Effect of dietary carbohydrate and fat supplementation on the yield and chemical composition of fillet and the location of fat deposition in striped catfish (*Pangasius hypophthalmus*), African catfish (*Clarias gariepinus*) and snakehead (*Channa striata*)

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ARTICLE INFO

ABSTRACT

This study compared three different fish species, striped catfish (*Pangasius hypophthalmus*), African catfish (*Clarias gariepinus*) and snakehead (*Channa striata*), regarding the effect of dietary macronutrient composition on: 1. the fillet yield and the fillet chemical composition; 2. the location of fat deposition within the body (fillet, liver, viscera or rest fraction). The selected species were studied for the development of net energy formulas, in three different studies. The design of these studies and especially the diet formulation were similar. Diets were formulated according to a 2 × 2 factorial design: with or without extra carbohydrates supplementation; and with or without extra fat supplementation. Fillet yield of striped catfish (*P. hypophthalmus*), African catfish (*C. gariepinus*) and snakehead (*C. striata*) was not affected by the dietary macronutrient composition. Fillet fat and protein contents were changed by the dietary macronutrient composition. In all compartments (liver, viscera, fillet and the rest fraction), both dietary fat and dietary carbohydrates levels increased the fat content. The response to dietary carbohydrates in snakehead, a lowering of fillet fat content, is opposite to the response in both catfish species. The distribution of the total amount of body fat over the different compartments, was not influenced by dietary carbohydrates level, but did depend on dietary fat level. Dietary fat supplementation led to relatively more fat in viscera and fillet but less fat was stored in the rest fraction. In striped catfish (*P. hypophthalmus*), African catfish (*C. gariepinus*) and snakehead (*C. striata*), most of the body fat is stored in the rest fraction (head, skin, subcutaneous fat, scales, bones and air bladders).

1. Introduction

The increasing use of carbohydrates and fat in fish feed (Craig et al., 2017; Ytrestøyl et al., 2015) increases the variability in dietary non-protein energy content. Fish need energy for maintenance and growth. Energy can be acquired from either protein or non-protein sources, i.e., fat and carbohydrates. Because protein is the most expensive macronutrient in fish feed, fish farmers prefer that dietary protein is used for protein growth and especially muscle growth rather than for energy. In general digestible carbohydrates, mainly starch, is a cheaper energy source than fat. However, there are indications that too high

inclusion levels of starch in fish feed may limit the growth performance of Nile tilapia (*Oreochromis niloticus*) (Schrama et al., 2012), barramundi (*Lates calcarifer*) (Glencross et al., 2017), snakehead (*Channa striata*) (Phan et al., 2021a,b) and rainbow trout (*Oncorhynchus mykiss*) (Groot et al., 2021).

Among fish tissues, in most cases only muscles (i.e., fillets) are used for human consumption. Liver, viscera, head, bones and skin are regularly used as by-products for feed production. In various fish species, the yield of the fillet is low, being around 30%, *e.g.* in striped catfish (*Pangasius hypophthalmus*) (Asemani et al., 2019; Da et al., 2012), African catfish (*Clarias gariepinus*) (Jantrarotai et al., 1998) and snakehead

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(*C. striata*) (Tan and Azhar, 2014). From a food efficiency perspective, it is important to know if changing dietary macronutrient composition can increase the fillet yield. In addition, fish consumers have diversified preferences of tastes and firmness for the fish fillet. The tastes and firmness of the fillet are related to its chemical composition, *i.e.*, protein, fat. If the fillet composition can be modified by dietary macronutrient composition, *e.g.* by adding dietary energy from either fat or carbohydrates, the preferred composition of the fillet can be obtained by tailoring the feed (i.e., functional feeds for fillet). Likewise, if the amount of energy in the body compartments used as by-products can be reduced, this will contribute to the resource use efficiency. Thus, the information about the effect of dietary macronutrient composition on fillet composition might enable the formulation of balanced feeds for optimal fillet yield, fillet nutrient content and resource use efficiency. Yet, only few studies have assessed the impact of dietary macronutrient composition on the nutrient partitioning over different compartments (fillet, liver, viscera and the rest fraction) in fish (Salze et al., 2014; Teodósio et al., 2021; Van der Meer et al., 1997).

Biologically, dietary protein, fat and carbohydrates can be converted to somatic fat and partly stored at different locations in the body, for example in the liver, viscera, fillet and the rest fraction, which in this study are defined as different body compartments. However, publications regarding the impact of dietary macronutrient composition on fat deposition mainly focus on the whole body and/ or fillet composition (Aliyu-Paiko et al., 2010; Rodehutscord and Pfeffer, 1999). Hence, limited information is available on the location of fat storage in specific body compartments for fish. The location of fat storage differs between fish species. European eel mainly stores fat in muscle (Otwell and Rickards, 1981), while African catfish accumulates fat in the abdominal cavity (Matter et al., 2004) and cod (*Gordus morhua*) accumulates fat in the liver (Hemre et al., 1989). These differences between fish species may lead to variation in impact of dietary macronutrient composition on the location of fat deposition in liver, viscera, fillet and the rest fraction.

This study compared three different fish species, striped catfish (*P. hypophthalmus*), African catfish (*C. gariepinus*) and snakehead (*C. striata*), regarding the effect of dietary macronutrient composition on: 1. the fillet yield and the fillet chemical composition; 2. the location of fat deposition within the body (fillet, liver, viscera or rest fraction). The selected species were studied for the development of net energy formulas, in three different studies (striped catfish - *P. hypophthalmus*, Phan et al., 2021b; snakehead - *C. striata*, Phan et al., 2021a). The design of these studies and especially the diet formulation were similar. Diets were formulated according to a 2 × 2 factorial design: with or without extra carbohydrates supplementation; and with or without fat extra supplementation; and fed at two different feeding levels. The data used in the current study are from fish sampled at the highest feeding level.

2. Materials and methods

2.1. Experimental diets

This study had a 3 × 2 × 2 factorial design with the following factors: species including striped catfish (*P. hypophthalmus*), African catfish (*C. gariepinus*) and snakehead (*C. striata*); dietary carbohydrates level (low versus high); and dietary fat level (low versus high). For all fish species, firstly, a basal diet was formulated that covered the nutritional requirements for the fish. This diet had a low carbohydrates and low fat content. The other 3 experimental diets were formulated according to the 2 by 2 design by diluting 575 units of basal diet with 300 units of a carbohydrates source and or 125 units of a fat source (Table 1). Since all 4 experimental diets within species were aimed to be on average within the range of commercial diets, the protein content of the basal diet was set lower for striped catfish (*P. hypophthalmus*), compared to snakehead (*C. striata*) and African catfish (*C. gariepinus*) (Table 1). Soybean oil was used as a fat source for striped catfish (*P. hypophthalmus*) and snakehead (*C. striata*) and a blend of rapeseed oil and soybean oil (1: 1) for African

Table 1
Chemical composition of diets (g. kg^{-1}, on a dry matter basis) fed to striped catfish, African catfish and snakehead.

	Diet			
	Low carbohydrates		High carbohydrates	
	Low fat	High fat	Low fat	High fat
Mixing ratios				
Fat source[1]	0	125	0	125
Carbohydrates source[2]	0	0	300	300
Basal mixture[3]	575	575	575	575
Analyzed chemical composition				
Crude protein				
Striped catfish	380	300	260	243
African catfish	502	412	329	284
Snakehead	535	435	419	367
Fat				
Striped catfish	62	236	44	165
African catfish	83	251	57	182
Snakehead	70	195	54	162
Carbohydrates[4]				
Striped catfish	426	348	597	501
African catfish	319	260	552	479
Snakehead	224	226	407	365
Starch				
Striped catfish	209	187	375	334
African catfish	171	140	436	396
Snakehead	147	139	346	298
Ash				
Striped catfish	132	117	99	91
African catfish	96	77	63	55
Snakehead	171	144	120	106
Energy (MJ. kg^{-1})				
Striped catfish	18.3	21.8	17.9	20.7
African catfish	20.5	24.4	19.5	22.1
Snakehead	18.4	21.3	18.4	21.1

[1] Fat source is the blend of soybean oil and rapeseed oil (1:1) for African catfish or soya oil for striped catfish and snakehead.
[2] Carbohydrates source is cassava for striped catfish or wheat flour for African catfish and snakehead.
[3] For striped catfish, basal mixture includes soybean meal 17.4%, rice bran full fat 17.4%, fishmeal 15.7%, wheat flour 13.9%, wheat 12.2%, rapeseed meal 7%, feather meal 7%, premix 9.6%. For African catfish, basal mixture includes fishmeal 13.9%, soya protein concentrate 13.9%, pea protein 13.9%, wheat gluten 13.9%, wheat 15.4%, wheat bran 17.4%, premix 11.5%. For snakehead, basal mixture includes fishmeal 34.8%, soy protein concentrate 20.9%, meat bone meal 13.9%, wheat 17.4%, wheat flour 5.2%, premix 7.8%.
[4] Total carbohydrates = 1000 – (protein + fat + ash). Starch, protein, fat and ash were determined based on chemical analysis.

catfish (*C. gariepinus*). Wheat flour was used as carbohydrates source for African catfish (*C. gariepinus*) and snakehead (*C. striata*) and cassava for striped catfish (*P. hypophthalmus*). Both these carbohydrates sources are high in starch content, which is reflected in the large contrast in starch content between the experimental diets (Table 1).

All diets were produced by extrusion into 3 mm pellets. Diets for snakehead (*C. striata*) and striped catfish (*P. hypophthalmus*) were produced by De Heus (Vinh Long, Vietnam). Diets for African catfish (*C. gariepinus*) were produced by Research Diet Service (Wijk bij Duurstede, The Netherlands). For details on pellet production for striped catfish (*P. hypophthalmus*) and snakehead (*C. striata*) see Phan et al. (2021a,b).

All species were fed restrictively by hand twice daily at 9.00 and 15.00. The fish sampled in this study for compartment analysis were fed a level of 22 g.kg$^{-0.8}$.d^{-1} for striped catfish (*P. hypophthalmus*) and African catfish (*C. gariepinus*) and 20 g.kg$^{-0.8}$.d^{-1} for snakehead (*C. striata*).

These feeding levels were close to apparent satiation. On the first day of feeding, the feed given was calculated by multiplying the initial biomass of fish in tank with the feeding level, i.e., 20 or 22 g.kg$^{-0.8}$.d^{-1}. The feed given to fish was adjusted per day based on the predicted daily body weight. The predicted daily body weight was calculated by adding the predicted daily weight gain to the initial body weight on a day basis. The predicted daily weight gain at the first day was calculated by dividing the amount of feed consumed in the previous meal by the assumed FCR, which was 1.1, 1.0 and 1.2 for striped catfish (*P. hypophthalmus*), African catfish (*C. gariepinus*) and snakehead (*C. striata*), respectively.

To avoid suppressed growth, the more carnivorous the studied fish species (snakehead (*C. striata*) > African catfish (*C. gariepinus*) > striped catfish (*P. hypophthalmus*), the higher the protein content of the diets, but the supplementation of carbohydrate and fat sources for the three species were comparable (Fig. 1).

2.2. Animal ethics

The African catfish (*C. gariepinus*) study was conducted in the research facility of CARUS-ARF at Wageningen University (The Netherlands) in accordance with the Dutch law on the use of animals

Fig. 1. The composition of the four diets: low carbohydrates low fat (LCLF), low carbohydrates high fat (LCHF), high carbohydrates low fat (HCLF), high carbohydrates high fat (HCHF) fed to striped catfish, African catfish and snakehead on a weight basis (A) and on an energy basis (B).

(Act on Animal Experiments) for scientific purposes and was approved by the Central Animal Experiments Committee (CCD) of The Netherlands (project number: 2018.W-0021.001). The snakehead (*C. striata*) and striped catfish (*P. hypophthalmus*) studies were conducted at the research and development centre of De Heus in Vinh Long (Vietnam) in compliance with Vietnamese law. Additionally, the experimental procedures were internally evaluated by the Ethical Committee judging Animal Experiments of Wageningen University (The Netherlands) and approved for meeting the EU regulations for the care and use of laboratory animals conform to Directive 2010/63/EU. These fish were kept and handled in agreement with EU-legislation and Vietnamese laws.

2.3. Fish handling

The experiments on striped catfish (*P. hypophthalmus*) and snakehead (*C. striata*) were conducted in 500-L round tanks (0.6 m in height and 1 m in diameter), which were integrated in a recirculating aquaculture system (RAS) with a water flow per tank of 30 L.min^{-1}. The experiment on African catfish (*C. gariepinus*) was conducted in 70-L rectangular glass tanks (70 × 35 × 40 cm, length x width x height), which were integrated into a RAS with a water flow per tank of 7 L.min^{-1}. In all experiments, the four experimental diets were randomly assigned to one of 12 tanks (three replicates per diet). The initial body weight of striped catfish (*P. hypophthalmus*), African catfish (*C. gariepinus*) and snakehead (*C. striata*) were, respectively 29.1 g, 71.6 g, and 29.1 g. In the striped catfish, African catfish (*C. gariepinus*) and snakehead (*C. striata*) experiment, respectively 120, 35 and 100 fish were stocked per tank and the experimental length was 63, 30 and 42 days, respectively.

Average water quality parameters during the striped catfish experiment for temperature, oxygen, pH, conductivity, NH_4-N, NO_2-N and NO_3-N, respectively, 28.6 ± 0.49 °C, 5.01 ± 1.04 mg.L^{-1}, 7.2 ± 0.22, 2.8 ± 0.59 mS.m^{-1}, <0.5 mg.L^{-1}, < 0.5 mg.L^{-1}, and < 50 mg.L^{-1}. Average water quality parameters during the African catfish (*C. gariepinus*) experiment for temperature, oxygen, pH, conductivity, NH_4-N, NO_2-N and NO_3-N, were respectively 27.7 ± 0.2 °C, 6.3 ± 0.3 mg.L^{-1}, 7.2 ± 0.3, 4 ± 0.4 mS.m^{-1}, 0.4 ± 0.2 mg.L^{-1}, 0.3 ± 0.2 mg.L^{-1} and 304.2 ± 78.2 mg.L^{-1}. Average water quality parameters during the snakehead (*C. striata*) experiment for temperature, oxygen, pH, conductivity, NH_4-N, NO_2-N and NO_3-N, were respectively, 28.5 °C ± 0.8, 8 ± 0.17 mg.L^{-1}, 6.9 ± 0.3, 2.6 ± 0.69 mS.m^{-1}, < 1 mg.L^{-1}, <1 mg.L^{-1}, and < 50 mg.L^{-1}. To measure temperature and oxygen, the device HQ40 (Hach co., United Kingdom) was used. To measure conductivity and pH, the device HI8633 (Hanna co., Rumani) was used. Other parameters were measured by using the test kit (Sera GmbH, Germany).

2.4. Sample preparation and chemical analysis

The day prior to the end of each experiment, fish were not fed. At the end of the experiment 20 striped catfish (*P. hypophthalmus*) per tank, seven African catfish (*C. gariepinus*) per tank and five snakehead (*C. striata*) per tank were randomly selected for body compartment measurements. Striped catfish (*P. hypophthalmus*) and snakehead (*C. striata*) were euthanized by using an overdose of Aqui-S (Aqui-S New Zealand Ltd., Lower Hutt, New Zealand) and African catfish (*C. gariepinus*) were euthanized by using an overdose of 2-phenoxyethanol. The selected fish were batch weighed per tank. Striped catfish (*P. hypophthalmus*) were dissected directly after ending the experiment; African catfish (*C. gariepinus*) and snakehead (*C. striata*) were first frozen at −20 °C for later dissection. Fish were separated into four compartments: 1) fillets; 2) livers (without gallbladder); 3) viscera including pancreas, stomach, intestine, gonadal glands, abdominal fat tissue and gallbladder; and 4) the rest fraction including head, skin, subcutaneous fat, scales, bones and air bladders. First fish were gutted and livers were separated from the viscera excluding the gallbladder. The viscera were collected including abdominal fat tissue and gallbladder. Thereafter, the
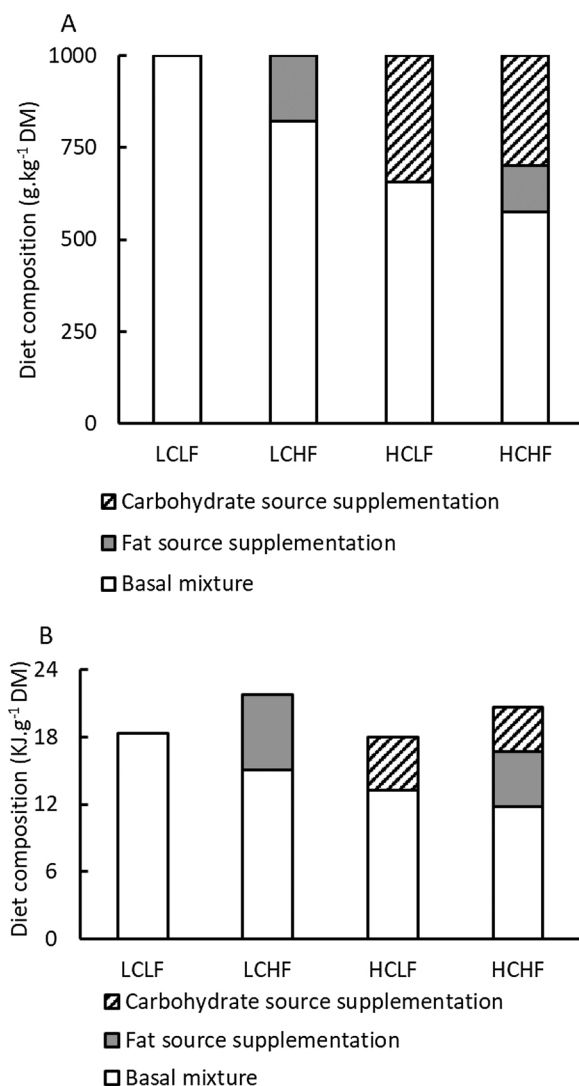
carcasses were skinned after which fish were filleted. The four defined compartments were pooled and weighed per tank and thereafter frozen at -20 °C for later analysis. The sample preparation before chemical analysis was according to the methods reported for body composition measurement by Saravanan et al. (2012).

Before chemical analysis, fish compartment samples were thawed and minced to ensure sample homogeneity. In thawed samples dry matter (DM) was measured fresh material. For ash, crude protein (CP), fat and gross energy (GE) analyses samples were first oven-dried (60 °C). Proximate composition of the compartments were analysed 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 98,311,998).

2.5. Calculations

The organ somatic indices (%) were calculated by dividing the organ weight by the mean body weight of sampled fish. The absolute amount of protein and fat in compartments (i.e., liver, viscera, fillet and the rest) were determined by multiplying the protein or fat content by the respective organ somatic indices and the total body weight of the sampled fish. The total amount of protein and fat in the body is the sum of the absolute amounts of nutrients in the four compartments. The protein and fat deposition (as % of total protein and fat amount in the body) in compartments is calculated by dividing the absolute amounts of protein and fat in the compartments by the total amount of protein and fat in the body. The fat deposition in fillet, liver, viscera and the rest fraction was used to indicate the location of fat deposition in the present study.

2.6. Statistical analysis

Data analysis was conducted using statistical analysis systems statistical software package version 9.1 (SAS Institute). Three-way ANOVA was used to investigate the effect of species, dietary carbohydrates, fat supplementation and their interaction on the organ somatic indices, compartment chemical composition and location of protein and fat deposition. Tank was used as the experimental unit in the statistical analysis. Tukey's test was used for post hoc pairwise comparison of means. Significance was set at $P < 0.05$.

3. Results

3.1. Growth performance

At the end of the experiments, a total of 240 striped catfish (*P. hypothalamus)* (20 fish.tank^{-1}), 84 African catfish (*C. gariepinus*) (7 fish.tank^{-1}), and 60 snakehead (*C. striata*) (5 fish.tank^{-1}) were sampled with a mean final body weight (BW) of 109.3 g, 212.0 g and 122.7 g, respectively. Increasing dietary carbohydrates decreased the final body weight of the studied fish species. The final body weight of striped catfish (*P. hypophthalmus*), African catfish (*C. gariepinus*) and snakehead (*C. striata*) (110.6 g, 198.1 g, and 103.3 g, respectively) at the high dietary carbohydrates levels was lower than that of fish (134.8 g, 225.9 g, 115.3 g, respectively) at the low dietary carbohydrates levels ($P < 0.05$) (Supplementary table S1). Increasing dietary fat did not affect the final body weight of the studied fish species.

3.2. Compartment somatic indices

Fillet yield differed between species ($P < 0.05$). Snakehead (*C. striata*) had the highest fillet yield (33.3%) and African catfish (*C. gariepinus*) had the lowest fillet yield (29.8%) (Table 2). Neither carbohydrates nor fat supplementation affected the fillet yield for the three species ($P > 0.05$) (Table 3,4, Supplementary table S1).

Table 2

Differences between the studied fish species regarding somatic indices, composition (g. kg^{-1}, on a wet weight basis) of body compartments and location of deposited protein and fat over body compartments averaged over the four experimental diets.

	Species			SEM	P values
	Striped catfish	African catfish	Snakehead		
Somatic indices (%)					
Hepato-somatic index	2.0	2.0	2.1	0.11	ns
Viscera somatic index	6.2[a]	4.9[b]	3.7[c]	0.33	***
Fillet yield	31.8[ab]	29.8[c]	33.3[a]	1.54	***
Rest fraction	55.8[ab]	57.2[a]	53.9[c]	1.25	***
Liver composition (g. kg^{-1})					
Dry matter	228[b]	298[a]	–	6.2	***
Ash	16[a]	10[b]	–	1.5	***
Protein	141[a]	98[b]	135[a]	8.0	***
Fat	46[c]	138[b]	200[a]	10.1	***
Viscera composition (g. kg^{-1})					
Dry matter	498[a]	463[ab]	401[c]	38.2	***
Ash	15	8	12	8.4	ns
Protein	81[b]	99[b]	147[a]	10.6	***
Fat	394[a]	331[a]	215[b]	40.8	***
Fillet composition (g. kg^{-1})					
Dry matter	242	238	244	4.4	ns
Ash	13[a]	12[b]	12[b]	0.5	***
Protein	166[c]	180[b]	206[a]	2.3	***
Fat	65[a]	51[a]	17[c]	3.8	***
Composition of rest fraction (g. kg^{-1})					
Dry matter	354[a]	310[b]	340[a]	6.4	***
Ash	44[b]	41[b]	78[a]	3.2	***
Protein	143[c]	161[b]	191[a]	3.5	***
Fat	147[a]	102[b]	64[c]	3.7	***
Location of deposited protein (%)					
Liver	2.1[a]	1.3[c]	1.5[b]	0.12	***
Viscera	3.6	3.1	3.0	0.39	ns
Fillet	37.6[a]	35.1[b]	38.1[a]	1.38	***
Rest fraction	56.8[b]	60.5[a]	57.3[b]	1.59	***
Location of deposited fat (%)					
Liver	0.8[c]	3.1[b]	8.0[a]	0.54	***
Viscera	19.3	17.5	15.6	2.66	ns
Fillet	15.8[a]	15.8[a]	8.9[b]	1.59	***
Rest fraction	64.2	63.5	67.5	2.58	ns

Means within rows lacking a common superscript are significantly different. SEM and P values are based on three−way ANOVA.***, P<0.001; ns, non-−significantly different.

Only carbohydrates supplementation showed an interaction effect on the hepato-somatic index (HSI) between species ($P < 0.05$) (Fig. 2). Except for the HSI, the response of the other somatic indices to differences in the dietary macronutrient content was similar for the studied species. Details about the effect of species, carbohydrates and fat supplementation on the organ somatic indices are presented in Tables 2,3 and 4, respectively. Increasing the dietary carbohydrates content increased the HSI and VSI (P < 0.01) (Table 3), but increasing the dietary fat content only increased the VSI ($P < 0.05$) (Table 4). Neither carbohydrates nor fat supplementation affected the percentage of the rest fraction ($P > 0.05$) (Table 3,4).

3.3. Chemical composition of compartments

There was interaction between species and carbohydrates on fillet

Table 3

Effect of dietary carbohydrates supplementation on somatic indices, composition (g. kg^{-1}, on a wet weight basis) of body compartments and location of deposited protein (% total body protein) and fat (% total body fat) over body compartments averaged over the three studied fish species.

	Carbohydrates			
	Low	High	SEM	*P* values
Somatic indices (%)				
Hepato-somatic index	1.9^b	2.2^a	0.11	***
Viscera somatic index	4.7	5.3	0.33	***
Fillet yield	32.1	31.1	1.54	ns
Rest fraction	56.2	55.0	1.25	ns
Liver composition (g. kg^{-1})				
Dry matter	259	268	6.2	ns
Ash	13	13	1.5	ns
Protein	122	127	8.0	ns
Fat	139	116	10.1	***
Viscera composition (g. kg^{-1})				
Dry matter	447	461	38.2	ns
Ash	14	9	8.4	ns
Protein	109	109	10.6	ns
Fat	301	326	40.8	ns
Fillet composition (g. kg^{-1})				
Dry matter	238	244	4.4	*
Ash	12	12	0.5	ns
Protein	185	183	2.3	ns
Fat	41	48	3.8	***
Composition of rest fraction (g. kg^{-1})				
Dry matter	330	339	6.4	*
Ash	55	53	3.2	ns
Protein	166	164	3.5	ns
Fat	95	113	3.7	***
Location of deposited protein (%)				
Liver	1.5^b	1.8^a	0.12	***
Viscera	3.0	3.5	0.39	ns
Fillet	37.2	36.7	1.38	ns
Rest fraction	58.4	58.0	1.59	ns
Location of deposited fat (%)				
Liver	4.1	3.7	0.54	ns
Viscera	17.1	17.8	2.66	ns
Fillet	14.1	13.0	1.59	ns
Rest fraction	64.7	65.5	2.58	ns

Means within rows lacking a common superscript are significantly different. SEM and P values are based on three−way ANOVA.. ***, P<0.001; *, P<0.05; ns, non−significantly different.

Table 4

Effect of dietary fat supplementation on somatic indices, composition (g. kg^{-1}, on a wet weight basis) of body compartments and location of deposited protein (% total body protein) and fat (% total body fat) over body compartments averaged over the three studied fish species.

	Fat			
	Low	High	SEM	*P* values
Somatic indices (%)				
Hepato-somatic index	2.0	2.0	0.11	ns
Viscera somatic index	4.4^b	5.6^a	0.33	***
Fillet yield	32.2	31.0	1.54	ns
Rest fraction	55.9	55.3	1.25	ns
Liver composition (g. kg^{-1})				
Dry matter	258	268	6.2	*
Ash	12	14	1.5	*
Protein	130	119	8.0	*
Fat	106	149	10.1	***
Viscera composition (g. kg^{-1})				
Dry matter	384^b	523^a	38.2	***
Ash	11	12	8.4	ns
Protein	118	100	10.6	***
Fat	238^b	389^a	40.8	***
Fillet composition (g. kg^{-1})				
Dry matter	229^b	254^a	4.4	***
Ash	12	12	0.5	ns
Protein	188	180	2.3	***
Fat	29^b	60^a	3.8	***
Composition of rest fraction (g. kg^{-1})				
Dry matter	313^b	356^a	6.4	***
Ash	55	53	3.2	ns
Protein	169	161	3.5	***
Fat	86^b	123^a	3.7	***
Location of deposited protein (%)				
Liver	1.7	1.6	0.12	ns
Viscera	3.0	3.5	0.39	ns
Fillet	37.3	36.6	1.38	ns
Rest fraction	58.0	58.4	1.59	ns
Location of deposited fat (%)				
Liver	4.2	3.7	0.54	ns
Viscera	15.4	19.5	2.66	***
Fillet	11.2^b	15.8^a	1.59	***
Rest fraction	69.2^a	61.0^b	2.58	***

Means lacking a common superscripts differ significantly (*P* < 0.05). SEM and P values were based on three-way ANOVA. ***, P<0.001; *, P<0.05; ns, non-significantly different.

protein and fat content (Figs. 3 and 4, Supplementary S2) (*P* < 0.05). Increasing the dietary carbohydrates content increased the fillet fat content in African catfish (*C. gariepinus*) and striped catfish (*P. hypophthalmus*) by 16 g.kg^{-1} from 50 g.kg^{-1} at the low carbohydrates diets to 66 g.kg^{-1} at the high carbohydrates diets averaged over the two species and fat levels. In contrast, the increase in dietary carbohydrates decreased fillet fat in snakehead (*C. striata*) by 10 g.kg^{-1} from 22 g.kg^{-1} at the low carbohydrate diets to 12 g.kg^{-1} at the high carbohydrates diets averaged over fat levels (*P* < 0.05). Dietary fat supplementation increased the fillet fat content from 29 g.kg^{-1} at low fat diets to 60 g.kg^{-1} at high fat diets averaged over species and carbohydrates levels (Table 4, Supplementary table S2). The chemical composition of the liver (Figs. 5 and 6), viscera and the rest fraction are given in supplementary table S3, S4 and S5, respectively.

3.4. The location of deposited fat

Snakehead (*C. striata*) had the highest fat deposition in the liver and the lowest fat deposition in the fillet amongst the three studied fish

species (Table 2). The fat deposition is comparable in viscera and the rest fraction amongst the three studied fish species (*P* > 0.05) (Table 2). Averaged over the three studied fish species, the highest fat deposition was in the rest fraction (65%) and the lowest fat deposition was in the liver (4.6%) while the deposition of fat in viscera (17.5%) and fillet (13.5%) were similar (Table 2).

Changes in dietary macronutrient composition, *e.g.* supplementation of non-protein energy sources from either carbohydrates or fat did not affect the location of protein deposition in fillet, viscera and the rest fraction (Supplementary table S6). The supplementation of carbohydrates and fat affected the location of fat deposition in the liver, viscera, fillet and the rest fraction. There were effects of interaction between species and carbohydrates supplementation on the fat deposition in the liver (Fig. 7) and the rest fraction (Supplementary table S7) (*P* < 0.05). The deposition of fat in the liver was about ten times higher in snakehead (*C. striata*) than in striped catfish (*P. hypophthalmus*) (*P* < 0.05) (Fig. 7).

In fillet, the deposition of fat was comparable between striped catfish (*P. hypophthalmus*) and African catfish (*C. gariepinus*), which was almost
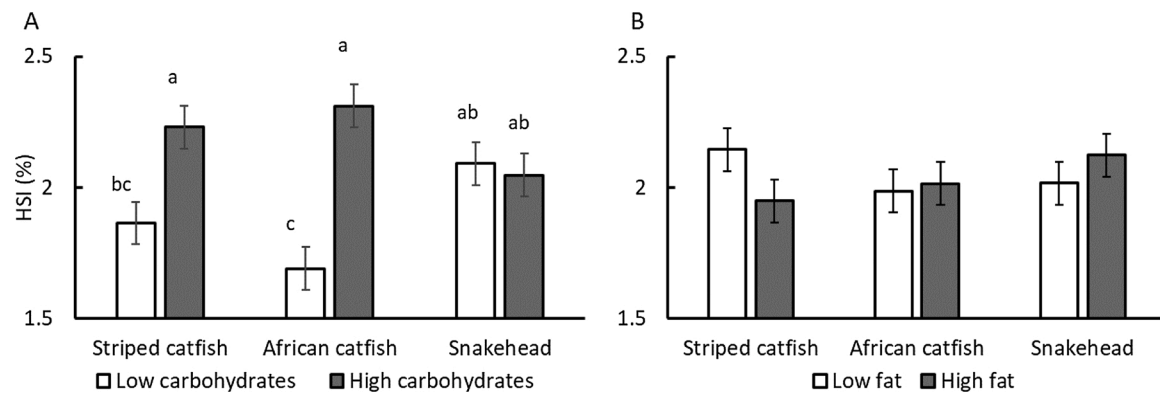
Fig. 2. Interaction effect between fish species (striped catfish, African catfish and snakehead) and carbohydrates supplementation (low vs. high) (A), and between fish species and fat supplementation (low vs. high) (B) on the hepato−somatic index (HSI). In the case of a significant interaction effect, means lacking a common superscript within a panel differ ($P < 0.05$).
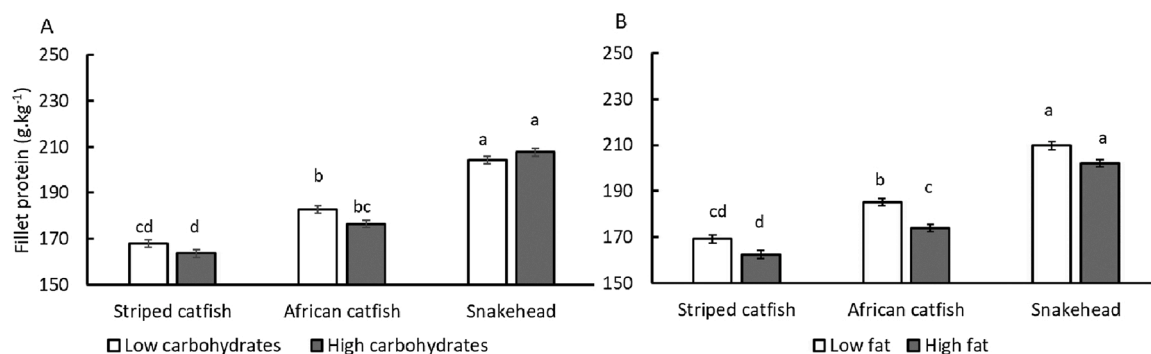
Fig. 3. Interaction effect between fish species (striped catfish, African catfish and snakehead) and carbohydrates supplementation (low vs high) (A), and between fish species and fat supplementation (low vs high) (B) on fillet protein content. In the case of a significant interaction effect, means lacking a common superscript within a panel differ ($P < 0.05$).
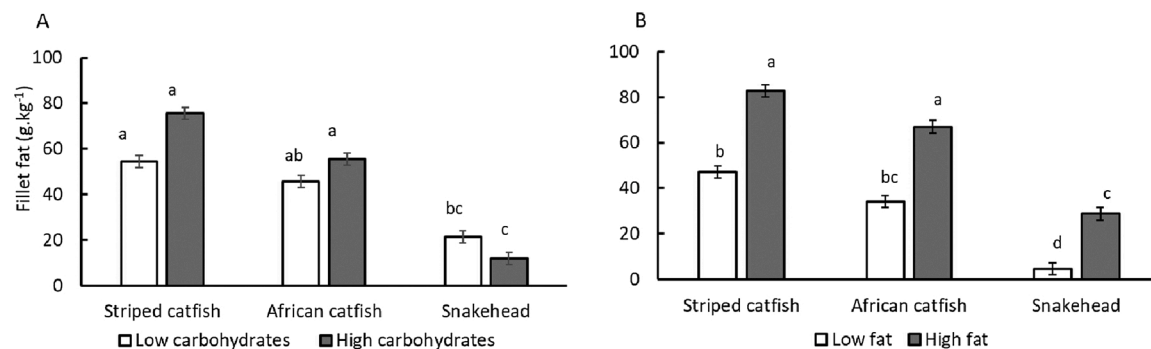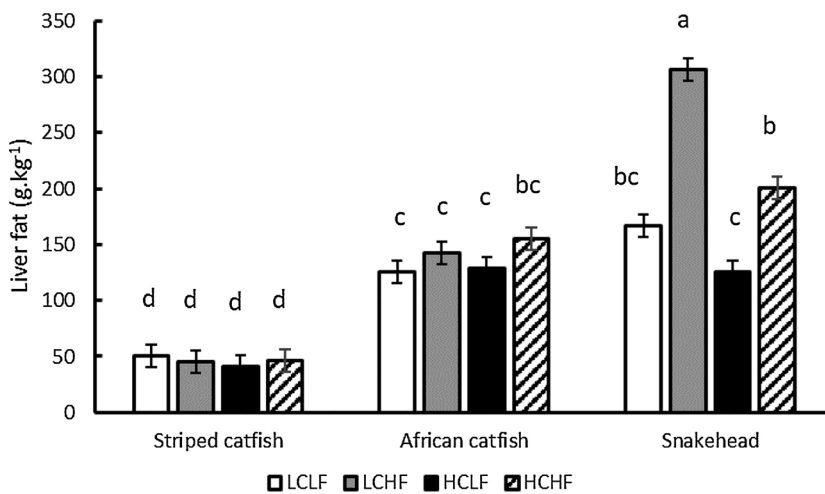

Fig. 4. Interaction effect between fish species (striped catfish, African catfish and snakehead) and carbohydrates supplementation (low vs. high) (A), and between fish species and fat supplementation (low vs. high) (B) on fillet fat content. In the case of a significant interaction effect, means lacking a common superscript within a panel differ ($P < 0.05$).

double the value found in snakehead (*C. striata*) ($P < 0.05$) (Table 2). There was interaction between species and fat supplementation on the deposition of fat in fillet ($P < 0.05$) (Fig. 8, Supplementary table S7). Fat supplementation had a stronger effect on the deposition of fat in the fillet of snakehead (*C. striata*) than in the fillet of striped catfish (*P. hypophthalmus*) and African catfish (*C. gariepinus*). In snakehead (*C. striata*), increasing the dietary fat content from the low fat to the high fat averaged over carbohydrates levels increased the fat deposition in the fillet by about 300% (Fig. 8). In striped catfish (*P. hypophthalmus*) and African catfish (*C. gariepinus*), increasing the dietary fat from the low fat to the high fat averaged over the two species and carbohydrates levels only increased the fat deposition in the fillet by 16%

(Supplementary table S7). Fat suplementation increased the deposition of fat by 26.6% in the viscera and decreased the fat deposition by 11.8% in the rest fraction ($P < 0.01$) (Table 4).

4. Discussion

In most cases, fillet is for human consumption, thus this study aimed to investigate the effect of dietary macronutrient composition on the fillet yield. Averaged over the fish species studied in this study, the fillet yield was 31.8%, which is similar to values found in other studies on striped catfish (*P. hypophthalmus*) (Asemani et al., 2019; Da et al., 2012), African catfish (*C. gariepinus*) (Jantrarotai et al., 1998) and snakehead

Fig. 5. Interaction effect between species (striped catfish, African catfish and snakehead), carbohydrates supplementation (low vs. high) and fat supplementation (low vs. high) ($P < 0.05$) on liver fat content of studied fish fed one of the four diets: low carbohydrates low fat (LCLF), low carbohydrates high fat (LCHF), high carbohydrates low fat (HCLF), high carbohydrates high fat (HCHF). In the case of a significant interaction effect, means lacking a common superscript within a panel differ ($P < 0.05$).
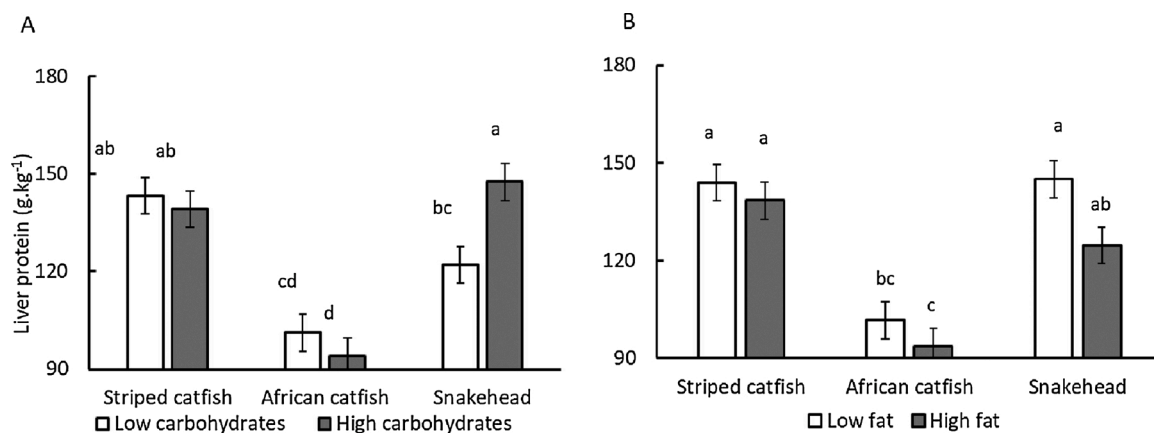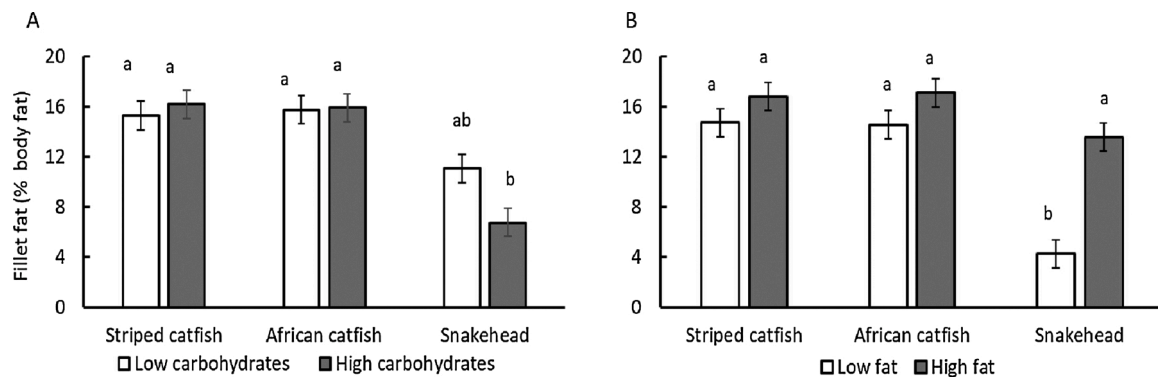

Fig. 6. Effect of interaction between species (striped catfish, African catfish and snakehead) and carbohydrates supplementation (low vs. high) (A), and between species and fat supplementation (low vs. high) (B) on liver protein content. In the case of a significant interaction effect, means lacking a common superscript within a panel differ ($P < 0.05$).
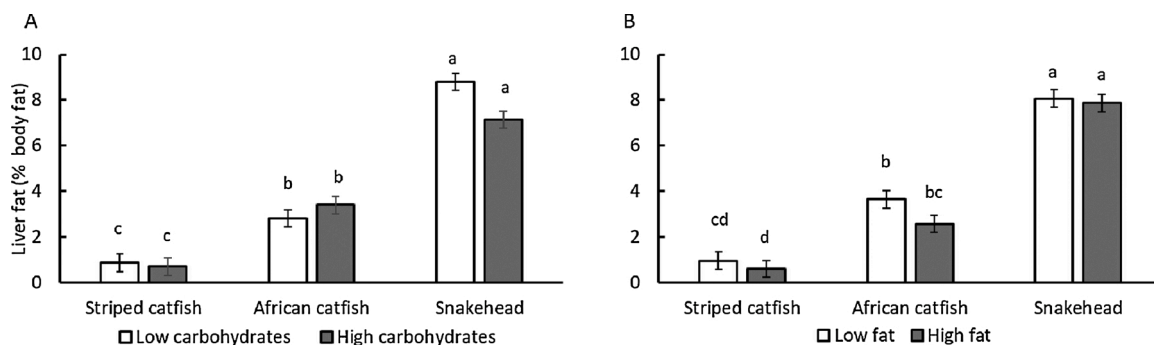
Fig. 7. Interaction effect between fish species (striped catfish, African catfish and snakehead) and carbohydrates supplementation (low vs high) (A), and between fish species and fat supplementation (low vs high) (B) on the amount of fat deposition in the liver as percentage of total body fat. In the case of a significant interaction effect, means lacking a common superscript within a panel differ ($P < 0.05$).

(*C. striata*) (Tan and Azhar, 2014). In the present study, fillet yield was unaffected by dietary macronutrient composition. Neither dietary carbohydrates nor fat supplementation altered the fillet yield of striped catfish (*P. hypophthalmus*), African catfish (*C. gariepinus*) and snakehead (*C. striata*). The absence of a dietary effect on fillet yield is comparable with previous studies assessing dietary impacts on fillet yield in African catfish (*C. gariepinus*) (Jantrarotai et al., 1998) and striped catfish (*P. hypophthalmus*) (Asemani et al., 2019; Da et al., 2012).

The observed range in chemical composition of fillets in the present study was comparable to earlier studies on striped catfish (*P. hypophthalmus*) (Da et al., 2012; Orban et al., 2008), African catfish (*C. gariepinus*) (Szabo et al., 2009), and snakehead (*C. striata*) (Aliyu--Paiko et al., 2010). Only, the fillet fat content of striped catfish (*P. hypophthalmus*) in the current study was higher compared to the value found by (Orban et al., 2008). The effect of dietary carbohydrates supplementation on the fillet fat content in snakehead (*C. striata*) was

Fig. 8. Interaction effect between fish species (striped catfish, African catfish and snakehead) and carbohydrates supplementation (low vs high) (A), and between fish species and fat supplementation (low vs high) (B) on the amount of fat deposition in the fillet as percentage of total body fat. In the case of a significant interaction effect, means lacking a common superscript within a panel differ ($P < 0.05$).

different from the effect in the other two fish species. In both catfish species, carbohydrates supplementation increased fillet fat content, whereas carbohydrates supplementation decreased fillet fat content in snakehead (*C. striata*) (Fig. 4). This might relate to carnivorous nature of snakehead (*C. striata*) and or its low capacity to utilize energy from digested carbohydrates (Phan et al., 2021b). In grass carp (*Ctenopharyngodon idella)*, a herbivorous fish, diets with a high carbohydrates content increases fillet fat content (Guo et al., 2015). In contrast to this, in cod (*Gordus morhua*), a carnivorous fish, dietary carbohydrates had no impact on fillet fat content (Hemre et al., 1989).

In contrast to carbohydrates, the impact of fat supplementation on fillet fat content was uniform across the three studied species. With increasing dietary fat supplementation, the fillet fat content increased (Fig. 4). This effect of dietary fat on the fillet fat content is consistent to literature for a large range of species: African catfish (*C. gariepinus*) (Jantrarotai et al., 1998; Lim et al., 2001), channel catfish (*Ictalurus punctatus*) (Stowell and Gatlin, 1992), grass carp (*C. idella*) (Guo et al., 2015; Regost et al., 2001). These findings demonstrate that the dietary fat supplementation can increase the fillet fat content of the studied fish species. In contrast, the fillet fat content of European seabass (Peres and Oliva-Teles, 1999) and hybrid striped bass (Gaylord and Gatlin Iii, 2000) were unaffected by increasing dietary fat levels. The differences between species might be due to the applied contrast in the dietary fat. However, this explanation seems unlikely since the contrast in dietary fat level in European seabass study (Peres and Oliva-Teles, 1999) and hybrid striped bass study (Gaylord and Gatlin Iii, 2000) was comparable to those applied in the current study. Most likely the absence and the size of the impact of dietary fat on fillet fatness is species-dependent. At similar dietary fat levels, the source of fat can also influence the fillet fat content in some fish species. Using linseed oil caused a higher fillet saturated fatty acid content than using echium oil in snakehead (*C. striata*) diets (Jaya-Ram et al., 2016). Rainbow trout fed the soybean oil diet had the highest fillet fat content, compared to rainbow trout fed salmon oil, linseed oil, chicken fat, pork lard and beef tallow (Greene and Selivonchick, 1990). However, in some other fish species, the dietary fat source affected the fillet fat composition but not the fillet fat content. The fillet fat composition of Atlantic salmon (*Salmo salar*), i.e. the ratio between n-6 and n-3, the percentage of monounsaturated fatty acids and n-6 fatty acid was related to the dietary inclusion level of canola oil and poultry fat (Higgs et al., 2006). The level of long chain HUFA in the fillet of sunshine bass (*Morone chrysops x M saxatylis*) was higher in the marine oil diet than in the corn oil diet (Lane et al., 2006). The dietary fat source can also affect the aroma of fish fillet. The higher dietary soybean oil rich in n-6 fatty acids increased n-6 derived volatile aldehydes which in turn raise the sensory value of the off-flavour in the fillet of tench (*Tinca tinca*) (Turchini et al., 2007). The level of 3-hexen-1-ol causing the fresh grass odour in fillet of brown trout (*Salmo trutta*) was ten times higher in the fish oil diet than in the pork lard diet (Turchini et al., 2004). In the

present study, the difference in dietary fat source (rapeseed oil vs soybean oil) might also partly cause the difference in the fillet fat content. It remains a question whether the absolute difference in the fillet fat content may have affected, either positive or negative, the taste and texture of the fillet. This will depend on the preference and tolerance of the fish consumers. Furthermore, fillet fat content may also affect further processing. *e.g.,* the high fillet fat content can hamper the smoking process due to the high risk of fat oxidation during preservation (Nortvedt and Tuene, 1998).

This study also evaluated whether the fat deposition in the liver, viscera, fillet and the rest fraction was affected by the dietary macronutrient composition. Differences in the fat deposition were first indicated by the organ somatic indices and the chemical composition of the liver, viscera, fillet and the rest fraction. The impact of both dietary fat and carbohydrates supplementation on the rest fraction was similar to the impact on fillet yield and composition (Table 3,4). The HSI of the three species in the current study were similar to the values found in other studies on striped catfish (*P. hypophthalmus*) (Asemani et al., 2019; Da et al., 2012), African catfish (*C. gariepinus*) (Jantrarotai et al., 1998; Serrano et al., 1992) and snakehead (*C. striata*) (Aliyu-Paiko et al., 2010). In the present study, dietary carbohydrates supplementation increased the HSI from 1.9 to 2.2% averaged over species, except for snakehead (*C. striata*). Dietary carbohydrates level did not increase the HSI in snakehead (*C. striata*) (Supplementary table S1). According to literature excessive metabolised carbohydrates may be accumulated in the form of glycogen or fat in the liver, which in turn results in a higher HSI (Mohanta et al., 2009) or whole body fat in the fish (Jiang et al., 2014; Tian et al., 2012; Azaza et al., 2015). In addition, African catfish (*C. gariepinus*) also had the lowest HSI when fed high protein diets (*i.e.* low carb and or fat diets) in previous observations (Jantrarotai et al., 1998; Serrano et al., 1992). A higher HSI and/or VSI in the fish fed high dietary carbohydrates levels was also found in silver barb (*Puntius gonionotus*) (Mohanta et al., 2009), *Catla catla* (Yengkokpam et al., 2006), cod (*G. morhua*) (Hemre et al., 1989) and trout (*O. mykiss*) (Groot et al., 2021). These observations were confirmed in the current study for striped catfish (*P. hypophthalmus*) and African catfish (*C. gariepinus*), but this was not the case for snakehead (*C. striata*). In snakehead (*C. striata*), dietary carbohydrates supplementation decreased the effect of fat supplementation on the liver fat content in snakehead (Fig. 5) as these effects interacted with each other. In snakehead (*C. striata*) fed the low carbohydrates diets, fat supplementation increased the liver fat content by 139 g.kg^{-1}, but by only 75 g.kg^{-1} in high carbohydrates diets (Supplementary table S3). These findings indicate the large differences in the location of fat deposition within the body, especially regarding the liver and viscera between snakehead (*C. striata*) (a strict carnivore) and the other two fish species (African catfish - *C. gariepinus* and striped catfish - *P. hypophthalmus*), which are more omnivorous species.

The difference in the effect of dietary carbohydrates

supplementation on the fillet fat content and the fat deposition in the liver, viscera, fillet and the rest fraction between snakehead (*C. striata*) and the other two species (striped catfish - *P. hypophthalmus* and African catfish - *C. gariepinus*) may be because of differences in the process of lipogenesis in the liver and viscera in these fish. An increased body fat content was observed in silver barb (Mohanta et al., 2009), tilapia and carp (*C. carpio*) using diets high in carbohydrates (levels > 260 g.kg⁻¹ DM). In addition, plasma glucose levels were more than twice as high in trout (about 1.6 g.l⁻¹) compared to tilapia (about 0.7 g.l⁻¹) when fed high carbohydrates diets (Figueiredo-Silva et al., 2013). This indicates that trout has a limited ability to metabolise digested carbohydrates (*i.e.*, glucose) and/or convert glucose to adipose efficiently. The limited utilisation of digested carbohydrates on trout was also observed by Groot et al. (2021). The difference in the process of lipogenesis, e.g., the presence of lipogenic enzymes, could explain the difference in the carbohydrates utilisation between these fish species. Malic (a lipogenic enzyme) in the blood of trout was found to be unaffected by dietary macronutrient composition while in tilapia the level of this enzyme was higher in the blood of fish fed diets high in carbohydrates compared to fish fed diets low in carbohydrates (Figueiredo-Silva et al., 2013). These studies on trout and tilapia may also explain the differences found between snakehead (*C. striata*) and the other two studied fish: striped catfish (*P. hypophthalmus*) and African catfish (*C. gariepinus*) because of the similar contrast in their feeding habits. Looking at the differences of their natural feeding habits, it is reasonable to assume that striped catfish (*P. hypophthalmus*) and African catfish (*C. gariepinus*) are able to utilize and convert glucose from digested carbohydrates into somatic fat in the liver and viscera. Yet, it seems that the ability to use this strategy is limited in snakehead (*C. striata*).

The effect of either carbohydrates or fat supplementation on the location of fat deposition within the fish (i.e., the distribution of body fat over different compartments) has to the best of our knowledge not been addressed in literature regarding fish. In all fish species, the effects of dietary carbohydrates and fat were additive, but only dietary fat altered the distribution of fat over the body (Supplementary table S7, Fig. 8). Dietary fat increased the fat deposition in the fillet and viscera and reduced the amount of fat in the rest fraction. The impact of dietary fat on the amount of fat stored in fillets, differed between the fish species. The increase in fat stored in fillet in response to the dietary fat supplementation was larger for snakehead (*C. striata*) than for striped catfish (*P. hypophthalmus*) and African catfish (*C. gariepinus*) (Fig. 8). This stronger response in snakehead (*C. striata*) to dietary fat may link to the fact that digested fat is efficiently used for energy gain whereas digested carbohydrates has a minimal energetic value (Phan et al., 2021b). More carnivorous species such as salmonids have a lower capacity to utilize carbohydrates, and thus rely more on fat, compared to omnivorous or herbivorous species such as Nile tilapia (*Oreochromis niloticus*), African catfish (*C. gariepinus*) and carp (*Cyprinus carpio*) (Hemre et al., 2002; Molina-Poveda, 2016). These findings in the present study imply that the possibility to formulate functional diets to modify the fat deposition in the fillet is higher for snakehead (*C. striata*) than for striped catfish (*P. hypophthalmus*) and African catfish (*C. gariepinus*).

Though the present study found a difference in the location of fat deposition caused by the dietary macronutrient composition in snakehead (*C. striata*), the major location of fat deposition in striped catfish (*P. hypophthalmus*), African catfish (*C. gariepinus*) and snakehead (*C. striata*) is still in the rest fraction (head, skin, subcutaneous fat, scales, bones and air bladders) independent whether carbohydrates or fat is supplemented. Yet, the fat deposition in the fillet and viscera is comparable over three studied species in response to the change in dietary macronutrient composition. In salmonids, fat is mainly deposited in the viscera and to a lesser extent in the fillet (Gélineau et al., 2001; Sheridan, 1994). In Atlantic cod (*Gadus morhua*), the liver can store more than 80% of the whole body fat content as their muscle has a very low capacity to deposit fat (Hansen et al., 2008; Kjær et al., 2009). In turbot (*Psetta maximus*), the main location of fat is under the skin and

carcass, with little or no fat deposition in the viscera (Andersen et al., 1993; Regost et al., 2001). Surprisingly, the fat deposition in the fillet, the part of striped catfish (*P. hypophthalmus*), African catfish (*C. gariepinus*) and snakehead (*C. striata*) most consumed by humans, is from 3.1 to 17.3% of the total fish body fat even when supplementing large amounts of either carbohydrates or fat to diet. This indicates that the large amount of fat in striped catfish (*P. hypophthalmus*), African catfish (*C. gariepinus*) and snakehead (*C. striata*) is not for human consumption and changing the dietary macronutrient composition cannot improve the inefficient way of using fish fat source for human consumption.

5. Conclusions

Fillet yield of striped catfish (*P. hypophthalmus*), African catfish (*C. gariepinus*) and snakehead (*C. striata*) was not affected by the dietary macronutrient composition. However, fillet fat and protein contents were influenced by the dietary macronutrient composition. In all fish body compartments, both dietary fat and dietary carbohydrates levels increased the fat content. The response to dietary carbohydrates in snakehead (*C. striata*), a lowering of fillet fat content, is opposite to the response in both catfish species. The distribution of the total amount of body fat over the different compartments, was not influenced by dietary carbohydrates level, but did depend on dietary fat level. Dietary fat supplementation led to relatively more fat in viscera and fillet but less fat was stored in the rest fraction. In snakehead (*C. striata*), striped catfish (*P. hypophthalmus*) and African catfish (*C. gariepinus*), most of the body fat is stored in the rest fraction (head, skin, subcutaneous fat, scales, bones and air bladders).

Author statement

L.T.T. Phan: Conceptualization, Methodology, Formal analysis, Investigation, Project administration, Writing – original draft, Writing - review & editing, Visualization; **J. Kals:** Conceptualization, Resources, Supervision, Writing - review & editing; **K. Masagounder:** Conceptualization, Resources, Writing - review & editing; **J. Mas-Muñoz:** Conceptualization, Resources, Supervision, Writing - review & editing; **N.T.H. La:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – Review & Editing; **J.W. Schrama:** Conceptualization, Methodology, Formal analysis, Investigation, Project administration, Writing - review & editing, Supervision, Funding acquisition.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.aqrep.2021.100806.

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