

Differences in energy utilization efficiency of digested protein, fat and carbohydrates in snakehead (*Channa striata*)

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ABSTRACT

This study aimed to assess if the relationship between digestible energy (DE) intake and energy retention (RE) is dependent on dietary macronutrient composition; and to quantify the energy utilization efficiency of digested protein (dCP), fat (dFat) and carbohydrates (dCarb) for growth in snakehead (*Channa striata*). This was achieved by conducting a 42-day feeding trial on snakehead (29.1 g) with a 4×2 factorial design. Nutrient digestibility, nitrogen and energy balances were measured. Four diets were formulated, which had contrasting levels of protein, fat and carbohydrates. Each diet was tested at 2 feeding levels, in order to estimate within each feeding level the relationship between DE and RE. Snakehead (a carnivore) is well able to digest starch. Starch digestibility ranged from 93% to 99.5%. The linear relationship between RE and DE intake was affected by diet. Diets with a high Carb content had a lower slope (0.45 versus 0.53 to 0.56), which indicates that DE is less efficiently used for RE. Multiple regression of RE as a function of dCP, dFat and dCarb (in $\text{g} \cdot \text{kg}^{-0.8} \cdot \text{d}^{-1}$) intake was also applied to estimate the energy utilization efficiency of each digestible macronutrient. A linear relationship was found between RE and dCP, dFat and dCarb. Snakehead are a lean fish, which we observed prioritise digestible protein intake for protein gain. The estimated energy efficiencies of dCP, dFat and dCarb (respectively, $k_{\text{NE;dCP}}$, $k_{\text{NE;dFat}}$ and $k_{\text{NE;dCarb}}$) for energy retention were 53, 79 and 5%, respectively. Snakehead can digest starch at the high level but cannot metabolise digested starch for growth efficiently.

1. Introduction

Snakehead (*Channa striata*), also called murrel or haruan, recently became a widely farmed freshwater fish in South East Asia due to its rapid growth. This carnivorous species is an aggressive predator, which, as an obligate air-breather, can survive in shallow and slow moving waters (Shafri and Abdul Manan, 2012). It uses the foraging strategy of hiding and suddenly attacking its preys (i.e., small fish, frogs, birds) (Shafri and Abdul Manan, 2012; Liu et al., 2000). Its rapid growth and high foraging activities need to be fuelled by energy. Energy can be yielded from digested protein, fat and carbohydrates via different metabolic pathways (NRC, 2011), resulting in the different energy utilization efficiencies of these macronutrients. The ingredients used for snakehead feed appear to be increasingly diversified (Zehra and Khan, 2012; Hien et al., 2017; Aliyu-Paiko and Hashim, 2012). This also leads to an increased variation in the composition of digested macronutrients. However, effects of this diversification on the energy metabolism has not been assessed for snakehead.

Various animal feed energy evaluation systems have been used to quantify the dietary energy available for growth after being ingested, ranging from digestible (DE) and metabolisable (ME) to net energy (NE) systems (NRC, 1981). Fish energy demands for maintenance and growth has been assessed by using the factorial approach on a digestible basis (Lupatsch et al., 2003; Glencross, 2008; Glencross and Bermudes, 2012). In such an approach, the digestible energy utilization efficiency for energy gain (k_{gDE}), which is the regression slope of retained energy on DE intake, is assumed to be independent on the feed composition. In other words, the composition of DE (digested protein, fat and carbohydrates) is assumed to have no effect on k_{gDE} . However, variation in the dietary macronutrient content altered k_{gDE} in barramundi (*Lates calcarifer*) (Glencross et al., 2017), Nile tilapia (*Oreochromis niloticus*) (Schrama et al., 2012) and rainbow trout (*Oncorhynchus mykiss*) (Rodehutscord and Pfeffer, 1999). This increased variation in k_{gDE} , which can be induced by the larger variability in ingredients used in practical feed formulation, will result in imprecise/biased estimation of energy value of feeds when using a DE evaluation system. The need for

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alternative fish feed energy evaluation systems has been suggested in various studies (Glencross et al., 2014; Hua et al., 2010; Azevedo et al., 2005). While the fish feed energy evaluation is still mainly based on a DE basis, the pig feed energy evaluation has evolved to a NE basis since several decades ago, making a distinction in the energy utilization efficiency for each macronutrient (i.e., protein, fat and starch) (Noblet et al., 1994).

Recently, the first steps towards a NE approach of feed evaluation were made for Nile tilapia, rainbow trout (Schrama et al., 2018), carp and barramundi (Phan et al., 2019). Among these four fish species, the differences in energy utilization efficiencies for growth of digestible fat (dFat; $k_{NE;dFat}$) were small (86–94%) and slightly larger for digestible protein (dCP; $k_{NE;dCP}$) (47–64%). The energy utilization efficiency of digestible carbohydrate (dCarb; $k_{NE;dCarb}$) showed the largest differences between the fish species (18–70%). These studies found that common carp and Nile tilapia can utilise digested carbohydrates very efficiently according to a linear relationship over a wide range of digestible carbohydrate intakes. In contrast, rainbow trout (Schrama et al., 2018) and barramundi (Phan et al., 2019) exhibited a curvilinear relationship between digestible carbohydrate intake and energy retention. With increasing carbohydrates levels, the response in growth diminishes, implying that rainbow trout and barramundi, both carnivorous fish, have limited capacity to utilise carbohydrates. This raises the question, whether this low energy utilization efficiency of digested carbohydrates is specific for carnivorous fish species.

Therefore, this study was done in snakehead: (1) to investigate the effect of macronutrient composition on the relationship between DE intake and energy retention (DE approach), (2) to quantify energy utilization efficiency of digested protein, fat and carbohydrates by multiple regression on energy retention (NE approach).

2. Materials and methods

2.1. Experimental diets

Four diets were used with different proportions of crude protein (36.7–53.5%), crude fat (5.4–19.5%) and carbohydrates (22.4–40.7%). This large range in dietary macronutrient composition was created by varying mainly the content of wheat flour (carbohydrate rich ingredient) and soya oil (Table 1). Due to this large range in macronutrients, diets were formulated to have a constant ratio between protein and premix content. Details on amino acid requirements of snakehead are scarce. Therefore diets were optimized using the amino acid requirements (on g per kg crude protein) average over freshwater teleost fish (NRC, 2011). The analysed amino acid composition of the experimental diets were close to the planned values and are shown in Supplementary Table S1.

By applying the triangle approach of Raubenheimer (2011), a wide range of macronutrient contents (i.e., crude protein, fat and total carbohydrates) was created between the four experimental diets (Table 1). First a diet was formulated having high protein level (PROT-diet). This PROT-diet was diluted with wheat flour (30%) to get a diet high in starch content (CARB-diet), with soya oil (12.5%) to get a diet high in lipid content (LIPID-diet) or with both wheat flour and soya oil to get a diet high in lipid and starch content (MIX-diet). Each diet was studied at 2 feeding levels, which resulted in a 4×2 factorial design with a total of 8 treatments. This design aimed to achieve large contrasts in digested macronutrient intake among the 4 diets. These large contrasts enabled multiple regression analysis of energy retention (i.e., growth response) as a function of dCP, dFat and dCarb intake.

Diets were produced by De Heus (Vinh Long, Vietnam). Fishmeal, soy protein concentrate, meat and bone meal and wheat gluten were hammer-milled through a 0.9 mm screen at 1470 rpm. All ingredients, expect soy oil in the LIPID- and MIX-diet, were mixed in a 60-L batch mixer for 240 s. Prior to extrusion, these mixtures were conditioned for 10 s at a temperature between 85 and 100 °C. Diets were processed by

Table 1

Formulation and composition in each of four experimental diets.

	PROT	CARB	LIPID	MIX
	“protein”	“protein”	“protein”	“protein”
		+ Carb	+ Lipid	+ Carb + Lipid
Diet formulation (g.100 g ⁻¹ , as-is):				
Wheat flour	5.2	37.7	4.3	33.0
Soybean oil	0.0	0.0	17.9	12.5
Fishmeal	34.8	22.9	28.5	20.0
Soy protein concentrate	20.9	13.7	17.2	12.0
Meat bone meal	13.9	9.2	11.4	8.0
Wheat gluten	17.4	11.4	14.3	10.0
Methionine 99%	0.5	0.3	0.4	0.3
L-lysine-HCl 79%	1.6	1.0	1.3	0.9
Threonine 98%	0.4	0.2	0.3	0.2
Squid oil	0.9	0.6	0.7	0.5
Mono ammonium phosphate	2.6	1.7	2.1	1.5
Premix*	1.9	1.3	1.6	1.1
Chemical composition (g.kg ⁻¹ DM):				
DM	931	934	965	950
Crude protein	535	419	435	367
Crude fat	70	54	195	162
Total carbohydrate	224	407	226	365
Starch	147	346	139	298
NSP	78	61	86	66
Crude ash	171	120	144	106
Yttrium (mg.kg ⁻¹ DM)	346	222	259	225
Gross energy (kJ.g ⁻¹ DM)	18.4	18.4	21.3	21.1

PROT, diet with a high protein content; CARB, the PROT diet supplemented with starch; LIPID, the PROT diet supplemented with lipid; MIX, the PROT diet supplemented with starch and lipid; Carb, carbohydrates; DM, dry matter; NSP, non-starch polysaccharides. *De Heus Animal Nutrition B.V. closed formula vitamin and trace mineral premix to meet NRC 2011 requirements of fresh water fish

extrusion on a twin-screw extruder with a capacity of 150 kg/h using a 2 mm die at 95–110 °C. This resulted in 3 mm floating pellets, which were dried at 95 °C for 10 min. Thereafter pellets of the LIPID- and MIX-diet were vacuum coated with soy oil. After coating, pellets were cooled at 30–33 °C for 10 min.

2.2. Fish handling

The experiment was run from December 2018 to January 2019 at the research and development centre of De Heus (Vinh Long, Vietnam) and was done in compliance with Vietnamese law. Additionally, the experimental procedures were internally evaluated and approved for meeting the EU regulations for the care and use of laboratory animals conform to Directive 2010/63/EU. This evaluation was done by the Ethical Committee judging Animal Experiments of Wageningen University, The Netherlands.

A total of 2400 snakehead (*Channa striata*) (mixed sex), with a mean body weight (BW) of 29.1 g (SD 0.07), were obtained from the local snakehead hatchery of An Giang (Mekong Delta, Vietnam). The experiment was conducted using twenty four 500-L tanks, which were part of one RAS system. The water flow per tank was 30 L/min. During the experiment outlet water had a temperature of 28.5 °C (SD 0.8) and the dissolved oxygen level ranged from 7.6 to 8.4 ppm. The outlet water had pH 6.9 (SD 0.3), $NH_4 < 1$ mg/L, $NO_2 < 1$ mg/L, and $NO_3 < 50$ mg/L. At the start of the experiment, groups of one hundred fish were batch-weighed and randomly assigned to one of the twenty four tanks.

Snakehead were hand-fed one of the four diets and one of the two feeding levels of approximately 10 and 20 g.kg^{-0.8}.d⁻¹. Fish were fed twice daily for 42 days from 09:00 to 10:00 h and from 16:00 to 17:00 h.

2.3. Sample preparation and chemical analysis

At the start of experiment, ten fish from the initial population were euthanized by overdose of Aqui-S (Aqui-S New Zealand Ltd., Lower Hutt, New Zealand) for determining initial body composition. At the end of the experiment, ten fish per tank were similarly euthanized to determine final body composition. The fish were then frozen at -20 °C. The sample preparation for chemical analysis was followed the methods reported by Saravanan et al. (2012).

After sample collection, fish were frozen and minced to ensure the sample homogeneity. In thawed, fresh fish samples, dry matter (DM) was measured, whereas fish samples for ash, crude protein (CP), fat and gross energy (GE) analyses were first oven-dried (60 °C). Diets and oven-dried (70 °C) faecal samples were analysed for DM, yttrium, P, CP, fat, starch and gross energy contents.

Proximate composition of fish, feed and faeces were assessed according to ISO-standard analysis for determination of dry matter (DM; ISO 6496: 2009), crude ash (ISO 5984:2002), crude fat (ISO 6492, 1999), crude protein (ISO 16634-2:2009, crude protein = Kjeldahl-N × 6.25), starch (ISO 6493: 2000), and energy (bomb calorimeter, ISO 9831,1998). Total carbohydrates content of feed and faeces were calculated as DM minus crude protein minus crude ash minus crude fat.

2.4. Nutrient digestibility measurement

Yttrium oxide was added as an inert marker to experimental diets. The water outlet of each tank was connected to a separate faeces settling unit of 70 L (Tu Quang Ltd., Can Tho, Vietnam). The settling column has a diameter of 50 cm, a height of 90 cm and a conical bottom angled at 50 cm from the bottom. Settling columns were equipped with an ice-cooled glass bottle at the bottom to prevent bacterial degradation of faecal nutrients. Faeces settled in the column overnight were collected daily prior to the morning feeding during the last 2 weeks of the experiment and pooled per tank. The procedure of faeces collection was identical to the study of Meriac et al. (2014).

Apparent nutrient digestibility coefficients (ADC_{nutrient}) of the diets were calculated using the following equation:

$$ADC_{\text{nutrient}} = (1 - (Y_{\text{diet}}/Y_{\text{faeces}}) \times (\text{Nutrient}_{\text{faeces}}/\text{Nutrient}_{\text{diet}})) \times 100\%$$

where Y_{diet} and Y_{faeces} are the yttrium oxide concentration of the diet and faeces, respectively, and $\text{Nutrient}_{\text{diet}}$ and $\text{Nutrient}_{\text{faeces}}$ are the DM, crude ash, phosphorus, calcium crude protein, fat, starch, non-starch polysaccharides total carbohydrates or energy content of diet and faeces, respectively.

2.5. Nutrient balances calculations

To standardise for differences in body weight and digestible macronutrient intake, nitrogen and energy balance parameters were expressed per unit of metabolic body weight. Metabolic body weight was calculated as the average of initial and final metabolic body weight (calculated as $BW^{0.8}$, in $kg^{0.8}$). The calculations of energy and nitrogen balances parameters per tank were based on those described by Saravanan et al. (2012). Nutrient intake was determined by multiplying the feed intake per tank with the analysed dietary nutrient content. Digestible nutrient intake (dCP, dFat or dCarb) was determined by multiplying nutrient intake with the measured nutrient digestibility coefficient per tank. Energy and nitrogen retention (respectively RE and RN) were determined from net gain, calculated by difference between initial and final whole-body content. Branchial and urinary N losses (BUN) were calculated based on difference between digestible N intake and RN. Branchial and urinary energy losses (BUE) was measured by multiplying BUN by 24.85, which is the energy content (in kJ) of 1 g excreted nitrogen with the assumption that $NH_3\text{-N}$ is the only form of this excretion (Bureau et al., 2003). ME intake was determined by difference between DE intake and BUE. Heat production was measured

by deducting ME from RE.

3. Data analysis

Data analysis was conducted using statistical analysis systems (SAS Institute) statistical software package version 9.1. Two-way ANOVA was used to investigate the effect of diet, feeding level and their interaction on digestibility, performance, nitrogen and energy balances data. Significance was set at $P < 0.05$.

Linear regression of RE (in $kJ.kg^{-0.8}.d^{-1}$) as a function of DE intake DE (in $g.kg^{-0.8}.d^{-1}$) was applied to estimate the energy utilization efficiency (k_{eDE}) of each diet using the following model:

$$RE_i = \mu + \beta \times DE + e_i$$

where μ is the intercept, β is the energy utilization efficiency; e_i is error term and $i = 1, \dots, n$ with $n = 6$ per diet.

The difference in the slopes of the regressions among different diets was tested for significance using a general linear model with RE as dependent parameter, DE as covariate and diet as a fixed factor. If the interaction effect between diet and DE was significant ($P < 0.05$), the slopes differed between diets.

Multiple regression of RE (in $kJ.kg^{-0.8}.d^{-1}$) 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:

$$RE_i = \mu + \beta_1 \times dCP_i + \beta_2 \times dFat_i + \beta_3 \times dCarb_i + e_i \quad (1)$$

where μ is the intercept, being an estimate for fasting heat production (FHP); $\beta_1, \beta_2, \beta_3$ are the energy utilization efficiency of dCP ($k_{NE;dCP}$), dFat ($k_{NE;dFat}$) and dCarb ($k_{NE;dCarb}$); e_i is error term and $i = 1, \dots, n$ with $n = 24$.

4. Results

For almost all measured parameters in the current study, the interaction effect between feeding level and diet was significant. In general, the order of the response to the different diets were comparable at both feeding levels, but the absolute differences between the effect of diets were larger at the high feeding level compared to the low feeding levels. In other words the diet effect was more amplified at the high compared to the low feeding level.

Snakehead used in this study grew well, with a daily weight gain ranging from 8.0 to 16.6 $g.kg^{-0.8}.d^{-1}$ (Table 2). At the end of experiment, final BW was almost double the initial BW at the low feeding level and triple the initial BW at the high feeding level. Daily digestible nutrient intakes (dCP, dFat and dCarb), final body weight and growth were all affected by both feeding level, diet and their interaction ($P < 0.001$; Table 2 & supplementary Table S3).

Data on initial and final body composition of snakehead are given in Supplementary Table S2. At the start of the experiment, body fat content of snakehead was 32 $g.kg^{-1}$ (on wet basis). Final body fat content was affected by the interaction effect between diet and feeding level ($P < 0.05$). This interaction effect was reflected in larger differences between dietary treatments at the high compared to the low feeding level (Fig. 1). Fat content was slightly higher at the high feeding level than at the low feeding level (averaged over diets 59 versus 51 $g.kg^{-1}$). Averaged over both feeding levels, the differences in final fat content between snakehead fed the PROT-diet and CARB-diet (38 versus 35 $g.kg^{-1}$) and between snakehead fed the LIPID-diet and MIX-diet (76 versus 71 $g.kg^{-1}$) were marginal. These pairs of diets differed mainly in the amount of carbohydrate, which suggested that dietary carbohydrate content had a marginal effect on body fat content (Fig. 1). Comparing pairs of diets, which differed only in the amount of soya oil inclusions, showed a doubling of the final body fat content when soya oil was added. Snakehead fed the PROT-diet and CARB-diet remained almost as lean as at the start of the experiment. In other words, body fat content

Table 2Performance data in snakehead (*C. striata*), means per experimental diet and feeding level (FL) during the 42-d experimental period ($n = 3$).

	PROT	CARB	LIPID	MIX	SEM	Diet	P-value	
	“protein”	“protein”	“protein”	“protein”			FL	Diet x FL
FL		+ Carb	+ Lipid	+ Carb + Lipid				
Final BW (g)								
Low	62 ^{de}	56 ^f	66 ^d	58 ^{ef}	0.93	< 0.001	< 0.001	< 0.001
High	109 ^a	95 ^b	99 ^b	88 ^c				
Feed intake (g.d ⁻¹)								
Low	0.74	0.74	0.74	0.74	— ^a	— ^a	— ^a	— ^a
High	1.74	1.74	1.46	1.46				
Daily weight gain (g.kg ^{-0.8} .d ⁻¹)								
Low	9.4 ^e	8.0 ^g	10.1 ^d	8.6 ^f	0.12	< 0.001	< 0.001	< 0.001
High	16.6 ^a	14.9 ^b	15.4 ^b	13.8 ^c				
FCR								
Low	0.94 ^d	1.18 ^a	0.85 ^e	1.06 ^c	0.01	< 0.001	0.001	< 0.001
High	0.92 ^d	1.11 ^b	0.87 ^e	1.05 ^c				
Survival (%)								
Low	99.7	98.7	98.7	98.7	0.74	0.537	0.351	0.991
High	99.0	98.3	98.3	98.0				

PROT, diet with a high protein content; CARB, the PROT diet supplemented with starch; LIPID, the PROT diet supplemented with lipid; MIX, the PROT diet supplemented with starch and lipid; carb, carbohydrates; FL, feeding level; P values for effects of diet, feeding level or the interaction, respectively; BW, body weight; FCR, feed conversion rate.

^aNo statistical analysis was conducted on feed intake, because it was controlled at two levels.

^{abcde}For parameters with a significant interaction effect between diet and feeding level, means within parameters lacking a common superscript differ significantly ($P < 0.05$).

at these diets remained similar to the initial body fat content (32 g.kg⁻¹ on wet basis).

The relatively lean growth of snakehead was also reflected in the ratio of energy retention as fat versus protein (RE as fat: RE as protein). Averaged over feeding levels and diets, RE as fat was 30 kJ.kg^{-0.8}.d⁻¹ and RE as protein was 52 kJ.kg^{-0.8}.d⁻¹ (Table 4). On an energetic basis,

the ratio between fat and protein gain was 0.29, 0.25, 0.91 and 0.89 J.J⁻¹ for snakehead fed the PROT-, CARB-, LIPID- and MIX-diet, respectively. On a weight basis (g fat gain:g protein gain), the ratio between fat and protein gain was 0.22, 0.19, 0.58 and 0.57 g.g⁻¹ for snakehead fed the PROT-, CARB-, LIPID- and MIX-diet, respectively.

Within all experimental treatments, starch had the highest ADC

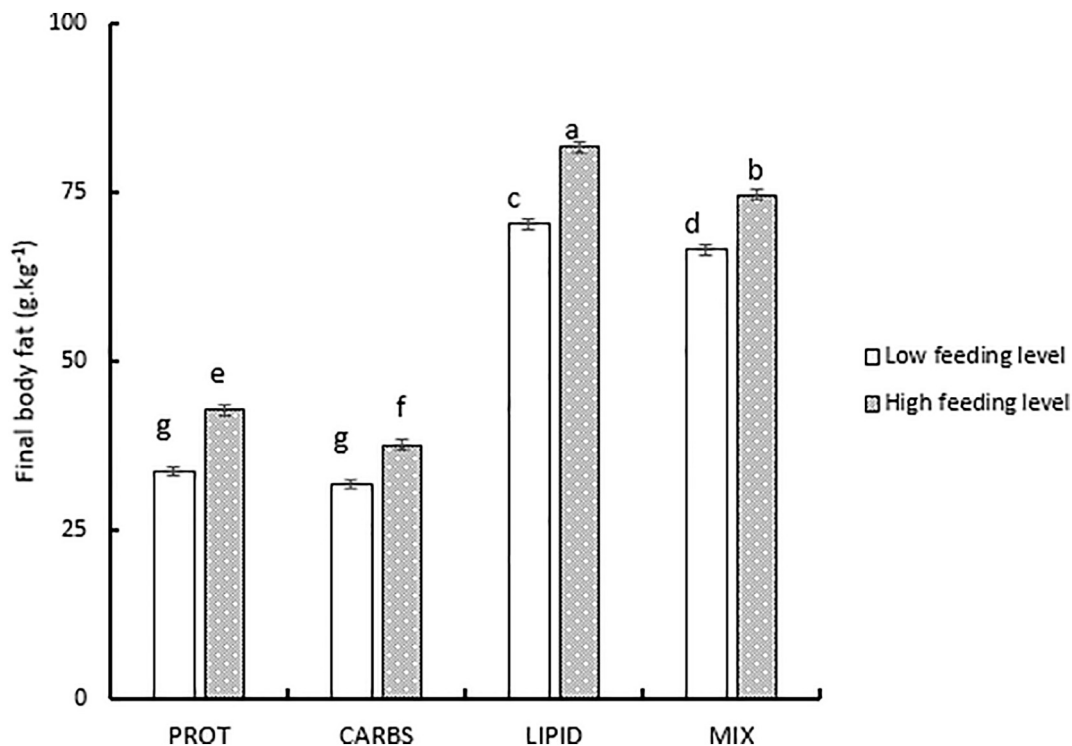


Fig. 1. Final body fat content (g.kg⁻¹, wet basis) of snakehead (*C. striata*) as affected by diet and feed level ($n = 3$): Diet effect, $P < 0.001$; Feeding level effect, $P < 0.001$; Diet x Feeding level interaction effect, $P = 0.012$. PROT, diet with a high protein content; CARB, the PROT diet supplemented with starch; LIPID, the PROT diet supplemented with lipid; MIX, the PROT diet supplemented with starch and lipid. Means lacking a common superscript differ significantly ($P < 0.05$).

Table 3

Apparent digestibility coefficient (ADC; in %) of nutrients in snakehead, means per experimental diet and feeding level (FL) during the 42-d experimental period (n = 3).

Nutrient	FL	PROT	CARB	LIPID	MIX	SEM	Diet	FL	Diet x FL
		“protein”	“protein”	“protein”	“protein”				
			+ Carb	+ Lipid	+ Carb + Lipid				
Energy	Low	86.7 ^{cd}	87.6 ^{bc}	89.0 ^a	88.0 ^{ab}	0.24	< 0.001	< 0.001	0.035
	High	84.4 ^g	83.8 ^g	86.0 ^{de}	85.3 ^{ef}				
Protein	Low	92.5 ^a	91.7 ^a	92.1 ^a	89.8 ^{bc}	0.17	< 0.001	< 0.001	0.003
	High	90.3 ^b	89.5 ^{bc}	90.1 ^b	89.0 ^c				
Fat	Low	93.0 ^c	89.3 ^c	95.9 ^a	94.3 ^b	0.25	< 0.001	< 0.001	< 0.001
	High	90.4 ^e	84.7 ^f	91.7 ^d	92.1 ^{cd}				
Carbohydrates	Low	63.2 ^g	81.5 ^a	67.0 ^e	79.1 ^b	0.34	< 0.001	< 0.001	< 0.001
	High	62.5 ^g	75.8 ^c	65.1 ^f	73.6 ^d				
Starch	Low	99.5 ^a	97.7 ^c	99.3 ^a	96.4 ^d	0.10	< 0.001	< 0.001	< 0.001
	High	99.2 ^a	95.2 ^c	98.3 ^b	93.0 ^f				

PROT, diet with a high protein content; CARBS, the PROT diet supplemented with starch; LIPID, the PROT diet supplemented with lipid; MIX, the PROT diet supplemented with starch and lipid; carb, carbohydrates; DM, dry matter; NSP, non-starch polysaccharides.

^{abcde}For parameters with a significant interaction effect between diet and feeding level, means lacking a common superscript differ significantly ($P < 0.05$).

with the values ranging from 93.0 to 99.5% for different diets between the two FL (Table 3). Averaged over feeding levels and diets, ADC of starch, energy, CP, fat and total carbohydrates were, respectively, 97.3, 86.4, 90.6, 91.4 and 71.0%. For all macronutrients, ADC was lower for snakehead fed the high compared to the low feeding level ($P < 0.001$). Furthermore, ADC of macronutrients were dependent on the dietary treatment ($P < 0.001$), but the differences in ADC between diets were

affected by feeding levels, which was indicated by the significant interaction effect ($P < 0.01$). In general, the differences in macronutrient ADCs between diets were larger in snakehead at the high compared with the low feeding level (Table 3). For example regarding starch ADC, post hoc pairwise comparison of mean showed that at the low feeding level starch ADC of the PROT-diet (99.5%) was not different from the LIPID-diet (99.3%), but differed from the CARB-diet

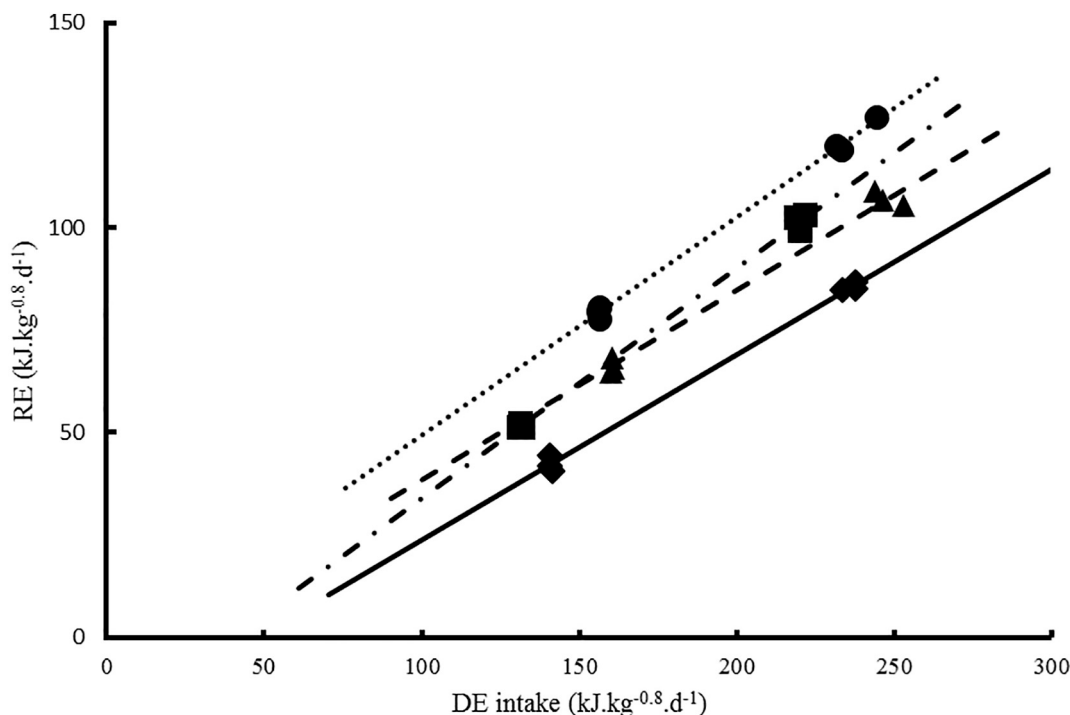


Fig. 2. Relationship between retained energy (RE) and digestible energy intake (DE) for snakehead fed one of four experimental diets: PROT, diet with a high protein content; CARB, the PROT diet supplemented with starch; LIPID, the PROT diet supplemented with lipid; MIX, the PROT diet supplemented with starch and lipid. (■ PROT: RE = -22 (SE 2.6) + 0.56 (SE 0.014) DE ($R^2 = 0.99$); ♦ CARB: RE = -22 (SE 2.9) + 0.45 (SE 0.014) DE ($R^2 = 0.99$); • LIPID: RE = -4 (SE 2.8) + 0.53 (SE 0.014) DE ($R^2 = 0.99$); ▲ MIX: RE = -8 (SE 5.8) + 0.46 (SE 0.028) DE ($R^2 = 0.99$)). The estimated energy requirements for maintenance was 40, 48, 7 and 18 $\text{kJ.kg}^{-0.8}.\text{d}^{-1}$ at PROT, CARB, LIPID and MIX diet, respectively.

Table 4

Energy balance parameters ($\text{kJ.kg}^{-0.8}.\text{d}^{-1}$) and protein efficiency (%) in snakehead (*C. striata*), means per experimental diet and feeding level (FL) during the 42-d experimental period ($n = 3$).

	FL	PROT	CARB	LIPID	MIX	SEM	Diet	P-value	
		“protein”	“protein”	“protein”	“protein”			FL	Diet x FL
			+ Carb	+ Lipid	+ Carb + Lipid				
GE intake	Low	151 ^e	161 ^e	175 ^d	182 ^d	2.1	< 0.001	< 0.001	0.001
	High	261 ^c	282 ^{ab}	275 ^b	290 ^a				
DE intake	Low	131 ^f	141 ^e	156 ^d	161 ^d	1.8	< 0.001	< 0.001	0.006
	High	220 ^c	236 ^b	236 ^b	248 ^a				
Branchial urinary energy losses	Low	9 ^c	7 ^c	6 ^f	5 ^g	0.1	< 0.001	< 0.001	< 0.001
	High	14 ^a	12 ^b	9 ^c	8 ^d				
ME intake	Low	122 ^f	133 ^e	150 ^d	155 ^d	1.7	< 0.001	< 0.001	0.008
	High	206 ^c	225 ^b	227 ^b	240 ^a				
Heat production	Low	70 ^d	91 ^c	71 ^d	89 ^c	1.6	< 0.001	< 0.001	0.001
	High	104 ^b	139 ^a	105 ^b	133 ^a				
RE	Low	52 ^f	42 ^g	79 ^d	66 ^e	1.2	< 0.001	< 0.001	0.010
	High	102 ^b	86 ^c	122 ^a	107 ^b				
RE as protein	Low	41 ^d	35 ^e	41 ^d	35 ^e	0.5	< 0.001	< 0.001	< 0.001
	High	76 ^a	67 ^b	65 ^b	57 ^c				
RE as fat	Low	10 ^g	7 ^g	38 ^c	31 ^d	1.0	< 0.001	< 0.001	0.007
	High	26 ^c	19 ^f	57 ^a	50 ^b				
Fat gain: protein gain (g/g)	Low	0.19 ^{de}	0.17 ^e	0.59 ^a	0.59 ^a	0.007	< 0.001	0.025	< 0.001
	High	0.24 ^c	0.21 ^{cd}	0.58 ^{ab}	0.55 ^b				
RE as fat: RE as protein (J/J)	Low	0.25 ^{bc}	0.21 ^c	0.92 ^a	0.90 ^a	0.019	< 0.001	0.052	0.008
	High	0.34 ^b	0.28 ^{bc}	0.89 ^a	0.88 ^a				
Protein efficiency*	Low	43 ^c	44 ^c	53 ^a	52 ^a	0.5	< 0.001	< 0.001	0.003
	High	47 ^b	49 ^b	54 ^a	54 ^a				

PROT, diet with a high protein content; CARB, the PROT diet supplemented with starch; LIPID, the PROT diet supplemented with lipid; MIX, the PROT diet supplemented with starch and lipid; carb, carbohydrates; GE, gross energy; DE, digestible energy; ME, metabolisable energy; RE, retained energy.

*Protein efficiency is retained protein divided by digestible protein intake (%).

^{abcde}For parameters with a significant interaction effect between diet and feeding level, means within parameters lacking a common superscript differ significantly ($P < 0.05$).

(97.7%) and MIX-diet (96.4%). The latter two diets had the highest dietary starch content due to the inclusion of wheat flour (Table 1). At the highest feeding level, starch ADC was different between all diets, but especially at the CARB- and MIX-diet starch ADC was reduced compared to the PROT- and LIPID-diet, respectively 95.2 and 93.0% versus 99.2 and 98.3% (Table 3). Data on DM, crude ash, phosphorous and non-starch polysaccharides are given in Supplementary Table S3.

The first aim of this study was to assess the effect of diet composition (i.e., macronutrient content) on the relationship between DE intake and RE. The measured linear relationships between DE and RE differed between the experimental diets (Fig. 2). RE was affected by the interaction effect between diet and DE intake. This implies that the slopes of the lines (i.e., digestible energy utilization efficiency for energy gain, k_{gDE}) were different between diets ($P = 0.001$). The CARB- and MIX-diet, in which wheat flour was included (high in starch level) had the lowest k_{gDE} , respectively, 0.45 and 0.46. Whereas, the LIPID- and PROT-diet had the highest k_{gDE} , respectively, 0.53 and 0.56. With increasing DE intake, the differences in RE between the diets became larger (Fig. 2).

The second aim of this study was to quantify energy utilization efficiency of digested protein, fat and carbohydrate for growth (i.e., estimating the NE equation for snakehead). Energy and nitrogen balances were measured based on digested nutrient intake (dCP, dFat and dCarb), which were presented in Supplementary table S3 and S4 respectively. The wide range of digestible nutrient intake brought about a large variability of RE from 42 to 122 $\text{kJ.kg}^{-0.8}.\text{d}^{-1}$ (Table 4).

By conducting the multiple linear regression of RE (i.e., NE) (in $\text{kJ.kg}^{-0.8}.\text{d}^{-1}$) as a function of dCP, dFat and dCarb (in $\text{g.kg}^{-0.8}.\text{d}^{-1}$), the following equation was generated for snakehead:

$$\text{RE} = -14.2 (\text{SE } 2.46) + 12.5 (\text{SE } 0.46) \text{dCP} + 31.0 (\text{SE } 0.85) \text{dFat} + 0.9 (\text{SE } 0.51) \text{dCarb} \quad R^2 = 0.99 \quad (2)$$

By dividing the coefficients of dCP, dFat and dCarb (respectively, 12.5, 31.0 and 0.9 kJ.g^{-1}) in Eq. (2) by energy values of these macronutrients (23.6 kJ.g^{-1} CP, 39.5 kJ.g^{-1} fat and 17.2 kJ.g^{-1} carbohydrates), the energy utilization efficiency of dCP, dFat and dCarb (respectively, $k_{NE;dCP}$, $k_{NE;dFat}$ and $k_{NE;dCarb}$) were determined as 53%,

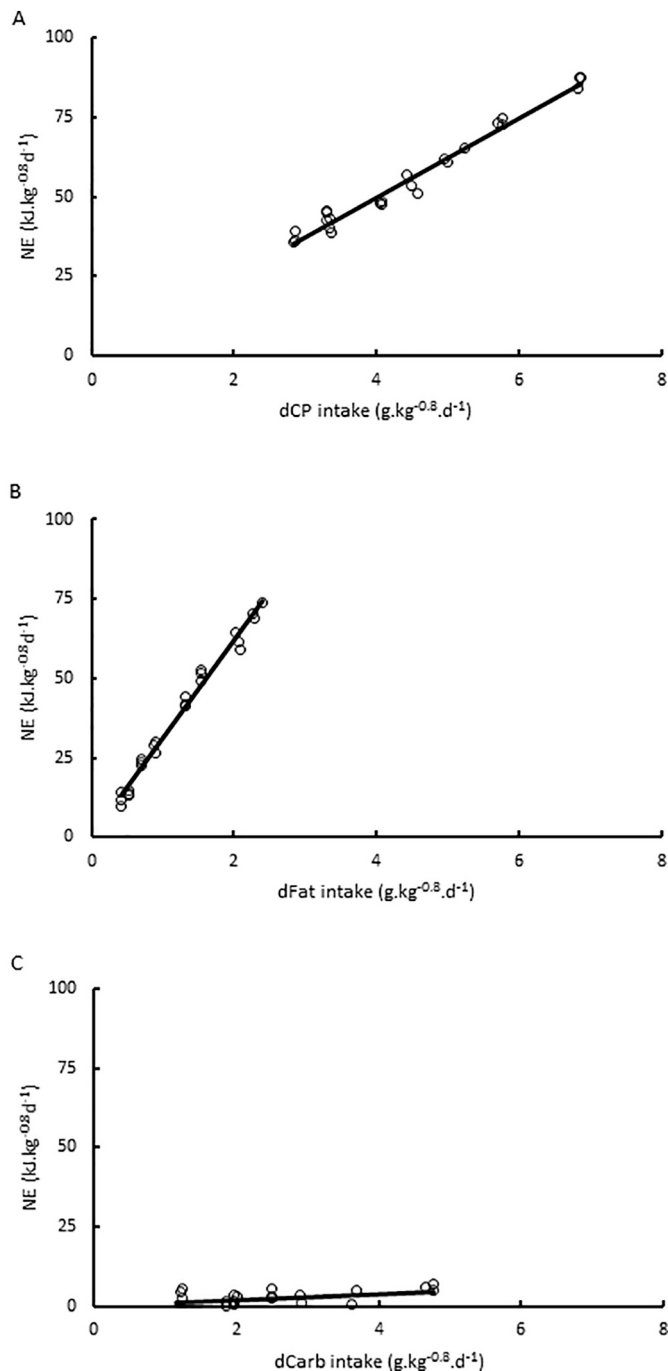


Fig. 3. Relationship between net energy (NE) and digestible protein (dCP) intake (A), NE and digestible fat (dFat) intake (B), NE and digestible carbohydrates (dCarb) intake (C). The NE values are corrected for variation in dFat and dCarb intake in panel (A); for variation in dCP and dCarb intake in panel (B) and for variation in dCP and dFat intake in panel (C). This was performed as follows: the measured retained energy value for each data point in the data set was increased with the estimated fasting heat production to obtain the NE value, which was then corrected towards zero dFat and dCarb in order to have only the effect of dCP on NE in panel (A); zero dCP and dCarb in order to have only the effect of dFat on NE in panel (B); and zero dCP and dFat in order to have only the effect of dCarb on NE in panel (C). This was conducted using Eq. (2) given in Table 5.

79% and 5%, respectively. In Fig. 3, the relationships between NE and respectively dCP, dFat and dCarb derived from the estimated Eq. (2) were visualized. It clearly shows that increasing dCarb (Fig. 3c) only

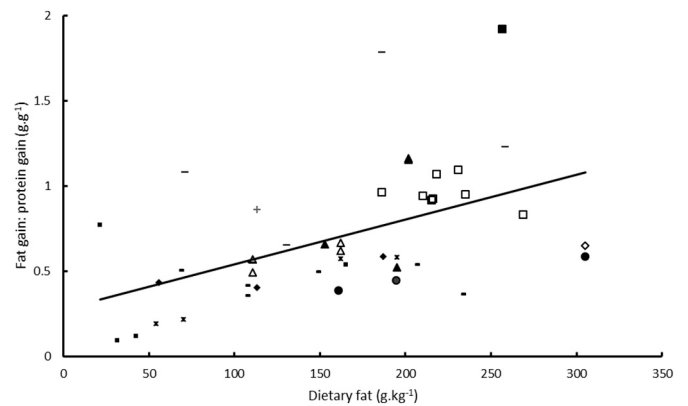


Fig. 4. Relationship between fat gain-to-protein gain ratio and dietary fat content. Data are derived from Table 5 in study of Schrama et al. (2012) and supplemented with the data of Glencross et al. (2017), Phan et al. (2019) and the present study (○ *Argyrosomus japonicus*, ◇ *Salmo salar*, □ *Oncorhynchus mykiss*, - *Lates calcarifer*, △ *Seriola lalandi*, ▲ *Dicentrarchus labrax*, ● *Gadus morhua*, ■ *Anguilla anguilla*, + *Pangasianodon hypophthalmus*, _ *Cyprinus carpio*, ◆ *Oreochromis niloticus*, ■ *Ctenopharyngodon idella*, * *Channa striata* in present study).

marginally increased NE compared to increasing dFat (Fig. 3b) and increasing dCP (Fig. 3a).

5. Discussion

This study demonstrates that snakehead have a very low body fat content (between 32 and 82 g.kg⁻¹) even at diets with the high dietary lipid content (LIPID- and MIX-diet; Fig. 1, Supplementary Table S2). This low body fat content is consistent with other studies on snakehead, but the fatness of the snakehead in the present study of the LIPID- and MIX-diet was in the higher range of reported values (Aliyu-Paiko and Hashim, 2012; Arockiaraj et al., 1999; Dayal et al., 2012; Mohanty and Samantaray, 1996; Wee and Tacon, 1982). Compared to other species, snakehead is a relatively lean fish. The current body fat content of snakehead was e.g., lower compared to barramundi (Glencross et al., 2017), rainbow trout (Glencross et al., 2007; Glencross et al., 2008; Glencross, 2009; Azevedo et al., 1998; Bureau et al., 2006), Nile tilapia (Schrama et al., 2012) and common carp (Phan et al., 2019). Comparison between species (i.e., studies) can be affected by differences in dietary fat content. Therefore, in Fig. 4 the fat gain to protein gain across fish species as a function of dietary fat content is depicted. Averaged over feeding levels, the fat gain to protein gain ratio of snakehead in the current study ranged from 0.2 to 0.6 g/g. This is comparable to barramundi (0.4 to 0.5 g.g⁻¹) (Glencross, 2008; Glencross et al., 2017) and yellowtail kingfish (0.5 to 0.7 g.g⁻¹) (Booth et al., 2010), but lower than common carp (0.6 to 1.8 g.g⁻¹) (Nwana et al., 2007; Phan et al., 2019) and rainbow trout (0.8 to 1.1 g.g⁻¹) (Glencross et al., 2007; Glencross et al., 2008; Glencross, 2009; Azevedo et al., 1998; Bureau et al., 2006). Even considering the differences in dietary fat content between studies on different fish species, snakehead has the higher priority for protein versus fat gain compared to most fish species (Fig. 4).

To formulate aquaculture feeds, it is necessary to have information about energy requirements of fish species and data regarding (1) nutrient digestibility of ingredients, (2) energy requirements for maintenance and (3) energy utilization efficiency for growth on a digestible basis (DE, k_{gDE}) or on a metabolisable basis (ME, k_{gME}). The evaluation of dietary energy evaluation for fish is influenced by two main factors: (1) digestion of dietary nutrients and (2) utilization of these digested nutrients.

In the current study of all macronutrients, starch had the highest digestibility (97.3% averaged over diets, Table 3). This is comparable to

Table 5
Estimated net energy equations in snakehead, common carp, barramundi, Nile tilapia, rainbow trout and pigs.

Species	Equation*	R ²	References
Snakehead	NE = 12.5 dCP + 31.0 dFat + 0.9 dCarb	0.99	Present study (2)
Carp	NE = 11.2 dCP + 34.1 dFat + 10.4 dCarb	0.99	Phan et al., 2019 (3)
Barramundi	NE = 15.2 dCP + 37.1 dFat + 3.1 dCarb	0.99	Phan et al., 2019 (4)
Barramundi	NE = 15.9 dCP + 35.2 dFat + 9.4 dCarb - 1.9 (dCarb) ²	0.99	Phan et al., 2019 (5)
Trout	NE = 15.1 dCP + 35.0 dFat + 12.1 dCarb	0.91	Schrama et al., 2018 (6)
Trout	NE = 13.5 dCP + 33.0 dFat + 34.0 dCarb - 3.64 (dCarb) ²	0.92	Schrama et al., 2018 (7)
Tilapia	NE = 11.5 dCP + 35.8 dFat + 11.3 dCarb	0.99	Schrama et al., 2018 (8)
Pig	NE = 11.3 dCp + 35.0 dFat + 14.4 ST + 12.1 dRest		Noblet et al., 1994 (9)
Pig	NE = 10.8 dCp + 36.1 dFat + 13.5 dST _e + 9.5 dST _f + 9.5 dNSP		CVB, 1993 (10)

NE, net energy; RE, retained energy; dCP, digestible protein; dFat, digestible fat; dCarb, digestible carbohydrates (comprising of starch, sugars and NSP); dRest, the remaining dietary fraction being digestible (dRest = DM - dCP - dFat - ST - digestible ash) (see Noblet et al., 1994); dST_e, enzymatically digestible starch; dST_f, the amount of starch that is digested after microbial fermentation; ST, starch (both enzymatically and fermentable degradable); dNSP, digestible NSP.

*In the estimated equation of the present study, NE is expressed in kJ.kg^{-0.8}.d⁻¹ and digestible nutrient intakes (dCP, dFat and dCarb) in g.kg^{-0.8}.d⁻¹. In the NE equations for pigs, NE is expressed in MJ.kg⁻¹ feed and digestible nutrients in g.kg⁻¹ feed.

observations in European sea bass (98%) (Peres and Oliva-Teles, 2002), rainbow trout (98%) (Burel et al., 2000) and common carp (99%) (Phan et al., 2019), all using gelatinised starch in diets and applying similar faeces collection methods. However compared to other carnivorous fish species the starch ADC of snakehead is higher than in barramundi (88%) (Glencross et al., 2017b) and turbot (82%) (Burel et al., 2000). The current high starch ADC in snakehead is most likely due to the high gelatinization degree of starch since pellets were produced by extrusion. Gelatinization has been shown to improve starch digestibility in a variety of fish species (Krogdahl et al., 2005; Peres and Oliva-Teles, 2002). Other processing conditions including heating during pressing and extruding, wet or dry conditioning and fermentation can also increase starch digestibility (Krogdahl et al., 2005). In the current study, starch digestion in snakehead decreased with increasing dietary starch inclusion, which parallels to the finding of suppressed starch ADC at high dietary starch levels in carnivorous fish like barramundi (Glencross et al., 2017; Glencross et al., 2012) and rainbow trout (Meriac et al., 2014).

For diet formulation on digestible energy (DE) the energy utilization efficiency for growth of DE (k_{gDE} , the slope of the lines in Fig. 2) is an important parameter, which is often considered constant within a fish species. The current estimates of k_{gDE} in snakehead ranged between 45 and 56% of snakehead, which is low compared to estimates in other fish species: barramundi, 55 to 79% (Glencross, 2008; Glencross, 2006); common carp, 49 to 66% (Phan et al., 2019); rainbow trout, 62 to 74% (Glencross, 2009); European seabass (64–82%) (Lupatsch et al., 2010; Lupatsch et al., 2001; Lupatsch et al., 2003; Peres and Oliva-Teles, 2005). The current study clearly demonstrates that k_{gDE} is affected by dietary macronutrient composition, with the lowest k_{gDE} (45 and 46%) being observed that diets with the high starch inclusion (CARB- and MIX-diet; Fig. 2). This reduction in k_{gDE} in snakehead at diets with a high starch inclusion is fully in line with observations in tilapia (Schrama et al., 2012), barramundi (Glencross et al., 2017) and rainbow trout (Rodehutsord and Pfeffer, 1999; Schrama et al., 2018). The relatively low k_{gDE} for all diets might be an indication of a poor ability to utilise starch as energy source for ATP production and fat synthesis. However the observed lower k_{gDE} in snakehead can also be partially related to its relative lean growth (Fig. 1). The energy deposition of fat is more efficient than the energy deposition of protein (Bureau et al., 2006).

Like suggested in early studies on Nile tilapia, rainbow trout, common carp and barramundi (Schrama et al., 2018; Phan et al., 2019) the impact of dietary macronutrient composition on k_{gDE} does support the use of a net energy (NE) approach in snakehead. In a NE approach of energy evaluation, the energy utilization efficiency is differentiated into the energy utilization efficiency of digested protein ($k_{NE;dCP}$), digestible fat ($k_{NE;dFat}$) and digestible carbohydrates ($k_{NE;dCarb}$). A comparison of these estimated partial energetic efficiencies of digestible

nutrients across species is given in Table 5.

The estimated $k_{NE;dCP}$ of 12.5 kJ.g⁻¹ in snakehead was lower than the values found for two other carnivorous fish species, trout and barramundi (Table 5). In fact the $k_{NE;dCP}$ of snakehead was only slightly higher than the values estimated for common carp and Nile tilapia both herbivorous fish (Table 5). This is opposite to the expectation that strict carnivorous fish would be highly adapted to protein rich diets and thus having a higher energetic utilization of digested protein. However, evolution on a protein abundant diet might also have led to a larger proportion of protein being used as energy source (for ATP and fat deposition). The lower $k_{NE;dCP}$ can also be due to an imbalanced amino acid profile of diets. However this is unlikely considering the observed high protein retention efficiency (retained protein as percentage of digestible protein; Table 4) in the current study. At the high feeding level, the protein efficiency ranged between 47 and 54%. These values are in the high ranges of values reported for barramundi (from 37 to 59%; Glencross et al., 2017), but higher than for common carp (from 39 to 46%; Phan et al., 2019) and Nile tilapia (from 32 to 53%; Haidar et al., 2018)..

Compared to other fish species and pigs, the energy utilization efficiency of digestible fat ($k_{NE;dFat}$) was lower for snakehead (31.1 kJ.g⁻¹ vs. 35 to 37.1 kJ.g⁻¹ based on linear relationships; Table 5). This lower $k_{NE;dFat}$ is most likely related to the low body fat content of snakehead (i.e., low ratio between fat and protein at gain). As a consequence of the low fat gain relative to protein gain, a larger proportion of the digested fat may be used to supply ATP for the prioritised protein deposition in snakehead, especially since utilization of digestible carbohydrates ($k_{NE;dCarb}$) was almost nihil. It appears that dietary fat supplementation elicited a protein sparing effect through increased protein efficiency in the LIPID and MIX diets. In contrast, dCarb did not contribute to energy retention and ATP production. The energy expenditure for protein deposition is generally estimated about 50 mmol ATP per g of protein deposited (about 10 mmol for transport) (Houlihan et al., 1986), which costs 11 to 24% and 19 to 42% of the total energy budget at maintenance and in growing fish, respectively (Carter and Houlihan, 2001). However, it maintains to be clarified whether snakehead uses fat as the major energy source for protein synthesis.

The estimated energy utilization efficiency of dCarb ($k_{NE;dCarb}$) in snakehead was 0.9 kJ.g⁻¹. This indicates that only 5% of the digested carbohydrates was retained as energy in the body, which implies that snakehead is almost unable to metabolise dCarb. The $k_{NE;dCarb}$ of snakehead is much lower than that of herbivorous fish (tilapia and common carp) (Schrama et al., 2018; Phan et al., 2019) and other carnivorous fish like rainbow trout ~70% (Schrama et al., 2018) and barramundi ~18% (Phan et al., 2019). This may indicate that the liver is unable to downregulate the blood glucose level for yielding energy in carnivores, especially in snakehead.

Most surprisingly, the highly digested starch (93.0–99.5%) did not

contribute to the energy gain of snakehead due to the very low $k_{NE;dCarb}$. This again confirms that starch is not utilised efficiently in some carnivorous fish despite its high digestibility, which was also observed in a previous study (Kamalam et al., 2017). More specifically, the starch-rich diet (CARB-diet) had the lowest energy retention at both feeding levels (Table 2). This indicates that glucose, originated from dietary starch is also minimally used for its physical activities and is not the reason for the observed high protein efficiency retention. What is the fate of this absorbed glucose in snakehead requires further investigations. It is arguable that part of the blood glucose is lost via urine (Sha et al., 2011) or by osmoregulation via the gills (Thompson et al., 1989). The extremely low $k_{NE;dCarb}$ and the lowest energy retention as fat in snakehead fed the starch-rich diet (CARB-diet) at both feeding levels might indicate that part of the digested starch (i.e., glucose) is lost. Therefore, studying blood glucose levels of snakehead in relation to dietary starch content is required in comparison to other fish species.

6. Conclusion

Snakehead is a lean fish which has relative higher priority for protein deposition over fat deposition. Part of the digested fat is used as energy source (ATP production) to facilitate this lean growth. This is reflected by the slightly lower energetic utilization efficiency of digested fat ($k_{NE;dFat}$) in snakehead compared to trout, barramundi, common carp and Nile tilapia. Starch is well digested by snakehead. However, digested carbohydrates are poorly utilised as energy source. The energetic utilization efficiency of digestible carbohydrates ($k_{NE;dCarb}$) was only 5%. This reflects the limited capacity of snakehead to metabolise digested carbohydrates (i.e., starch).

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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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2020.736066>.

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