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Effect of temperature on the energy utilization efficiencies of digested protein, fat, and carbohydrates in Nile tilapia (*Oreochromis niloticus*)

B.K. Dey [a,b], M.C.J. Verdegem [a], M.A.J. Nederlof [a], K. Masagounder [c], J. Mas-Muñoz [d], J. W. Schrama [a,*]

[a] Aquaculture and Fisheries group, Wageningen University, Wageningen, The Netherlands
[b] Sustainable Aquaculture Program, WorldFish, Bangladesh
[c] Evonik Operations GmbH, Hanau-Wolfgang, Germany
[d] De Heus Animal Nutrition B.V., The Netherlands

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ABSTRACT

Most effective feed formulations for fish have historically relied mainly on digestible energy (DE) but recently also on net energy (NE) approaches, which indicate how energy or macronutrients are utilized for growth or energy retention (RE). This study aimed to determine the effect of temperature on 1) DE-based feed formulations that explored the relationship between DE intake and RE, and 2) NE-based feed formulations that examined the energy utilization efficiency of macronutrients, specifically digested protein (dCP), fat (dFat), and carbohydrate (dCarb) for growth in Nile tilapia. The study involved two separate experiments, one at 24 °C and one at 32 °C for Nile tilapia (initial weight ~ 38 g). Four diets having contrasting levels of protein, fat, and carbohydrates were used; and two feeding levels (FL; 10 *vs.* 18 $g.kg^{-0.8}.d^{-1}$) were applied. Digestibility of protein differed more between FLs at 32 °C. In contrast, fat and carbohydrate digestibility differed more between FLs at 24 °C. The slope of the relationship of RE to DE intake differed between 24 and 32 °C at one of the four diets ($P < 0.05$), which implies that the DE value of diets/ingredient is dependent on temperature. RE was related to dCP, dFat, and dCarb intake at 24 °C as RE = −27.6 (SE 2.86) + 13.3 (SE 0.53) dCP + 32.0 (SE 0.81) dFat +10.9 (SE 0.43) dCarb, $R^2 = 0.99$ and at 32 °C as RE = −54.4 (SE 5.43) + 14.7 (SE 0.96) dCP + 32.7 (SE 1.46) dFat +10.6 (SE 0.77) dCarb, $R^2 = 0.98$. Fasting heat production increased with temperature (27.6 *vs.* 54.4 $kJ.kg^{-0.8}.d^{-1}$; $P < 0.05$). The energy utilization efficiencies of dCP, dFat, and dCarb were similar at 24 and 32 °C ($P > 0.05$); being 56, 81, and 63% at 24 °C and 62, 83, and 62% at 32 °C. The practical implication of this is that the NE approach of feed evaluation for Nile tilapia is independent of water temperature.

1. Introduction

Fish need energy for physiological maintenance processes and for the deposition of biomass in the form of protein, fat, and minerals. Energy in diets originates from lipids, carbohydrates, and proteins (macronutrients), which are metabolized through different biochemical processes to yield energy (NRC, 2011). Differences in these metabolic pathways result in different values of efficiency in generating energy from digested proteins, lipids, and carbohydrates. The overall energy efficiency of fish diets is therefore influenced by the relative ratios of dietary macronutrients, which can thus have an impact on the growth of fish (Glencross et al., 2017; Phan et al., 2019; Phan et al., 2021a, 2021b; Phan et al., 2022; Schrama et al., 2018). Besides dietary macronutrient composition, environmental factors such as rearing temperature may influence the energy efficiency for growth (Cho and Slinger, 1980).

Growth or energy retention (RE) can be quantified as a function of either digestible energy (DE) intake, as is done in the DE approach of feed evaluation, or as a function of digestible protein, fat, and carbohydrate intake (dCP, dFat, and dCarb, respectively), which is the NE approach of feed evaluation. In the DE approach, RE is described as a linear function of DE intake [$RE = \mu + \beta \times$ DE intake; β is the energy utilization efficiency of DE for growth (k_{gDE})] (*e.g.*, Glencross et al., 2008; Glencross and Bermudes, 2012; Lupatsch et al., 2003) and it is assumed that k_{gDE} is independent of the dietary macronutrient content (as discussed by Hua et al., 2010; Glencross and Bermudes, 2012). In the NE approach, RE is described as a discrete function of dCP, dFat, and

* Corresponding author.
E-mail addresses: johan.schrama@wur.nl, AFI.Office@wur.nl (J.W. Schrama).

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dCarb intake [NE = RE − μ = β_1 × dCP + β_2 × dFat + β_3 × dCarb; β_1, β_2, and β_3 are the energy utilization efficiencies of dCP ($k_{NE;dCP}$), dFat ($k_{NE;dFat}$), and dCarb ($k_{NE;dCarb}$), respectively]. Estimating NE equations have been done recently for various fish species such as barramundi (*Lates calcarifer*), striped catfish (*Pangasius hypophthalmus*), African catfish (*Clarias gariepinus*), snakehead (*Channa striata*), rainbow trout (*Oncorhynchus mykiss*), and Nile tilapia (*Oreochromis niloticus*) (Schrama et al., 2018; Phan et al., 2019; Phan et al., 2021a, 2021b; Phan et al., 2022).

Temperature strongly determines the metabolic rate in fish (Clarke and Johnston, 1999; Clarke and Fraser, 2004). Nile tilapia, an important aquaculture species, can tolerate a wide range of temperatures; having reported optimal temperatures ranging from 20 to 30 °C (Azaza et al., 2008; El-Sayed and Kawanna, 2008; Mjoun et al., 2010; Mirea, 2013). Tilapia faces stress, disease, and increased mortality at temperatures of 37–38 °C and higher, while the lethal lowest temperature for tilapia is 10–11 °C (reviewed in Abd El-Hack et al., 2022). Commercial production of Nile tilapia has been documented in 74 nations throughout both hemispheres, indicating that it is cultivated all over the world (FAO, 2022) at a wide range of temperature. Therefore, for feed evaluation it is important to know if and how temperature alters the relationships used in either the DE and NE approaches of feed evaluation. Various studies have assessed the impact of temperature on the relationship between RE and DE (*e.g.*, Azevedo et al., 1998; Cho and Slinger, 1980; Hepher et al., 1983), but none considered assessing it at various types of diets except for Hepher et al. (1983) in red tilapia. Regarding NE equations, studies on the effect of temperature are completely lacking.

Therefore, the present study was undertaken to investigate the effect of temperature on the utilization of energy (macronutrients) for growth in Nile tilapia, a globally important aquaculture species. The main aims of the study were 1) to determine the effect of temperature on the efficiency of energy use for growth (k_{gDE}), and 2) to determine the effect of temperature on the efficiency of energy derived from dCP, dFat, and dCarb for growth ($k_{NE;dCP}$, $k_{NE;dFat}$, and $k_{NE;dCarb}$, respectively) in Nile tilapia.

2. Materials and methods

2.1. Fish and fish housing

To assess the effect of temperature on the utilization of digested macronutrients in Nile tilapia (natural whole male silver strain, Silver NMT™, Til-Aqua International, Someren, The Netherlands), two separate experiments were serially performed at 24 and 32 °C. The optimal temperature for tilapia is 28 °C (El-Sayed and Kawanna, 2008). Previously, the NE equation had been developed for tilapia kept at 28 °C (Schrama et al., 2018). Therefore, the temperatures for the current trials were chosen to be 4 °C below and 4 °C above the 28 °C as being the optimal temperature. The experiments were approved by the Ethical Committee adjudicating Animal Experiments of Wageningen University, the Netherlands (DEC number 2018.W-0021.003) and carried out according to the Dutch law on animal experiments. The origin of the experimental fish was the same for both experiments. The experiments were carried out in the Aquaculture Research Facility of Wageningen University. Twenty-four 70-L rectangular glass aquaria were connected to the same recirculation system that had a common reservoir, a sedimentation tank for solids removal, a trickling filter for gas exchange and NH_4^+ nitrification, and an oxygen reactor. The outflow of each aquarium was connected to a separate feces settling unit which was equipped with an ice-cooled glass bottle at the bottom to minimize bacterial degradation of fecal nutrients. Each experiment was run for 6-weeks. At the beginning of each experiment, 35 fish were randomly assigned and stocked per aquarium. For the 24 °C experiment, average start weight was 37.6 g, which resulted in an initial stocking density of 18.8 kg.m^{-3} and for the 32 °C experiment it was 39.0 g with an initial stocking density of 19.5 kg.m^{-3}.

In both experiments, the water flow per aquarium was set at 7 L. min^{-1}, which was checked at least once a week using a hand-held liquid rotameter. The dissolved oxygen (DO) concentration of the inlet water was adjusted to 9 mg.L^{-1} (by adding pure oxygen when necessary) to ensure the DO of >4 mg.L^{-1} inside the fish tanks, *i.e.*, outlet water. Water quality was maintained within the optimal range for tilapia and was measured daily during the first week and twice a week thereafter. Water quality parameters, *i.e.*, temperature, pH, conductivity and DO were recorded daily using a digital meter (WTW-Multi 3430 for temperature, pH, and conductivity; WTW 340i OXY for DO), total ammonia (TAN), nitrite (NO_2-N), and nitrate (NO_3-N) were measured using colorimetric kits (Merck). The mean measured water quality parameters during the 24 and 32 °C experiments were, respectively: temperature, 24.2 and 31.8 °C; pH, 7.5 and 7.7; conductivity, 3249 and 3441 μS.cm^{-1}; DO, 7.9 and 5.4 mg.L^{-1}; TAN, 0.1 and 0.7 mg.L^{-1}; NO_2-N, 0.3 and 0.6 mg.L^{-1}; and NO_3-N, 300 and 352 mg.L^{-1}. For both experiments, the same photoperiod was applied with light being on between 08:00 and 20:00.

2.2. Diets and feeding

Four diets having different levels of macronutrients (*i.e.*, crude protein, lipid, and carbohydrates) were made. Diets from the same batch were applied in both experiments. A wide range of macronutrients in the four experimental diets was generated by following the triangle approach of Raubenheimer (2011). Creating this large contrast in dietary macronutrient composition was achieved by diluting a basal diet, which had a high protein content (HP_{diet}; Table 1), with either a starch rich source (HC_{diet}; gelatinized wheat starch), a vegetable oil mixture (HF_{diet}; soyabean and rapeseed oil; 1:1) or both (HFC_{diet}; Table 1). Diets were formulated to meet requirements for vitamins, minerals and essential fatty acids and to have a balanced amino acids profile for

Table 1
Formulation and composition in each of four experimental diets for Nile tilapia.

Diet formulation and proximate composition	HP_{diet}	HC_{diet}	HF_{diet}	HFC_{diet}
Formulation (g.kg^{-1}, as-is)				
Fishmeal LT70	139.1	91.4	114.3	80.0
Soy protein concentrate	139.1	91.4	114.3	80.0
Pea protein concentrate	139.1	91.4	114.3	80.0
Wheat gluten	139.1	91.4	114.3	80.0
Wheat meal	167.5	110.1	137.6	96.3
Wheat bran	173.9	114.3	142.9	100.0
Wheat starch (pre-gelatinised)	–	342.9	–	300.0
Fish oil	34.8	22.9	28.6	20.0
Soybean oil	–	–	89.3	62.5
Rapeseed oil	–	–	89.3	62.5
Vitamin and mineral premix PV02	3.5	2.3	2.9	2.0
Vitamin C35	0.5	0.3	0.4	0.3
Vitamin E50	0.3	0.2	0.3	0.2
Monocalcium phosphate	31.3	20.6	25.7	18.0
Calcium carbonate ($CaCO_3$)	17.4	11.4	14.3	10.0
L-Lysine HCl 99%	5.2	3.4	4.3	3.0
L-Threonine	3.5	2.3	2.9	2.0
DL-Methionine	5.2	3.4	4.3	3.0
Yttrium oxide	0.35	0.23	0.29	0.2
Composition				
Dry matter (g.kg^{-1})	895	921	912	894
Crude protein (g.kg^{-1} DM)	538	348	419	297
Crude fat (g.kg^{-1} DM)	78	52	267	186
Carbohydrates (g.kg^{-1} DM)	286	536	237	461
Gross energy (kJ.g^{-1} DM)	20.46	19.53	24.30	21.95
Ash (g.kg^{-1} DM)	98.0	64.5	77.7	56.1
Calcium (g.kg^{-1} DM)	24.3	15.9	19	13.7
Phosphorus (g.kg^{-1} DM)	16.1	10.7	12.7	9.2
Magnesium (g.kg^{-1} DM)	1.9	1.3	1.5	1.1
Yttrium (g.kg^{-1} DM)	0.30	0.25	0.26	0.18

HP_{diet}, high protein diet; HC_{diet}, high carbohydrates diet; HF_{diet}, high fat diet; HFC_{diet}, high fat and carbohydrates diet; DM, dry matter.

tilapia based on the available knowledge in NRC (2011). Yttrium oxide was added as an inert marker to measure digestibility. The amounts of yttrium oxide, which were added, are given in Table 1. Diets were produced by SPAROS LDA (Olhao, Portugal). Pellets with a size of 3 mm were manufactured with a twin-screw extruder (model BC45, Clextral, France) with a screw diameter of 55.5 mm. Before feeding, pellets were sieved (2.5 mm) to remove dust and small particles.

Each experiment had a 4 by 2 factorial design: four diets and two feeding levels (FL, low *vs.* high). Per experiment, triplicate aquaria were randomly allocated to one of the eight experimental treatments. Fish were fed restrictively. Two FLs were applied to estimate the utilization of energy per kJ of digestible energy intake (slope, k_{gDE}) and the energy requirements for maintenance (DE_m). The low FL aimed at about 50% and the high FL at about 90% of satiation intake. The 50% and 90% satiations were previously determined as 10 $g.kg^{-0.8}.d^{-1}$ and 18 $g.kg^{-0.8}.d^{-1}$, respectively, for about 40-g Nile tilapia. Fish were hand fed twice per day (09:00 and 15:00). The fish were given 1 h each time to consume their ration. During the feeding period, feed was gradually given in small portions in intervals of about 5 min and was attuned to the demand of the fish to ensure the minimum spillage of pellets. The daily feeding ratio per aquarium was calculated based on the mean initial fish weight, the FL of the treatment (in $g.kg^{-0.8}.d^{-1}$) and the daily expected growth of the fish. The calculation of daily expected growth in relation to feed ration was estimated from the expected feed conversion ratio (FCR). The FCR was assumed to be 1 for the prediction of daily growth at both FL.

2.3. Sampling

Throughout the 6-weeks experimental period, 50 g of each experimental diet were sampled each week and stored at 4 °C until analyzing the proximate compositions.

The overnight settled feces in the settling units were collected daily prior to the morning feeding. This was done 5 days per week during the last 5 weeks of the experiment. All feces collected over this period was pooled per tank. The procedure of feces collection was according to Meriac et al. (2014). Feces were stored at −20 °C until oven-drying and later analysis.

One day prior to weighing, fish were not fed. For initial and final body weight measurements fish were mildly sedated using 2-phenoxyethanol solution (0.3 $mL.L^{-1}$). For measuring body composition, twenty randomly selected fish from the initial population were euthanized with 2-phenoxyethanol at a dose of 3 $mL.L^{-1}$. Similarly at the end of the experiment 10 fish per tank were randomly selected and euthanized. The fish were stored at −20 °C until analysis of body composition.

2.4. Sample preparation and chemical analysis

Fish samples were prepared for chemical analysis in accordance with the methods reported by Saravanan et al. (2012). Fish were sawn into slices and minced to ensure the homogeneity of the samples. Fresh fish samples were used for dry matter (DM), crude protein (CP), and ash analysis. Fish samples were first freeze dried for fat and gross energy (GE) analyses. Diets and oven-dried (70 °C) fecal samples were analyzed for DM, yttrium, phosphorus, CP, fat, starch, and GE content.

Proximate composition analysis of fish, feed, and feces were performed following the ISO-standard: determination of DM (ISO 6496, 1983), crude ash (ISO 5984, 1978), crude protein (ISO 5983, 1997, crude protein = Kjeldahl-N × 6.25), crude fat (ISO 6492, 1999), GE (ISO 9831, 1998) (Meriac et al., 2014). Yttrium was analyzed using inductively coupled plasma-mass spectrometry (ICP-OES). Total carbohydrates content of feed or feces was calculated as DM minus crude protein minus crude fat minus crude ash.

2.5. Nutrient digestibility measurement

The following equation was used to calculate apparent nutrient digestibility coefficients ($ADC_{nutrient}$):

$$ADC_{nut}\ (in\%) = \left[1-\left(Y_{diet}/Y_{feces}\right) \times \left(Nutrient_{feces}/Nutrient_{diet}\right)\right] \times 100$$

where, Y_{diet}, yttrium concentration of the diet ($g.kg^{-1}DM$); Y_{feces}, yttrium oxide concentration of the feces ($g.kg^{-1}DM$); $Nutrient_{diet}$, DM, protein, fat, carbohydrates or energy content of diet ($g.kg^{-1}DM$ or $kJ.kg^{-1}DM$); $Nutrient_{feces}$, DM, protein, fat, carbohydrates or energy content of feces ($g.kg^{-1}DM$ or $kJ.kg^{-1}DM$).

2.6. Nutrient balances calculations

Calculations of energy and nitrogen balances were done as described by Saravanan et al. (2012). Nitrogen and energy balance parameters were expressed per unit of metabolic body weight (in $kg^{0.8}$). This was done to standardize the differences in body weight. Intake of each nutrient (gross basis) was determined by multiplying the averaged feed intake for each treatment by the inclusion level of the nutrient in the diet. Digestible nutrient intake was determined by multiplying gross nutrient intake with the diet-specific ADC of the nutrient. Energy- and nutrient retention rates were determined from net gain, which is the difference between final and initial whole-body content. Branchial and urinary nitrogen losses (BUN) were calculated by subtracting nitrogen retention from digestible nitrogen intake. Branchial and urinary energy (BUE) was measured by multiplying BUN with 24.85, which is the energy content (in kJ) of 1 g excreted nitrogen, assuming that NH_3-N is the only form of this excretion (Bureau et al., 2003). Metabolizable energy (ME) intake was determined by the difference between digestible energy intake and BUE. Heat production was measured by subtracting ME from retained energy (RE).

2.7. Data analysis

Statistical package for social sciences (SPSS) version 22 and statistical analysis systems (SAS Institute) software package version 9.1 was used to conduct data analysis. The effect of diet, FL, temperature, and their interactions on digestibility, growth performance, nitrogen balance, and energy balance data were tested by a three-way ANOVA. For many parameters, the three-way interaction was significant and therefore the effects of diet, FL, and their interaction were also tested by a two-way ANOVA for each temperature separately, to enable identification of differences in relationship between diet and feeding level at the different temperatures. In the case of a significant interaction effect between feeding level and diet, Tukey *post hoc* comparison of means was done.

Simple linear regression between RE (in $kJ.kg^{-0.8}.d^{-1}$) and DE intake (in $g.kg^{-0.8}.d^{-1}$) was performed to determine the energy utilization efficiency (k_{gDE}) of each diet using the following model:

$$RE_i = \mu + \beta \times DE_i + e_i \quad (1)$$

where μ is the intercept that indicates an estimate for fasting heat production (FHP); β is the energy utilization efficiency; e_i is the error term where $i = 1, \ldots, n$ with $n = 6$ per diet. The difference in the slopes of the regression lines between diets was tested using a general linear model with RE as a dependent variable, DE as a covariate and diet as a fixed factor. If the interaction effect 'diet × DE' is significant ($P < 0.05$), the slopes are different across diets.

Multiple regression of RE as a function of dCP, dFat, and dCarb (in $g.kg^{-0.8}.d^{-1}$) was applied to estimate the energy utilization efficiency of each digestible macronutrient using the following model:

$$RE = \mu + \beta_1 \times dCP + \beta_2 \times dFat + \beta_3 \times dCarb + e_i \quad (2)$$

where μ is the intercept that indicates an estimate for FHP; β_1, β_2, and β_3 are the energy utilization efficiencies of dCP ($k_{NE;dCP}$), dFat ($k_{NE;dFat}$), and dCarb ($k_{NE;dCarb}$); e_i is the error term where i = 1, …, n with $n = 24$ for each temperature. To assess differences in β_1, β_2, and β_3 (*i.e.*, $k_{NE;dCP}$, $k_{NE;dFat}$, $k_{NE;dCarb}$) between temperatures, a combined mixed model in SAS was used with the inclusion of a fixed effect of temperature and 2-way interaction of temperature with each type of digestible macronutrient intake (dCP, dFat or dCarb). Significance level was set at $P < 0.05$.

3. Results

3.1. Performance of fish

Feed intake was slightly higher at 32 °C compared to 24 °C (1.3 *vs.* 1.2 g DM.d^{-1}; $P < 0.05$; Table 2; Supplementary Table S1). The average weight gains (WG) were 1.38 and 1.44 g.d^{-1} at 24 °C and 32 °C, respectively ($P < 0.05$). However, there was an interaction effect between diet, FL, and temperature on WG ($P < 0.05$). At the high FL, HFC_{diet} had a similar WG while the other diets had a slightly higher WG at 32 °C compared to 24 °C (Table 2). At the low FL, HFC_{diet} again had a similar WG, but for the other diets the WG was lower at 32 °C than at 24 °C (Table 2). The final body weight of tilapia did not differ much within diets between temperatures at the low FL, but at the high FL the final body weight within diets was higher at 32 °C, with the exception for HFC_{diet}. Regardless of temperature and FL, the overall growth was found to be the highest at HF_{diet} (Table 2). At 24 °C, FCR ranged from 0.72 to 1.01 with an average of 0.88 and at 32 °C from 0.84 to 1.09 with an average of 0.94 (Table 2). Survival was 99% at 24 °C and 98% at 32 °C, though survival was lower at HFC_{diet}, especially at 32 °C compared to 24 °C (95% *vs.* 98%; Supplementary Table S1).

3.2. ADC of nutrients

All nutrient ADC ($ADC_{nutrient}$), except ADC_{ash}, were affected by the 3-way interaction of diet, FL, and temperature (Supplementary Table S2). To clarify this 3-way interaction, a 2-way ANOVA for the effect of diet and FL was performed for each temperature separately (Fig. 1; Supplementary Table S2). The 3-way interaction was mainly due to the fact that an interaction effect between diet and FL was different between temperatures. ADC_{prot} decreased with FL at 24 °C only for HFC_{diet}, while it increased with FL at 32 °C for all diets (Fig. 1A, B). Hence, ADC_{prot} of HFC_{diet} was appeared to be dependent on FL and temperature. The average ADC_{prot} was slightly higher at 24 °C (93.7%) than at 32 °C (93.5%). ADC_{fat} declined with FL averaged over all diets at both temperatures. However, the decline was larger at 24 °C than at 32 °C, especially for the diets with a high fat content (HF_{diet} and HFC_{diet}; Fig. 1C, D). Hence, ADC_{fat} was more sensitive to FL at the lower temperature (*i.e.*, 24 °C) compared to that at the higher temperature (*i.e.*, 32 °C). For example, in HF_{diet}, the ADC_{fat} declined with increasing FL from 97.7 to 92.2% at 24 °C while it declined from 97.6 to 96.3% at 32 °C. The average ADC_{fat} was lower at 24 °C (93.9%) than at 32 °C (94.7%). Similar to ADC_{fat}, the decline in ADC_{carbs} with increasing FL was enhanced at 24 °C compared to 32 °C (Fig. 1E, F). In HF_{diet} for example, the ADC_{carbs} declined with increasing FL from 72.2 to 59.9% at 24 °C while it only declined from 66.8 to 62.2% at 32 °C. The average ADC_{carbs} was higher at 24 °C (76.9%) compared to that at 32 °C (75.9%). ADC_{prot}, ADC_{fat}, and ADC_{carbs} did not follow a same pattern within temperature, as was discussed above. As a result, the priority of ADC_{prot}, ADC_{fat}, or ADC_{carbs} will determine which diet is chosen.

3.3. Energy balance

Body composition, energy balance, and nitrogen balance measurements were not the main aim of this study, but were measured to

Table 2
Performance data in Nile tilapia (*Oreochromis niloticus*) at 24 or 32 °C, means ($n = 3$) per experimental diet, feeding level (FL), and temperature during 45- or 43-d experimental period, respectively.

T (°C)		Diet					
	FL	HP_{diet}	HC_{diet}	HF_{diet}	HFC_{diet}	SEM	Significance[#]
Final BW (g)							
24	Low	82	75	83	71	1.89	D***; FL***; T***; D × FL**; FL × T**
	High	126	114	126	112		
32	Low	83[a]	74[a]	83[a]	73[a]		
	High	134[c]	121[b]	137[c]	112[b]		
Feed intake (g DM.d^{-1})							
24	Low	0.7	0.7	0.7	0.7	0.022	FL***; T***
	High	1.7	1.7	1.7	1.7		
32	Low	0.8	0.8	0.8	0.8		
	High	1.9	1.9	1.8	1.7		
Weight gain (g.d^{-1})							
24	Low	1.02	0.84	1.03	0.76	0.04	D***; FL***; T**; D × FL**; FL × T***; D × FL × T*
	High	1.98	1.71	1.99	1.67		
32	Low	0.99[a]	0.78[a]	1.00[a]	0.77[a]		
	High	2.21[c]	1.87[b]	2.26[c]	1.66[b]		
FCR							
24	Low	0.73[a]	0.89[b]	0.72[a]	0.98[c]	0.014	D***; FL***; T***; D × FL**; D × T*; FL × T***
	High	0.85[b]	0.99[c]	0.84[b]	1.01[c]		
32	Low	0.85	1.07	0.84	1.09		
	High	0.85	1.00	0.80	1.05		

BW, body weight; FCR, feed conversion ratio on dry matter feed intake basis; T, temperature; FL, feeding level; HP_{diet}, high protein diet; HC_{diet}, high carbohydrates diet; HF_{diet}, high fat diet; HFC_{diet}, high fat and carbohydrates diet; SEM, standard error of mean.
[abc] If interaction effect of diet x feeding level is significant within temperature, means lacking a common superscript within temperature differ significantly ($P < 0.05$).
[#] Significance is shown if there is effect of diet, feeding level, temperature or any of their interactions based on the 3-way ANOVA. Specification of significance level: *** = ≤0.001; ** ≥0.001 to <0.010; * = ≥0.010 to ≤0.050.

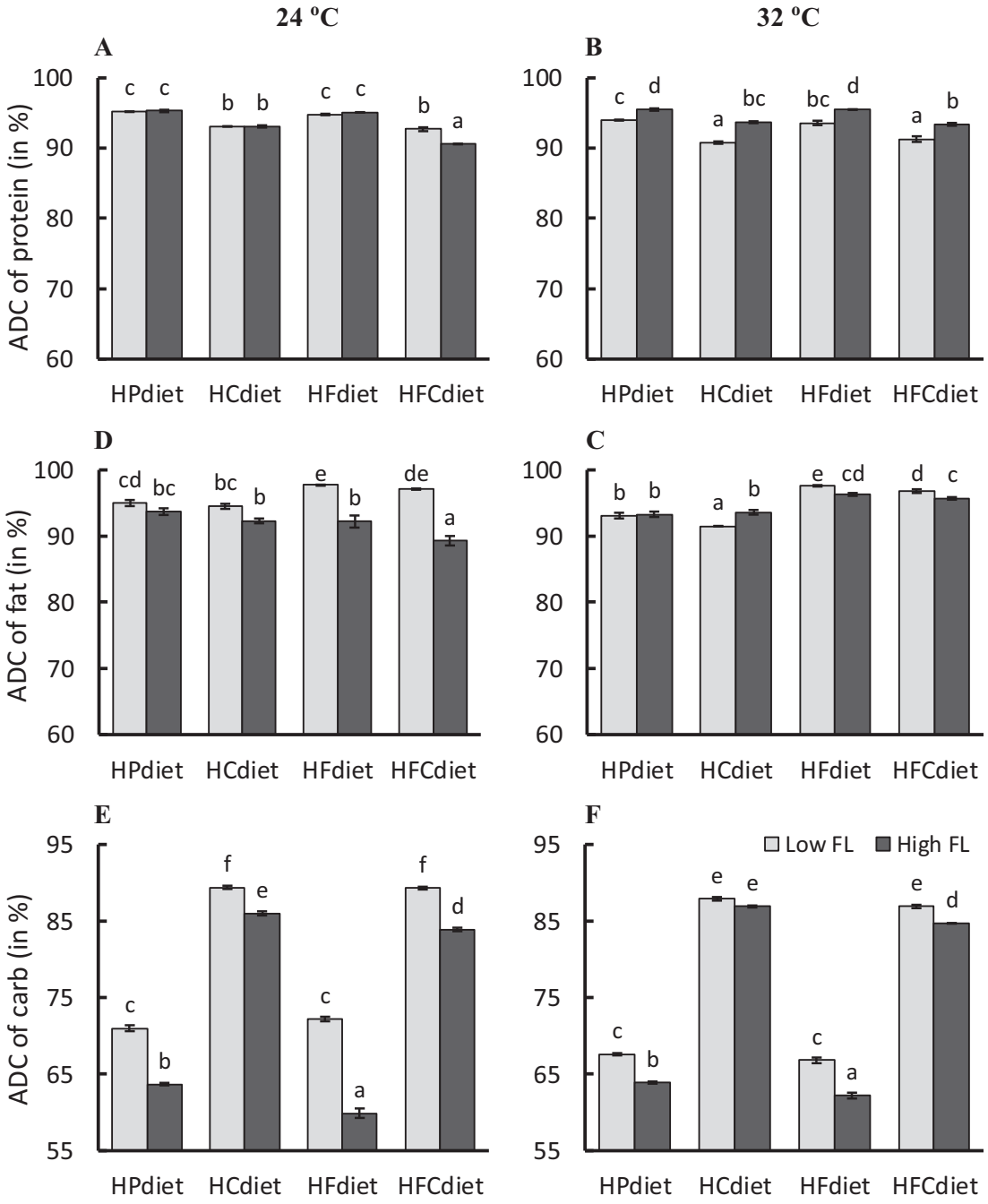

Fig. 1. Apparent digestibility coefficient (ADC) of protein (Panel A and B), fat (Panel C and D) and carbohydrates (Panel E and F) in Nile tilapia (*Oreochromis niloticus*) at 24 or 32 °C. The bars express means ($n = 3$) per experimental diet, feeding level (FL) and temperature. The error bars indicating the standard errors. [abc] were mentioned if interaction effect of diet and feeding level is significant at 95% confidence level within temperature. Bars within panels having a different letter are different ($P < 0.05$). HP_{diet}, high protein diet; HC_{diet}, high carbohydrates diet; HF_{diet}, high fat diet; HFC_{diet}, high fat and carbohydrates diet.

determine total energy retention (RE), RE as fat (RE_{fat}), and RE as protein (RE_{prot}). Therefore, data on the initial and final body composition, energy balance, and nitrogen balance are not discussed but only presented in Supplementary Tables S3, S4, and S5. Total RE (RE_{tot}), RE_{fat}, and RE_{prot} were all influenced by the 3-way interaction ($P < 0.05$). Average RE_{tot} was higher at 24 °C than at 32 °C (103 *vs.* 90 $kJ.kg^{-0.8}.d^{-1}$; Fig. 2A, B). Except for HFC_{diet}, the increase in RE_{tot} with FL at all diets was larger at 32 °C than at 24 °C. The average RE_{prot} was 45 and 42 $kJ.kg^{-0.8}.d^{-1}$ respectively at 24 °C and 32 °C ($P < 0.001$). In other words, the lower temperature resulted in higher protein deposition compared to the higher temperature. At 24 °C, RE_{prot} was not affected by the interaction between diet and FL, while this interaction effect was present at 32 °C ($P < 0.05$). As such, at the higher temperature FL is necessary to be tuned with diet type to get optimum RE_{prot}. The increase in RE_{prot} with FL was smaller at 24 °C than at 32 °C, especially for HP_{diet}, HC_{diet}, and HF_{diet}. HP_{diet}, HC_{diet}, and HF_{diet} performed better in terms of RE_{prot} compared to HFC_{diet} at both temperatures (Fig. 2C, D). The difference between temperatures in RE_{fat} were larger than that in RE_{prot}. The average RE_{fat} was 58 and 47 $kJ.kg^{-0.8}.d^{-1}$ at 24 °C and 32 °C, respectively. At all diets and FLs, RE_{fat} was always higher at the low

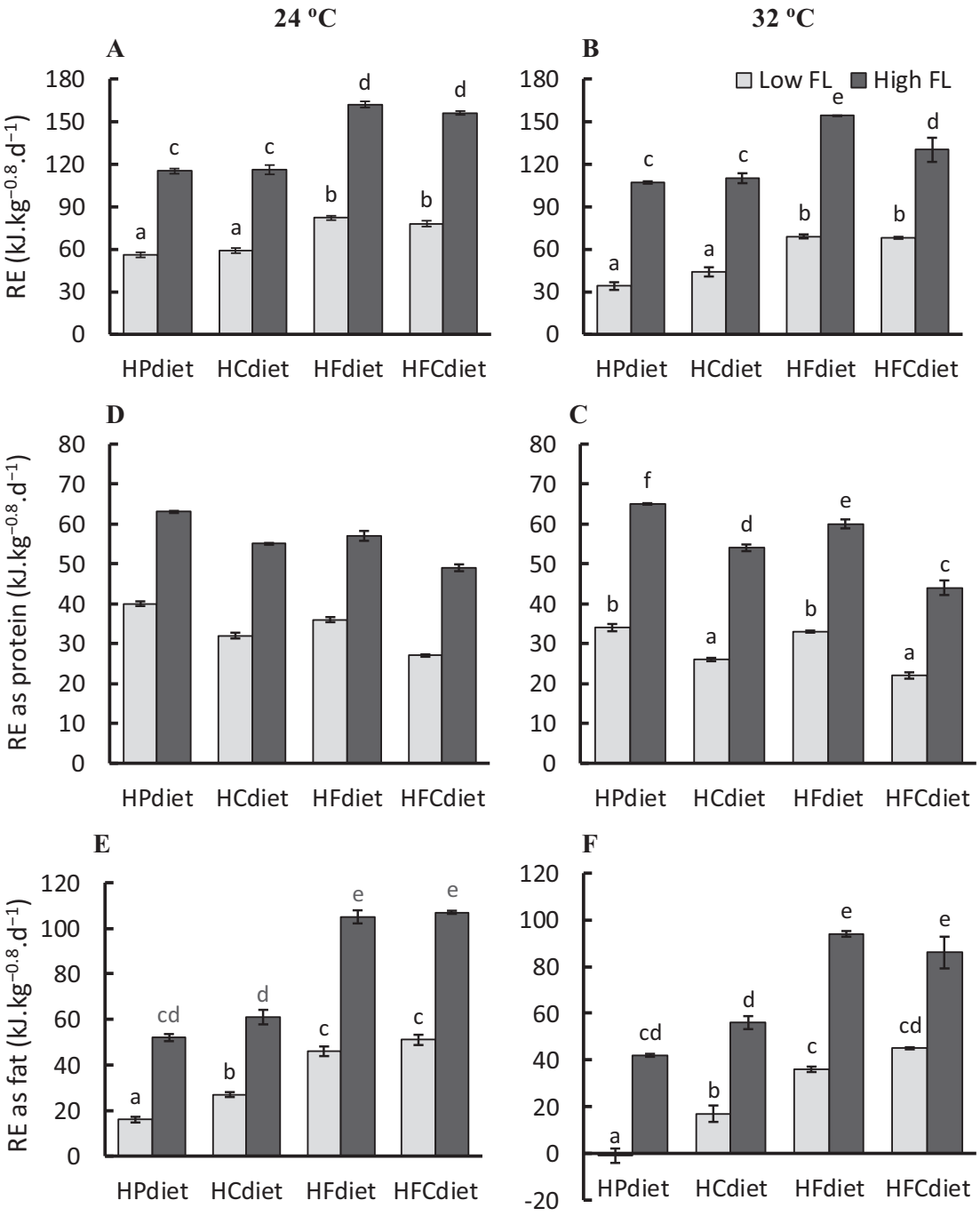

Fig. 2. Energy retention (RE; Panel A and B), RE as protein (Panel C and D) and RE as fat (Panel E and F) in Nile tilapia (*Oreochromis niloticus*) at 24 or 32 °C. The bars express means ($n = 3$) per experimental diet, feeding level (FL) and temperature. The error bars indicating the standard errors. ^{abc} were mentioned if interaction effect of diet and feeding level is significant at 95% confidence level within temperature. Bars within panels having a different letter are different ($P < 0.05$). HP_{diet}, high protein diet; HC_{diet}, high carbohydrates diet; HF_{diet}, high fat diet; HFC_{diet}, high fat and carbohydrates diet.

temperature (Fig. 2E, F). At the low FL and at 32 °C, fish fed HP_{diet} used a small amount of body fat for protein gain, which is indicated by the slightly negative RE_{fat} (−1 $kJ.kg^{-0.8}.d^{-1}$). The increase in RE_{fat} with FL between the different diets was dependent on temperature. For example for HP_{diet} the increase with FL in RE_{fat} was 36 $kJ.kg^{-0.8}.d^{-1}$ at 24 °C and 43 $kJ.kg^{-0.8}.d^{-1}$ at 32 °C, while for HFC_{diet} this increase was 56 $kJ.kg^{-0.8}.d^{-1}$ at 24 °C and only 41 $kJ.kg^{-0.8}.d^{-1}$ at 32 °C (Fig. 2E, F). However, considering the RE_{tot}, HF_{diet} performed best regardless of temperature and FL (Fig. 2A, B).

3.4. RE-DE relationship vs. temperature

The first study aim was to assess the effect of temperature on the relationship between digestible energy (DE) intake and RE in tilapia. In Table 3, the estimated relationship between RE and DE at each diet and temperature is given. The relationship between DE and RE was affected by an interaction effect between temperature and diet ($P < 0.01$; Fig. 3). The slopes of these lines (k_{gDE}), which represent the energetic utilization efficiency of DE for energy gain, were thus influenced by an interaction

Table 3
Estimated equations of retained energy (RE) related to digestible energy (DE) intake for different diets in Nile tilapia.

Diet	T (°C)	Equation	R^2	DE_m (kJ.kg$^{-0.8}$.d^{-1})
HP_{diet}	24	RE = −25 (SE 3.9) + 0.61 (SE 0.02) DE	0.99	41
	32	RE = −66 (SE 5.8) + 0.71 (SE 0.029) DE	0.99	92
HC_{diet}	24	RE = −16 (SE 5.2) + 0.56 (SE 0.03) DE	0.99	29
	32	RE = −47 (SE 10.1) + 0.63 (SE 0.05) DE	0.98	75
HF_{diet}	24	RE = −35 (SE 5.9) + 0.72 (SE 0.03) DE	0.99	49
	32	RE = −69 (SE 4.4) + 0.80 (SE 0.019) DE	0.99	86
HFC_{diet}	24	RE = −41 (SE 4.9) + 0.76 (SE 0.02) DE	0.99	54
	32	RE = −34 (SE 11.3) + 0.60 (SE 0.05) DE	0.97	57

HP_{diet}, high protein diet; HC_{diet}, high carbohydrates diet; HF_{diet}, high fat diet; HFC_{diet}, high fat and carbohydrates diet; T, temperature; RE, retained energy; DE, digestible energy; SE, standard error; DE_m, digestible energy demand for maintenance.

between diet and temperature ($P < 0.01$). For HFC_{diet}, k_{gDE} declined with temperature, while for the other diets k_{gDE} increased with temperature. HFC_{diet} and HF_{diet} showed the highest growth (*i.e.*, k_{gDE}) at 24 and 32 °C, respectively (Table 3). Averaged over all 4 diets, RE was related to DE as follows:

$$\text{at } 24^{\circ}\text{C}: \ RE = -36\ (SE\ 7.0) + 0.70\ (SE\ 0.034)\ DE \quad R^2 = 0.95 \tag{3}$$

$$\text{at } 32^{\circ}\text{C}: \ RE = -60\ (SE\ 7.4) + 0.72\ (SE\ 0.034)\ DE \quad R^2 = 0.95 \tag{4}$$

Averaged over all diets, temperature did not affect k_{gDE} ($P > 0.05$). Temperature only altered the relation between RE and DE by having different intercepts ($P < 0.001$), which are a reflection of the fasting heat production (FHP). Thus temperature increased FHP from 36 kJ.kg$^{-0.8}$.d^{-1} at 24 °C to 60 kJ.kg$^{-0.8}$.d^{-1} at 32 °C. As such, to achieve the same growth at both temperatures, tilapia needed 1.7 fold more energy at 32 °C than that at 24 °C. Except for HFC_{diet}, all other diets have a lower FHP at 24 °C compared to 32 °C (Table 3). The DE requirements for maintenance (DE_m), being the DE intake at which RE is zero, increased with temperature ($P < 0.001$) and was not different between diets ($P > 0.05$). For all diets, DE_m was lower at 24 °C than at 32 °C, although the difference between temperatures were small for HFC_{diet} (Table 3). Estimated from Eqs. (3) and (4), increasing the temperature from 24 to

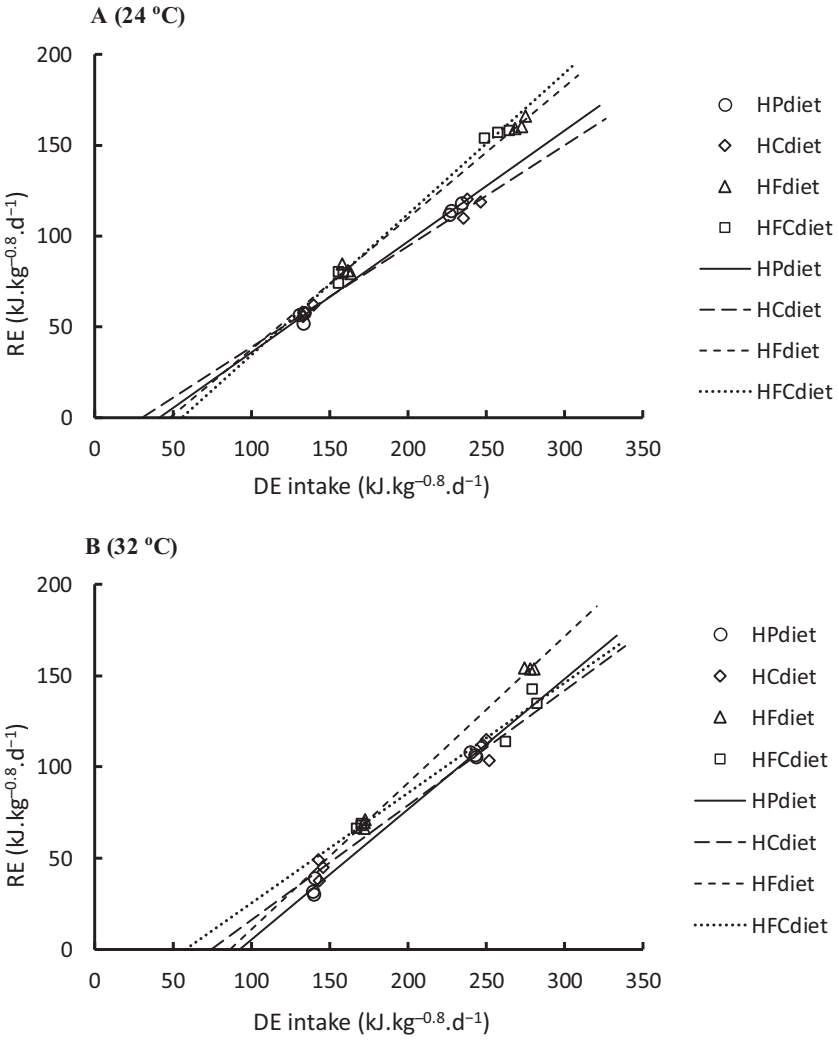

Fig. 3. Relationship between retained energy (RE) and digestible energy intake (DE) for Nile tilapia (*Oreochromis niloticus*) fed one of four experimental diets at 24 °C (Panel A) or 32 °C (Panel B). HP_{diet}, high protein diet; HC_{diet}, high carbohydrates diet; HF_{diet}, high fat diet; HFC_{diet}, high fat and carbohydrates diet.

32 °C increased DE_m by 63%, from 51 to 83 $kJ.kg^{-0.8}.d^{-1}$.

3.5. NE equations vs. temperature

The second study aim was to assess the effect of temperature on the energy utilization efficiency of digestible macronutrients, *i.e.*, digestible protein (dCP), digestible fat (dFat), and digestible carbohydrates (dCarb), for growth in tilapia. Multiple regression between RE (in $kJ.kg^{-0.8}.d^{-1}$) and dCP, dFat, and dCarb (in $g.kg^{-0.8}.d^{-1}$) was conducted to quantify the energy utilization efficiencies of dCP, dFat, and dCarb for growth in tilapia at 24 and 32 °C. This yielded the following equation at 24 °C:

$$RE = -28\ (SE\ 2.9) + 13.3\ (SE\ 0.53)\ dCP + 32.0\ (SE\ 0.81)\ dFat + 10.9\ (SE\ 0.43)\ dCarb \quad (5)$$

and at 32 °C:

$$RE = -54\ (SE\ 5.4) + 14.7\ (SE\ 0.96)\ dCP + 32.7\ (SE\ 1.46)\ dFat + 10.6\ (SE\ 0.77)\ dCarb \quad (6)$$

The R^2 value of the equation at 24 and 32 °C was 0.99 and 0.98, respectively.

The energy utilization efficiency of dCP, dFat, and dCarb ($k_{NE;dCP}$, $k_{NE;dFat}$, and $k_{NE;dCarb}$, being the respective regression coefficients) were not affected by temperature ($P > 0.05$). The differences in $k_{NE;dCP}$, $k_{NE;dFat}$, and $k_{NE;dCarb}$ between temperatures was very small. In other words, once the energy demand for maintenance (*i.e.*, intercept) is met, consumption of one gram extra dCP, dFat, or dCarb deposits similar amount of energy in tilapia at both rearing temperatures. From Eqs. (5) and (6), the $k_{NE;dCP}$, $k_{NE;dFat}$, and $k_{NE;dCarb}$ values in percentage were calculated by dividing the beta coefficients of dCP, dFat, and dCarb by the energetic value of the respective macronutrients (23.6, 39.5, and 17.2 $kJ.g^{-1}$ for CP, fat, and carbohydrates, respectively). The energy utilization efficiency was 56% and 62% for dCP, 81% and 83% for dFat, and 63% and 62% for dCarb at respectively 24 and 32 °C. The only difference between the equations at 24 and 32 °C was the intercept ($P < 0.05$; Eqs. (5) and (6)). This intercept, which is also an estimation of FHP for tilapia, was 97% higher for fish kept at 32 °C than those at 24 °C (28 *vs.* 54 $kJ.kg^{-0.8}.d^{-1}$, at 24 *vs.* 32 °C).

4. Discussion

4.1. Nutrient digestion

In this study, it was hypothesized that the temperature had an impact on $ADC_{nutrient}$ (*i.e.*, ADC_{prot}, ADC_{carb}, ADC_{fat}) because the temperature is claimed to affect the efficiency of digestive enzymes in several studies (Miegel et al., 2010; Bowyer et al., 2012; Mazumder et al., 2018). The measured ADCs in tilapia in this study were different between temperatures, but this effect differed between dietary macronutrients. Many previous studies on different fish species at different temperatures (*i.e.*, 0.6–25 °C) reported diverse levels of effects of temperature on $ADC_{nutrient}$ (Windell et al., 1978; Bendiksen et al., 2003; Bowyer et al., 2012; Huguet et al., 2015; Olsen and Ringø, 1998; Yamamoto et al., 2007). Besides temperature, $ADC_{nutrient}$ also depends on feeding level (FL), nutrient composition in diet (*e.g.*, Phan et al., 2019, 2021a, 2021b; Schrama et al., 2012) and fish age (Windell et al., 1978) which often varies between studies. This makes it difficult to draw precise comparisons between the current study and previous investigations.

The present study recorded a 0.2% higher average ADC_{prot} in tilapia at lower temperature (24 °C) compared to higher temperature (32 °C). This difference in ADC_{prot} is apparently not big, but for a big farm a little difference in ADC_{prot} may result in a noticeable difference in overall growth and profitability at farm level, as protein digestibility is correlated with growth. Larger and even opposite effects of temperature on ADC_{prot} have been reported for other species, *e.g.,* in common carp (*Cyprinus carpio*) a 4.9% higher ADC_{prot} was found at 20 °C compared to 30 °C (Révész et al., 2020), while another study found 2–2.9% higher ADC_{prot} at 25 °C compared to 17 °C (Yamamoto et al., 2007). These inconsistencies between and also within fish species may related to the temperature range examined in the different studies. In fact, common carp thrive and use nutrients best in water temperatures between 20 and 25 °C (Watanable et al., 1996), whereas the optimum temperature for tilapia is 28 °C (El-Sayed and Kawanna, 2008). Hence, the optimal temperature range for each species may explain inter-species differences in ADC. Inter study differences in ADC may vary with the ingredients in diets. Considering the carnivorous fish species, lower temperature increased ADC_{prot} by 1–2% in brook trout (*Salvelinus fontinalis*) reared at 15 or 19 °C (Amin et al., 2014), by 1% in Atlantic salmon (*Salmo salar* L.) reared at 10 or 20 °C (Huguet et al., 2015), and by 3–9% in yellowtail kingfish reared at 13 or 21 °C where a significant increase in protease enzyme activity in the posterior intestine was recorded at lower temperature (Miegel et al., 2010). These results in carnivorous species are in line with the present study on omnivorous tilapia. In contrast to the findings of the present study, some other studies on carnivorous Atlantic salmon reared at 2 or 8 °C (Bendiksen et al., 2003), rainbow trout (*Oncorhynchus mykiss*) reared at 11 or 18 °C (Yamamoto et al., 2007), and Arctic charr (*Salvelinus alpinus* L.) reared at 0.6 or 10 °C (Olsen and Ringø, 1998) provided higher ADC_{prot} at higher temperature. In this case, ADC_{prot} seems to be more dependent on the age of fish and rearing temperature range rather than the species or trophic level they occupy.

The average ADC_{carb} in the present study was 1% higher at lower temperature (24 *vs.* 32 °C), indicating that the higher ADC_{carb} may assist the protein sparing more at 24 °C compared to that at 32 °C, and thus may ensure less protein requirement (Shiau and Peng, 1993). However, this finding of the present study contrasts with that of Yamamoto et al. (2007), who reported a 1.4–1.7% lower ADC_{carb} at lower temperature (17 *vs.* 25 °C) in common carp (*C. carpio*). ADC_{carb} was 12–22% higher at lower temperature (15 *vs.* 19 °C) in carnivorous brook trout (*S. fontinalis*; Amin et al., 2014). This finding is in line with the present study, however, the value is much higher compared to that in the present study. In other carnivorous fish, such as yellowtail kingfish (*Seriola lalandi*) reared at 18 or 22 °C (Bowyer et al., 2012), rainbow trout (*O. mykiss*) reared at 11 or 18 °C (Yamamoto et al., 2007), and Arctic charr (*S. alpinus* L.) reared at 0.6 or 10 °C (Olsen and Ringø, 1998), ADC_{carb} increased with temperature. In Arctic charr, the increase was notably higher (20–23%) at higher temperature (Olsen and Ringø, 1998). These results in carnivorous fish are in contrast to that of the present study in tilapia. Hence, the ADC_{carb} may not always be explained by the trophic level of fish, but it may be more dependent on the ingredients (*e.g.*, type of carbohydrates) and nutrient composition in diets (*e.g.*, Phan et al., 2019, 2021a, 2021b; Schrama et al., 2012). The type of carbohydrates plays an important role in digestion. For example, non-starch polysaccharides get fermented by intestinal bacteria (Maas et al., 2020), and the bacterial activities are influenced by several factors like gut pH and temperature (reviewed in Ray et al., 2012; Volkoff and Rønnestad, 2020). The optimum pH and/or temperature for carbohydrate-fermenting bacteria may vary among the bacterial species and that may result in differences in ADC_{carb} regardless of fish species and trophic level of fish. In addition, the interaction of carbohydrate type and rearing temperature may influence the digestion of nutrients by regulating the evacuation rate of digesta (reviewed in Volkoff and Rønnestad, 2020). The food, for instance carbohydrates, get less time to be fermented in the intestine when the evacuation rate is high. The evacuation rate could be impacted by the type of carbohydrates because some of them are more viscous compared to others that impact the evacuation rate of digesta. Therefore, in order to achieve better digestibility, the best choice of the types of carbohydrates may depend on the actual water temperature.

This study estimated a 0.8% lower average ADC_{fat} at 24 °C compared to 32 °C. This finding is similar to most of the studies on different fish species like yellowtail kingfish (Bowyer et al., 2012), Arctic charr (*Salvelinus alpinus*; Olsen and Ringø, 1998), and Atlantic salmon (*Salmo*

salar; Bendiksen et al., 2003); but in contrast to the studies by Amin et al. (2014) on brook trout reared at 15 or 19 °C and by Miegel et al. (2010) on yellowtail kingfish reared at 13 or 21 °C where the ADC_{fat} was higher at the lower temperature. Miegel et al. (2010) also observed a significant increase in lipase activity in the anterior intestine of yellowtail kingfish at lower temperature. Hence, species and temperature-dependent enzymatic activity may impact ADC_{fat} (Volkoff and Rønnestad, 2020). At lower temperatures, the activity of lipase-producing microbes in the intestine (reviewed in Ray et al., 2012) of tilapia may also be decreased which may turn into lower ADC_{fat} at lower temperature. In addition, the type and melting point of fat (Austreng et al., 1979), differences in diet manufacturing techniques (Opstvedt et al., 2003), and temperature-affected bile acid (fat emulsifier) production may cause the observed differences in ADC_{fat} between studies. The water quality of tilapia farms, especially in intensive culture farms, could decline due to the lower fat digestion at lower temperatures. To ensure good water quality and little water treatment/change in intensive tilapia farms at relatively lower temperatures, dietary supplementation with emulsifiers may be advantageous.

Along with temperature, feeding level (FL) and diet composition also had an impact on $ADC_{nutrient}$, which had already been shown by multiple authors in several fish species (*e.g.*, Phan et al., 2019, 2021a, 2021b; Windell et al., 1978). The present study relates the effect of FL and/or diet with rearing temperature. The effect of FL on ADC_{prot}, or ADC_{carb} in tilapia was less varied between 24 and 32 °C, however that was not the case for ADC_{fat}. For instance, the trends in ADC_{fat} at high FL are entirely reversed between 24 and 32 °C, with the highest ADC_{fat} in fat-rich diets at 32 °C (HF_{diet} and HFC_{diet}; Fig. 1C, D). These variations may occur due to temperature- and pH-influenced enzymatic reactions in the gut (reviewed in Ray et al., 2012; Volkoff and Rønnestad, 2020) of tilapia, as described previously. HFC_{diet}, which is high in fat and carbohydrates, had the lowest ADC_{fat} at 24 °C but had a 6% higher ADC_{fat} at 32 °C. Hence, FL and diet resulted in different effects, especially on fat digestion, between 24 and 32 °C in tilapia. The findings indicated that at 32 °C tilapia can handle fat better than that at 24 °C. Therefore, it would be good to take these variations into account when determining the macronutrient compositions of tilapia diets. For example, tilapia diets in tropical and subtropical areas can be supplemented with fat when it is particularly summer and the water temperature is substantially higher.

4.2. Effect of diet and temperature on RE-DE relationship

The relationship between retained energy (RE) and digestible energy (DE) intake in the present study differed between diets. In other words, dietary macronutrient composition affected the k_{gDE} (*i.e.*, the slope of the RE *vs.* DE linear regression; $P < 0.05$) (Fig. 3; Table 3). Within temperature, HFC_{diet} showed the fastest growth with a k_{gDE} of 76% at 24 °C, whereas high-fat diet (HF_{diet}) showed the fastest growth with a k_{gDE} of 80% at 32 °C. Hence, the DE approach of feed formulation demands for different macronutrient compositions depending on rearing temperatures. In this study, the observed effect of diet at 32 °C is in line with the study of Schrama et al. (2012) on Nile tilapia reared at 28 °C, where high-fat diet provided a higher k_{gDE} (64%) compared to high-starch diet (59%). Similar findings were reported by Glencross et al. (2011), Glencross et al. (2017), and Phan et al. (2019) in Tra Catfish (*Pangasianodon hypothalamus*) at 32 °C, barramundi (*L. calcarifer*) at 30 °C, and common carp at 23 °C, respectively. Hepher et al. (1983) reported considerable differences in the efficiency of metabolizable energy based feed utilization for growth (k_{gME}) between diets given to red tilapia reared at 24 °C, with the most efficient k_{gME} at high-protein diet compared to medium-protein or low-protein diets. Similarly, snakehead (*C. striata*) reared at 29 °C provided highest k_{gDE} at high-protein diet (Phan et al., 2021b); but that is not the case in striped catfish (*Pangasius hypophthalmus*) reared at 29 °C where high-carbohydrate diet provided the highest k_{gDE} (Phan et al., 2021a). Next to the impact of dietary macronutrient composition, variability in k_{gDE} between fish species could also be due to the inter-species differences in feeding habits and anatomical and physiological features.

In the present study, the k_{gDE} and DE_m were affected by temperature. At 24 °C, HP_{diet}, HC_{diet}, and HF_{diet} each showed substantially lower k_{gDE} values than their respective k_{gDE} values at 32 °C (Table 3). The difference between k_{gDE}s in HFC_{diet} at 24 and 32 °C was highest, indicating the most temperature sensitive performance of this diet type. Hepher et al. (1983) recorded the highest k_{gME} at high-protein diet at 24 °C, but the same diet provided the second highest k_{gME} at 21 °C, followed by medium-protein diet in red tilapia. The study of Hepher et al. (1983) is not entirely comparable to the present study because the species, diets, and temperature range are not the same between the experiments. However, the effect of temperature on k_{gDE} or k_{gME} was clearly observable in both of the experiments. The effect of temperature on k_{gDE} or k_{gME} could be due to the temperature-dependent digestion, absorption, and utilization of nutrients that have been reviewed by Volkoff and Rønnestad (2020). In addition to k_{gDE}, DE_m is also dependent on temperature in the present study. The positive correlation between DE_m and temperature had been established long ago (Clarke and Fraser, 2004; McNab and Morrison, 1963). In the present study, at 24 °C, DE_m was 55% lower in HP_{diet}, 61% lower in HC_{diet}, 43% lower in HF_{diet}, and 5% lower in HFC_{diet} compared to the respective DE_ms at 32 °C. Hence, in terms of DE_m values, temperature minimally affected the diet supplemented with carbohydrates and fat (HFC_{diet}). At 24 °C, HFC_{diet} resulted in the highest DE_m (54 $kJ.kg^{-0.8}.d^{-1}$) and at 32 °C in the lowest DE_m (57 $kJ.kg^{-0.8}.d^{-1}$) in comparison to the other 3 diets in the present study. Finally, once the DE_m is met, HFC_{diet} and HF_{diet} resulted in the fastest growth of tilapia at 24 and 32 °C, respectively.

There was an interaction effect of diet and temperature on k_{gDE} in the present study. For instance, HFC_{diet} resulted in the highest k_{gDE} (76%) at 24 °C while the fat-rich diet (HF_{diet}) resulted in the highest k_{gDE} (80%) at 32 °C in Nile tilapia. Furthermore, HFC_{diet} provided higher k_{gDE} at lower temperature, but in the other 3 diets the k_{gDE}s are higher at higher temperature. A similar interaction effect on k_{gDE} in red tilapia was reported by Hepher et al. (1983) where a 'high-protein and low-carbohydrate' diet was more effective in promoting growth at 24 °C than it was at 21 °C, whereas 'medium-protein and medium-carbohydrate' and 'low-protein and high-carbohydrate' diets were more effective in promoting growth at 21 °C than they were at 24 °C (Hepher et al., 1983). However, in their experiment the fat contents were similar among the diets, which was not the case in the present study. There is currently little evidence available on the interaction of diet and temperature on k_{gDE} in different fish species.

Temperature did not affect the average k_{gDE} over all diets ($P > 0.05$) but did affect the average DE_m ($P < 0.05$; Eqs. (3) and (4)) in the present study. In addition, the estimated energy utilization efficiencies for tilapia were 70% at 24 °C and 72% at 32 °C in the present study, which are comparable to 71% at 29 °C in striped catfish (Phan et al., 2021a). The average DE_m was 51 $kJ.kg^{-0.8}.d^{-1}$ at 24 °C and 83 $kJ.kg^{-0.8}.d^{-1}$ at 32 °C, indicating a 63% increase at the higher temperature in tilapia in the current study. Accordingly, a tilapia diet prepared using the DE approach would need to contain about 63% more maintenance energy at 32 °C than it would at 24 °C in order to achieve the same growth between 24 and 32 °C. Hepher et al. (1983) reported specific routine metabolism 25.4 and 36.7 $cal.d^{-1}$ in red tilapia when reared at 21 and 24 °C, respectively, which indicates a 31% difference in specific routine metabolism between temperatures (Hepher et al., 1983). However, the fat content in the diets used by Hepher et al. (1983) did not considerably differ where the influence of diet on DE_m has been found in several studies discussed before in this article. In striped catfish reared at 29 °C, the calculated DE_m over all 4 diets was 50 $kJ.kg^{-0.8}.d^{-1}$ (Phan et al., 2021a) which appeared to be much lower compared to that in the present study on Nile tilapia. This difference could be due to the combined variations among species, nutrient ratio in diets, and rearing temperatures between studies. The lower DE_m at 24 °C in the present study resulted in the higher energy deposition in tilapia at 24 °C compared to

32 °C (*i.e.*, RE_{tot}; 103 *vs.* 90 $kJ.kg^{-0.8}.d^{-1}$ at 24 *vs.* 32 °C; Fig. 2A, B) following the similar trend in RE_{prot} and RE_{fat} (Fig. 2B, C, D, F; Supplementary Table S4). Although the ADC_{fat} was higher at 32 °C, the deposition of fat in the body, *i.e.*, RE_{fat}, was lower, with a negative value at HP_{diet} and a low FL. Possibly this is because at the higher temperature more fat was used to produce more energy for maintenance as more ATP is needed to maintain cellular functions at higher temperatures (Clarke and Fraser, 2004). In fact, the breakdown of one unit of fat generates the highest level of energy (39.5 $kJ.g^{-1}$) compared to the breakdown of one unit of protein (23.6 $kJ.g^{-1}$) or carbohydrates (17.2 $kJ.g^{-1}$) (Einen and Roem, 1997; NRC, 2011; Phan et al., 2021a).

4.3. Temperature-NE relationship

The main aim of this study was to compare the NE equations in tilapia at different temperatures, *i.e.*, 24 and 32 °C. In NE approach, estimating RE in relation to the intake of dCP, dFat and dCarb [RE = intercept + (β_1 × dCP) + (β_2 × dFat) + (β_3 × dCarb) = intercept + NE], a discrete utilization efficiency of dCP ($k_{NE,dCP}$), dFat ($k_{NE,dFat}$) and dCarb ($k_{NE,dCarb}$) for growth is used. This fine-tuning of macronutrient utilization basically makes the NE approach preferable to the DE approach [RE = intercept + (k_{gDE} × DE intake)] (Schrama et al., 2012, 2018; Phan et al., 2019, 2021a, 2021b). In the present study, NE equations were formed by multiple regression, and the large contrast between dCP, dFat, and dCarb intake (Supplementary Table S1) at 24 or 32 °C enabled the performance of multiple regression between RE and dCP, dFat, and dCarb intake. Ultimately, this allowed us to assess the $k_{NE,dCP}$, $k_{NE,dFat}$ and $k_{NE,dCarb}$.

The utilization efficiency of dCP, dFat, and dCarb for growth, in other words, the values of $k_{NE,dCP}$, $k_{NE,dFat}$, and $k_{NE,dCarb}$ did not differ between 24 and 32 °C, which was also followed by the unaffected ratio of fat gain to protein gain ($g.g^{-1}$) by temperature ($P > 0.05$; 0.72 *vs.* 0.70 at 24 *vs.* 32 °C; Supplementary Table S4). This means that the NEs are not different in tilapia reared at 24 or 32 °C ($P > 0.05$; Eqs. (5) and (6)). Table 4 presents a list of NE equations from different experiments on different fish species including tilapia. From Table 4, once the DE_m is met, the protein deposition (*i.e.*, $k_{NE,dCP}$) is numerically highest in tilapia at 32 °C (62%; present study) compared to 56% in tilapia at 24 °C (present study), and 49% in tilapia at 27–28 °C (Schrama et al., 2018). Hence, protein deposition in tilapia did not exponentially increase with temperature. The protein deposition in a carnivorous fish barramundi was 64% at 30 °C (Phan et al., 2019), which is closest to the value of 62% in tilapia at 32 °C in the present study compared to the other species and temperatures mentioned in Table 4. The protein deposition values numerically indicate that the rearing temperature 32 °C is suitable for the best protein deposition in Nile tilapia compared to other temperatures discussed above, *i.e.*, 24, 27 or 28 °C (Table 4). On the other hand, once the energy demand is met, the fat deposition (*i.e.*, $k_{NE,dFat}$) in tilapia at 32 °C in the present study is 83% which is lower than 90% in tilapia at 27–28 °C (Schrama et al., 2018), 86% in common carp at 23 °C and 94% in barramundi at 30 °C (Phan et al., 2019). This value (83%) is slightly higher than 81% in tilapia at 24 °C in the present study. Furthermore, as extra energy from carbohydrates or glucose resulted in glycogen and/or fatty acid in the body, the $k_{NE,dCarb}$ values represent a fat deposition of 62% at 32 °C and 63% at 24 °C in the present study. Once the energy demand for maintenance is met, the overall macronutrient deposition numerically indicates 6% higher protein energy deposition and 1% higher non-protein energy deposition in tilapia at 32 °C compared to 24 °C in the present study (Eqs. (5) and (6)). Hence, the efficiency of protein energy deposition is higher than the efficiency of non-protein energy deposition in Nile tilapia at 32 °C compared to 24 °C.

Temperature did not affect NE equations (NE = RE – FHP), but did affect FHP (*i.e.*, intercepts of Eqs. (5) and (6)) in tilapia. FHP was 48% higher at 32 °C compared to 24 °C in this study. Table 4 shows the FHPs in different fish species including tilapia at various temperatures, where FHP in omnivorous fish species, *i.e.*, common carp and tilapia, increased with temperature. However, the FHP in barramundi at 30 °C was much lower and not in the same flow of temperature-FHP relationship as in common carp and tilapia (Table 4). This could be due to the inter-species differences in several factors such as their region of origin (*i.e.*, tropical or temperate) and physiology. In fact, the feeding habit of barramundi (carnivorous) is different from common carp and tilapia (omnivorous). In addition, very low $k_{NE,dCarb}$ value for barramundi in Table 4 indicates the less capability of handling the digested glucose (Phan et al., 2019), indicating the physiological differences between barramundi and common carp or tilapia. In the present study, temperature did not affect the energy utilization efficiency of macronutrients but only affected FHP.

5. Conclusion

This study on Nile tilapia confirms that the relationship between RE and DE intake is dependent on the dietary macronutrient composition, which is reflected in differences in the energy utilization efficiency of DE for growth (k_{gDE}). However, the current study showed that the effect of diet composition on k_{gDE} is temperature dependent. This interaction effect between temperature and diet on the relationship between RE and DE intake indicates that energy evaluation on DE basis is influenced by temperature. In contrast, temperature only affected the fasting heat production (*i.e.*, intercept in RE equation), but did not affect the energetic efficiency of dCP, dFat, and dCarb (respectively, $k_{NE;dCP}$, $k_{NE;dFat}$, and $k_{NE;dCarb}$) in the NE based evaluation. This implies for tilapia when using a NE based feed evaluation, that the energy value of feed/ingredients are not dependent of water temperature. Practically it means that the feed industries can apply one standard NE equation for tilapia irrespective of climatic region and/or season (for sure between 24 and 32 °C). NE calculations are done using the estimated digested macronutrients contents. As the digestibility of macronutrients were shown to be affected by temperature, this should be accounted for in NE calculations. In other words, testing/quantification of digestibilities of ingredients for optimal diet formulations for Nile tilapia should be done at the relevant temperature(s).

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Table 4
Estimated net energy equations in two omnivorous fish common carp and Nile tilapia, and a carnivore barramundi.

Species*	T (°C)	FHP ($kJ.kg^{-0.8}.d^{-1}$)	Equation	R^2	Eq. No.	Reference
Common carp	23	22	NE = 11.2 dCP + 34.1 dFat +10.4 dCarb	0.99	7	Phan et al., 2019
Nile tilapia	24	28	NE = 13.3 dCP + 32.0 dFat +10.9 dCarb	0.99	8	Present study
Nile tilapia	27–28	44	NE = 11.5 dCP + 35.8 dFat +11.3 dCarb	0.99	9	Schrama et al., 2018
Nile tilapia	32	54	NE = 14.7 dCP + 32.7 dFat +10.6 dCarb	0.98	10	Present study
Barramundi	30	18	NE = 15.2 dCP + 37.1 dFat +3.1 dCarb	0.99	11	Phan et al., 2019

T, temperature; FHP, fasting heat production.
* Common carp and tilapia are omnivorous; barramundi is carnivorous.

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CRediT authorship contribution statement

B.K. Dey: Conceptualization, Formal analysis, Writing – original draft. **M.C.J. Verdegem:** Conceptualization, Methodology, Writing – review & editing. **M.A.J. Nederlof:** Investigation, Formal analysis, Writing – review & editing. **K. Masagounder:** Funding acquisition, Conceptualization, Writing – review & editing. **J. Mas-Muñoz:** Funding acquisition, Conceptualization, Writing – review & editing. **J.W. Schrama:** Funding acquisition, Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The WorldFish's funders had no role and as such did not influence the study design, the collection, analysis and interpretation of data, the writing of the report, and the decision to submit the article for publication.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.739876.

References

Abd El-Hack, M.E., El-Saadony, M.T., Nader, M.M., Salem, H.M., El-Tahan, A.M., Soliman, S.M., Khafaga, A.F., 2022. Effect of environmental factors on growth performance of Nile tilapia (*Oreochromis niloticus*). Int. J. Biometeorol. 66 (11), 2183–2194. https://doi.org/10.1007/s00484-022-02347-6.

Amin, M.N., Barnes, R.K., Adams, L.R., 2014. Effect of temperature and varying level of carbohydrate and lipid on growth, feed efficiency and nutrient digestibility of brook trout, *Salvelinus fontinalis* (Mitchill, 1814). Anim. Feed Sci. Technol. 193, 111–123. https://doi.org/10.1016/j.anifeedsci.2014.04.016.

Austreng, E., Skrede, A., Eldegard, Å., 1979. Effect of dietary fat source on the digestibility of fat and fatty acids in rainbow trout and mink. Acta Agric. Scand. 29 (2), 119–126. https://doi.org/10.1080/00015127909435220.

Azaza, M.S., Dhraïef, M.N., Kraïem, M.M., 2008. Effects of water temperature on growth and sex ratio of juvenile Nile tilapia *Oreochromis niloticus* (Linnaeus) reared in geothermal waters in southern Tunisia. J. Therm. Biol. 33 (2), 98–105. https://doi.org/10.1016/j.jtherbio.2007.05.007.

Azevedo, P.A., Cho, C.Y., Leeson, S., Bureau, D.P., 1998. Effects of feeding level and water temperature on growth, nutrient and energy utilization and waste outputs of rainbow trout (*Oncorhynchus mykiss*). Aquat. Living Resour. 11 (4), 227–238. https://doi.org/10.1016/S0990-7440(98)89005-0.

Bendiksen, E.Å., Berg, O.K., Jobling, M., Arnesen, A.M., Måsøval, K., 2003. Digestibility, growth and nutrient utilisation of Atlantic salmon parr (*Salmo salar* L.) in relation to temperature, feed fat content and oil source. Aquaculture 224 (1–4), 283–299. https://doi.org/10.1016/S0044-8486(03)00218-7.

Bowyer, J.N., Qin, J.G., Adams, L.R., Thomson, M.J., Stone, D.A., 2012. The response of digestive enzyme activities and gut histology in yellowtail kingfish (*Seriola lalandi*) to dietary fish oil substitution at different temperatures. Aquaculture 368, 19–28. https://doi.org/10.1016/j.aquaculture.2012.09.012.

Bureau, D.P., Kaushik, S.J., Cho, C.Y., 2003. Bioenergetics. In: Fish Nutrition, Third edition. Elsevier, pp. 1–59. https://doi.org/10.1016/B978-012319652-1/50002-1.

Cho, C.Y., Slinger, S.J., 1980. Effect of water temperature on energy utilization in rainbow trout (*Salmo gairdneri*). In: Proceedings of the Eighth Symposium on Energy Metabolism Held at Churchill College, Cambridge, September, 1979, pp. 287–291. https://doi.org/10.1016/B978-0-408-10641-2.50063-X.

Clarke, A., Fraser, K.P.P., 2004. Why does metabolism scale with temperature? Funct. Ecol. 18 (2), 243–251. https://www.jstor.org/stable/3599364.

Clarke, A., Johnston, N.M., 1999. Scaling of metabolic rate with body mass and temperature in teleost fish. J. Anim. Ecol. 68 (5), 893–905. https://doi.org/10.1046/j.1365-2656.1999.00337.x.

Einen, O., Roem, A.J., 1997. Dietary protein/energy ratios for Atlantic salmon in relation to fish size: growth, feed utilization and slaughter quality. Aquac. Nutr. 3 (2), 115–126. https://doi.org/10.1046/j.1365-2095.1997.00084.x.

El-Sayed, A.F.M., Kawanna, M., 2008. Optimum water temperature boosts the growth performance of Nile tilapia (*Oreochromis niloticus*) fry reared in a recycling system. Aquac. Res. 39 (6), 670–672. https://doi.org/10.1111/j.1365-2109.2008.01915.x.

FAO, 2022. FishStatJ: Universal Software for Fishery Statistical Time Series: Aquaculture Production 1950–2020. FAO, Rome. https://www.fao.org/fishery/en/statistics/software/fishstatj.

Glencross, B.D., Bermudes, M., 2012. Adapting bioenergetic factorial modelling to understand the implications of heat stress on barramundi (*Lates calcarifer*) growth, feed utilisation and optimal protein and energy requirements–potential strategies for dealing with climate change? Aquac. Nutr. 18 (4), 411–422. https://doi.org/10.1111/j.1365-2095.2011.00913.x.

Glencross, B., Hawkins, W., Evans, D., Rutherford, N., Dods, K., McCafferty, P., Sipsas, S., 2008. Evaluation of the influence of *Lupinus angustifolius* kernel meal on dietary nutrient and energy utilization efficiency by rainbow trout (*Oncorhynchus mykiss*). Aquac. Nutr. 14 (2), 129–138. https://doi.org/10.1111/j.1365-2095.2007.00512.x.

Glencross, B., Hien, T.T.T., Phuong, N.T., Cam Tu, T.L., 2011. A factorial approach to defining the energy and protein requirements of Tra Catfish, *Pangasianodon hypothalamus*. Aquac. Nutr. 17 (2), e396–e405. https://doi.org/10.1111/j.1365-2095.2010.00774.x.

Glencross, B.D., Blyth, D., Bourne, N., Cheers, S., Irvin, S., Wade, N.M., 2017. An analysis of partial efficiencies of energy utilisation of different macronutrients by barramundi (*Lates calcarifer*) shows that starch restricts protein utilisation in carnivorous fish. Br. J. Nutr. 117 (4), 500–510. https://doi.org/10.1017/S0007114517000307.

Hepher, B., Liao, I.C., Cheng, S.H., Hsieh, C.S., 1983. Food utilization by red tilapia—effects of diet composition, feeding level and temperature on utilization efficiencies for maintenance and growth. Aquaculture 32 (3–4), 255–275. https://doi.org/10.1016/0044-8486(83)90223-5.

Hua, K., Birkett, S., De Lange, C.F.M., Bureau, D.P., 2010. Adaptation of a non-ruminant nutrient-based growth model to rainbow trout (*Oncorhynchus mykiss* Walbaum). J. Agric. Sci. 148 (1), 17–29. https://doi.org/10.1017/S0021859609990037.

Huguet, C.T., Norambuena, F., Emery, J.A., Hermon, K., Turchini, G.M., 2015. Dietary n-6/n-3 LC-PUFA ratio, temperature and time interactions on nutrients and fatty acids digestibility in Atlantic salmon. Aquaculture 436, 160–166. https://doi.org/10.1016/j.aquaculture.2014.11.011.

Lupatsch, I., Kissil, G.W., Sklan, D., 2003. Comparison of energy and protein efficiency among three fish species gilthead sea bream (*Sparus aurata*), European sea bass (*Dicentrarchus labrax*) and white grouper (*Epinephelus aeneus*): energy expenditure for protein and lipid deposition. Aquaculture 225 (1–4), 175–189.

Maas, R.M., Verdegem, M.C., Wiegertjes, G.F., Schrama, J.W., 2020. Carbohydrate utilisation by tilapia: a meta-analytical approach. Rev. Aquac. 12 (3), 1851–1866. https://doi.org/10.1111/raq.12413.

Mazumder, S.K., Das, S.K., Rahim, S.M., Abd Ghaffar, M., 2018. Temperature and diet effect on the pepsin enzyme activities, digestive somatic index and relative gut length of Malabar blood snapper (*Lutjanus malabaricus* Bloch & Schneider, 1801). Aquac. Rep. 9, 1–9. https://doi.org/10.1016/j.aqrep.2017.11.003.

McNab, B.K., Morrison, P., 1963. Body temperature and metabolism in subspecies of peromyscus from arid and mesic environments. Ecol. Monogr. 33 (1), 63–82. https://doi.org/10.2307/1948477.

Meriac, A., Eding, E.H., Schrama, J., Kamstra, A., Verreth, J.A.J., 2014. Dietary carbohydrate composition can change waste production and biofilter load in recirculating aquaculture systems. Aquaculture 420–421, 254–261. https://doi.org/10.1016/j.aquaculture.2013.11.018.

Miegel, R.P., Pain, S.J., Van Wettere, W.H.E.J., Howarth, G.S., Stone, D.A.J., 2010. Effect of water temperature on gut transit time, digestive enzyme activity and nutrient digestibility in yellowtail kingfish (*Seriola lalandi*). Aquaculture 308 (3–4), 145–151. https://doi.org/10.1016/j.aquaculture.2010.07.036.

Mirea, E.T., 2013. Influence of Different Water Temperature on Intensive Growth Performance of Nile Tilapia in a Recirculation Aquaculture System, 60. Lorena Dediu University of Agriculture Science and Veterinary Medical. http://193.231.26.6/firaa/Pdf/Pdf_Vol_60/Catalina_Mirea2.pdf.

Mjoun, K., Rosentrater, K., Brown, M.L., 2010. Tilapia: Environmental Biology and Nutritional Requirements. https://openprairie.sdstate.edu/cgi/viewcontent.cgi?article=1163&context=extension_fact.

NRC, 2011. Nutrient Requirements of Fish and Shrimp. National Academies Press. https://doi.org/10.17226/13039.

Olsen, R.E., Ringø, E., 1998. The influence of temperature on the apparent nutrient and fatty acid digestibility of Arctic charr, *Salvelinus alpinus* L. Aquac. Res. 29 (10), 695–701. https://doi.org/10.1046/j.1365-2109.1998.29100695.x.

Opstvedt, J., Nygård, E., Samuelsen, T.A., Venturini, G., Luzzana, U., Mundheim, H., 2003. Effect on protein digestibility of different processing conditions in the production of fish meal and fish feed. J. Sci. Food Agric. 83 (8), 775–782. https://doi.org/10.1002/jsfa.1396.

Phan, L.T.T., Groot, R., Konnert, G.D., Masagounder, K., Figueiredo-Silva, A.C., Glencross, B.D., Schrama, J.W., 2019. Differences in energy utilisation efficiencies of digestible macronutrients in common carp (*Cyprinus carpio*) and barramundi (*Lates calcarifer*). Aquaculture 511, 734238. https://doi.org/10.1016/j.aquaculture.2019.734238.

Phan, L.T.T., Kals, J., Masagounder, K., Mas-Munoz, J., Schrama, J.W., 2021a. Energy utilisation efficiencies of digested protein, fat and carbohydrates in striped catfish (*Pangasius hypophthalmus*) for whole body and fillet growth. Aquaculture 544, 737083. https://doi.org/10.1016/j.aquaculture.2021.737083.

Phan, L.T.T., Masagounder, K., Mas-Muñoz, J., Schrama, J.W., 2021b. Differences in energy utilization efficiency of digested protein, fat and carbohydrates in snakehead

(*Channa striata*). Aquaculture 532, 736066. https://doi.org/10.1016/j.aquaculture.2020.736066.

Phan, L.T.T., Kals, J., Masagounder, K., Mas-Muñoz, J., Schrama, J.W., 2022. Energy utilisation efficiencies of digestible protein, fat and carbohydrates for African catfish (*Clarias gariepinus*). Aquac. Rep. 23, 101051 https://doi.org/10.1016/j.aqrep.2022.101051.

Raubenheimer, D., 2011. Toward a quantitative nutritional ecology: the right-angled mixture triangle. Ecol. Monogr. 81, 407–427. https://doi.org/10.1890/10-1707.1.

Ray, A.K., Ghosh, K., Ringø, E.J.A.N., 2012. Enzyme-producing bacteria isolated from fish gut: a review. Aquac. Nutr. 18 (5), 465–492. https://doi.org/10.1111/j.1365-2095.2012.00943.x.

Révész, N., Kumar, S., Bogevik, A.S., Fazekas, G., Jeney, Z., Hegyi, Á., Sándor, Z.J., 2020. Effect of temperature on digestibility, growth performance and nutrient utilization of corn distiller's dried grains with soluble (DDGS) in common carp juveniles. Aquac. Res. 51 (2), 828–835. https://doi.org/10.1111/are.14432.

Saravanan, S., Schrama, J.W., Figueiredo-Silva, A.C., Kaushik, S.J., Verreth, J.A., Geurden, I., 2012. Constraints on energy intake in fish: the link between diet composition, energy metabolism, and energy intake in rainbow trout. PLoS One 7, e34743. https://doi.org/10.1371/journal.pone.0034743.

Schrama, J.W., Saravanan, S., Geurden, I., Heinsbroek, L.T.N., Kaushik, S.J., Verreth, J.A.J., 2012. Dietary nutrient composition affects digestible energy utilisation for growth: a study on Nile tilapia (*Oreochromis niloticus*) and a literature comparison across fish species. Br. J. Nutr. 108 (2), 277–289. https://doi.org/10.1017/S0007114511005654.

Schrama, J.W., Haidar, M.N., Geurden, I., Heinsbroek, L.T., Kaushik, S.J., 2018. Energy efficiency of digestible protein, fat and carbohydrate utilisation for growth in rainbow trout and Nile tilapia. Br. J. Nutr. 119 (7), 782–791. https://doi.org/10.1017/S0007114518000259.

Shiau, S.Y., Peng, C.Y., 1993. Protein-sparing effect by carbohydrates in diets for tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture 117 (3–4), 327–334. https://doi.org/10.1016/0044-8486(93)90329-W.

Volkoff, H., Rønnestad, I., 2020. Effects of temperature on feeding and digestive processes in fish. Temperature 7 (4), 307–320. https://doi.org/10.1080/23328940.2020.1765950.

Watanable, T., Takeuchi, T., Satoh, S., Kiron, V., 1996. Digestible crude protein contents in various feedstuffs determined with four freshwater fish species. Fish. Sci. 62, 278–282. https://doi.org/10.2331/fishsci.62.278.

Windell, J.T., Foltz, J.W., Sarokon, J.A., 1978. Effect of fish size, temperature, and amount fed on nutrient digestibility of a pelleted diet by rainbow trout, *Salmo gairdneri*. Trans. Am. Fish. Soc. 107 (4), 613–616. https://doi.org/10.1577/1548-8659(1978)107<613:eofsta>2.0.co;2.

Yamamoto, T., Shima, T., Furuita, H., Sugita, T., Suzuki, N., 2007. Effects of feeding time, water temperature, feeding frequency and dietary composition on apparent nutrient digestibility in rainbow trout *Oncorhynchus mykiss* and common carp *Cyprinus carpio*. Fish. Sci. 73 (1), 161–170. https://doi.org/10.1111/j.1444-2906.2007.01314.x.