Effects of temperature and salinity on survival, growth and utilization of energy, protein and amino acids in red hybrid tilapia *Oreochromis mossambicus* x *O. niloticus* at different feeding rates

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Abstract. We assessed the effect of two sets of water conditions, (i) freshwater (0‰ salinity) at normal ambient temperature (28°C) and, (ii) brackish water with a salinity of 12‰ at an elevated temperature of 34°C, on survival, growth and the utilization of energy, protein and amino acids in fingerlings of red hybrid tilapia (*Oreochromis mossambicus* x *O. niloticus*) at feeding rates of 0 (fasting), 25%, 50%, 75%, 85% and 100% (apparent satiation) for 28 days. Fingerlings of uniform weight (13±0.09 g) were acclimated to the water conditions for six days before being assigned randomly to 250 L composite tanks with the designed temperature and salinity, at a density of 36 fingerlings per tank. Fingerlings were fed commercial formulated for tilapia. Fingerling weight and content in the fish body of crude fat, crude protein and amino acids were measured at the start and at the end of the feeding experiment. Survival rates and growth rates increased with increasing feeding rate, but at most feeding rates were marginally lower at 34°C-12‰ than at 28°C-0‰. Energy, crude fat and crude protein in the fish body all increased with increasing feeding rate. The maintenance requirements for energy and for protein were both significantly higher at 34°C-12‰ than at 28°C-0‰, but there was almost no difference in the utilization rate of either energy or protein between water treatments. The maintenance requirements for all sampled amino acids were also higher at 34°C-12‰ than at 28°C-0‰. The utilization rate of most amino acids was slightly higher at 28°C-0‰ than at 34°C-12‰, but it is unclear if these differences between the two treatments are functionally significant. Our results suggest that red hybrid tilapia was cultured in the throughout condition close to their isotonic threshold at a temperature of 34°C and salinity of 12‰, resulting in lower feed intake, lower apparent digestibility coefficients for key amino acids, lower body levels of crude fat, crude protein, and energy, which, in turn, reduce survival rate and growth performance.

Key Words: amino acids, feed utilization, high temperature, maintenance requirement, tilapia.

Introduction. Coastal freshwater bodies in many parts of the world are experiencing rising water temperatures and salt intrusion as a consequence of global warming, with potentially adverse impacts on the culture of fish and other freshwater aquatic animals in these environments (Cloern & Jassby 2012). Elevated salinities affect the ability of stenohaline fish species to osmoregulate, and early indicators of osmorgulatory stress include reduced feed intake and evacuation rates (MacLeod 1977; Imsland et al 2007), which have been reported at salinities as low as 4‰ in walking catfish (*Clarias batrachus*) (Sahoo et al 2003), 9‰ in snakehead (*Channa striata*) (Lan et al 2020), and 14‰ for striped catfish (*Pangasionodon hypophthalmus*) (Nguyen et al 2014). Elevated temperature and salinity have also been reported to affect a range of other processes directly or indirectly related to digestion and feed utilization (Evans 1993; Giffard-Mena et al 2006; Psochiou et al 2007), including the secretion of digestive enzymes (Woo & Kelly 1995; Moutou et al 2004; Hamed et al 2016; Mozanzadeh et al 2021), emptying time and absorption ability of chyme (Vinagre et al 2007; Noor et al 2018), feed digestibility (Ferraris et al 1986; Wang et al 1997; Barman et al 2005; Nitzan et al 2017;

Tran-Ngoc et al 2018), and fish performance (Kangómbe & Brown 2008; Nguyen et al 2014; Lan et al 2020).

For euryhaline fish species, the effect of elevated temperature and salinity on survival, feed utilization and growth is less clear and appears to be somewhat species dependent. Growth of Nile tilapia (*Oreochromis niloticus*) has been reported to be reduced at a temperature of 28-32ºC and salinities of 8‰ and above (Likongwe et al 1996; Azaza et al 2008), while Florida red hybrid tilapia (*O. urolepis hornorum* x *O. mossambicus*) grew best at a salinity of 18‰ and temperatures in the range of 26-30ºC (Watanabe et al 1993), and another hybrid of red tilapia, *O. niloticus* x *O. aureus* has been reported to still grow well at an unrealistically high salinity of 52‰ and temperature of 24-28ºC (Hassanen et al 2014), although there appear to be adverse effects on performance and some metabolic indices in this hybrid at salinities above 34‰ (Barreto-Curiel et al 2015). Up to now, the document on combined effects of temperature and salinity on nutrients requirements especially amino acids is limited.

Red hybrid tilapia (*Oreochromis* spp.) is a promising aquaculture species (Jayaprasad et al 2011); production doubled from about 0.5 million tonnes in 2010 to around 1 million tonnes in 2018, and is predicted to increase in the next ten years worldwide (FAO 2020). However, in order to realize its potential and formulate appropriate feeding and management strategies, it is important to understand how temperature and salinity affect its requirements for amino acids and other nutrients. The objective of the present study was to assess the combined effects of temperature and salinity on survival, growth and the utilization of amino acids in red hybrid tilapia

Material and Method. Tilapia fingerlings were cultured for 28 days under two sets of water conditions, one consisting of ambient water temperature (27-28°C) and a salinity of 0‰ (treatment 28°C-0‰), and the other an elevated water temperature of 34°C coupled with a salinity of 12‰ (treatment 34°C-12‰). Within each set of conditions, fingerlings were either fasted (feeding rate of 0) or fed at one of four different feeding rates (25, 50, 75 and 100% of apparent satiation). Each treatment was triplicated. Fingerlings were given a period of acclimation, as described below, before imposing the experimental conditions. The study was conducted in July 2018 at Can Tho University, Vietnam.

Fish and acclimation conditions. About 2000 red hybrid tilapia (*Oreochromis mossambicus* x *O. niloticus*) fingerlings, with an initial body weight of 10-15 g fish^{-1}, were obtained from a commercial hatchery and acclimated for two weeks in four 0.5 m^3 tanks containing freshwater (0‰ salinity) at an ambient water temperature of 27-28°C. During this time, the fish were fed commercial 2 mm-sized pellets formulated for tilapia, containing 35.9% crude protein, 5.6% crude lipid, 10.9% total ash, and a calorific value of 19.1 KJ g^{-1} dry weight. Fish were fed twice a day (at 08:00 and 16:00) ad libitum. After two weeks of acclimation to the control ambient conditions, two of the tanks were kept at the original temperature and salinity (27-28°C, 0‰ salinity), while water temperature and salinity in the other two tanks were increased gradually to a final temperature of 34°C and salinity of 12‰ over a period of six days. Temperature was adjusted at the rate of 1°C per day (Hassanen et al 2014) using a heater, and salinity at the rate of 2‰ per day using saline water of 80‰ salinity. Fish in all tanks were fed to satiation during this adjustment phase. After this six-day adjustment period, fish in all four tanks were fed to satiation for a further seven consecutive days to determine the mean amount of "full" feed intake per fish daily for each water condition. Fish were then fasted for one day in preparation for the feeding experiment described below.

Feeding experiment. As indicated above, the experiment was conducted at two different water conditions, i) an ambient water temperature of 27-28°C and freshwater 0‰ (treatment 28°C-0‰), and ii) a higher water temperature of 34°C with a salinity of 12‰ (treatment 34°C-12‰). For the experiment, fish acclimated to each condition were randomly assigned to 15 aerated composite tanks (250 L) per water treatment, with the same water conditions as the acclimation tank, at a stocking density of 36 fish tank^{-1}. Fish in each tank, except those in the fasted treatment (0% feed), were fed twice a day (8:00 and 14:00) with the same commercial pellets used during the acclimation period, at one of the different feeding rates (25, 50, 75 and 100%). The feeding rates of 25%, 50% and 75% were calculated as a proportion of the maximum feed consumption at 100% apparent satiation (where 100% apparent satiation corresponded to 3.3% and 3.1% of body weight for treatment 28°C-0‰ and treatment 34°C-12‰, respectively) over the seven-day period at the end of the acclimation phase. We recorded the amount of feed given, and weighed and removed uneaten feed and dead fish daily.

Approximately 30-50% of the water in each tank was replaced every three days with water of the same salinity and at the same temperature. Water pH (7.0-7.4) and dissolved oxygen (4.12-5.63 mg L^{-1}) were measured twice a day using a pH meter (SevenGo, Mettler Toledo, USA) and an oxygen meter (SevenGo pro, Mettler Toledo, USA) respectively. The temperature of the 28°C-0‰ treatment was not controlled and was in the range 27.6-28.5°C, while the temperature of the 34°C-12‰ treatment was maintained at 33.4-34.1°C with a heater. Nitrite (NO_2^-) and total ammonia nitrogen were measured daily with a Sera test kit (Germany). Nitrate concentrations in all treatments throughout the feeding experiment were in the range of 0.11-0.21 mg L^{-1}, and total ammonia nitrogen levels were < 1 mg L^{-1}. Salinity was measured using a refractometer (Atago, Japan).

All fish were weighed at the start (mean initial weight = Wi) and at the end (mean final weight = Wf) of the feeding experiment. The survival rate (SR, %), daily weight gain (DWG, g day^{-1}), feed intake (FI, g fish^{-1}), and feed conversion ratio (FCR) were calculated as follows (where t = time in days):

$$SR\ (\%) = (\text{number of fish at the end of experiment}) / (\text{number of initial fish}) \times 100$$

$$DWG\ (g\ day^{-1}) = (Wf - Wi) / t$$

$$FI\ (g\ fish^{-1}) = \text{total dry weight of feed consumed} / \text{number of alive fish in tank}$$

$$FCR = \text{amount of consumed feed in dry matter} / \text{weight gain}$$

For chemical analyses, six fish from each tank were sampled at the start and at the end of the experiment. These were killed by placing in ice water for 30 minutes then in ice for two hours, minced, and stored at -20°C until needed for chemical analysis. The moisture content of the fish and feed was determined by drying the samples at 105°C to constant weight. Total nitrogen content in fish and feed was analysed by the Kjeldahl method. Crude protein was calculated as 6.25 x total nitrogen (AOAC 2016). Crude fat in the fish body were extracted with petroleum ether in a Soxhlet apparatus, and measured as the difference in weight of the fish body before and after the extraction. The ash content was determined by burning the samples in a muffle furnace at a temperature of 560-600°C for 6h. Carbohydrate (CHO) as a percentage was calculated as 100 – crude protein – crude fat – crude ash. The energy (KJ g^{-1}) of the sample was calculated using the formula [(Protein x 23.64) + (Fat x 39.54) + (CHO x 17.57)] / 100 (Halver & Hardy 2002). The amino acid content of feed, feces, and fish was determined by Upscience Ltd. Comp., Vietnam, following the sequence of oxidation by formic acid-hydrogen peroxide, incubation at 0ºC for 16 hours, hydrolysis at 110ºC for 23 hours, adjustment to pH 2.2, filtration, and then analysed with a Biochrom 30+ amino acid analyzer (Biochrom, England).

The efficiency of protein, amino acid, and energy utilization was based on the linear relationship; y = ax + b. Where y = daily gain in nutrient (or energy) g/kg live weight (LW)/day, where x = digestible intake of the nutrient (or energy), a = efficiency of utilization for that nutrient (or energy), and b is a constant representing the basal losses at zero intake. The value for y (daily gain of each nutrient) was calculated as the product of DWG (daily weight gain in g/kg LW/day) and the relative proportion (ratio) of that nutrient in the fish body. The maintenance requirement of each nutrient (or energy) was calculated based on the equation ax + b = 0 (y = 0) (Lupatsch 2003).

The live weight (LW) used in calculating maintenance requirements and utilization rates was based on metabolic weight and an exponent of 0.8 for energy, protein and amino acids in tilapia (Van Trung et al 2011). The digestible energy and protein intake values were calculated based on the digestibility results from Hien et al (2021). The digestibility of amino acids was calculated based on analyses of feed and faeces samples as reported by Hien et al (2021).

Statistical analyses. Before performing statistical analyses, data were checked for normality and homogeneity of variance. We ran a one-way ANOVA for each temperature and salinity. Duncan tests were employed for specific comparisons, where appropriate. Differences in digestibility of nutrients between the two water treatments were tested using a paired sample t-test. Differences were considered significant at $p < 0.05$. All statistical analyses were performed using the SPSS software (version 27.0, IBM, USA).

Results

Fish performance. The survival rates of fish cultured at 34°C-12‰ was 10-20% lower than those at 28°C-0‰ for all feeding rates below 100%; the fasting treatment at 34°C-12‰ had the lowest value survival rate of 66.7% (Table 1). Fish performance (i.e., growth and feed utilization efficiency) was affected by both culture conditions and feeding rates. Final weight and weight gain increased approximately linearly with feeding rate in both water treatments. Fish cultured at the lower temperature and salinity (28°C-0‰) had higher final weight and weight gain than those cultured in the higher temperature and salinity treatment (34°C-12‰) at the same feeding rate, but this difference was only significant at the highest feeding rate of 100% satiation (Table 1). Fish grown at 28°C-0‰ with a feeding rate of 100% satiation had the highest growth rate (0.41 g $fish^{-1}$ day^{-1}) (Table 1). Fish grown without feed lost slightly more weight (-0.08 g $fish^{-1}$ day^{-1}) in the 34°C-12‰ water treatment compared to those at 28°C-0‰ (-0.07 g $fish^{-1}$ day^{-1}) (Table 1).

Feed intake (FI) increased significantly and approximately linearly with increasing feeding rates, but there was no significant difference in feed intake between fish grown at 28°C-0‰ and 34°C-12‰ at the same feeding rate (Table 1). The FCR decreased with increasing feeding rate, and the FCR of fish cultured at 28°C-0‰ was significantly lower than that for fish at 34°C-12‰ at the same feeding rate (Table 1).

Chemical composition and amino acid composition. The proportions of crude protein and crude fat, as a percentage of total body weight, were both significantly lower in fish at 34°C-12‰ than those at 28°C-0‰ (Table 2).

This difference in composition was also apparent in fish at the start of the feeding experiment even though they were all from the same original batch, indicating that protein and fat levels had already been influenced by the different water conditions during the acclimation period. Similarly, the amino acids histidine, isoleucine, leucine and valine were lower in fish at 34°C-12‰ than those at 28°C-0‰, while the reverse was found for threonine (Table 2). There were no significant differences in the compositions of arginine, methionine, methionine+cysteine, phenylalanine, and tryptophan between water treatments (Table 2). Crude protein, crude fat and amino acid levels in fish increased with increasing feed intake (Table 2).

The digestibility of nutrients. The digestibility (ADC) values for amino acids in fish cultured at 28°C-0‰ were significantly higher ($p < 0.05$) than those at 34°C-12‰ (Table 3). The digestibility of phenylalanine was the most affected by water condition, followed by tryptophan and valine.

Table 1

The performance of red hybrid tilapia reared in different combinations of water temperature and salinity (28°C-0 ‰ and 34°C-12 ‰)

Parameters	*28°C-0 ‰*							*34°C-12 ‰*						
	Feeding treatments					*SEM*	*p values*	*Feeding treatments*					SEM	*p values*
	0	*25*	*50*	*75*	*100*			*0*	*25*	*50*	*75*	*100*		
Wi (g fish^{-1})	13.01^{a}	12.96^{a}	12.98^{a}	13.02^{a}	12.98^{a}	0.03	ns	13.08^{a}	13.03^{a}	13.03^{a}	13.03^{a}	13.02^{a}	0.02	ns
Wf (g fish^{-1})	11.1^{a}	14.6^{b}	18.7^{c}	21.4^{d}	24.5^{e}	1.28	**	10.9^{a}	14.3^{b}	17.4^{c}	21.2^{d}	22.6^{e}	1.16	**
DWG (g day^{-1})	-0.07^{a}	0.06^{b}	0.20^{c}	0.30^{d}	0.41^{e}	0.05	**	-0.08^{a}	0.05^{b}	0.16^{c}	0.29^{d}	0.34^{e}	0.04	**
Survival rate (%)	84.4^{a}	93.3^{a}	90.0^{a}	90.0^{a}	82.2^{a}	1.72	ns	66.7^{a}	80.0^{b}	72.2^{ab}	67.8^{ab}	77.8^{ab}	2.03	ns
Feed intake (g fish^{-1})	-	2.90^{a}	6.26^{b}	8.92^{c}	11.7^{d}	0.98	**	-	2.83^{a}	6.23^{b}	9.43^{c}	11.0^{d}	0.95	**
FCR	-	1.82^{b}	1.10^{a}	1.06^{a}	1.02^{a}	0.11	*	-	2.20^{c}	1.42^{b}	1.16^{a}	1.15^{a}	0.13	**

Wi: initial mean weight, Wf: final mean weight. Data are presented as means. Different letters in the same row represent significant difference ($p < 0.05$). Statistical analysis was done within the temperature-salinity group. ns: not significance; *: $p < 0.05$; **$p < 0.01$.

Table 2

Chemical and amino acid composition of the diet and the fish body of red hybrid tilapia reared in different combinations of water temperature and salinity (28°C-0 ‰ and 34°C-12 ‰)

Nutrients	*28°C-0 ‰*							*34°C-12 ‰*						
	Feeding treatments					*SEM*	*p values*	*Feeding treatments*					*SEM*	*p values*
	0	*25*	*50*	*75*	*100*			*0*	*25*	*50*	*75*	*100*		
Moisture (%)	76.4^{b}	75.5^{ab}	76.4^{b}	75.5^{ab}	74.5^{a}	0.25	ns	77.4^{c}	77.5^{c}	76.0^{b}	75.7^{ab}	75.2^{a}	0.27	**
Protein (%)	13.1^{a}	13.4^{a}	13.2^{a}	13.3^{a}	13.9^{a}	0.13	ns	12.0^{a}	11.9^{b}	12.6^{b}	12.9^{b}	13.2^{c}	0.16	*
Fat (%)	4.18^{a}	4.89^{bc}	4.72^{b}	4.95^{bc}	5.24^{c}	0.10	**	3.43^{a}	3.93^{b}	3.79^{b}	3.87^{b}	4.38^{c}	0.09	**
Ash (%)	6.06^{b}	5.88^{ab}	5.41^{a}	5.97^{b}	6.03^{b}	0.09	ns	6.84^{b}	6.46^{a}	7.19^{b}	7.15^{b}	6.91^{b}	0.09	*
GE (KJ g^{-1})	4.80^{a}	5.16^{bc}	5.04^{ab}	5.15^{bc}	5.42^{c}	0.07	*	4.25^{a}	4.41^{b}	4.69^{c}	4.80^{c}	5.06^{d}	0.08	**
Amino acids														
Arginine	2.54^{a}	3.35^{d}	3.32^{d}	3.17^{c}	2.85^{b}	0.10	**	2.34^{a}	3.45^{d}	3.29^{c}	2.93^{b}	2.85^{b}	0.08	**
Histidine	1.06^{c}	0.95^{a}	1.01^{b}	0.95^{a}	0.99^{b}	0.01	*	0.94^{bc}	0.99^{c}	0.89^{ab}	0.94^{bc}	0.88^{a}	0.01	**
Isoleucine	2.49^{a}	2.55^{a}	2.56^{a}	2.49^{a}	2.56^{a}	0.02	*	2.34^{a}	2.36^{a}	2.34^{a}	2.51^{b}	2.40^{ab}	0.01	ns
Leucine	4.06^{a}	4.17^{ab}	4.22^{b}	4.10^{ab}	4.11^{ab}	0.03	ns	3.88^{ab}	3.70^{a}	3.81^{ab}	3.97^{b}	3.83^{ab}	0.02	ns
Lysine	3.31^{a}	4.14^{d}	4.11^{d}	3.96^{c}	3.81^{b}	0.05	**	3.57^{ab}	4.01^{d}	3.73^{bc}	3.79^{c}	3.47^{a}	0.05	**
Methionine	1.43^{ab}	1.43^{ab}	1.41^{a}	1.43^{ab}	1.46^{b}	0.01	ns	1.37^{a}	1.40^{ab}	1.41^{ab}	1.43^{ab}	1.45^{b}	0.001	ns
Methionine+ cysteine	1.57^{a}	1.54^{a}	1.53^{a}	1.54^{a}	1.59^{a}	0.05	ns	1.51^{a}	1.53^{a}	1.55^{a}	1.58^{a}	1.58^{a}	0.04	ns
Phenylalanine	1.97^{a}	2.14^{c}	2.14^{c}	2.04^{b}	1.94^{a}	0.04	**	2.26^{c}	1.94^{b}	1.91^{ab}	1.89^{ab}	1.83^{a}	0.02	**
Threonine	2.38^{a}	2.58^{b}	2.61^{bc}	2.58^{b}	2.68^{c}	0.02	ns	2.44^{a}	2.44^{a}	2.51^{ab}	2.59^{b}	2.48^{ab}	0.03	**
Tryptophan	0.50^{c}	0.41^{a}	0.47^{b}	0.41^{a}	0.47^{b}	0.01	**	0.46^{c}	0.45^{c}	0.42^{b}	0.41^{b}	0.38^{a}	0.01	**
Valine	2.89^{c}	2.79^{b}	2.77^{ab}	2.68^{a}	2.79^{b}	0.02	ns	2.70^{b}	2.60^{ab}	2.53^{a}	2.70^{b}	2.58^{ab}	0.02	*

Data are presented as means. Different letters in the same row represent significant difference ($p < 0.05$). Statistical analysis was done within the temperature-salinity group of the final fish. Fish body compositions (protein, fat, ash, and gross energy) are shown in wet matter basis. The compositions of essential amino acids are presented in dry matter basis. ns: not significance; *: $p < 0.05$; **$p < 0.01$.

Table 3

Apparent digestibility coefficients (ADC) of essential amino acids for red hybrid tilapia reared at ambient conditions (28°C-0 ‰), and at elevated temperature and salinity (34°C-12 ‰)

ADC of essential amino acids (%)	*28°C-0 ‰*	*34°C-12 ‰*
Arginine	91.6±0.53[b]	89.6±0.75[a]
Histidine	86.1±0.88[b]	82.5±1.27[a]
Isoleucine	91.4±0.54[b]	89.7±0.75[a]
Leucine	94.0±0.38[b]	89.6±0.75[a]
Lysine	91.7±0.53[b]	89.7±0.75[a]
Methionine	88.8±0.71[b]	86.8±0.96[a]
Methionine + cysteine	88.1±0.75[b]	85.6±1.04[a]
Phenylalanine	87.0±0.83[b]	77.9±1.60[a]
Threonine	86.9±0.83[b]	83.7±1.18[a]
Tryptophan	83.0±1.08[b]	75.3±1.79[a]
Valine	85.1±0.95[b]	79.8±1.46[a]

Data are presented as mean ± standard error (n=3). Different letters in the same row represent significant difference ($p < 0.05$).

Maintenance requirement and the utilization rate of nutrients and amino acids. The maintenance requirement and the utilization rate of nutrients (energy, protein, fat, and amino acids) at 28°C-0 ‰ were higher than at 34°C-9 ‰ (Table 4, Figures 1 and 2). The maintenance requirements for energy, protein and amino acids were approximately 1.7 times higher for energy, 1.8 times higher for protein, and 1.1-1.6 times higher for amino acids in fish grown at 34°C-12 ‰ compared to fish grown at 28°C-0 ‰ (Table 4). The utilization rate for energy was marginally higher at 28°C-0 ‰ than at 34°C-12 ‰, but there was no difference in the utilization rate of protein between the two water treatments (Table 4). As for the utilization of amino acids, utilization rates for arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine and tryptophan were higher at 28°C-0 ‰ than at 34°C-12 ‰, while the utilization rates of methionine, methionine+cysteine, and valine were slightly lower at 28°C-0 ‰ than at 34°C-12 ‰ (Table 4, Figures 1 and 2).

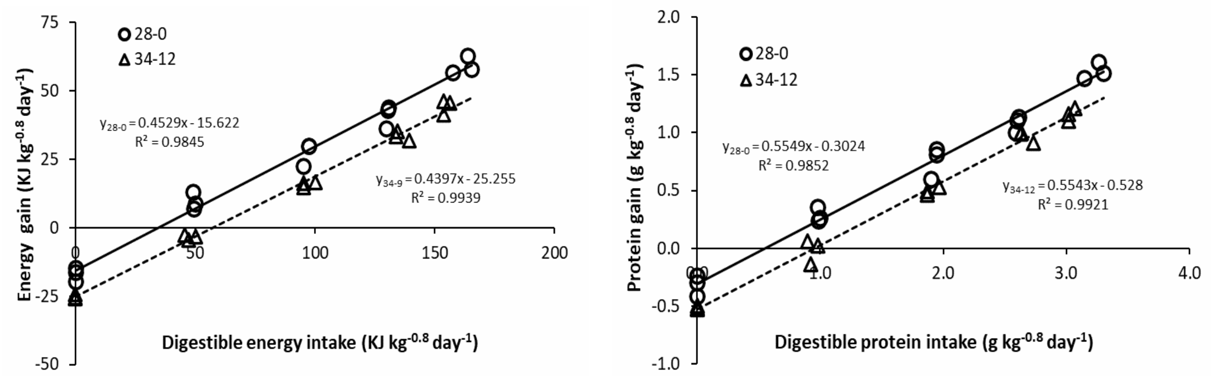

Figure 1. Relationship between (left) energy gain and digestible energy intake and (right) protein gain and digestible protein intake in red hybrid tilapia reared at ambient temperature and salinity (28°C-0 ‰) and elevated temperature and salinity (34°C-12 ‰). In each case the slope of the relationship is a measure of the utiization rate.

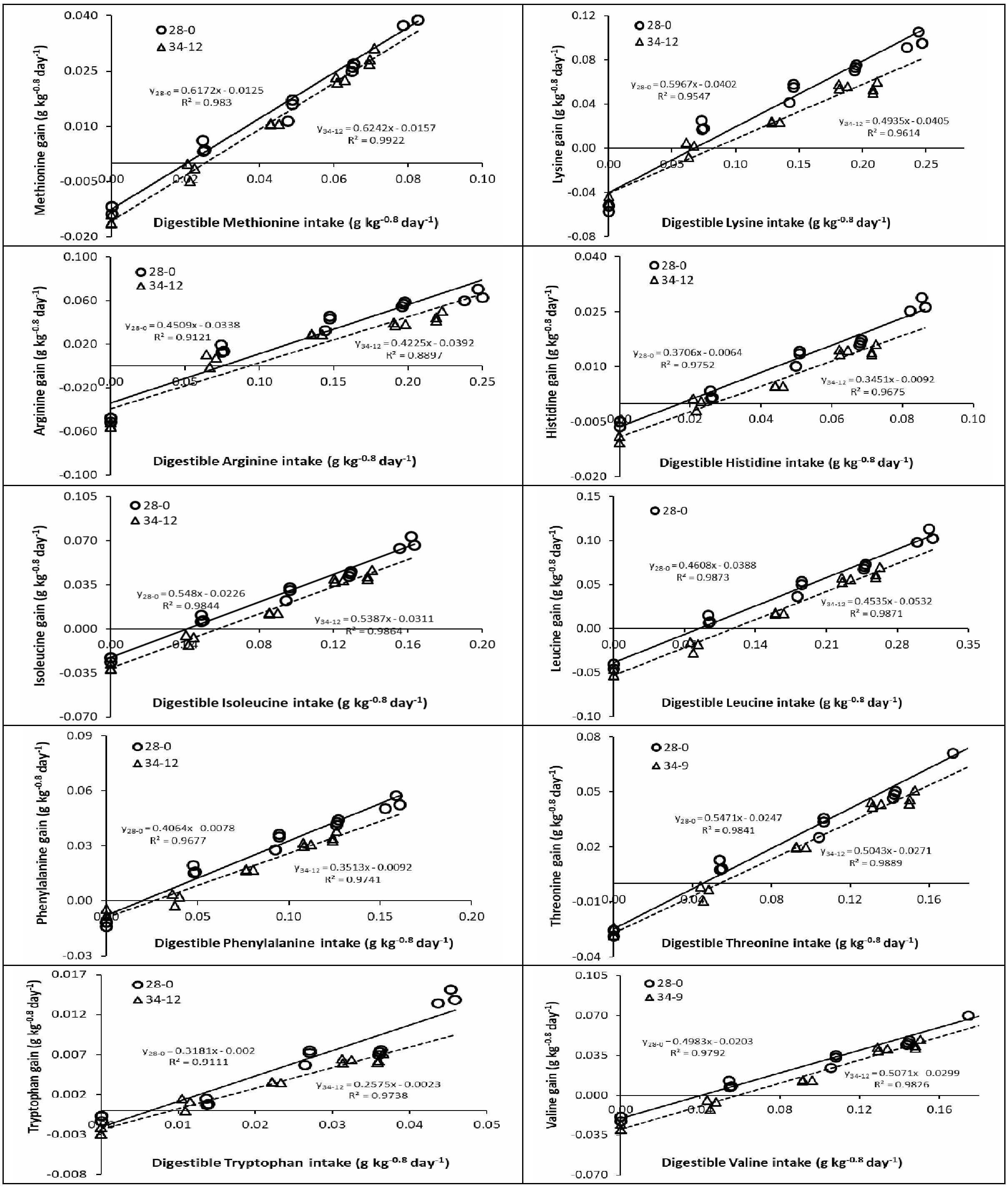

Figure 2. Relationship between amino acid gain and digestible amino acid intake for essential amino acids in red hybrid tilapia reared at ambient temperature and salinity (28°C-0 ‰) and elevated temperature and salinity (34°C-12 ‰). In each case the slope of the relationship is a measure of the utilization rate.

Table 4

Maintenance requirement and utilization rate for energy (KJ/kg$^{0.80}$/day), and protein and amino acid (g/kg$^{0.80}$/day) of red hybrid tilapia cultured at ambient temperature and salinity (28°C-0‰), and at elevated temperature and salinity (34°C-12‰)

Nutrients	*Maintenance requirement*		*Utilization rate*	
	28°C-0‰	*34°C-12‰*	*28°C-0‰*	*34°C-12‰*
Energy	34.49	57.44	0.453	0.440
Protein	0.545	0.953	0.555	0.554
Amino acid				
Arginine	0.075	0.093	0.451	0.423
Histidine	0.017	0.027	0.371	0.345
Isoleucine	0.041	0.058	0.548	0.539
Leucine	0.084	0.117	0.461	0.454
Lysine	0.067	0.082	0.597	0.494
Methionine	0.020	0.025	0.617	0.624
Methionine + cysteine	0.055	0.058	0.410	0.420
Phenylalanine	0.019	0.026	0.406	0.351
Threonine	0.045	0.054	0.547	0.504
Tryptophan	0.006	0.009	0.318	0.258
Valine	0.041	0.059	0.498	0.507

Discussion. Overall red hybrid tilapia cultured at an elevated temperature and salinity (34°C-12‰) had lower survival rates, slightly lower growth rates, much less body fat and significantly lower protein content than those cultured at an ambient temperature (28°C) and salinity of 0‰. Apparent digestibility coefficients for key amino acids were 2-10% lower at 34°C-12‰ than at 28°C-0‰, and consequently the amino acid content in the fish body was also lower at the elevated temperature and salinity. Elevated temperature and salinity also increased the maintenance requirements for energy and protein, and reduced the utilization efficiency for energy and some amino acids.

Tilapia are generally considered to be freshwater fish, although many species and hybrids appear to show some euryhaline characteristics that enable them to grow, though not necessarily optimally, at higher salinities (Watanabe et al 1993a, 1993b; Hassanen et al 2014; Barreto-Curiel et al 2015). The small but nonetheless significant reduction in survival and growth rate we observed at 34°C-12‰ suggests that the fingerlings might have been close to their isotonic threshold (equivalence between plasma osmolality and environmental osmolality), above which the energetic requirements for osmoregulation rise steeply (Mqolomba & Plumb 1992); an isotonic threshold of 12‰ has been reported for Nile tilapia (Hien et al 2021)

Unlike some studies with other freshwater fish (e.g., Lan et al 2019), we found only a small reduction in weight gain and feed intake by red hybrid tilapia cultured at elevated temperature and salinity (34°C-12‰) compared to those cultured in freshwater at ambient temperature (28°C-0‰). However, the energy content, and the levels of crude fat and crude protein as a proportion of body weight in fish grown at elevated temperature and salinity (34°C-12‰) were substantially lower than those in the body of fish cultured at 28°C-0‰. This is in line with the findings of Imsland et al (2001) and may be associated with osmoregulation (Evans 2010). Exposure of red hybrid tilapia to elevated temperature and salinity over a longer period of time are likely to amplify these differences and have a greater impact on weight gain and feed intake.

The apparent digestibility coefficients (ADC) of all amino acids analyzed were a little lower at elevated temperature and salinity (34°C-12‰) compared to freshwater at ambient temperature (28°C-0‰), and the similar findings were reported by Likongwe et al (1996), Lan et al (2020). The digestibility of feed ingredients depends on many factors, both exogenous and endogenous, but the rate of secretion of digestive enzymes and their activity, are two key factors reported to be influenced by salinity (Likongwe et al 1996; Wang et al 1997; Riche & Williams 2010; Lan et al 2020). This is a likely explanation for the significantly lower protein content we found at the higher water temperature and salinity.

Maintenance requirements for energy and protein vary widely between species and the conditions under which they are cultured. Our estimate of the maintenance requirement for energy in red hybrid tilapia (34.5 kJ $kg^{-0.80}$ day^{-1}) cultured in freshwater at a temperature of 28^oC is similar to that reported (35.1 kJ $kg^{-0.80}$ day^{-1}) by Van Trung et al (2011) for tilapia and for striped catfish (Glencross et al 2011) at the same temperature, but other species like cobia *Rachycentron canadum* have a much higher maintenance requirement for energy (74.3 kJ $kg^{-0.80}$ day^{-1}) at 28ºC (Van Tien et al 2016). In our study, the maintenance requirements for energy and protein at elevated temperature and salinity (34°C-12‰) were 1.7 times higher (energy), 1.8 times higher (protein) and 1.1-1.6 times higher (amino acids) than in freshwater at an ambient temperature of 28^oC, and so there were fewer resources available for fat and protein synthesis, hence the lower proportions of fat and protein in the body of fish at 34°C-12‰. Metabolic rates and energy requirements increase with temperature (Glencross & Bermudes 2010) and salinity (especially for osmoregulation; Evans 2010).

Nutrient use efficiency refers to the proportion of the nutrient intake that is retained in the body. It is determined graphically as the slope of the linear relationship between energy retention and energy intake (Figures 1 and 2), on a metabolic body weight basis (Lupatsch 2003; Glencross et al 2011; Glencross & Bermudes 2011; Van Trung et al 2011; Van Tien et al 2016). The utilization rate of energy, 0.453 (45.3%) at 28°C-0‰ (Figure 1), for red hybrid tilapia was similar to that reported by Van Trung et al (2011) for tilapia, but lower than that observed for other carnivorous fish species like gilthead seabream *Sparus aurata* (0.65), white grouper *Epinephelus aeneus* (0.66) and barramundi *Lates calcarifer* (0.68) (Lupatsch 2003; Lupatsch & Kissil 2005; Glencross & Bermudes 2011), or omnivores like striped catfish (0.510) (Glencross & Bermudes 2010). In theory, one might broadly expect the maintenance cost for energy (i.e., energy that is dissipated in maintaining an osmotic balance and other metabolic processes (Brett & Groves 1979)) to be inversely related in some form to nutrient use efficiency, which is a measure of energy gain as a function of feed intake and so reflects the long-term gain in energy embodied in the increase in body weight. This was broadly the case for energy in our study with red hybrid tilapia, where fish cultured at 34°C-12‰ had higher maintenance requirements, but a slightly lower energy use efficiency than their counterparts cultured in freshwater at a temperature of 28°C. However, it was not the case for protein, where the maintenance requirement for protein, like that for energy, was significantly higher for fish cultured at 34°C-12‰ than for those cultured at 28°C-0‰, but there was no statistical difference in the energy use efficiency. A similar result for protein was also observed in *Argyrosomus japonicus* (Pirozzi 2009), in barramundi (Glencross & Bermudes 2011).

Among amino acids, the greatest increases in maintenance requirement at 34°C-12‰ were found in histidine, tryptophan, valine, leucine, and isoleucine (1.40 to 1.55 times), while tryptophan, lysine, and phenylalanine had the largest decreases in utilization rate (14-19%). This implies that red hybrid tilapia exposed to high temperatures and high salinity, had sufficient of these amino acids in the diet to meet their maintenance requirement. Tryptophan has been reported/demonstrated to play an important role in stress tolerance and growth (Sahu et al 2020), so the large drop observed in its utilization rate at the higher temperature and salinity warrants further investigation.

Conclusions. The present study revealed that in red hybrid tilapia (*Oreochromis mossambicus* x *O. niloticus*), the lower survival rate and growth at 34°C-12‰ compared to the ambient temperature and freshwater 28°C-0‰ resulted in i) higher FCR, ii) lower apparent digestibility coefficients for amino acids, iii) lower body levels of crude protein, crude fat and energy, iv) higher maintenance requirements, which means that fish cultured in the throughout condition at 34°C-12‰ required more energy and other resources available for growth than those at 28°C-0‰, and lower utilization rates (except methionine, cysteine, and valine).

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Conflict of interest. The authors declare that there is no conflict of interest.

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