kg<sup>-1</sup> day<sup>-1</sup>) = (U<sub>N</sub>/17,031) \* (397/1.14)

Heat loss energy R = C - (P + F + U)

**Statistical analysis.** All results were calculated for mean and standard deviations of each treatment in MS Excel. Differences between the four treatments and the interactions between the two factors were determined by two-way ANOVA at a significant level of

0.05 using SPSS 27.0. All experiments were carried out in accordance with Vietnam's national guidelines for the protection and welfare of experimental animals (Law of Animal Health 2015).

## Results

**Ingested energy and retained energy for growth.** There was only a significant difference ( $p\text{-}T \times S < 0.05$ ) between the two salinities (0 and 12 ppt) in fish's final mean weight, feed intake, ingested energy, and production energy (Table 1). The fish cultured in high salinity (12 ppt) consumed less feed and grew slower, thus consuming and retaining less energy than the fish cultured in freshwater (0 ppt). However, the fish cultured at high temperature (34°C) tended to consume more feed so that more ingested energy thus higher growth compared to that in low temperature (28°C), with  $p$  values 0.055, 0.055, and 0.052, respectively.

Table 1  
Effect of temperature and salinity on ingested energy and production energy (retained energy for growth) of experimental fish

Treatment	Survival rate (%)	Initial mean weight ( $g\ fish^{-1}$ )	Final mean weight ( $g\ fish^{-1}$ )	Daily weight gain ( $g\ day^{-1}$ )	Feed intake ( $g\ fish^{-1}\ day^{-1}$ )*	Ingested energy ( $MJ\ kg^{-1}\ day^{-1}$ )	Production energy ( $MJ\ kg^{-1}\ day^{-1}$ )
34°C-12 ppt	91.1±6.9	10.7±0.2	54.2±5.3	0.74±0.1	0.97±0.2	0.76±0.1	0.20±0.0
28°C-12 ppt	92.2±1.9	10.8±0.2	44.7±1.2	0.58±0.1	0.78±0.1	0.61±0.0	0.17±0.0
34°C-0 ppt	91.1±5.1	10.8±0.1	62.8±5.8	0.88±0.1	1.04±0.1	0.82±0.1	0.25±0.0
28°C-0 ppt	86.7±3.3	10.7±0.2	61.3±2.7	0.86±0.1	1.01±0.1	0.79±0.0	0.23±0.0
<i>p values</i>							
T	0.557		0.052	0.057	0.055	0.055	0.093
S	0.337		< 0.001	< 0.001	0.017	0.017	0.004
T x S	0.337		0.142	0.142	0.139	0.139	0.479

\*dry matter basis; T = temperature (T); S = salinity (S); T x S = interaction between temperature and salinity. Values are means±SD (n = 3).

**Digestibility and fecal loss energy.** There was no significant difference ( $p > 0.05$ ) in the digestibility of the diet, the digestibility of nutrients of the diet, and fecal loss energy (Table 2). However, the digestibility of the diet tended to be lower at 12 ppt than that at 0 ppt ( $p = 0.073$ ). The energy loss from feces was the opposite relation to the digestibility of energy in the diet. As a result of this, there was a tendency that the higher temperature the fish had, the more energy the fish lost through feces ( $p = 0.069$ ).

Table 2  
Effect of temperature and salinity on dietary digestibility, nutrient digestibility, and fecal loss energy of experimental fish

Treatment	ADC <sub>diet</sub> (%)	ADC <sub>protein</sub> (%)	ADC <sub>energy</sub> (%)	Fecal loss energy ( $MJ\ kg^{-1}\ day^{-1}$ )
34°C-12 ppt	71.9±2.41	82.7±1.40	80.4±1.52	0.15±0.01
28°C-12 ppt	72.0±3.79	83.6±2.86	80.9±2.74	0.12±0.02
34°C-0 ppt	74.9±2.14	84.4±2.07	81.5±2.46	0.15±0.02
28°C-0 ppt	75.1±1.17	85.8±0.71	81.9±0.63	0.14±0.01
<i>p values</i>				
T	0.920	0.329	0.723	0.069
S	0.073	0.123	0.403	0.162
T x S	0.975	0.867	0.958	0.215

ADC = Apparent digestibility coefficient; T = temperature (T); S = salinity (S); T x S = interaction between temperature and salinity. Values are means±SD (n = 3).

**Excretory and heat loss energy.** The significant difference ( $p < 0.05$ ) was observed only between the two cultured salinities in the ingested nitrogen and production nitrogen while that was found between the two temperature levels in fecal loss nitrogen (Table 3). As similar to the energy expenditure, the fish cultured in high salinity (12 ppt) consumed and retained less nitrogen than the fish cultured in freshwater (0 ppt). However, the fecal loss nitrogen was only affected by the temperature ( $p < 0.05$ ). The higher temperature the fish have been cultured in the more nitrogen loss through feces. No significant difference was found in the nitrogen loss through excretion, energy loss through excretion, and energy loss through heat.

Table 3  
Effect of temperature and salinity on nitrogen balance in the fish body, excretory loss energy, and heat loss energy of experimental fish

Treatments	Ingested nitrogen ( $g\ kg^{-1}\ day^{-1}$ )	Production nitrogen ( $g\ kg^{-1}\ day^{-1}$ )	Fecal loss nitrogen ( $g\ kg^{-1}\ day^{-1}$ )	Excretory loss nitrogen ( $g\ kg^{-1}\ day^{-1}$ )	Excretory loss energy ( $MJ\ kg^{-1}\ day^{-1}$ )	Heat loss energy ( $MJ\ kg^{-1}\ day^{-1}$ )
34°C-12 ppt	2.33±0.37	0.55±0.04	0.40±0.03	1.37±0.33	0.028±0.007	0.38±0.09
28°C-12 ppt	1.87±0.07	0.50±0.05	0.31±0.06	1.06±0.06	0.022±0.001	0.31±0.02
34°C-0 ppt	2.49±0.12	0.79±0.15	0.39±0.06	1.31±0.14	0.027±0.003	0.39±0.03
28°C-0 ppt	2.42±0.10	0.74±0.05	0.34±0.02	1.33±0.08	0.027±0.002	0.39±0.03
<i>p values</i>						
T	0.055	0.342	0.036	0.213	0.213	0.220
S	0.017	0.001	0.664	0.381	0.381	0.152
T x S	0.139	0.913	0.392	0.170	0.170	0.230

T = temperature (T); S = salinity (S); T x S = interaction between temperature and salinity. Values are means±SD (n = 3).

**Energy budget.** There was no significant difference ( $p > 0.05$ ) in the energy partitioning of experimental fish (Table 4, Figure 1). However, the red hybrid tilapia cultured in high salinity (12 ppt) tended to retain a higher portion of energy for growth and lost lower portions through feces, excretion, and heat in comparison to the fish cultured in freshwater (0 ppt).

Table 4  
The energy budget of different fish species (as percentage of C)

Fish species	Mean weight ( $g\ fish^{-1}$ )	T ( $^{\circ}C$ )	S (ppt)	C ( $MJ\ kg^{-1}\ day^{-1}$ )	P (%)	F (%)	U (%)	R (%)	Reference
Red hybrid tilapia	10.8	34	12	0.76	26.7	19.6	3.65	50.0	This study*
	10.8	28	12	0.61	27.0	19.1	3.55	50.3	This study*
	10.8	34	0	0.82	30.0	18.5	3.29	48.2	This study*
	10.8	28	0	0.79	29.0	18.1	3.42	49.4	This study*
<i>p values</i>									
T				0.055	0.829	0.723	0.749	0.715	
S				0.017	0.139	0.403	0.358	0.520	
T x S				0.139	0.696	0.958	0.753	0.821	
Snakehead ( <i>Channa striata</i> )	6.6	34	9		32.1	15.0	5.45	47.4	Lan et al (2020)
	6.6	28	9		47.2	12.3	3.85	36.7	
	6.6	34	0		45.3	13.7	4.11	36.9	
	6.6	28	0		41.3	12.7	4.51	41.5	
Nile tilapia ( <i>Oreochromis niloticus</i> ) (GIFT)	12	25	0		29.9	9.6	2.4	52.4	Xie et al (2011)
	12	28	0		31.1	9.4	2.1	53.3	
	12	31	0		30.7	8.4	2.0	56.1	
	12	34	0		27.0	10.6	1.1	59.2	
	12	37	0		12.4	10.1	0.4	76.5	

T = temperature; S = salinity; C = ingested energy; P = production energy; F = fecal loss energy; U = excretory loss energy; R = heat loss energy (R). \*Values are means (n = 3).

Feces energy (F) was for 18.1-19.6% of food energy, with no significant differences among treatments. The proportion of food energy allocated to excretion (U) ranged from 3.29 to 3.65%. The energy intake retained as growth (P) varied from 26.7% to 30.0% and was highest in the 34°C-0ppt treatment. A large portion of food energy was spent on the metabolism (R) with a range from 48.2 to 50.3% and highest in the 12 ppt treatments.

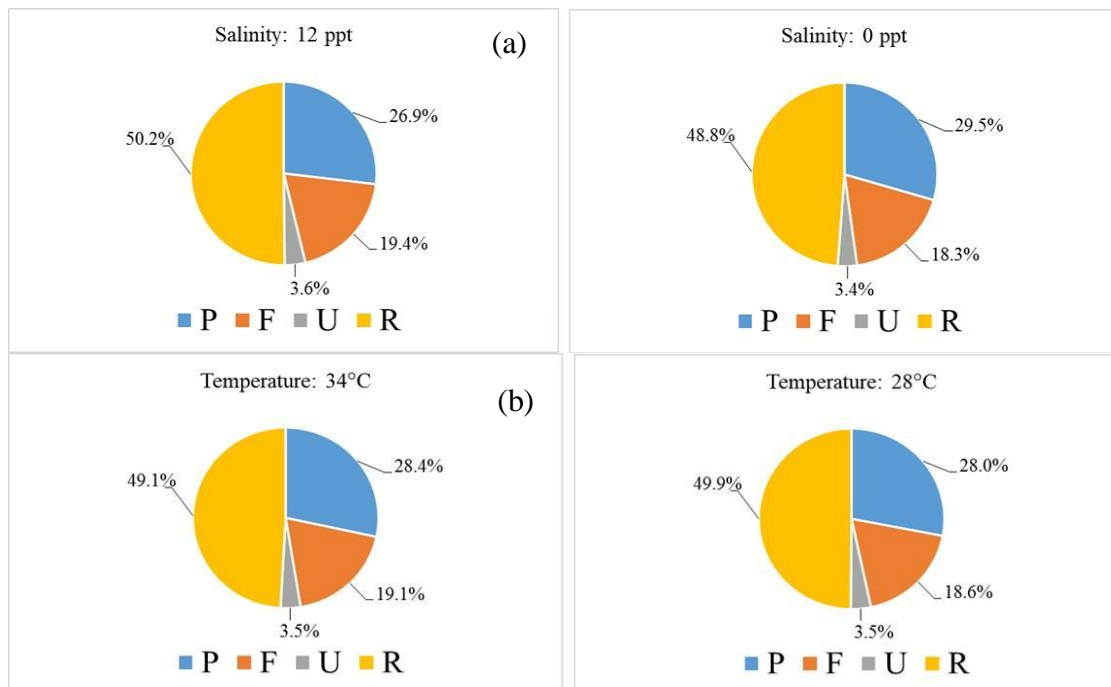


Figure 1. The energy budget of experimental red tilapia (*Oreochromis mossambicus* x *O. niloticus*) at 12 ppt vs 0 ppt (a) and at 34°C vs 28°C (b); P = production energy, F = fecal loss energy, U = excretory loss energy, and R = heat loss energy.

**Discussion.** This study showed how the red tilapia responds to the single or combined effect of salinity and temperature using energy budget as an indicator of performance of nutritional physiology. The results revealed that the cultured salinity reduced the feed intake thus decreased the ingested energy. Also, the salinity negatively affected the growth of the experimental red tilapia and the production energy. Whereas, only the high cultured temperature increased the amount of nitrogen loss through feces (Table 3). In comparison between 28 and 34°C, the high cultured temperature still tended to increase the feed intake, the fish growth, and the digestibility (Tables 1 and 2). When the red hybrid tilapia was cultured at low temperature and high salinity (28°C-12 ppt), the fish revealed the lowest energy intake, the lowest energy loss (through feces, excretion, and heat), and the lowest production energy or the amount of energy accumulated for growth (Tables 1, 2, and 3).

In this study, the effect of temperature or salinity on fish growth and feed intake was found (Table 1). We found that the feed intake and the fish growth tended to increase with the increase of cultured temperature but the salinity 12 ppt reduced feed intake thus hampered the red tilapia growth. The salty taste affecting the feed appetite leading to less feed intake thus the feed consumption not enough for the growth could be a reason. Previous studies have indicated that fish cultured at different salinities may have different food intakes, for *Clarias batrachus* feed intake in 10 ppt was significantly lower compared to lower salinity treatments (Sahoo et al 2003). In common carp (*Cyprinus carpio*), the daily feed consumption rate of the fingerlings was high in freshwater and diminished with an increase in salinity up to 6.5 ppt (Wang et al 1997). However, there was an opposite effect of salinity on the feed intake between the freshwater and marine species. In marine species, the fish cultured in high salinities had high feed intake, e.g., sole (*Solea solea* and *Solea senegalensis*) (Vinagre et al 2007) and

hybrid grouper (*Epinephelus fuscoguttatus* × *E. lanceolatus*) (Noor et al 2018). The feed intake is related to the osmoregulation of the fish. The euryhaline fish cultured in high salinity would drink more water thus increasing the movement of chyme fluid and followed by the increase of evacuation rate (Vinagre et al 2007). These resulted in an increase in feed intake.

The energy accumulation or production energy for the growth of fish increased with increasing temperature and salinity but when the temperature and salinity rose above the threshold, the accumulated energy of the fish decreased. It means that if the freshwater fish cultured in low salinity would consume lower energy for osmotic pressure regulation. So, the fish would get less stress thus grow better (Mqolomba & Plumb 1992). Also, the study of Henken et al (1986) on catfish (*Clarias gariepinus*) reported that the energy intake increased with the increase of temperature. According to the study of Likongwe et al (1996) on Nile tilapia, the cultured temperature (32°C) increased the fish growth but the increasing salinity (0, 8, 12, and 16 ppt) inhibited the fish growth. The growth energy percentage and energy utilization decreased in response to higher energy demand at water temperatures that were too high or low (Peres & Oliva-Teles 1999). In the present study, the red tilapia showed good growth (production nitrogen) at 0 ppt (28°C and 34°C) and lower production nitrogen at 12 ppt level (28°C-34°C), which is explained by the reduced energy intake so it affected the energy accumulation of fish. In addition, different tilapia species had different salinity tolerance, i.e., red tilapia (*O. niloticus* × *O. aureus*) could tolerate high salinity 52 ppt but at low temperature between 24 and 28°C (Hassanen et al 2014). Nile tilapia juvenile could promote rapid growth if they were cultured at 28-32°C and 0-12ppt (Linkongwe et al 1996).

We further explored the effect of temperature and salinity on feed digestibility and fecal loss energy (Table 2). We just found the tendency of decreased diet digestibility at the high salinity of red tilapia. When the cultured salinity increased, the ability of secreted digestive enzymes as well as the activity of these enzymes decreased thus reducing the feed digestibility of fish. Riche & Williams (2009) stated that the digestibility of protein and essential amino acids of *Trachinotus carolinus* decreased from 93.6% and 92.2% at 3 ppt to 86.5% and 87.1% at 28 ppt, respectively. A similar result was also found in the study on carp cultured at 0.5-14.5 ppt (Wang et al 1997). Whereas, previous studies on rainbow trout (*Oncorhynchus mykiss*) (Cho & Slinger 1979; Choubert et al 1982) confirmed the digestibility increased with the increase of cultured temperature due to the increase of digestive enzyme activities. However, if the cultured temperature was too high, the digestive enzymes are easily denatured, reducing the digestibility of fish (Volkoff & Rønnestad 2020). The apparent digestibility of fish increases with increases in temperature within a suitable temperature range (Wang & Qiu 2000). However, in *Spinibarbus denticulatus*, digestibility decreased with an increase in temperature (Zhang et al 2017). The digestibility curve decreased with temperature increases in juvenile sole *Cynoglossus semilaevis* (Fang et al 2010). Some research suggested that digestibility might not be affected by temperature (Beamish 1972). In this study, fecal loss energy tended to increase with the increase of temperature. When the cultured temperature increased, the feed intake of fish would increase followed by the increase of fish body metabolism thus the fish produced more feces (Table 2) and excreted more nitrogen (Table 3). Besra (1997) reported that the fecal loss energy in climbing perch *Anabas testudineus* (13 g) was 3,600 Kcal in summer compared to 1,300 Kcal in winter.

In general, living in high salinity and high temperature, the fish must use energy to regulate the body temperature, the osmosis. It means that the fish coped with stress instead of growth. The study on *Mugil liza* of Lisboa et al (2015) proved that the concentration of glycogen in the liver tended to increase with increasing salinity. The increase of glycogen concentration represents fish stress. In the estimation of an energy budget, an important factor is water temperature, which affects feeding, basal metabolism, growth, excretion, and defecation (Brafield & Llewellyn 1982). In this study, the red hybrid tilapia cultured in high salinity (12 ppt) tended to retain a higher portion of energy for growth, lost lower portions through feces, excretion and heat in comparison to the fish cultured in freshwater (0 ppt). The isotonic point in chyme of the red tilapia was

measured at the fish cultured in 12 ppt. So, in this salinity, the fish would expense the lowest energy for osmosis regulation.

There was no combined effect between cultured temperature and salinity on the energy partitioning of fish. The high salinity 12 ppt strongly decreased the feed intake, growth, ingested energy, production energy, ingested nitrogen, and production nitrogen of experimental fish. Hassanen et al (2014) recommended that red tilapia fish (*Oreochromis niloticus* x *O. aureus*) can be reared at high salinities up to 52 ppt with retention at temperatures between 24 and 28°C. It is clear to see that different hybrid red tilapia responded to salinity and temperature at different levels. The hybrid red tilapia in this study were hybrid between *Oreochromis mossambicus* and *O. niloticus*, which is dominant in Southeast Asia (Mather et al 2001). Barreto-Curiel et al (2015) concluded that there were no significant differences in growth performance (weight gain) and biological indices (e.g., feed conversion efficiency and survival) in red tilapia rearing in seawater 34 ppt and freshwater, that was observed after 90 days of experimentation. The hybrid red tilapia in Vietnam were mixed between different hybrid red tilapia, thus, the identical species was unknown, however, it had the better growth at the isosmotic point at 34°C as in freshwater at different water temperatures. Winberg (1956) suggested, in general, the fish spent 44% of ingested energy for heat loss and 20% of ingested energy for fecal loss, and these values depend on species and dietary chemical compositions. Feed intake energy (IE), recovered energy (RE), faecal energy (FE), excretory energy (UE + ZE) and heat energy (HE) were calculated to obtain the energy budget (Xie et al 2011). Results showed that effects of water temperature on growth, feed use, and energy distribution of *O. niloticus* showed that fish raised at 37°C, the ratio of IE to RE and UE + ZE is lower while HE is higher than that lower temperature condition. The optimal growth temperature was estimated as 30.1°C based on the regression of specific growth rate and water temperature. Energy budget at maximum growth (34°C) was: 100 IE = 27.0 RE + 1.1 (ZE + UE) + 10.6 FE + 59.2 HE. HE accounted for 69.3% and RE for 30.7% of metabolizable energy. These variations may be the results of different fish species and experimental conditions (Xie et al 2011). The present study showed that the recovered energy of red tilapia is the same as Nile tilapia (27-30%) (Xie et al 1997).

Brett & Groves (1979) provided general energy budget for carnivores and herbivores as: carnivores: 100 C = 29 RE + 7 (ZE + UE) + 20 FE + 44 HE; herbivores: 100 C = 20 RE + 2 (ZE + UE) + 41 FE + 37 HE. The present result showed that the energy budget of red tilapia at maximum growth was 30% (100 C = 30.0 P + 18.5 F + 3.29 U + 48.2) in 34°C-0 ppt. As omnivores, the recovered energy in a proportion of the energy intake of red tilapia was between carnivores and herbivores (Xie et al 2011). However, the recovered energy in a proportion of metabolizable energy was reported much higher in carnivores 50.9% in Chinese long snout catfish (*Leiocassis longirostris*) (Han et al 2004), 31-47% in *Channa striata*, and lower in herbivores 21.2% in grass carp (*Ctenopharyngodon idella*) (Cui et al 1994). Comparing the effects of temperature and salinity on snakehead vs red tilapia, snakehead was strongly affected by temperature more than red tilapia. For example, the lowest production energy of snakehead was at 34°C and 9 ppt while that of red tilapia was at 28°C and 12 ppt (Table 4). The energy budget of fish depends on species, size, environmental conditions, especially water temperature (NRC 2011). Different results in Cui & Liu (1990) showed lower recovered energy in carnivores. Therefore, there are still some difficulties in the comparison of energy budgets from different results because too many factors could affect the energy budget.

**Conclusions.** There was no interaction effect of cultured temperature and salinity on the energy partitioning of red tilapia. The high salinity 12 ppt strongly decreased the feed intake, growth, ingested energy (C), production energy (P), ingested nitrogen, and production nitrogen of experimental fish. The high temperature of 34°C only significantly reduced fecal loss nitrogen. The lowest values of feed intake, growth, C, P, F (fecal loss energy), U (excretory loss energy), and R (heat loss energy) were found at 28°C-12 ppt. The energy budget of red tilapia at different temperatures and salinity is as following:



34°C-12 ppt:  $100 C = 26.7 P + 19.6 F + 3.65 U + 50.0 R$   
28°C-12 ppt:  $100 C = 27.0 P + 19.1 F + 3.55 U + 50.3 R$   
34°C-0 ppt:  $100 C = 30.0 P + 18.5 F + 3.29 U + 48.2 R$   
28°C-0 ppt:  $100 C = 29.0 P + 18.1 F + 3.42 U + 49.4 R$ .

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