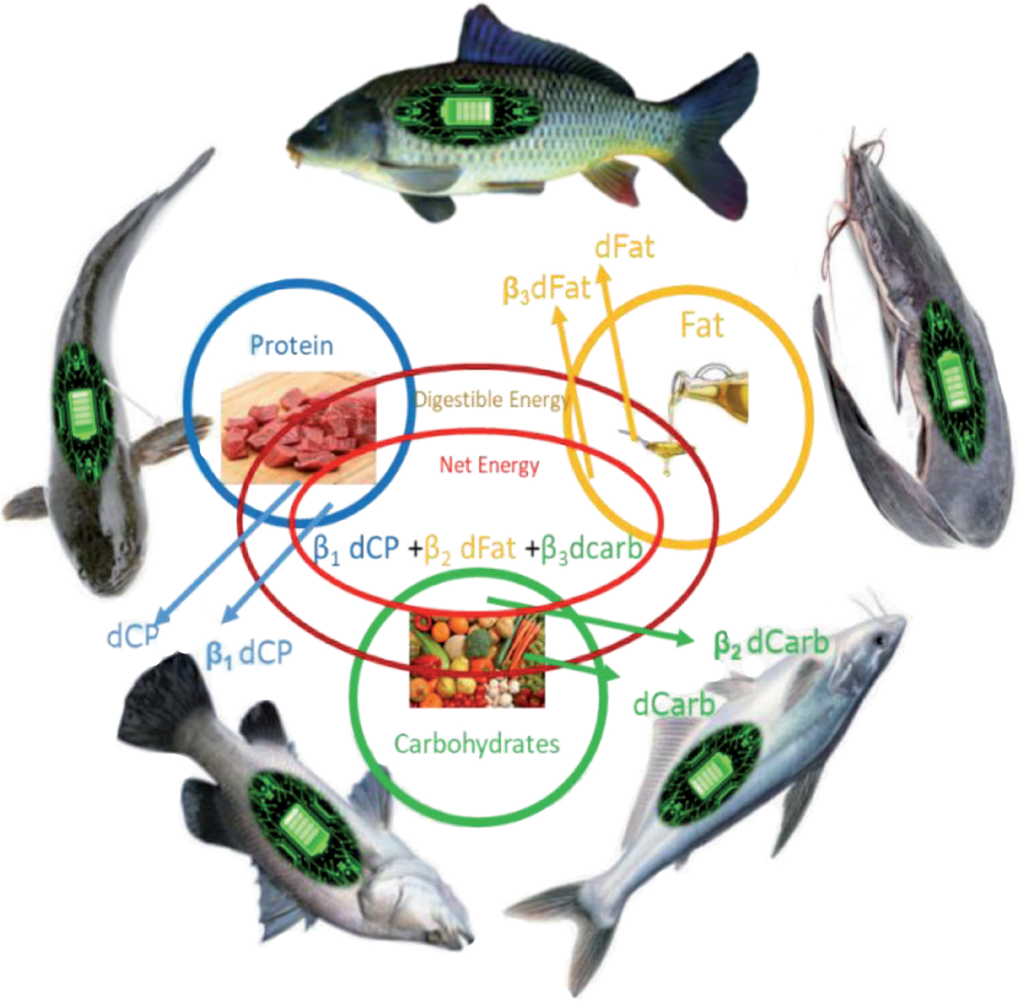
Net energy evaluation for fish feed



Phan Lê Thiện Thuật

Propositions

1. Moving from a digestible to a net energy evaluation of feeds for fish improves the resource use efficiency.
   (this thesis)

2. Even when digested, carbohydrates have a negligible nutritional value for barramundi and snakehead.
   (this thesis)

3. Research for moon colonization puts extra pressure on earth’s natural resources.

4. Opposite to filing patents, sharing knowledge and raw data via open access stimulates scientific innovations.

5. Artificial intelligence improves the living standard of employees with dangerous tasks.

6. Making black and white needs more than black and white.

Propositions belonging to the thesis, entitled
Net energy evaluation for fish feed
Phan Le Thien Thuat
Wageningen, 20 May 2022

Net energy evaluation for fish feed

Phan Le Thien Thuat

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Net energy evaluation for fish feed

Phan Le Thien Thuat

Thesis

submitted in fulfilment of the requirements for the degree of doctor
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For my parents

Abstract

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The major aims of this thesis are: 1. To investigate the effect of dietary macronutrient composition on the relationships between retained energy (RE) and digestible energy (DE) for African catfish, barramundi, carp, snakehead and striped catfish, 2. To quantify the net energy equations for the five selected fish species, 3. To investigate whether the net energy equations are similar across the five fish species studied and 4. To assess the impact of dietary macronutrient composition on the proximate composition and location of fat deposits in the different body compartments of African catfish, striped catfish and snakehead. The minor aims of this thesis are: 1. To investigate differences in the digestibility of carbohydrates between the selected fish species and 2. To study the effect of dietary starch levels on plasma glucose and triglycerides values in snakehead and striped catfish. The major aims were achieved by performing experiments on African catfish, common carp, snakehead and striped catfish and by re-analysing data of a recent published study on barramundi. For every experiment a total of four diets were formulated following a 2×2 factorial design with 2 digested protein to digested fat ratios and 2 digested protein to digested carbohydrates ratios. For African catfish, carp, striped catfish and snakehead, 2 feeding levels were applied such that the overall experimental design was a 2×2×2 factorial design, however for barramundi, three feeding levels were applied, satiation, 80% initial satiation, and 60% initial satiation, respectively resulting in a 2×2×3 factorial design. For each fish species, regression of retained energy and digestible energy intake was performed to assess if the effect of dietary macronutrient composition on the relationships between retained energy and digestible energy exists. In addition, for each species, multiple regression of retained energy as a function of digested protein, fat and carbohydrates intake (in $g.kg^{-0.8}.d^{-1}$) was applied to estimate the energy utilization efficiency of each digestible macronutrient and determine the net energy equation. The net energy equations were compared among fish species studied. This study found that the digestibility of starch is high in all the fish species studied and that of non-starch polysaccharides (NSP) is species related and only digested by striped catfish and common carp. The energy utilization efficiencies of digested protein and fat are comparable between the fish species studied. The energy utilization efficiency of digested carbohydrates is different across the fish species studied causing the net energy equations to be different between the fish species studied. This different contribution of carbohydrates is due to the difference in the ability to use carbohydrates, *i.e.*, the ability to convert plasma glucose to plasma triglycerides. Striped catfish has a better ability to convert plasma glucose to plasma triglycerides than snakehead. Dietary fat supplementation increased the fat content of liver, viscera, fillet and the rest fraction in striped catfish, African catfish and snakehead. Finally, the impact of digestibility of nutrients on the energy evaluation, the motivations to move from a digestible energy system to a net energy system and next steps for the energy evaluation of fish feed, such as the energy utilisation efficiency of non-starch polysaccharides, the optimal balance between digestible protein and net energy for fillet and whole body growth and net energy equations for different production systems are discussed.

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General introduction

1. Development of global aquaculture and aqua-feed production

Global aquaculture production, of both aquatic animals and plants, increased from approximately 40 to 112 million tonnes between 2000 and 2017 (Tacon *et al*., 2020). The expansion of production areas and the intensification of the aquaculture sector have facilitated this growth. Between 2000 and 2018, global finfish production, which uses compound feeds, increased 6.8 % per year from approximately 14 to 45 million tonnes (Figure 1). Consequently, global finfish feed production during this period increased 7.7 % per year, from approximately 14 to 53 million tonnes (Figure 1).

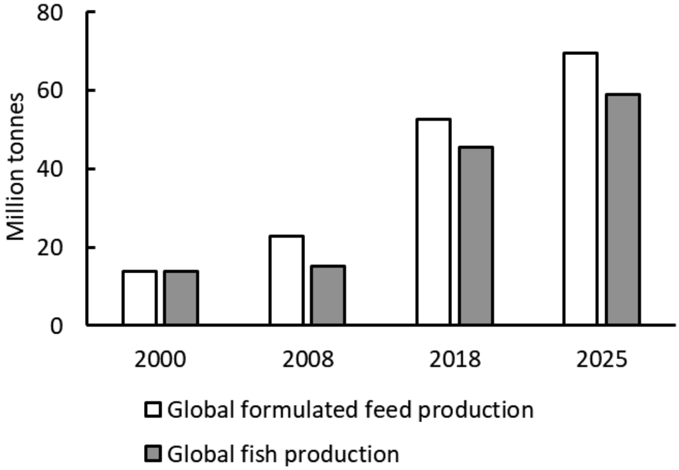

Figure 1. The development of global finfish production using formulated feeds, and the increase in formulated feed production (Tacon & Metian, 2008; Tacon *et al*., 2021).

To facilitate the growth of global aqua-feed production, technology has evolved over time. Historically, pond culture was practiced in an extensive way without feed supplementation. The intensification of pond culture was, next to the use of aeration, mainly related to the introduction of compound feeds and the increased proportion of finfish species farmed using formulated feeds. Fish species of a higher trophic level (*i.e*., snakehead, barramundi) were initially fed with baitfish, which was later replaced by compound feeds. Barramundi feed can be taken as an example of the evolution in fish feed technology. Barramundi diets evolved from baitfish to simply pressed pellets to modern extruded energy-dense pellets (Glencross, 2006).

Over time, the dietary ingredient composition has also changed to meet the demands of the increasing dependency of finfish culture on formulated feeds. It is challenging to search, find and have sufficient raw materials to fulfil the increasing demand for fish feed. This is because the supply of conventional protein and fat sources for fish feed, *i.e*., fish meal and fish oil, has been unstable for years in absolute amounts, and is expected to remain so in the near future (Tacon & Metian, 2008). To reduce the dependency of the aqua feed industry on fishmeal and fish oil, the use of alternative ingredients, mainly of animal (*i.e*., processing by-products offal, trimmings) and terrestrial plant origin, has increased to become the principle ingredients in aqua-feeds (Tacon & Metian, 2008).

Norwegian salmon feed is an example of this trend. Between 1990 and 2013, the inclusion levels of fish meal and fish oil decreased, while plant oil and plant protein increased significantly (Figure 2).

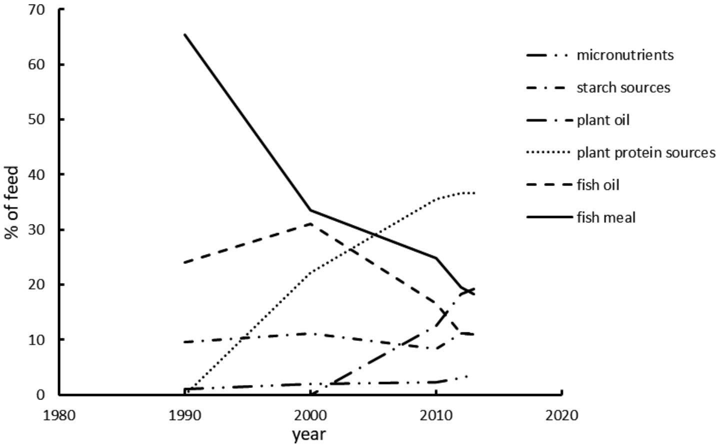

Figure 2. The trend in the inclusion level (% of feed) of different ingredients in an average Norwegian salmon feed, from 1990 to 2013. Micronutrients are pigments, vitamins, minerals and crystalline amino acids. Starch sources are wheat, pea and cassava. Plant oil mainly consists of rapeseed oil. Plant protein sources are soybean concentrate, sunflower expeller, wheat gluten, fava beans, pea protein, maize gluten and hoarse beans (Ytrestøyl *et al*., 2015).

The change in dietary ingredient composition resulted in a change in the quantity and quality of the dietary macronutrient composition. This change evolved differently between species of different trophic levels, *i.e.* between the herbivorous, omnivorous and carnivorous fish species. For herbivorous and/or omnivorous fish, the increasing use of plant protein sources in fish feeds also increased the carbohydrate content. This is due to the fact that, in general, the level of protein is lower and the level of carbohydrate is higher in plant-based ingredients, compared to animal-based ingredients. In addition, starch these fish species is used as energy source to spare protein for growth. For carnivorous fish, the trend is to use higher levels of fat in the diet, as this can reduce the cost of feed by partially sparing protein for growth (Craig *et al*., 2017). Carnivorous species, such as salmonids, have a lower capacity to utilise carbohydrates (Eddy & Handy, 2012) and in general rely more on fat as an energy source, compared to omnivorous or herbivorous species such as Nile tilapia (*Oreochromis niloticus*), African catfish (*Clarias gariepinus*) and carp (*Cyprinus carpio*) (Hemre *et al*., 2002; Molina-Poveda, 2016). In feeds for carnivorous fish, fat can replace up to 20% of the protein, while in feeds for herbivorous fish carbohydrates can replace up to 30% of the protein without affecting growth (Eddy & Handy, 2012). In summary, the demand for formulated fish feeds is rising, which coincides with diversification of feed ingredients. The current trend in fish feed is for an increasing carbohydrate or fat content. Moreover, the increasing competition with human food, as well as the production of feed for other animals, *i.e*., pig, poultry or pets, and the use of biomass for the production of bio-fuel, will stimulate the use of by-products from the human food processing industries, *i.e*., animal- or plant-based by-products. As a consequence, this will increase variability in the quality of fish feed ingredients.

2. Requirements of a balanced diet

To formulate a balanced diet for optimal growth, it is essential to understand the quantity of nutrients, *i.e.*, protein, fat and carbohydrates that a fish requires for maintenance and growth (Figure 3). Maintenance includes vital life processes, like swimming, protein turnover, tissue repair and oxygen uptake. The growth of fish is mainly the sum of protein and fat synthesis. Protein synthesis requires amino acids and energy. Fat synthesis requires fatty acids, glycerol and energy. The fatty acids and glycerol can originate from dietary fat or from *de novo* synthesis from protein and carbohydrates (Figure 3).

Fish under intensive culture conditions obtain these macronutrients from the feed ingredients in the diet. For farmed fish to have a balanced diet, the inclusion levels of dietary macronutrients, *i.e.*, protein, fat, and carbohydrates (*i.e.*, starch and non-starch polysaccharides), have to match the requirements of the fish (Figure 3). After ingestion of feed, the digestion process breaks down the macronutrients, protein, fat and carbohydrates into smaller fractions using various enzymes. Pepsin breaks down dietary protein into peptides, which are broken down by peptidases into amino acids. Lipase breaks down fat into free fatty acids. In addition, to support fat digestion, bile acids emulsify fat by breaking down the large fat droplets into smaller droplets to increase the surface area for lipase to react with triglycerides and to facilitate the absorption of fatty acids. Carbohydrates consist mainly of starch and non-starch polysaccharides. Amylase breaks down starch into glucose. The non-starch polysaccharides are often considered inert in most fish species, but in some fish species they can be partly digested by exogenous enzymes (*e.g.*, xylanase) or fermented by intestinal bacteria, which generates volatile fatty acids, amongst others (Romano *et al.*, 2018).

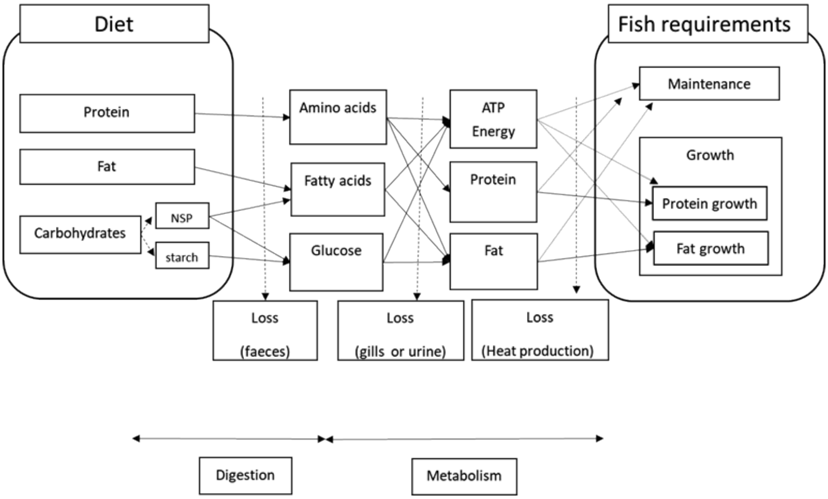

Figure 3. The match of dietary macronutrients with fish requirements

3. The current energy evaluation system in fish feed

Protein, fat and carbohydrates in feed can all supply energy for fish. In Figure 4, a schematic diagram shows the partitioning of energy in fish. After the ingestion of feed, part of the dietary energy (*i.e.*, gross energy) is excreted via the faeces, because it is indigestible. This loss is called the faecal energy loss. The first step of feed evaluation is to determine the digestibility of the different macronutrients, *i.e.*, protein, fat and carbohydrates. Historically, studies on digestibility often focused on the macronutrients that had a major impact on the feed prices, *i.e.*, protein and fat, while information about the digestibility of carbohydrates in many fish species was neglected. The digestion process can differ between species depending on the morphology, pH and enzymes available in the digestive tract. Between fish species, *i.e.*, trout and tilapia, the digestibility of carbohydrates, especially of non-starch polysaccharides is very different, while the digestibility of protein and fat is comparable (Haidar *et al*., 2016; Staessen *et al*., 2019).

The digested energy (DE) consists of digested protein, fat and carbohydrates and can be converted into metabolisable energy (ME). During the use of digestible energy for anabolic and catabolic processes, ammonia and/or urea are formed. These metabolites are excreted through the gills and/or urine and the related energy loss is termed branchial and urine energy loss. The ME available for a fish is used for maintenance processes and for energy gain (*i.e*., retained energy; RE). Subtracting the total heat production from ME gives RE. The total heat production is comprised of the heat increment of feeding and the heat production due to voluntary activity and basal metabolism. The fraction of energy that remains after deducting the heat increment of feeding from ME is called net energy (NE) (Figure 4). NE is for: maintenance, which is equal to fasting heat production; and for fish growth, which is equal retained energy (RE). In animal nutrition, dietary energy is widely expressed as either DE, ME or NE.

ME is rarely used in fish feed to express its energy potential, because it is difficult to measure. Currently, the energy demand for fish growth and maintenance is mainly expressed as DE, *i.e*., the DE evaluation system. In this method, the relationship between the intake of digestible energy (DE) and the retained energy (RE) needs to be estimated (Figure 5). This is most commonly done by linear regression using the following equation:

$$\text{RE} = \text{intercept} + \text{slope} \times \text{DE intake} \qquad \text{(Equation 1)}$$

The slope of the relationship reflects the conversion efficiency of digestible energy into retained energy, *i.e.*, the $k_{\mathrm{g,DE}}$. The intercept of the regression line with the x-axis is the amount of digestible energy required for maintenance. This DE requirement for maintenance is calculated by dividing the intercept of the regression line with the Y axis (being equal to the fasting heat production) by the slope of the line. From this relationship, the growth of fish on an energetic basis, *i.e.*, RE, can also be predicted from the DE intake. Similarly, the amount of DE needed to achieve a certain RE can be calculated. The latter approach is used to calculate the required DE content of a diet and is called the factorial approach, as shown in various studies on striped catfish (Glencross *et al*., 2011), European seabass (Lupatsch *et al*., 2010) and barramundi (Glencross, 2008).

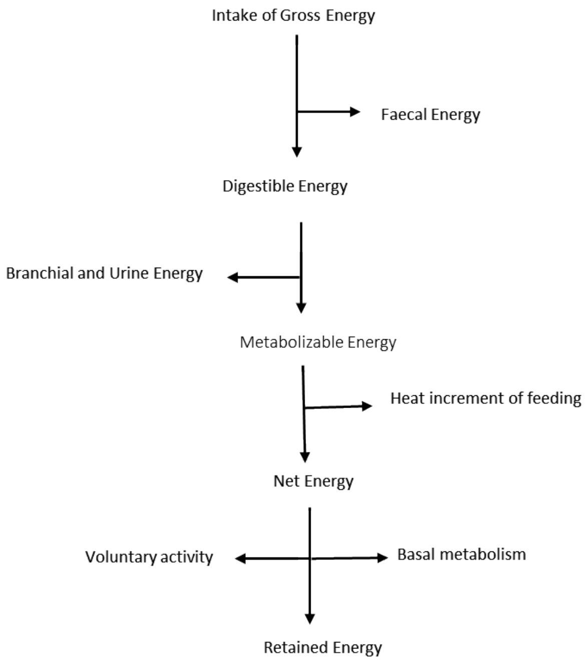

Figure 4. Energy partitioning in fish

The relationship between the digested energy intake and the retained energy thus currently forms the basis for formulation of balanced fish feeds. This relationship, *i.e.*, the energy demand for maintenance and growth, differs between fish species (Table 1).

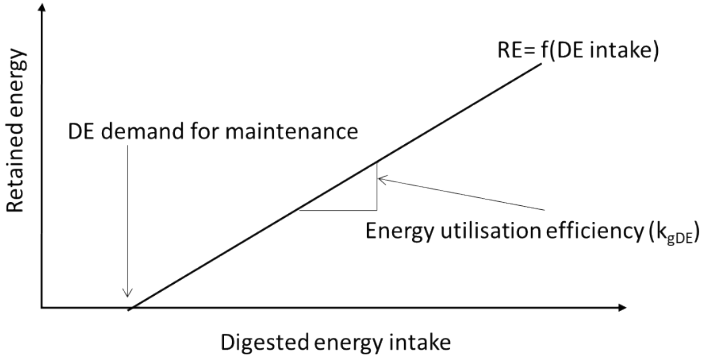

Figure 5. Components of relationship between retained energy (RE) and digested energy (DE) intake

For new culture species, the relationship between RE and DE needs to be estimated to enable optimisation of feed formulas in terms of DE content. When using the DE system for a specific fish species, it is assumed that k_{gDE} is independent of the dietary macronutrient composition. However, this seems debatable as within a species changes in the dietary macronutrient composition can affect the energy utilisation efficiency (Figure 6). This was shown for barramundi (Glencross *et al*. 2017), carp (Schwarz & Kirchgessner, 1995), trout (Rodehutscord and Pfeffer, 1999), and tilapia (Schrama *et al*., 2012). This impact on k_{gDE} hampers a proper estimation of both the dietary DE requirement for maintenance and growth and the prediction of growth (RE), for specific fish species.

Table 1. The relationship between retained energy (RE) and digestible energy (DE) intakes for different fish species

Species	**DE equations**	**R^2**	**References**
Striped catfish	RE = - 20.5 + 0.55 DE intake	0.95	Glencross *et al*., 2011
Barramundi	RE = - 26.8 + 0.68 DE intake	0.91	Glencross *et al*., 2008
European seabass	RE = - 32.6 + 0.64 DE intake	0.99	Lupatsch *et al*., 2010

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Insights into the effect of dietary macronutrient composition on the relationship between RE and DE intake, show that the starch-rich diets limit growth in barramundi (Glencross *et al*., 2017) and tilapia (Schrama *et al*., 2012). In fact, the utilisation of energy from carbohydrates, *i.e.*, starch, differs between trout versus tilapia (Figueiredo-Silva *et al*., 2013) and grass carp versus Chinese long snout catfish (Su *et al*., 2020). These studies show the differing abilities of fish species to synthesise *de novo* fat from carbohydrates. Besides, fat can also be synthesised from dietary fat or protein. The fatness levels of different fish species fed similar amounts of energy in the form of digested protein, fat and carbohydrate consequently differ. This can also affect the relationship between RE and the intake of DE, sourced from different macronutrients. Consequently, the increasing use of carbohydrate or fat in fish diets can affect the value of DE, due to the resulting differences in the relationship between RE and DE between fish species. It is important to investigate these unknown differences in order to be able to accurately predict the dietary DE value and the growth of fish, *i.e.*, RE.

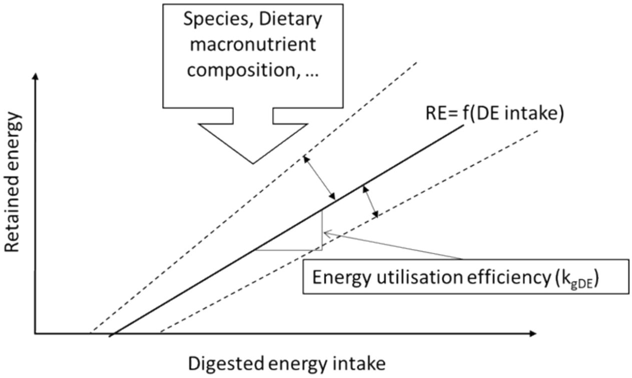

Figure 6. Variability in the relationship between RE and DE intake

Moreover, there has been a rapid expansion of new fish species in aquaculture, *e.g.,* striped catfish, yellowtail kingfish and snakehead. To formulate balanced diets for these newly cultured fish species, it is necessary to understand the energy required for maintenance and growth. However, such information is still lacking. In addition, it is unknown, but likely, that changes in the dietary macronutrient composition can affect the energy utilisation efficiency in new culture fish species, as already shown for barramundi and tilapia.

4. The future energy evaluation system for fish feed

The energy evaluation system currently used for fish feed, the DE approach, may be discontinued in the future. This is because of the increasing variability in values for both the energy demand for maintenance and the energy utilisation efficiency for growth, when a wide range of new ingredients are introduced into aqua-feed. As addressed above, the potential impact of dietary macronutrients on k_{gDE} is to reduce the accuracy of DE evaluation of aqua-feeds, when the formulation becomes more variable. Therefore, various aqua-feed producers are currently looking for an alternative energy evaluation for aqua-feeds.

Compared to fish nutrition, pig nutrition in many countries moved many decades ago from a DE approach to a NE approach, due to variability in the ingredients used in the formulation and to the related variability in macronutrient composition. Examples of such NE approaches are the French (Noblet *et al.*, 1994) and Dutch systems (CVB, 1993). Both the French and Dutch NE system are quite similar. These systems make NE (the demand for maintenance and energy gain) a function of the digested macronutrient intake (Table 2). The major difference between these systems is how they divide the digested carbohydrates into different fractions. A simplified version of the NE equations was first applied in fish using the following equation to relate RE to the digested protein, fat and carbohydrate intake (Schrama *et al.* 2018):

$$RE_i = \mu + \beta_1 \times dCP + \beta_2 \times dFat + \beta_3 \times dCarb \qquad \text{(Equation 2)}$$

where μ is the intercept, being an estimate for fasting heat production (FHP); β_1, β_2, β_3 are the energy utilisation efficiencies of dCP ($k_{NE;dCP}$), dFat ($k_{NE;dFat}$) and dCarb ($k_{NE;dCarb}$). The NE system can quantify and separate the energy utilisation efficiencies of digested macronutrients, *i.e.*, protein, fat and others (Table 2, Figure 7). The energy utilisation efficiency ($k_{g,DE}$) in the DE system becomes a function of $k_{NE;dCP}$, $k_{NE;dFat}$, and $k_{NE;dCarb}$ in the NE system. In the NE system for pigs, digested fat is used most efficiently for growth, compared to other digested macronutrients (CVB, 1993; Noblet *et al.*, 1994). In these pig NE systems the energy utilisation efficiency of digested starch ($k_{NE;dCarb}$) is higher than that of digested protein ($k_{NE;dCP}$). Carbohydrates mainly include starch and the rest fraction. Starch can be digested by either amylases or fermentation by bacteria. In the French NE system (Noblet *et al.*, 1994), the energy utilisation efficiency of carbohydrates was quantified as a function of the energy utilisation efficiencies of starch and the remaining dietary fraction being digestible (Table 2). This rest fraction is total carbohydrates minus starch (on digestible basis). In the Dutch NE system (CVB, 1993), the energy utilisation efficiency of carbohydrates was quantified as a function of the energy utilisation efficiencies of enzymatic digested starch, fermentable starch, and fermented non-starch polysaccharides (Table 2).

In fish, the energy utilisation efficiencies of digested protein, fat and carbohydrates have been quantified for tilapia and trout (Schrama *et al.*, 2018)(Table 2). In the study of Schrama *et al*. (2018), a meta-analysis was used and the fraction of carbohydrates was not separated into starch and non-starch polysaccharides. The latter is explained due to limited studies on the digestibility of non-starch polysaccharides in fish. In the linear relationship between NE and digested carbohydrates, trout and tilapia are similar in their ability to use digested carbohydrates for retaining energy (Table 2). However, a significant curvilinear relationship between digested carbohydrate and energy gain was found in trout, but not in tilapia. This indicates that the ability to use carbohydrates is limited for trout, but this is not the case in tilapia (Schrama *et al.*, 2018). The study of Schrama *et al.* (2018) showed large variations between studies in the experimental conditions, *i.e.*, water quality parameters, experimental diets and fish weight. Consequently, this may have caused the high variation in the estimated energy utilisation efficiency values for digested protein, fat and carbohydrate (respectively, $k_{NE;dCP}$, $k_{NE;dFat}$ and $k_{NE;dCarb}$). In addition, the rapid expansion of new fish species, *i.e.*, striped catfish or yellowtail kingfish, requires the validation of the NE system per species. The response of different fish species to diets may vary and affect the energy utilisation efficiencies of digested protein, fat and carbohydrate. This is true for both for new and established farmed fish species, such as striped catfish, snakehead, African catfish and carp.

Table 2. Estimated net energy equations for Nile tilapia, rainbow trout and pigs in the NE system

Species	Equation*	References
Pig	NE = 11.3 dCp + 35.0 dFat + 14.4 ST + 12.1 dRest	Noblet *et al.*, 1994
Pig	NE = 10.8 dCp + 36.1 dFat + 13.5 dST_e + 9.5 dST_f + 9.5 dNSP	CVB, 1993
Trout	NE = 15.1 dCP + 35.0 dFat + 12.1 dCarb	Schrama *et al,* 2018*.*
Trout	NE = 13.5 dCP + 33.0 dFat + 34.0 dCarb – 3.64 $(dCarb)^2$	Schrama *et al.,*2018
Tilapia	NE = 11.5 dCP + 35.8 dFat + 11.3 dCarb	Schrama *et al.* 2018

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, dgestible NSP.
*In the estimated equations for fish, 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.

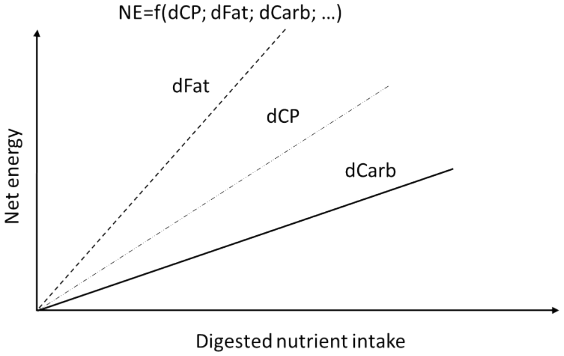

Figure 7. The relationship between net energy (NE) and digested protein (dCP), digested fat (dFat) and digested carbohydrates (dCarb).

5. A balanced diet formulation for goals other than fish growth

In addition to matching the requirements of the fish, a balanced formulation might also meet other goals, *i.e.*, low environmental impact of aquaculture, or cost-effective production. To meet the goal of low environmental impact of aquaculture, some parameters, *i.e.*, the amount of phosphorus and nitrogen excreted by the fish into the environment can be considered. To reach the goal of cost-effective production, the profitability of farming is the most important factor. To achieve the goal of profitability, the key product, *i.e.*, fish fillet, needs to be improved in terms of its quantity and quality. Fillet yield is an important quantitative standard, used to evaluate the quantity of fillets produced (Asemani *et al*., 2019; Da *et al*., 2012). Regarding the quality of the fillet, the chemical composition, *e.g*. the fat deposition, is the measurable standard as the chemical composition of the fillet can determine its texture, firmness, taste and shelf-life.

Data on the potential of digested macronutrients to contribute to fillet growth, fillet quality and/ or composition, and growth of other defined body compartments, *i.e.*, liver, viscera, and the rest fraction (head, bones and skin), can improve the quantity and quality of fillet production and mitigate filleting waste. More importantly, it might facilitate the development of an alternative feed evaluation system, considering the economic priority of fillet production. Currently, fish feed formulation is mainly focuses on defining the optimal macronutrient composition to achieve the best growth at the individual fish level. An alternative feed evaluation system, which would focus on growth or energy utilisation efficiency at the fillet level, could be a useful tool for tailor-made feed formulations, which meet the diverse expectations of the fish market, *i.e.*, the low fat vs. high fat fillet for different markets. However, information about effect of digested macronutrients on the gain and composition of the different body compartments is lacking. Insights into the effects of dietary macronutrient composition on fillet yield and chemical composition, and the location of fat deposits, are important in achieving the most efficient use of macronutrients.

6. Rationale and aims of the thesis

Fish feed formulation requires precise information about the energy needed for growth and maintenance. Currently, evaluation of ingredients and diet formulations, regarding energy, is commonly done on a DE basis. Determination of the optimal dietary DE content requires an estimation of the relationship between RE and DE (Equation 1). In this DE-approach to feed evaluation, it is assumed that the relationship between RE and DE is not affected by the dietary macronutrient composition. However, as aforementioned in some fish species, it has been shown that this is not correct. In barramundi (Glencross *et al*. 2017), trout (Rodehutscord and Pfeffer, 1999), tilapia (Schrama *et al*., 2012) and carp (Schwarz & Kirchgessner, 1995), the k_{gDE} is affected when dietary starch is replaced by dietary fat. However, for most other cultured fish species the effect of dietary macronutrient composition has not been studied. Due the increasing global demand for fish feeds, together with diversification of the ingredients used, the variability in macronutrient composition of fish diets will increase. Therefore, the first major aim of this thesis was to assess, for different fish species, whether the relationship between RE and DE is affected by dietary macronutrient content.

Analogous to the historical development of energy evaluation in pigs, it is a logical next step that fish feed formulation should move from a DE- to a NE-approach, driven by the diversification of ingredients used. The first NE equations estimated for fish were done for Nile tilapia and rainbow trout, according to equation 2 (Table 2). This first step, regarding the development of a NE evaluation for fish, indicated that the equations differed between Nile tilapia and rainbow trout. In particular, the energetic value of digested carbohydrate ($k_{NE;dCarb}$) was different. Therefore, the second major aim of the current study was to quantify NE equations for different fish species. More specifically, the energetic utilisation efficiencies of digestible protein ($k_{NE;dCP}$), digestible fat ($k_{NE;dFat}$) and digestible carbohydrate ($k_{NE;dCarb}$) for energy gain are estimated and compared between different fish species.

Digested protein, fat and carbohydrates sourced from feeds can be converted to somatic fat, although dietary protein is generally spared for protein growth as protein sources are expensive. The increasing use of ingredients high in carbohydrates for herbivorous and/or omnivorous fish, and ingredients high in fat for carnivorous fish, can also change the chemical composition and location of fat deposits in the different body compartments of fish. 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, *e.g*. being around 30% in striped catfish (Asemani *et al*., 2019; Da *et al*., 2012), African catfish (Jantrarotai *et al*., 1998) and snakehead (Tan & Azhar, 2014). This means around 70% weight of these fish species is used as by-products. If the proportion of the body compartments used as by-products, on an energy basis, can be reduced by changing the dietary macronutrient composition, this will contribute to increased energy resource use efficiency. However, only a few studies have investigated the effect of dietary composition on nutrient partitioning in 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). In addition, the rapid expansion of fish species, *e.g.*, striped catfish, African catfish and snakehead, requires novel data on the response of these fish to the diets. It is unknown whether the responses to the changes in dietary macronutrient composition in terms of the chemical composition and location of fat deposits in the different body compartments are similar or not across fish species. This study compares the influence of supplemented carbohydrates or fat on the chemical composition and location of fat deposits, in the different body compartments of different fish species.

African catfish, barramundi, carp, striped catfish and snakehead were selected to be studied in this thesis, because it is unknown whether the dietary macronutrient composition affects the relationship between RE and DE intake. In addition, the net energy equations have not been quantified for these fish species. The representative variability in feeding habits of these fish species can be used to test the effect of the “species” factor on the relationship between RE and DE intake; and the relationship between RE and intake of digested protein, fat and carbohydrates. In addition, this representative variability can clarify the transparency of the influence of supplemented carbohydrates or fat on the chemical composition and location of fat deposits in the different body compartments of African catfish, striped catfish and snakehead.

To summarise, the major aims of this thesis are:
1. To investigate the effect of dietary macronutrient composition on relationships between RE and DE for the selected fish species.
2. To quantify the net energy equations for the selected fish species.
3. To investigate whether the net energy equations are similar across fish species studied.
4. To assess the impact of dietary macronutrient composition on the chemical composition and location of fat deposits in the different body compartments of African catfish, striped catfish and snakehead.

The minor aims of this thesis are:
1. To investigate differences in the digestibility of carbohydrates between fish species
2. To study the effect of dietary starch levels on plasma glucose and triglycerides in snakehead and striped catfish.

7. The thesis outline

To fulfil the major aims, the following parameters were measured: retained energy (RE) and intake of digested protein, fat, carbohydrate and energy. Thereafter, the relationship between retained energy and digested energy (DE) intake, and the relationship between RE and the intake of digested protein, digested fat and digested carbohydrates were determined for barramundi, carp (**Chapter 2**), African catfish (**Chapter 3**), striped catfish (**Chapter 4**) and snakehead (**Chapter 5**) (Figure 8). To explain the observed differences in the utilisation of carbohydrates, the postprandial plasma glucose and triglyceride levels of striped catfish and snakehead, fed the same diets using a low *vs*. high carbohydrate diet (*i.e.*, starch inclusion level at a similar feeding rate), were studied (**Chapter 6**). In addition, the effect of increasing levels of fat and carbohydrate in the diet on the location of fat deposits in liver, viscera, fillet and the rest fraction (head, bones and skin) was studied, with the focus on fillet production. The effect of the increasing levels of carbohydrate and fat in the diet on the composition of these compartments are described in **Chapter 7**. **Chapter 8,** the general discussion, includes the overall results of this thesis, integrated with current literature, the possible practical applications for improving current fish feed formulations and suggestions for further research.

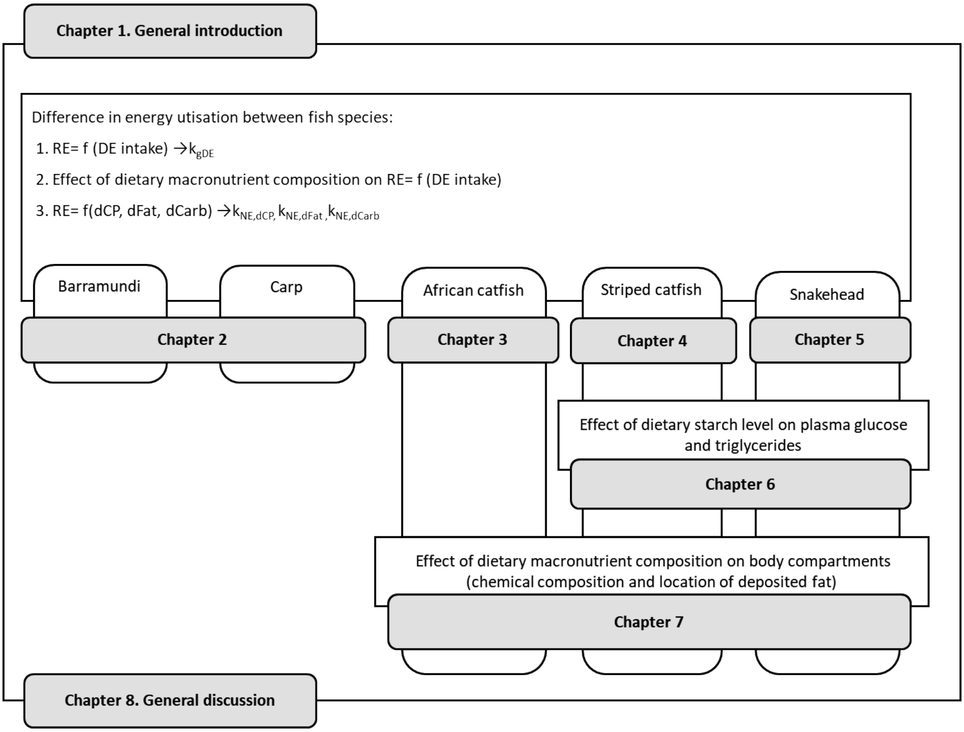

Figure 8. The thesis outline

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Differences in energy utilisation efficiencies of digestible macronutrients in common carp (*Cyprinus carpio*) and barramundi (*Lates calcarifer*)

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Abstract

This study aimed to assess macronutrient-specific energy utilisation efficiency (*i.e.*, protein, lipid and carbohydrate) for growth in common carp (an omnivorous species) and barramundi (a carnivorous species) and to assess if species-specific differences exist in energetic efficiency of digestible protein (dCP), digestible fat (dFat) and digestible carbohydrates (dCarb). This was achieved by conducting a feeding trial experiment on common carp and by re-analysing data of a recent study on barramundi. A total of four diets were formulated following a 2×2 factorial design with 2 dCP-to-dFat ratios and 2 dCP-to-dCarb ratios. For carp, 2 feeding levels were applied such that the overall experimental design was a 2×2×2 factorial design, however for barramundi, three feeding levels were applied (satiation, 80% initial satiation and 60% initial satiation), resulting in a 2×2×3 factorial design. For each fish species, multiple regression of retained energy (RE) as a function of dCP, dFat and dCarb (in $g.kg^{-0.8}.d^{-1}$) was applied to estimate the energy utilization efficiency of each digestible macronutrient. For carp, dCP, dFat and dCarb show linear relationships to RE, however for barramundi, dCP and dFat were linearly related to RE, but dCarb was curvilinearly related to RE. The estimated energetic efficiencies of dCP, dFat and dCarb (respectively, $k_{NE;dCP}$, $k_{NE;dFat}$, and $k_{NE;dCarb}$) for energy retention were 47, 86 and 60%, respectively, showing large degree of similarity with Nile tilapia and pigs. Carp and barramundi had similar $k_{NE;dFat}$ (86 *vs.* 94%), but different $k_{NE;dCP}$ (47 *vs.* 64%) and $k_{NE;dCarb}$ (60 *vs.* 18%). The net energy equations were NE = 11.2 x dCP + 34.1 x dFat + 10.4 x dCarb for carp, and NE = 15.9 x dCP + 35.2 x dFat + 9.4 x dCarb – 1.9 x $(dCarb)^2$ for barramundi.

Key words: Energy evaluation; Energy metabolism; Bioenergetics: Net energy; Energy efficiency; Digestible nutrients; *Cyprinus carpio*; *Lates calcarifer*

2.1. Introduction

Growth in fish, like other animals, requires amino acids, essential fatty acids and minerals, but also energy. Fish needs to consume energy for the accretion of fish biomass (*i.e.*, protein, fat and bone structures) and for maintenance processes. Dietary energy is provided in the form of lipids, carbohydrates and proteins. Each of these macronutrients is metabolised using different biochemical processes to yield energy (NRC, 2011). Differences in these metabolic pathways lead to distinct values of efficiency in deriving energy from digested proteins, lipids, and carbohydrates. The respective proportions of each dietary macronutrient therefore affect the overall energy efficiency of fish feeds. The effect of dietary macronutrient composition on the energy utilisation values have been shown in Nile tilapia (Schrama *et al.*, 2018), rainbow trout (Saravanan *et al*., 2012) and barramundi (Glencross *et al.*, 2014, Glencross *et al.*, 2017). Observations of the energetic utilisation of these macronutrients by these fish species for maintenance and growth, appears to be species-specific and/or trophic level-specific (*i.e.,* herbivorous, omnivorous or carnivorous fish) (Schrama *et al.*, 2012).

In the evaluation of animal feed, various systems have been used to estimate dietary energy availability after being ingested, ranging from digestible (DE) and metabolisable (ME) to net energy (NE) systems (NRC, 1981). The DE-based factorial approach has been widely applied to estimate fish dietary energy requirements (Glencross, 2006, Williams *et al.*, 2003, Williams *et al.*, 2006, Glencross, 2008, Glencross and Bermudes, 2012). In such factorial approaches (*i.e.,* DE approach), the efficiency of digestible energy utilisation for growth (k_{gDE}) is given by the regression slope of retained energy and DE intake. This k_{gDE} is assumed to be independent of feed composition and thus the composition of DE (*i.e.*, digested protein, fat and carbohydrates). However, the k_{gDE} varies in barramundi (Glencross *et al.*, 2017), Nile tilapia (Schrama *et al.*, 2012) and rainbow trout (Rodehutscord and Pfeffer, 1999) when fed diets with different macronutrient profiles. This indicates that k_{gDE} is affected by dietary macronutrient profile. Moreover, Schrama *et al.* (2012) found k_{gDE} to be correlated to species' trophic level, although this might be related to variation in dietary nutrient content. This variability in k_{gDE} demonstrates the limitation of DE feed evaluation systems. With the diversification of ingredients used in fish feeds, also the composition of the digestible macronutrient profile will become more variable. Consequently, in practical feed formulation using DE evaluations will introduce a potential bias due to this variability in k_{gDE}. Others have addressed the necessity for alternative energy evaluation of fish feeds (Glencross *et al.*, 2014, Hua *et al.*, 2010, Azevedo *et al.*, 2005). A NE approach might be such an alternative. NE evaluation has been applied for pig feed for several decades. In NE evaluation systems, each macronutrient (*i.e.*, protein, lipid and starch) has its own partial efficiency for growth, which is estimated by multiple regression between retained energy and digested protein, lipid and starch respectively (Noblet *et al.*, 1994). In other words, k_{gDE} is considered to be a function of the underlying specific energy utilization efficiencies of each type of digestible macronutrients.

The first steps to develop a NE evaluation for fish feed were recently undertaken for Nile tilapia and rainbow trout using a meta-analysis approach (Schrama *et al.*, 2018). The estimated energy utilisation efficiencies for growth of digestible protein (dCP; $k_{NE;dCP}$), digestible fat (dFat; $k_{NE;dFat}$) and digestible carbohydrate (dCarb; $k_{NE;dCarb}$) were respectively, 49%, 91%, 66% for Nile tilapia and 64%, 89% and 70% for rainbow trout (Schrama *et al.*, 2018). Digestible protein was utilised for energy retention more efficiently in rainbow trout (64%) than in Nile tilapia (49%) suggesting that the energetic efficiency of digestible protein ($k_{NE;dCP}$) is also dependent on the trophic level in the NE approach (Schrama *et al.*, 2018). Species-specific effect of individual macronutrient inclusion level was also shown by the curvilinear relationship found between retained energy and digestible carbohydrate intake in rainbow trout (Schrama *et al.*, 2018).

Aims of this study were: (1) to assess macronutrient-specific energy utilisation efficiency (*i.e.*, protein, lipid and carbohydrate) for growth in common carp (an omnivorous species) and barramundi (a carnivorous species); and (2) to assess if species-specific differences exist in energetic efficiency of dCP, dFat and dCarb. This was achieved by conducting a feed trial experiment on common carp and by re-analysing data of a recent study on barramundi (Glencross *et al*., 2017), which had the similar setup but different data analysis method from that of the study on common carp.

2.2. Materials and methods

2.2.1. Carp experiment

Experimental diets. A total of four diets were used in the carp feed trial, with different proportions of crude protein (28.5 – 52.9%), crude lipid (7.1 -25.8%) and carbohydrates (17.2 -47.1%). This large variability in dietary macronutrient composition was created using a wide range of ingredients (Table 1). Despite this large variability, diets were formulated to meet requirements for vitamins, minerals, essential fatty acids and amino acids of common carp. Despite the differing levels of dietary protein, amino acid ratios were kept constant meeting the ideal ratios based on the available knowledge (NRC, 2011). The analysed amino acid composition of the experimental diets are given in Supplementary Table S1

The triangle approach (Raubenheimer, 2011) was applied to create a wide range of macronutrient (*i.e.*, crude protein, lipid and total carbohydrates) inclusion levels in the four experimental diets (Table 1). Diets were formulated following a 2×2 factorial design with 2 dCP-to-dFat ratios and 2 dCP-to-dCarb ratios. For each diet, 2 feeding levels were applied such that the overall experimental design was a 2×2×2 factorial design with a total of 8 treatments. This design was required to achieve large contrasts in digestible macronutrient intake among the 4 diets. This facilitated multiple regression analysis of energy retention (*i.e.*, growth response) as a function of dCP, dFat and dCarb intake.

Fish handling. The experiment started December 2014. It was approved by the Ethical Committee judging Animal Experiments of Wageningen University, The Netherlands (DECnr 2014109b) and carried out according to the Dutch law on animal experiments.

A total of 840 common carps (*Cyprinus carpio)*, with a mean body weight (BW) of 28.9 g (SE 0.25) were obtained from the carp population (Strain: R3R8 F12, Breed: FxM, mixed sex) of Wageningen Aquatic Research Facility (CARUS-ARF, Wageningen, the Netherlands). The experiment was conducted at the aquatic respiration unit of Wageningen Aquatic Research Facility (CARUS-ARF, Wageningen, the Netherlands), which includes a total of twelve 200-L tanks with a water flow of 7 L/min. Water temperature was maintained at 23 ± 0.5°C and the dissolved oxygen level of inlet water ranged from 8 to 11 ppm. At the start of the experiment, groups of thirty five fish were batch-weighed and randomly assigned to one of the twelve tanks.

Carp were hand-fed one of the four diets and one of the two feeding levels of approximately 12 and 20 g.kg$^{-0.8}$.d^{-1}. Fish were fed twice daily for 28 days from 09:00 to 10:00 hours and from 16:00 to 17:00 hours. To obtain 3 replicates per treatment (*i.e.*, 24 tanks in total), 2 consecutive trials were run in the 12 tanks aquatic respiration unit under identical conditions.

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

	Diet 1	Diet 2	Diet 3	Diet 4
	"P"	"P"	"P"	"P"
		"+ C"	"+ F"	"+C+F"
Diet formulation (g.kg^{-1}, as-is)				
Gelatinised Maize Starch	-	342.9	-	300
Oil blend*	-	-	178.6	125
Fish meal (CP>680)	156.5	102.9	128.6	90
Wheat gluten	156.5	102.9	128.6	90
Pea protein concentrate	156.5	102.9	128.6	90
Soya protein concentrate	156.5	102.9	128.6	90
Wheat	234.8	154.3	192.9	135
Fish oil	34.8	22.9	28.6	20
Soy oil	34.8	22.9	28.6	20
Monocalciumphosphate	34.8	22.9	28.6	20
Lime ($CaCO_3$)	8.7	5.7	7.1	5
L-Lysine sulphate	3.68	1.79	3.00	2.05
DL-Methionine	4.69	2.89	3.92	2.90
Premix	17.4	11.4	14.3	10
Yttrium oxide	0.2	0.2	0.2	0.2
Nutrient composition (g.kg^{-1} DM)				
DM	926	887	940	918
Crude protein	529	339	430	285
Digestible protein	506	319	412	273
Total fat	110	71	258	186
Digestible fat	103	65	231	168
Total carbohydrates	265	528	234	474
Digestible carbohydrates	191	471	172	428
Total starch	152	330	126	391
Digestible starch	149	329	125	390
Gross energy (kJ.g^{-1} DM)	21.20	19.64	24.92	22.39
Digestible energy (kJ.g^{-1} DM)	19.06	17.92	22.24	20.46
Ash	96	62.4	78.7	54.8
Phosphorus (total)	16.8	10.9	13.5	9.3

P, protein; C, carbohydrates; F, fat.

Diet 1, high protein diet; Diet 2, supplemental starch diet; Diet 3, supplemental fat diet; Diet 4, supplemental starch and fat diet; Carb, Carbohydrates. DM, dry matter.

*Equal amount of rapeseed, soya and palm oil.

Sample preparation and chemical analysis. At the beginning of each trial, ten fish from the initial population were euthanized by overdose of 2-phenoxyethanol for the analysis of initial body composition. At the end of each trial, ten fish from each tank were euthanized in the same way to determine final body composition. The fish were then frozen at -20°C. The samples were prepared for chemical analysis according to the methods reported by Saravanan *et al.* (2012).

After sample collection, fish were sawn into slices and minced to ensure sample homogeneity. Fresh fish samples were used for dry matter (DM), ash and crude protein (CP) analysis whereas fish samples for fat and gross energy (GE) analyses were first freeze dried. Diet and oven-dried (70 °C) faecal samples were analysed for DM, yttrium, Ca, P, CP, fat, starch and gross energy contents.

Proximate composition of fish, feed and faeces were determined according to ISO-standard analysis for determination of dry matter (DM; ISO 6496, 1983), crude ash (ISO 5984, 1978), crude fat (ISO 6492, 1999), crude protein (ISO 5983, 1997, crude protein = Kjeldahl-N × 6.25), energy (ISO 9831,1998), and starch (NEN/ISO 15914) (Meriac *et al.*, 2014). Total carbohydrates content of feed and faeces was calculated as DM minus crude protein minus crude ash minus crude fat.

Nutrient digestibility measurement. Yttrium oxide was added as an inert marker to experimental diets at 0.02% (as-is). Each of the twelve tanks was connected to a separate faeces settling unit. 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:

$$ADC_{nutrient} = (1- (Y_{diet}/ Y_{faeces}) \times (Nutrient_{faeces}/Nutrient_{diet})) \times 100\%,$$

where Y_{diet} and Y_{faeces} are the yttrium oxide concentration of the diet and faeces, respectively, and $Nutrient_{diet}$ and $Nutrient_{faeces}$ are the DM, protein, fat, carbohydrates or energy content of diet and faeces, respectively.

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 $BW/1000)^{0.8}$)). The calculations of energy and nitrogen balances were based on those described by Saravanan *et al.* (2012). Intake of each nutrient on a gross basis was determined by multiplying the averaged feed intake for each treatment by the nutrient inclusion level in the diet. Digestible nutrient intake was determined by multiplying gross nutrient intake with the diet-specific nutrient digestibility coefficient. Energy and nutrient retention rates 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 N and N retention. Branchial and urinary energy (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-N is the only form of this excretion (Bureau *et al.*, 2003). Metabolisable energy intake was determined by difference between digestible energy intake and BUE. Heat production was measured by deducting ME from retained energy.

2.2.2. Barramundi data set

The barramundi data set was derived from a study by Glencross *et al.* (2017), which assessed the impact of dietary macronutrient composition on energy, nitrogen and fat balances in juvenile barramundi weighing 69.6 g (SD 0.75). The unit and measurement of body weight, digestible macronutrient intake, nitrogen and energy balance parameters in the barramundi dataset are identical to those employed in the analysis of the carp experiment. The design of the barramundi study was similar to the study on carp, where four diets were formulated having contrasting protein, fat and starch levels. However, in the barramundi study, three feeding levels were applied (satiation, 80% initial satiation and 60% initial satiation), resulting in a 2x2×3 factorial design. All treatments were duplicated using 24 tanks (Glencross *et al.*, 2017). Digestibility measurements were based on faeces collected after the growth experiment. Only fish fed to satiation during the growth experiment (8 tanks) were used to collect faeces. Faecal collection was conducted by manual stripping once a day at about 6 hours post-feeding (Glencross *et al.*, 2017). Since crude ash content was not measured in faeces, the carbohydrates content of both feed and faeces was calculated from the measured energy, crude protein and crude fat content as described by Schrama *et al.* (2018). In this calculation, 23.6, 39.5 and 17.2 $kJ.g^{-1}$ were used as the combustible energy content of CP, fat and carbohydrates, respectively (NRC, 2011).

2.2.3. Data analysis

Statistical analysis systems (SAS Institute) statistical software package version 9.1 was used to conduct data analysis. For carp, the effect of diet, feeding level and their interaction on digestibility, growth performance, nitrogen and energy balances data were tested by two-way ANOVA. For the barramundi dataset, no ANOVA analyses were done as the data are published elsewhere (Glencross *et al.*, 2017).

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

$$RE_i = \mu + \beta_1 \times dCP_i + \beta_2 \times dFat_i + \beta_3 \times dCarb_i + e_i \qquad \text{(Equation 1)}$$

where μ isthe intercept, being an estimate for fasting heat production (FHP); β_1, β_2, β_3 are the energy utilisation efficiencies of dCP ($k_{NE;dCP}$), dFat ($k_{NE;dFat}$) and dCarb ($k_{NE;dCarb}$); e_i is error term and i =1,..., n with n = 24 for both carp and barramundi data. The linearity and curve-linearity were checked in the relationship of RE with dCP, dFat and dCarb. Analyses were implemented separately for each species. To assess differences in β_1, β_2 and β_3 (*i.e.*, $k_{NE;dCP}$, $k_{NE;dFat}$, $k_{NE;dCarb}$) between carp and barramundi, a combined mixed model was used with the inclusion of a fixed effect of species and 2-way interaction of species with each type of digestible macronutrient intake (dCP, dFat or dCarb). Significance was set at $P<0.05$.

2.3. Results

2.3.1. Carp experiment

Overall growth performance was good in the carp experiment with daily weight gain ranging from 9.9 to 22.4 g.kg$^{-0.8}$.d^{-1} (Supplementary Table S2). Both feeding level and diet significantly (P<0.001) influenced daily digestible nutrient intake (Table 2 and Supplementary table S3) and ultimately final BW and daily body weight gain (Supplementary Table S2). Information on the impact of feeding level and diet on N balance parameters and body composition is given in Supplementary Table S4 and S5.

Table 2. Digestible nutrient intake and energy balance of carp (n =3) and barramundi (n=2), fed 4 different diets (mean values and standard deviations)

	Carp				Barramundi			
Variables	Mean	SD	Min	Max	Mean	SD	Min	Max
Digestible nutrient intake (g.kg$^{-0.8}$.d^{-1})								
dCP	4.95	1.56	2.82	7.96	5.08	2.56	2.59	9.63
dFat	1.86	0.93	0.66	3.59	1.12	0.65	0.39	2.60
dCarb	4.22	2.24	1.71	7.88	0.94	1.15	0.04	4.16
Energy balance parameters (kJ.kg$^{-0.8}$.d^{-1})								
GE intake	291	71	204	399	224	113	120	407
DE intake	263	62	186	352	180	90	98	328
BUE losses	11.4	3.8	6.4	19.2	9.9	4.8		19.3
ME intake	251	60	178	341	170	86	92	309
Heat production	111	21	86	139	67	28	43	137
Energy retention (total)	140	41	86	207	103	61	45	209
Energy retention as protein	49	15	27	79	61	33	29	115
Energy retention as fat	91	35	37	152	44	29	12	98
FRE	1.27	0.5	0.77	2.35	0.94	0.31	0.45	1.59

Min, minimum; Max, maximum; dCP, digestible protein intake; dFat, digestible fat intake; dCarb, digestible carbohydrates intake; GE, gross energy; DE, digestible energy; BUE, branchial urinary energy; ME, metabolisable energy; FRE, Fat retention efficiency (% of digestible intake).

Overall average ADCs (*i.e.*, regardless of treatment) were 86% for DM, 90% for energy, 95% for CP, 91% for fat, 81% for carbohydrates, 99% for starch, 49% for NSP and 33% for ash. The carbohydrates fraction showed the largest between-diets variability with ADCs ranging from 68 to 91% in diets 1 (high FL) and 4 (low FL) respectively. Averaged over both feeding levels, protein ADC was lowest for the 2 carbohydrates-supplemented diets (Diet 2 and 4), while fat ADC was lowest for the 2 fat-supplemented diets (Diet 3 and 4). Total carbohydrates ADC was highest in the 2 carbohydrates-supplemented diets (Diet 2 and 4) (Table 3). Except for ash and total phosphorus, all nutrient ADCs were affected by both diet, feeding level and their interaction (P<0.001; Table 3). Overall, nutrients ADC declined when the feeding level was raised. However, the significant interaction effect between diet and feeding level indicated that this decline with feeding level differed between diets. The decline in protein, total carbohydrates and starch ADC with feeding level was largest for Diet 1 (high protein content, no starch neither fat supplementation). In contrast, the decline in fat ADC with feeding level was largest for Diet 3 (fat-supplemented diet) (Table 3).

2.3.2. NE equations

The main aim of this paper was to assess differences in energy utilisation efficiency for growth (*i.e.*, quantifying NE equations) among digested macronutrients (*i.e.*, protein, lipid and carbohydrates). Energy and nitrogen balances were calculated based on digestible nutrient intake (dCP, dFat and dCarb) for both the carp and barramundi experiments. Energy and nitrogen balances are reported for the carp experiment in Supplementary table 2 and 3 respectively. These were reported by Glencross *et al.* (2017) for the barramundi dataset. The mean as well as the range of dCP and dFat daily intake (in g.kg$^{-0.8}$.d^{-1}) were comparable between carp and barramundi. However, the mean daily dCarb intake was much lower in barramundi compared to carp (0.94 *vs.* 4.22 g.kg$^{-0.8}$.d^{-1}). For both species, the large variability in digestible nutrient intake resulted in a large range in energy retention (RE). RE ranged from 86 to 207 kJ.kg$^{-0.8}$.d^{-1} in carp and from 45 to 209 kJ.kg$^{-0.8}$.d^{-1} in barramundi (Table 2).

The relationship between DE intake and RE in carp is given in Supplementary Figure S1 and by Glencross *et al.* (2017) for barramundi. The average energy utilisation efficiency of DE for growth (k_{gDE}) was lower in carp (0.62), ranging from 0.59 to 0.66 than in barramundi (0.68), ranging from 0.55 to 0.79 (Supplementary Fig S1).

Multiple linear regression of RE (*i.e.*, NE) as a function of dCP, dFat and dCarb yielded the following equations for carp:

$$RE = -22\ (SE\ 5) + 11.2\ (SE\ 0.8)\ dCP + 34.1\ (SE\ 1.2)\ dFat + 10.4\ (SE\ 0.5)\ dCarb \quad \text{(Equation 2)}$$

and for barramundi:

$$RE = -18\ (SE\ 3) + 15.2\ (SE\ 0.9)\ dCP + 37.1\ (SE\ 3.4)\ dFat + 3.1\ (SE\ 1.2)\ dCarb \quad \text{(Equation 3).}$$

The energetic utilisation efficiencies of dCP, dFat and dCarb (respectively, $k_{NE;dCP}$, $k_{NE;dFat}$, and $k_{NE;dCarb}$) were 15.2 kJ.g^{-1} (64%), 37.1 kJ.g^{-1} (94%) and 3.1 kJ.g^{-1} (18%) for barramundi and 11.2 kJ.g^{-1} (47%), 34.1 kJ.g^{-1} (86%), 10.4 kJ.g^{-1} (60%) for carp, respectively. Barramundi had a 36% higher energetic utilization efficiency of dCP for growth than carp (P=0.002). Conversely, carp had a 235% higher energetic utilization efficiency of dCarb for growth than barramundi (P<0.001). The energetic utilisation efficiency of dFat ($k_{NE;dFat}$) did not differ between the two species (P>0.05).

In carp, dCP, dFat and dCarb were all linearly related to RE (*i.e.*, no significant polynomial effect, P>0.05). Conversely, in barramundi, a significant quadratic component was found for dCarb (P<0.01), but not for dCP and dFat. Inclusion of the quadratic component for dCarb for barramundi resulted in the following relationship between RE and digestible nutrient intake:

$$RE = -22\ (SE\ 3) + 15.9\ (SE\ 0.87)\ dCP + 35.2\ (SE\ 3.11)\ dFat + 9.4\ (SE\ 2.71)\ dCarb - 1.9\ (SE\ 0.74)\ (dCarb)^2 \quad \text{(Equation 4)}$$

Inclusion of the quadratic component for dCarb into the equation had only a minor impact on the absolute values of $k_{NE;dCP}$ and $k_{NE;dFat}$ (Equation 3 and 4; Table 4).

Table 3. Apparent digestibility coefficient (ADC) (%) of dietary nutrients in carp (n=3) fed 4 diets (D) at 2 feeding levels (FL) over 28 days

		Diet 1	Diet 2	Diet 3	Diet 4		*P* values		
Nutrient	FL	"P"	"P"	"P"	"P"	SEM	D	FL	D x FL
			+C	+F	+C+F				
DM									
	Low	85.2^{bcd}	88.0^{ab}	84.7^{cd}	89.0^{a}	0.63	***	***	***
	High	81.4^{e}	87.6^{abc}	83.3^{de}	88.6^{a}				
Energy									
	Low	91.7^{a}	91.6^{a}	90.2^{ab}	91.7^{a}	0.42	***	***	***
	High	88.1^{c}	90.9^{a}	88.2^{bc}	91.0^{a}				
Protein									
	Low	96.4^{a}	94.3^{cd}	96.3^{a}	95.5^{abc}	0.26	***	***	***
	High	94.7^{bcd}	94^{d}	95.3^{abc}	95.7^{ab}				
Fat									
	Low	95.1^{a}	92.2^{ab}	91.5^{bc}	91.4^{bc}	0.62	***	***	***
	High	92.9^{ab}	91.3^{bc}	87.8^{d}	88.8^{cd}				
Carb									
	Low	76.4^{b}	89.7^{a}	73.6^{b}	90.5^{a}	0.94	***	***	***
	High	67.9^{c}	88.8^{a}	73.2^{b}	90.3^{a}				
Starch									
	Low	99.6^{a}	99.7^{a}	99.5^{a}	99.9^{a}	0.26	***	***	***
	High	96.7^{b}	99.3^{a}	98.6^{a}	99.8^{a}				
NSP									
	Low	45.3^{b}	73.0^{a}	43.5^{bc}	45.8^{b}	2.99	***	*	*
	High	29.3^{c}	71.1^{a}	43.6^{bc}	45.3^{b}				
Ash									
	Low	36.6	33.8	31.6	33.9	2.63	ns	ns	ns
	High	32.0	38.2	32.8	36.3				
Phosphorus									
	Low	47.5	46.3	48.5	49.8	2.20	ns	ns	ns
	High	42.9	49.8	49.5	51.7				

Diet 1, high protein (P) diet; Diet 2, supplemental starch (C) diet; Diet 3, supplemental fat (F) diet; Diet 4, supplemental starch and fat diet; carb, carbohydrates; DM, dry matter; NSP, non-starch polysaccharides

***, $P<0.01$; *, $P<0.05$; ns, non-significantly different

^{abc}If interaction effect is significant, means lacking a common superscript differ significantly ($P<0.05$)

Table 4. Estimated net energy equations in common carp, barramundi, Nile tilapia, rainbow trout and pigs

Species	Equation*	R^2	Equation
Carp	$NE = 11.2\ dCP + 34.1\ dFat + 10.4\ dCarb$	0.99	(2)
Barramundi	$NE = 15.2\ dCP + 37.1\ dFat + 3.1\ dCarb$	0.99	(3)
Barramundi	$NE = 15.9\ dCP + 35.2\ dFat + 9.4\ dCarb - 1.9\ (dCarb)^2$	0.99	(4)
Trout	$NE = 15.1\ dCP + 35.0\ dFat + 12.1\ dCarb$	0.91	(5)
Trout	$NE = 13.5\ dCP + 33.0\ dFat + 34.0\ dCarb - 3.64\ (dCarb)^2$	0.92	(6)
Tilapia	$NE = 11.5\ dCP + 35.8\ dFat + 11.3\ dCarb$	0.99	(7)
Pig	$NE = 11.3\ dCp + 35.0\ dFat + 14.4\ ST + 12.1\ dRest$		(8)
Pig	$NE = 10.8\ dCp + 36.1\ dFat + 13.5\ dST_e + 9.5\ dST_f + 9.5\ dNSP$		(9)

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, dgestible 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. Equation 2,3,4 (Present study), Equation 5, 6, 7 (Schrama *et al*., 2018), Equation 8 (Noblet *et al*., 1994), Equation 9 (CVB, 1993).

In Figure 1, the relationship between NE (corrected to zero dCP and dFat intake) and dCarb intake is given. For barramundi, inclusion of dCarb into the diet raised NE, but the increase in NE started to level off when dCarb intakes reached about 1.5 to 2.0 $g.kg^{-0.8}.d^{-1}$. For common carp, NE increased linearly over the full range of increasing dCarb from 2 to 8 $g.kg^{-0.8}.d^{-1}$. The linear relationships between NE and dFat and between NE and dCP in both common carp and barramundi are shown in Supplementary Figure S2.

2.4. Discussion

Feed formulation in aquaculture is currently based on the energy requirements of fish species and requires information on (1) nutrient digestibility of ingredients, (2) energy requirements for maintenance and (3) utilization efficiency of digestible energy (DE) or metabolizable energy (ME) for growth (k_{gDE} and k_{gME} respectively). Factors which can influence the evaluation of dietary energy are environmental conditions, choices of ingredients, nutrient digestibility and utilisation of digested nutrients. In this study, environmental conditions were identical across treatments for each species, therefore, this did not affect the feed evaluation.

The nutrient digestibility of raw materials are commonly used in feed formulation, assuming that these are additive. However, all macronutrient ADCs observed in the carp study (except ash and phosphorus) were affected by the interaction effect between diet and feeding level. This suggests that respective ingredients’ nutrient ADCs are not additive, which is in contrast to Cho and Kaushik (1990). This is possibly due to the higher quality of fish diets used in the past (*e.g.,* fishmeal-rich), which may have diminished the effects of feeding level on nutrient ADCs.

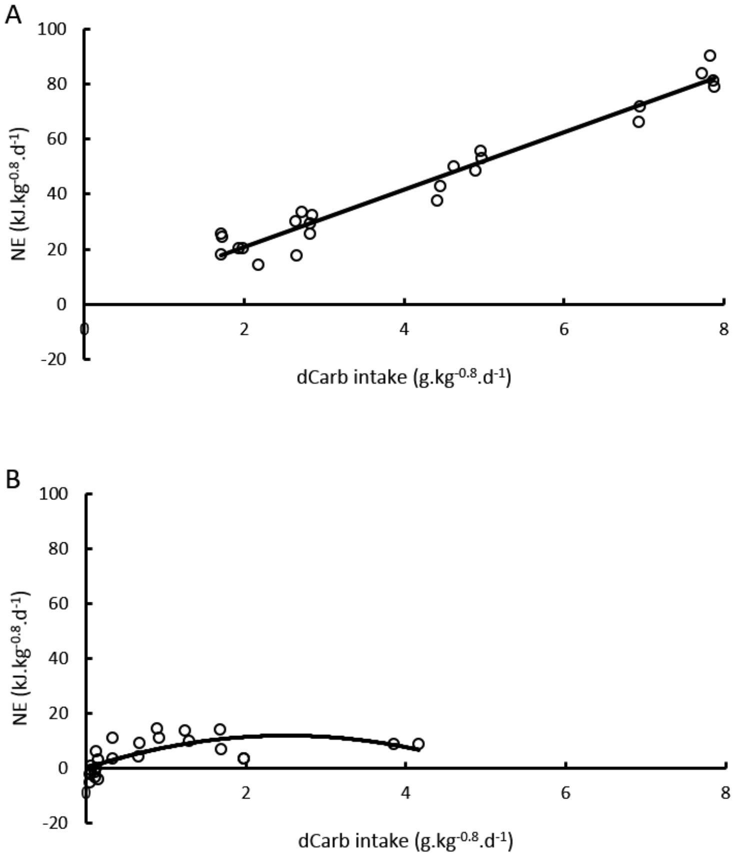

Figure 1. Relationship between net energy (NE) and digestible carbohydrates (dCarb) intake for carp (a) and barramundi (b). The NE values are corrected for variation in digestible protein (dCP) and digestible fat (dFat). This was performed as follows: the measured retained energy value for each data point in the data set was increased with the estimated fasting heat production to obtain the NE value, which was then corrected towards zero dCP and dFat intake in order to have only the effect of dCarb on NE. This was conducted using Equation (2) for Carp and Equation (4) for barramundi (Table 4).

The protein-rich diet (Diet 1) for carp was diluted with maize starch and/or a vegetable oil blend, both containing minimal protein. If ingredient nutrient ADC were additive, the ADC of protein in all 4 diets would be equal. However, ADC of protein differed among diets (Table 3). At the low feeding level, the negative effect of dietary carbohydrate level on ADC of protein is in line with a previous study (Takeuchi *et al.*, 1979). This was also found in African catfish possibly due to the increased chyme viscosity in the stomach (Harter *et al.*, 2015). Averaged over all diets, raising feeding level declined the protein ADC in carp (Table 3), which was in agreement with studies on African catfish (Henken *et al.*, 1985) and Nile tilapia (Haidar *et al.*, 2016). Decreasing dietary protein significantly increased protein digestibility only at the high feeding level, which was also observed at high feeding levels on mirror carp (Ufodike and Matty, 1983). In the current study, the largest protein ADC decline in the protein-rich diet may reflect an upper limit for protein digestion but may also be due to a larger fraction of the endogenous faecal nitrogen loss.

In the present study, the negative effect of dietary fat level on fat digestibility (Table 3) is in agreement with a previous finding on common carp (Yamamoto *et al.*, 2007). However it is in contrast to previous findings in various fish species such as Nile tilapia (Schrama *et al.*, 2012), African catfish (Harter *et al.*, 2015) and Atlantic salmon (Bendiksen *et al.*, 2003). This might reflect a lower capacity for fat digestion in common carp compared to other fish species. The lowered fat digestibility observed at the high feeding level in the present study, especially in fat-rich diet, indicating carp maximal fat digestion capacity reached.

Increasing crude fibre to the carbohydrate fraction reduced total carbohydrates digestibility while increasing the starch contribution leads to the opposite (Kirchgessner *et al.*, 1986). This is illustrated by the low NSP and high starch digestibility observed in the present study. Digestibility of NSP was increased by the addition of starch to Diet 2, but not by the addition of both starch and lipids to Diet 4. Digestion of NSP is assumed to result mainly from intestinal bacterial fermentation, as was suggested for Nile tilapia (Haidar *et al.*, 2016). The high digestibility of NSP in starch-rich diet (Diet 2) was thus most likely caused by a higher intestinal fermentation activity (Yamamoto *et al.*, 2007). However, NSP ADC did not improve in Diet 4 rich in starch and fat. Dietary fat inhibits intestinal microbial activities, which could have led to reduced NSP digestibility (Heinritz *et al.*, 2016).

The utilization efficiency of DE or ME for growth (k_{gDE} and k_{gME}) are commonly based on linear regression of RE with DE or ME intake (*i.e.*, the slope of the linear regression). This approach does not account for the possible effect of dietary macronutrient composition on k_{gDE} and k_{gME}. In NE approach (i.e., multiple regression between RE and intake of digested macronutrients including dCP, dFat, dCarb), a differentiation in energetic utilization efficiency of digested protein ($k_{\mathrm{NE,dCP}}$), fat ($k_{\mathrm{NE,dFat}}$) and carbohydrates ($k_{\mathrm{NE,dCarb}}$) is made. In the current study, such a NE approach was applied in two experiments with common carp and barramundi. The large contrast among digestible nutrients intake created in both experiments (Table 2) facilitated the multiple regression between RE and dCP, dFat and dCarb intake. This allowed to assess energetic utilisation efficiencies for growth for each type of digested macronutrient (respectively, $k_{\mathrm{NE;dCP}}$, $k_{\mathrm{NE;dFat}}$, and $k_{\mathrm{NE;dCarb}}$). In Table 4, a species comparison of NE formulas (*i.e.*, $k_{\mathrm{NE;dCP}}$, $k_{\mathrm{NE;dFat}}$, and $k_{\mathrm{NE;dCarb}}$) is made among barramundi and carp (this study), Nile tilapia and trout (Schrama *et al.*, 2018) and pigs (CVB, 1993; Noblet *et al.*, 1994). Table 4 shows that for all fish species studied, of all the nutrients digested, fat has the highest energetic efficiency ($k_{\mathrm{NE;dFat}}$), in line with results obtained for pigs. When considering the linear regression equations only, $k_{\mathrm{NE;dFat}}$ ranged from 86 to 94%, while $k_{\mathrm{NE;dCP}}$ ranged from 46 to 64% and $k_{\mathrm{NE;dCarb}}$ from 18 to 84%. This also shows that the variability in energetic efficiency was lowest for dFat and highest for dCarb. The lowest $k_{\mathrm{NE;dFat}}$ was observed for carp (this study), which is in line with the general statement that carp are less able to utilise dietary fat (NRC, 2011). However, although lower, the energetic efficiency of fat did not decrease with increasing fat intake (linear relationship between NE and dFat (Supplementary Figure S2). Even at high dietary fat levels (> 28%, Diet 3 and 4), RE was not reduced with increased dFat intake. This suggests that carps are not less able to handle fat at high dietary levels but that their overall efficiency of fat utilisation is lower than that of other fish species. However, the smaller variability in $k_{\mathrm{NE;dFat}}$ between fish species (Table 4) seems to demonstrate that the energetic efficiency of dFat is more conserved among fish species than that of dCP and dCarb.

Schrama *et al.* (2018) reported that the estimated energetic efficiency of protein ($k_{NE;dCP}$) in rainbow trout was reduced when the quadratic component of dCarb was included in the multiple regression analysis. The estimated $k_{NE;dCP}$ was then closer to that observed in Nile tilapia and pigs. In the current study, the relation of dCarb with NE was curvilinear for barramundi. Inclusion of the quadratic component in the NE formula (Equation 3 and 4; Table 4) for barramundi did not strongly affect the estimated $k_{NE;dFat}$ (35.2 *vs.* 37.1 kJ.g^{-1}) and $k_{NE, dCP}$ (15.9 *vs.* 15.2 kJ.g^{-1}) . This absence of inference is probably due to the low value of $k_{NE;dCarb}$ and the relatively low dCarb intake. When including the quadratic component into the equation for both barramundi and trout, it appears that the estimated $k_{NE, dCP}$ is higher than the estimates for $k_{NE;dCP}$ in common carp and Nile tilapia. This suggests that the energetic efficiency of digested protein might be different between fish species. This might suggest a higher $k_{NE;dCP}$ for fish having a higher trophic level (barramundi and trout *vs.* common carp and Nile tilapia). Among carnivorous species, there seems to be a difference in $k_{NE;dCP}$ as the efficiency was higher for barramundi than for rainbow trout (64 *vs.* 57%). This difference could be related to differences in glucose tolerance among carnivorous fish. Barramundi appeared to be less capable of handling hyperglycaemia than rainbow trout (Palmer and Ryman, 1972, Stone, 2003), although both of them are glucose intolerant. The limited capacity of barramundi to handle digested dietary glucose is confirmed by the extremely lower $k_{NE;dCarb}$, compared to other fish species (carp, trout, tilapia) and pigs (Table 4). This may cause protein and lipid to be used more efficiently by barramundi to compensate for the low energetic efficiency of carbohydrate in this study. Estimation of NE equations in other carnivorous fish species with low glucose tolerance would help better understanding the potential influence that glucose tolerance has on the estimation of $k_{NE;dCP}$ in carnivorous fish.

The linearity in the pig NE evaluation system facilitates estimation of diets NE value since feeding level does not affect the energetic efficiency of macronutrients. This also applies to evaluation of dietary NE values for Nile tilapia (Schrama *et al.*, 2018) and carp (present study). Conversely, estimation of diet NE value for barramundi is feeding level-dependent because of the curvilinear relation observed between dCarb intake and diet NE value. Curvilinearity in relationship between retained energy and dCarb shows that raising carbohydrate intake decreases the potential retention of energy (*i.e.*, NE value of diet), which illustrates that carnivorous fish have difficulties to handle carbohydrates (Glencross *et al.*, 2017). The potential use of carbohydrates, of which only sugars and starch are nutritionally available to fish (Stone, 2003, Kaushik, 2001), is dependent on the key enzymes involved in digesting starch, metabolising (Enes *et al.*, 2009, Krogdahl *et al.*, 2005) and transporting glucose (Krasnov *et al.*, 2001, Planas *et al.*, 2000, Teerijoki *et al.*, 2000) and inducible glucokinase (Panserat *et al.*, 2001a). Fish can efficiently absorb starch-derived glucose through the intestine (Furuichi and Yone, 1981). As a consequence of increasing dCarb intake, glucose levels increase in the blood stream in most species (Furuichi and Yone, 1981, Bergot, 1979a). If carbohydrate utilisation efficiency is assessed based on the rate of glucose distribution from absorption in digestive system to clearance in blood stream, carnivorous fish seems to be poor carbohydrate users (NRC, 2011). The rate of delivering glucose, its peak concentration in blood and clearing rate depend on species as well as carbohydrate sources and dietary inclusion levels (Stone, 2003, Bergot, 1979b, Hemre and Hansen, 1998, Wilson and Poe, 1987). In vertebrates, the role of liver in monitoring glucose homeostasis by being both consumer and producer of glucose is important. Several enzymes can be either turned on or off to dispose glucose, synthesize glycogen and lipid from glucose when the blood glucose pool increases, or to initiate de novo glucose synthesis and release glucose from glycogen when blood glucose decreases in order to meet fish glucose demand (Kamalam *et al.*, 2017). In fish, when blood glucose levels are over the threshold of glycaemia, glucose is released through urine and gills (Deng *et al.*, 2001, Hemre and Kahrs, 1997). Therefore, blood glucose concentration is dependent on the glucose flux as a result of producing and removing glucose simultaneously (Pilkis and Granner, 1992, Postic *et al.*, 2004). In carnivorous species, like rainbow trout, the liver is not able to downregulate the production of glucose in response to high dietary carbohydrates levels (Panserat *et al.*, 2001b). This contrasts with herbivorous and omnivorous species like carp and seabream (Panserat *et al.*, 2002). In the present study, carp also did not indicate any problems to handle dCarb. Barramundi on the other hand seems to be unable to handle any excess amount of digestible carbohydrate over 1.5 – 2.0 $g.kg^{-0.8}.d^{-1}$, which is lower than that of rainbow trout (3.0 – 3.5 $g.kg^{-0.8}.d^{-1}$) (Schrama *et al.*, 2018). This can be because the peak concentration of blood glucose when challenged with glucose input is lower in barramundi than in rainbow trout, indicating that barramundi is less tolerant to glucose than rainbow trout (Stone, 2003, Legate *et al.*, 2001). This observation validates that carnivorous fish have difficulties to handle carbohydrate-rich feeds. Carbohydrates were used less efficiently in carp (60%) than in tilapia (66%) (Schrama *et al.*, 2018). These $k_{NE,dCarb}$ were lower than that of dStarch in pigs using either French NE approach (84%) (Noblet *et al.*, 1994) or Dutch NE approach (78%) (CVB, 1993).

By using DE approach, the energy utilisation efficiency of barramundi and carp were determined based on the slope in the linear regression of RE as a function of DE intake. Though variations appeared in the slopes among diets between two species (Supplementary Fig S1) partly because of the diversification in protein, lipid and carbohydrates sources included in the feed formulation, the DE approach (*i.e.,* factorial approach) is unable to specify the differences and quantify energetic efficiency values of digested protein ($k_{NE,dCP}$), fat ($k_{NE,dFat}$) and carbohydrates ($k_{NE,dCarb}$). By using the NE approach (*i.e.*, the multiple regression between RE and digested macronutrients), these values however can be assessed for each species and significant differences in the energy utilisation efficiency of digested protein and carbohydrates between these two species can be clarified.

2.5. Conclusion

This study proves that the dietary energetic utilisation efficiency of fish is affected by the relative composition of dietary digestible macronutrients, which are dCP, dFat and dCarb. This effect on the energetic utilisation efficiency was distinct between carp and barramundi. For carp, dCP, dFat and dCarb show linear relationships to the energy retention. The estimated energetic efficiencies of dCP, dFat and dCarb for energy retention were 47, 86 and 60%, respectively, showing large degree of similarity with Nile tilapia and pigs. However, for barramundi, dCP and dFat were linearly related to NE, but dCarb was curvilinearly related to NE. Increasing dCarb intake results in an inflexion of dietary NE towards a plateau, illustrating the limited capacity of barramundi, a carnivorous, glucose-intolerant fish, to handle dietary starch/glucose. In this study, NE equations for carp and barramundi c were estimated to predict the potential for energy retention of diets/ingredients. The linearity in relationship between RE and intake of dCP, dFat and dCarb in carp implies that assessing the feed NE value for carp is applicable, regardless of feed intake. Conversely, the curvilinear relationship found between dCarb and NE in barramundi indicates that barramundi diet NE value depends on daily carbohydrate intake. Therefore, NE evaluation of barramundi feeds requires estimates of the feed intake, dietary carbohydrate content and digestibility.

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Supplementary data

Supplementary Table S1. Amino acid composition of diets for carp (*C. carpio*), fed 4 different diets at 2 feeding levels (FL) over 28 days

	Diet 1	Diet 2	Diet 3	Diet 4
	“protein”	“protein”	“protein”	“protein”
		+carb	+fat	+carb + fat
g.kg^{-1} Crude Protein				
Lys	55.8	53.6	56.2	56.1
Met	26.2	25.5	26.4	26.4
Met+Cys	40.2	39.6	40.9	40.6
Cys	14.0	14.1	14.5	14.2
Thr	34.4	33.6	34.8	32.8
Arg	61.2	60.0	61.9	58.7
Ile	41.5	41.0	42.5	39.8
Leu	74.1	72.7	75.2	71.2
Val	45.2	44.9	46.3	43.6
His	22.7	22.4	22.8	22.1
Phe	48.4	47.8	49.2	46.9
Gly	45.0	44.0	45.6	43.3
Ser	48.2	46.6	48.5	46.1
Pro	65.5	64.2	67.3	64.3
Ala	42.7	41.6	43.2	40.8
Asp	86.4	85.0	87.4	83.1
Glu	218.4	213.6	222.0	211.1

Diet 1, high protein diet; Diet 2, supplemental starch diet; Diet 3, supplemental fat diet; Diet 4, supplemental starch and fat diet; carb, carbohydrates.

Supplementary Table S2. Growth performance of carp (*C. carpio*) (n = 3), fed 4 different diets (D) at 2 feeding levels (FL) over 28 days

		Diet 1	Diet 2	Diet 3	Diet 4				
		“P”	“P”	“P”	“P”			*P* values	
	FL		+C	+F	+C+F	SEM	D	FL	D x FL
Initial BW (g)									
	Low	29.1	29.8	29.2	28.9	0.25	ns	***	ns
	High	28.8	28.7	28.8	28.2				
Final BW (g)									
	Low	60.3^{e}	51.9^{f}	59.9^{e}	49.5^{f}	1.08	***	***	***
	High	95.0^{a}	77.0^{c}	87.3^{b}	71.2^{d}				
Feed intake (g.fish^{-1}.d^{-1})									
	Low	0.9	0.9	0.9	0.9	0.17	***	***	ns
	High	1.8	1.8	1.7	1.6				
Daily weight gain (g.kg$^{-0.8}$.d^{-1})									
	Low	13.5	10.3	13.4	9.9	0.31	***	***	ns
	High	22.4	18.4	20.8	17.2				
Feed efficiency									
	Low	1.25	0.87	1.26	0.86	0.022	***	***	ns
	High	1.32	0.97	1.25	0.94				

Diet 1, high protein diet; Diet 2, supplemental starch diet; Diet 3, supplemental fat diet; Diet 4, supplemental starch and fat diet; carb, carbohydrates; Diet, D; FL, feeding level; *P* values for effects of diet, feeding level or the interaction, respectively; BW, body weight; Feed efficiency = Weight gain/Feed intake.

P, protein; C, carbohydrates; F, fat.

***, P<0.01; *, P<0.05; ns, non-significantly different

^{abcdef}If interaction effect is significant, means lacking a common superscript differ significantly (*P*<0.05).

Supplementary table S3. Digestible nutrient intake and energy balance of carp (*C. carpio*) (n = 3), fed 4 different diets at 2 feeding levels (FL) over 28 days

	FL	Diet 1	Diet 2	Diet 3	Diet 4				
		“P”	“P”	“P”	“P”			*P* value	
			+C	+F	+C+F	SEM	D	FL	D x FL
Digestible nutrient intake (g.kg$^{-0.8}$.d^{-1})									
dCP	Low	5.1^{c}	3.3^{f}	4.1^{e}	2.9^{g}	0.05	***	***	***
	High	7.9^{a}	5.3^{c}	6.4^{b}	4.6^{d}				
dFat	Low	1.0^{f}	0.7^{g}	2.4^{c}	1.8^{d}	0.03	***	***	***
	High	1.6^{e}	1.1^{f}	3.5^{a}	2.8^{b}				
dCarb	Low	2.0^{e}	4.9^{c}	1.7^{e}	4.5^{c}	0.10	***	***	***
	High	2.8^{d}	7.9^{a}	2.7^{d}	7.2^{b}				
Energy balance parameters (kJ. kg$^{-0.8}$.d^{-1})									
GEI	Low	212.2^{d}	205.0^{d}	248.3^{c}	234.8^{c}	2.95	***	***	***
	High	333.6^{b}	329.6^{b}	388.9^{a}	376.5^{a}				
DEI	Low	194.6^{d}	187.7^{d}	224.0^{c}	215.4^{c}	2.52	***	***	***
	High	294.0^{b}	299.5^{b}	343.1^{a}	342.6^{a}				
BUE	Low	12.4^{c}	7.7^{ef}	9.2^{ed}	6.5^{f}	0.35	***	***	***
	High	18.6^{a}	11.9^{c}	14.7^{c}	10.0^{d}				
MEI	Low	182.2^{e}	180.0^{e}	214.8^{d}	209.0^{d}	2.38	***	***	***
	High	275.4^{c}	287.5^{b}	328.4^{a}	332.6^{a}				
HP	Low	93.6^{b}	89.2^{b}	88.1^{b}	94.9^{b}	2.54	ns	***	ns
	High	125.9^{a}	129.4^{a}	131.7^{a}	134.9^{a}				
RE	Low	88.6^{d}	90.8^{d}	126.8^{c}	114.0^{c}	3.38	***	***	*
	High	149.5^{b}	158.1^{b}	196.7^{a}	197.7^{a}				
RE$_{prot}$	Low	46.9^{d}	32.9^{e}	42.8^{d}	29.1^{e}	1.46	***	***	*
	High	75.8^{a}	55.4^{c}	63.8^{b}	49.3^{cd}				
RE$_{fat}$	Low	41.1^{e}	57.3^{d}	86.4^{c}	86.7^{c}	2.88	***	***	***
	High	73.2^{c}	103.7^{b}	134.1^{a}	147.3^{a}				
FRE	Low	0.95^{bc}	1.96^{a}	0.85^{c}	1.13^{b}	0.05	***	*	ns
	High	1.07^{bc}	2.20^{a}	0.84^{c}	1.19^{b}				

^{abcde}If interaction effect is significant, means lacking a common superscript differ significantly ($P<0.05$).

Diet 1, high protein diet; Diet 2, supplemental starch diet; Diet 3, supplemental fat diet; Diet 4, supplemental starch and fat diet; carb, carbohydrates; GEI, gross energy intake; DEI, digestible energy intake; MIE, metabolisable energy intake; D, Diet; FL, feeding level. FRE, fat retention efficiency (g.g^{-1}).

***, P<0.01; *, P<0.05; ns, non-significantly different

Supplementary table S4. Nitrogen (mg.kg$^{-0.8}$.day^{-1}) balance analysis of carp (*C. carpio*) (n = 3), fed 4 different diets at 2 feeding levels (FL) over 28 days

		Diet 1	Diet 2	Diet 3	Diet 4		*P* value		
		"P"	"P"	"P"	"P"				
	FL		+C	+F	+C + F	SEM	D	FL	D x FL
GNI									
	Low	848.0^{d}	565.8^{g}	685.2^{f}	478.8^{h}	9.28	***	***	***
	High	1333.2^{a}	909.6^{c}	1073.1^{b}	767.7^{e}				
DNI									
	Low	817.2^{c}	533.7^{f}	659.8^{e}	457.1^{g}	8.72	***	***	***
	High	1262.0^{a}	855.2^{c}	1022.4^{b}	734.8^{d}				
FN									
	Low	30.7^{cd}	32.1^{c}	25.4^{cd}	21.7^{d}	2.08	***	***	***
	High	71.3^{a}	54.4^{b}	50.7^{b}	21.7^{c}				
RN									
	Low	318.1^{d}	222.7^{e}	290.2^{d}	197.5^{e}	9.92	***	***	*
	High	513.8^{a}	375.2^{c}	432.5^{b}	333.9^{cd}				
BUN									
	Low	499.1^{c}	311.0^{ef}	369.6^{de}	259.6^{f}	14.05	***	***	***
	High	748.2^{a}	480.0^{c}	590.0^{b}	400.8^{d}				
Protein efficiency									
	Low	38.9	41.7	44.0	43.2	1.32	*	ns	ns
	High	40.7	43.9	42.3	45.5				

Diet 1, high protein diet; Diet 2, supplemental starch diet; Diet 3, supplemental fat diet; Diet 4, supplemental starch and fat diet; carb, carbohydrates; Diet, D; FL, feeding level; *P* values for effects of diet, feeding level or the interaction, respectively; P, protein; F, fat; C, carbohydrates; GNI, gross nitrogen intake; DNI, digestible nitrogen intake; FN, faecal nitrogen; RN, retained nitrogen; BUN, branchial and urinary nitrogen, protein efficiency = retained protein/digestible protein intake (% of digestible intake).

***, P<0.01; *, P<0.05; ns, non-significantly different

^{abcefgh}If interaction effect is significant, means lacking a common superscript differ significantly (*P*<0.05).

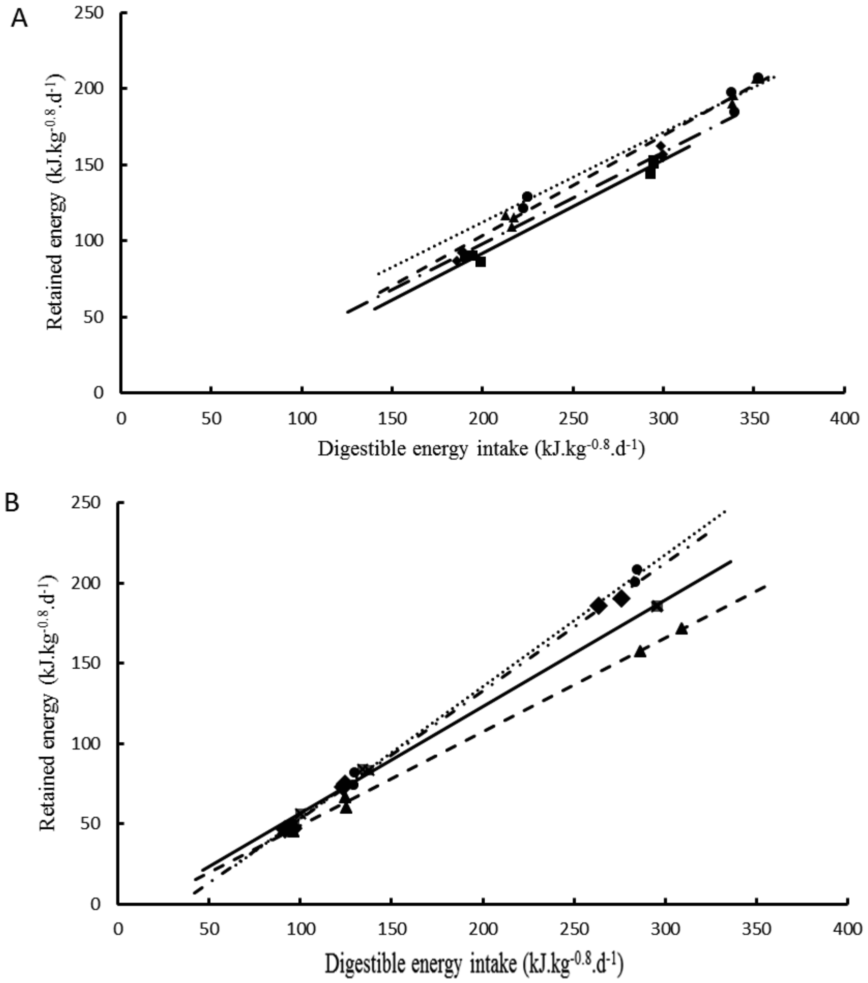
Supplementary table S5. Initial and final body composition ($g.kg^{-1}$, wet basis) of carp (*C. carpio*) (n = 3), fed 4 different diets at 2 feeding levels (FL) over 28 days

			Diet 1	Diet 2	Diet 3	Diet 4				
	FL		"P"	"P"	"P"	"P"		*P* value		
		Initial		+C	+F	+C + F	SEM	D	FL	D x FL
Dry matter		270								
	Low		256	280	298	309	4.0	***	*	Ns
	High		256	286	298	329				
Energy ($kJ.g^{-1}$)		7.4								
	Low		6.9^{f}	7.9^{e}	8.5^{cd}	9.2^{b}	0.11	***	***	*
	High		6.8^{f}	8.1^{de}	8.8^{bc}	9.9^{a}				
Protein		141								
	Low		145	139	138	135	2.0	***	***	Ns
	High		143	133	134	129				
Fat		104								
	Low		90^{f}	119^{e}	135^{cd}	154^{b}	2.4	***	***	*
	High		89^{f}	128^{de}	144^{bc}	173^{a}				
Ash		17								
	Low		19	20	20	19	1.3	ns	Ns	Ns
	High		18	19	18	19				

Diet 1, high protein diet; Diet 2, supplemental starch diet; Diet 3, supplemental fat diet; Diet 4, supplemental starch and fat diet; carb, carbohydrate, Diet, D; FL, feeding level. P, protein; F, fat; C, carbohydrates;

***, $P<0.01$; *, $P<0.05$; ns, non-significantly different

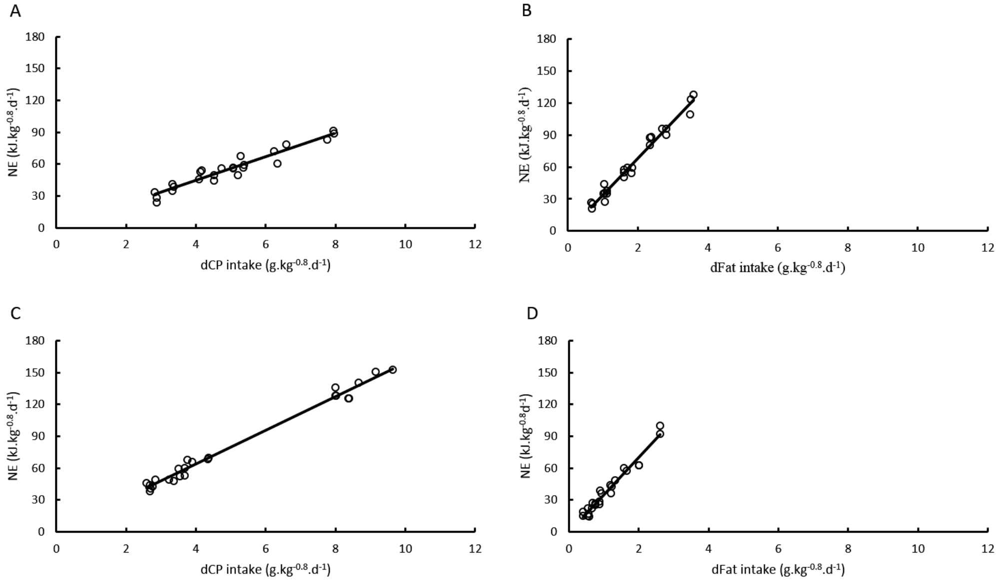
[abcdef] If interaction effect is significant, means lacking a common superscript differ significantly ($P<0.05$).



Supplementary Figure S1. Relationship between retained energy and digestible energy intake.

In panel (A) carp, ■ Diet 1: RE= -30.2 (SE 8.93) + 0.61 (SE 0.036) DE (R^2=0.986); ♦ Diet 2: RE= -22.3 (SE 6.25) + 0.60 (SE 0.025) DE (R^2=0.993); • Diet 3: RE= -5.8 (SE 12.4) + 0.59 (SE 0.043) DE (R^2=0.979); ▲ Diet 4: RE= -28.0 (SE 7.6) + 0.66 (SE 0.027) DE (R^2=0.994). The estimated energy requirements for maintenance was: 50, 37, 10 and 42 kJ.kg$^{-0.8}$.d^{-1} at Diet 1, 2, 3 and 4 respectively.

In panel (B) barramundi: ■ Diet Control: RE= -9.2 (SE 3.93) + 0.63 (SE 0.019) DE (R^2=0.996); ♦ Diet protein: RE= -27.1 (SE 2.86) + 0.75 (SE 0.015) DE (R^2=0.998); • Diet fat: RE= -29.1 (SE 3.66) + 0.79 (SE 0.019) DE (R^2=0.997); ▲ Diet starch: RE= -9.2 (SE 2.63) + 0.55(SE 0.013) DE (R^2=0.998). The estimated energy requirements for maintenance was: 15, 36, 37 and 17 kJ.kg$^{-0.8}$.d^{-1} at Diet control, protein, fat and starch respectively



Supplementary Figure S2. Relationship between net energy (NE) and digestible protein (dCP) intake (A), net energy (NE) and digestible fat (dFat) (B) for carp; net energy (NE) and digestible protein (dCP) intake (C), net energy (NE) and digestible fat (dFat) (D) for barramundi. The NE values are corrected for variation in digestible fat (dFat) and digestible carbohydrates (dCarb) (A,C); for variation in digestible protein (dCP) and digestible carbohydrates (dCarb) (B,D). 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 dCp and dCarb intake in order to have only the effect of dCP on NE (A,C) and towards zero dFat and dCarb intake in order to have only the effect of dFat on NE (B,D). This was conducted using Equation (2) for Carp and Equation (4) for Barramundi (Table 4).

3

Energy utilisation efficiencies of digested protein, fat and carbohydrates on African catfish (*Clarias gariepinus*)

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Abstract

This study aims to test the effect of dietary composition (*i.e.*, macro-nutrient content) on the energy utilisation efficiency of African catfish using the digestible energy (DE) approach and to estimate the energy utilization efficiencies of digested protein, fat and carbohydrates for growth on African catfish using the net energy (NE) approach. A total of 4 diets were studied on African catfish (71.6 g) at 2 feeding levels, low vs. high (12 vs. 22 $g.kg^{-0.8}.d^{--1}$) for 30 days, which resulted in a 4×2 factorial design with a total of 8 treatments. This design aimed to create large contrasts in the digested protein, fat and carbohydrates intakes among the 4 different diets to be able to run multiple regression analysis of energy retention (RE) (*i.e.*, growth response) as a function of dCP, dFat and dCarb intake. When using DE approach, diet composition (*i.e.*, macro-nutrient content) affects the energy utilisation efficiency (k_{gDE}) of African catfish. The supplementation of starch reduced the k_{gDE} from 93.5% to 78.5%, in low starch diets compared to high starch diets. When using the NE approach, energy utilization efficiencies of digested protein, fat and carbohydrates for growth on African catfish using the NE approach were estimated at 86%, 95% and 59%, respectively. In conclusion, in African catfish the relationship between DE and RE is affected by dietary macronutrient composition. This effect of diet composition on k_{gDE} implies that feed evaluation/formulation using a DE approach can be biased. Applying an NE approach for energy evaluation of feed/ingredients is advisable for African catfish.

3.1. Introduction

African catfish *(Clarias gariepinus)*, a freshwater fish, is widely farmed because of its good marketability, air-breathing characteristics, omnivorous feeding habit and rapid growth. Efficient conversion of feed into growth is a major factor determining the profitability of fish farming, which demands having access to nutritionally balanced diets. Formulation of nutritionally balanced diets requires knowledge about the nutrient requirements of the target species. Compared to other fish species, like salmon, trout and channel catfish, knowledge on the nutritional requirements of African catfish is relatively marginal. For example basic knowledge on the energy requirements (*e.g.*, demand for maintenance and growth) is absent for this fish species.

Currently, the use of digestible energy (DE) is a common approach to quantify the energy demand for maintenance and growth (Glencross, 2008; Glencross & Bermudes, 2012; Lupatsch *et al.*, 2003). In this approach, retained energy (RE) is commonly described as a linear function of DE (*i.e.*, RE = μ +β x DE, where μ is the intercept and β the energy utilisation efficiency of DE for growth, k_{gDE}) and k_{gDE} is assumed to be independent of dietary macronutrient composition. However, in barramundi (*Lates calcarifer*) (Glencross *et al.*, 2017), Nile tilapia (*Oreochromis niloticus*) (Schrama *et al.*, 2012a) and rainbow trout (*Oncorhynchus mykiss*) (Rodehutscord & Pfeffer, 1999) differences in the dietary macronutrient content altered k_{gDE}. For African catfish, estimates for k_{gDE} are not available and it is unknown if diet composition affects k_{gDE} in African catfish.

The potential impact of the dietary macro-nutrient content on the k_{gDE} value is a reason to move from the DE to a net energy (NE) evaluation approach in feed formulation. The NE approach has been applied successfully for many years for pig feed (CVB, 1993; Noblet *et al.*, 1994) and is recently developed for fish feed (Phan *et al.*, 2019; Phan *et al.*, 2021b; Schrama *et al.*, 2018). In a NE approach, RE is expressed as a function of the separate digestible macronutrient intakes, digested protein (dCP), fat (dFat) and carbohydrates (dCarb), respectively, [*i.e.*, RE= μ +β_1 x dCP + β_2 x dFat + β_3 x dCarb, where μ is the intercept, being an estimate for fasting heat production (FHP) and β_1, β_2, β_3 are the energy utilisation efficiencies of dCP ($k_{\mathrm{NE;dCP}}$), dFat ($k_{\mathrm{NE;dLipid}}$) and dCarb ($k_{\mathrm{NE;dCarb}}$), respectively]. The differences in the energy utilisation efficiencies of digested protein, fat and carbohydrates have been investigated recently for omnivorous fish (*i.e.*, Nile tilapia and common carp [C*yprinus carpio*], striped catfish [*Pangasius hypothalamus*]) and carnivorous fish (*i.e.*, barramundi, rainbow trout and snakehead [*Channa striata*]) (Phan *et al.*, 2019; Phan *et al.*, 2021b; Schrama *et al.*, 2018). In these studies, similarities were found in the energy utilization efficiencies of digested protein, fat and carbohydrates for pig (CVB, 1993; Noblet *et al.*, 1994) and omnivorous fish species studied. However, these energy utilization efficiencies of digested macronutrients have not been estimated for African catfish. In addition, it still remains to be investigated whether the energy utilization efficiencies of digested protein, fat and carbohydrates in African catfish are similar to the energy utilization of the other species mentioned.

Therefore, this study aimed to: 1. test the effect of diet composition (*i.e.*, macro-nutrient content) on the energy utilisation efficiency of African catfish for energy retention using the DE approach; 2. estimate the energy utilization efficiencies of digested protein, fat and carbohydrates for growth on African catfish using the NE approach.

3.2. Materials and methods

Animal ethics. This study (project number: 2018.W-0021.001) was carried out in accordance with the Dutch law on the use of animals (Act on Animal Experiments) for scientific purposes and was approved by the Central Animal Experiments Committee (CCD) of the Netherlands. This experiment was conducted in the research facility of CARUS-ARF at Wageningen University, the Netherlands. Fish were kept and handled in agreement with EU-legislation.

Experimental diets. Four diets were formulated with different contents of crude protein (284 – 502 $g.kg^{-1}$), crude fat (57 -251 $g.kg^{-1}$) and carbohydrates (260 – 552 $g.kg^{-1}$) by using the triangle approach (Raubenheimer, 2011) with the aim to have a wide contrast in macronutrient content between diets (*i.e.*, crude protein, fat and total carbohydrates). The range in dietary macronutrient composition was created by varying the inclusion level of gelatinized maize starch and a fat blend of 50% rape seed oil and 50% soya oil, further referred to as "fat blend" (Table 1). The amino acid profile was kept constant for all diets (Supplementary Table S1).

Diets were formulated to have a constant ratio between protein and premix content. Details on amino acid requirements of African catfish are not well investigated. Therefore, diets were optimized using the amino acid requirements averaged over freshwater teleost fish (NRC, 2011). These levels also met the Methionine (Elesho *et al.*, 2020), Lysine (Fagbenro *et al.*, 1998), Arginine (Fagbenro *et al.*, 1999) and Tryptophan (Fagbenro & Nwanna, 1999) requirements published for African catfish on a $g.kg^{-1}$ dietary protein basis. The analysed amino acid composition of the experimental diets is shown in Supplementary Table S1. First, the diet with the highest protein content (P-diet) was formulated using fish meal, wheat gluten, pea protein and soya protein concentrate as protein sources. This P-diet was diluted with gelatinised maize starch to create a high starch content diet (C-diet), with the fat blend to get a high fat content diet (F-diet), or with both to create a diet with a high fat and starch content (M-diet). All diets were fed at 2 feeding levels, low vs. high (12 vs. 22 $g.kg^{-0.8}.d^{-1}$). This resulted in a 4×2 factorial design. This design aimed to create large contrasts in the digestible macronutrient intake between the 8 treatments diets to enable multiple regression analysis of RE (*i.e.*, growth response) as a function of dCP, dFat and dCarb intake.

Diets were produced by Research Diet Service (Wijk bij Duurstede, The Netherlands). Fishmeal, pea protein, soy protein concentrate, wheat, wheat bran and wheat gluten were hammer-milled using a 1 mm screen. All ingredients, except the fat blend in the F- and M-diet, were mixed. Prior to extrusion, these mixtures were conditioned at a temperature between 85-100°C. Diets were processed by extrusion (Clextral BC45, Firminy, France) using a 2 mm die at 95-110°C. This resulted in 3 mm pellets, which were dried at 70°C for 3h. Thereafter, pellets were cooled to ambient temperature. After cooling, the F- and M-diets were vacuum-coated with the fat blend. All diets were stored at 4°C. Before feeding, pellets were sieved to remove dust and small particles. Fish were fed twice daily from 09:00 to 10:00 hours and from 16:00 to 17:00 hours for 30 consecutive days.

Fish handling. The experiment started July 2019. A total of 840 African catfish (*C. gariepinus)* (mixed sex), with a mean body weight (BW) of 71.6 g (se 0.1) were purchased from Fleuren en Nooijen (Nederweert, The Netherlands). At the start of the experiment, groups of 35 fish were batch-weighed and randomly assigned to one of twenty four 70-L tanks. These tanks were connected to one recirculating aquaculture system. The water flow per tank was 7 L/min. The measured water quality parameters during the experiment for temperature, oxygen, pH, conductivity, NH_4-N, NO_2-N and

Table 1. Experimental diet formulation and composition fed to African catfish

	P	C	F	M
	"protein"	"protein"	"protein"	"protein"
		+Carb	+Fat	+Carb+Fat
Ingredient composition (g.100 g^{-1}, as-is)				
Gelatinized maize starch	0	34.3	0	30
Fat blend*	0	0	17.9	12.5
Wheat	15.4	10.1	12.7	8.9
Wheat bran	17.4	11.4	14.3	10.0
Wheat gluten	13.9	9.1	11.4	8.0
Fishmeal	13.9	9.1	11.4	8.0
Soya protein concentrate	13.9	9.1	11.4	8.0
Pea protein concentrate	13.9	9.1	11.4	8.0
Fish oil	3.5	2.3	2.9	2.0
Lime ($CaCO_3$)	1.7	1.1	1.4	1.0
Monocalcium phosphate	3.1	2.1	2.6	1.8
L-lysine	0.5	0.3	0.4	0.3
DL-Methionine	0.5	0.3	0.4	0.3
L-Threonine	0.3	0.2	0.3	0.2
Premix	1.7	1.1	1.4	1.0
Analysed chemical composition (g.kg^{-1} DM)				
DM	949	925	954	935
Crude protein	502	329	412	284
Total fat	83	57	251	182
Total carbohydrate	319	552	260	479
Total starch	171	436	140	396
Gross energy (MJ.kg^{-1})	20.5	19.5	24.4	22.1
NSP	148	116	120	83
Ash	96	63	77	55
Acid-insoluble ash	1.49	0.92	1.21	0.85

P, the diet with a high protein content; C, the P diet supplemented with maize starch; F, the P diet supplemented with fat blend; M, the P diet supplemented with maize starch and fat blend; Carb, Carbohydrates; Fat blend (rapeseed oil: soybean oil = 1:1); Carb, Carbohydrates; (*) fat blend (rapeseed oil: soybean oil = 1:1); DM, dry matter.

NO_3-N were 27.7 ± 0.2°C, 6.3 ± 0.3 mg/L, 7.2 ± 0.3, 4 ± 0.4 mS/m, 0.4 ± 0.2 mg/L , 0.3 ± 0.2 mg/L , and 304.2 ± 78.2 mg/L, respectively.

Sampling and chemical analysis. At the start of the experiment, ten fish from the initial population were euthanized by an overdose of 2-phenoxyethanol for the analysis of the initial body composition. At the end of the trial, ten fish from each tank were euthanized similarly to determine the final body composition. After sampling, the fish were pooled and kept at -20°C. The preparation of the samples for the chemical analysis was according to Saravanan *et al.* (2012). Diet and oven-dried (70°C) faecal samples were analysed for DM, acid-insoluble ash, yttrium, Ca, P, CP, fat, starch and gross energy contents. Proximate composition of fish, feed and faeces were determined according to ISO-standard analysis for determination of dry matter (DM; ISO 6496, 1983), crude ash (ISO 5984, 1978), acid insoluble ash (AIA; ISO 5985, 1981), crude fat (ISO 6492, 1999), crude protein (ISO 5983, 1997, crude protein = Kjeldahl-N × 6.25), energy (ISO 9831,1998), and starch (NEN/ISO 15914) (Meriac *et al*., 2014). Yttrium, P and Ca were analysed by ICP-MS (NEN 15510, 2007). Total carbohydrates content of feed and faeces was calculated as DM minus crude protein minus crude ash minus crude fat and non-starch polysaccharide content total carbohydrates minus starch.

Nutrient digestibility estimation. Each tank as an experimental unit was connected to a separate faeces settling unit. Each settling unit was equipped with an ice-cooled glass bottle at the bottom to prevent bacterial degradation of faecal nutrients during collection. Faeces collection started after finishing the evening feeding. Faeces settled in the column overnight were collected daily prior to the morning feeding. 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:

$$ADC_{nutrient} = (1\text{- } (marker_{diet}/\ marker_{faeces}) \times (Nutrient_{faeces}/Nutrient_{diet})) \times 100\%,$$

where $marker_{diet}$ and $marker_{faeces}$ are the marker concentration of the diet and faeces, and $Nutrient_{diet}$ and $Nutrient_{faeces}$ are the DM, protein, fat, carbohydrates or energy content of diet and faeces, respectively. Originally, yttrium oxide was planned to be used as an inert marker. However, preliminary analysis of the relationship between RE and DE as well as the estimations of the NE equations gave some extremely high partial efficiency estimates (>100%) when using yttrium as an inert marker. It seemed that especially at the high feeding levels ADC values at some diets were low (See Supplementary Table 3a). Therefore, acid insoluble ash (AIA) was analysed in feed and faeces and used as a marker to estimate digestibility.

Faecal recovery calculations. Faecal recovery (in %) was calculated by dividing the total amount of AIA in the excreted faeces that was collected by the settling column for the total amount of AIA in the consumed feed (Amirkolaie *et al.*, 2005). The total amount of AIA in the excreted faeces was calculated by multiplying the amount of faecal DM collected by the AIA concentration in the faeces on DM basis. The total amount of AIA of the consumed feed was calculated by multiplying the total amount of consumed feed by the AIA concentration in the feed (on DM basis).

Nutrient balances calculations. To standardise for differences in body weight, digestible macronutrient intake, nitrogen and energy balance parameters were expressed per unit of metabolic body weight. Metabolic body weight was calculated as body weight (in kg) raised to the power of 0.8. The mean metabolic body weight was calculated as the average of the initial and final metabolic body weight. Intake of each macronutrient was determined by multiplying the averaged feed intake for each treatment by the nutrient inclusion level in the diet. The digestible macronutrient intake was calculated by multiplying the macronutrient nutrient intake with the ADC of the respective macronutrient. Calculations of energy and nitrogen balances are similar to those described by (Saravanan *et al.*, 2012a). The energy and nitrogen retention were derived from weight gain and initial and the final body energy and nitrogen composition. The branchial and urinary N losses (BUN) were calculated using the difference between digestible N, N intake and N retention. The branchial and urinary energy (BUE) was estimated by multiplying BUN by 24.85, which is the energy content (in kJ) of 1 g excreted nitrogen and the assumption that NH_3-N is the only form of N excreted (Bureau *et* *al.*, 2003). Metabolisable energy intake was calculated as the difference between digestible energy intake and BUE and heat production by subtracting RE from the metabolisable energy intake.

Data analysis

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

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

$$RE_i = \mu + \beta \times DE + e_i \qquad \text{(Equation 1)}$$

where μ is the intercept, β is the energy utilisation efficiency; e_i is error term and i =1,..., n with n = 6 per diet. To test the effect of carbohydrates and fat supplementation on the slopes, a general linear model was used with RE as dependent parameter, DE as covariate and carbohydrates and fat supplementation as fixed factors. Multiple regression of retained energy (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. The following model was used:

$$RE_i = \mu + \beta_1 \times dCP_i + \beta_2 \times dFat_i + \beta_3 \times dCarb_i + e_i \qquad \text{(Equation 2)}$$

where μ is the intercept, being an estimate for fasting heat production (FHP); β_1, β_2, β_3 are the energy utilisation efficiency of dCP ($k_{NE;dCP}$), dFat ($k_{NE;dFat}$) and dCarb ($k_{NE;dCarb}$); e_i is error term and i =1,..., 24. The linearity and curve-linearity were checked in the relationship of RE with dCP, dFat and dCarb.

3.3. Results

Daily weight gained of African catfish ranged from 11.2 to 24.2 $g.kg^{-0.8}.d^{-1}$ between treatments (Table 2). During the experiment, BW almost doubled at the low feeding level and tripled at the high feeding level. Both, final BW weight and daily weight gain were affected by feeding level, diet and their interaction ($P<0.001$; Table 2). Data on the initial and final body composition are presented in Supplementary Table S2. Averaged over all the treatments, RE as fat was 73 $kJ.kg^{-0.8}.d^{-1}$ and RE as protein was 66 $kJ.kg^{-0.8}.d^{-1}$ (Table 3). On energy basis, the ratio between fat and protein gain was influenced by diet ($P < 0.001$); being 0.5, 0.9, 1.3 and 1.9 $J.J^{-1}$ averaged over feeding levels, for the fish fed the P-diet, C-diet, F-diet and M-diet, respectively (Table 3).

Preliminary analysis showed that ADC values average over all treatments were lower when using yttrium as marker compared to ADC based on AIA as inert marker (Supplementary Table S3 a, b). Furthermore, estimates of partial energy efficiencies from the RE and DE relationship were outside the theoretical correct range (k_{gDE} > 100%). Therefore, ADC values using AIA as inert marker are presented and used for energy and nitrogen balance calculations. Averaged over the four diets, the ADC values of starch were the highest, followed by the ADC values of protein, fat and carbohydrates (Figure 1). Increasing the feeding level decreased the ADC of fat, carbohydrates and starch ($P <0.05$), but protein ADC was unaffected by feeding level ($P>0.05$). The decline in ADC with feeding level was smallest for starch, intermediate for fat and highest for carbohydrates. Averaged over all diets, the

ADC of fat was 90.1% and 87.1% at the low and high feeding level, respectively. Averaged over all diets, the difference in ADC of carbohydrates was 7.8% between feeding levels (Figure 1).

The recovery of African catfish faeces was low, being 18.5% averaged over all treatments (Figure 2). Diet affected the faecal recovery ($P<0.001$; Figure 2) whereas feeding level did not ($P>0.05$). Averaged over both feeding levels, the highest faecal recovery was found in the fish fed the P-diet (25.6%) followed by the F-diet (22.1%) and the C-diet (14.0%). The lowest faecal recovery was observed in fish fed the M-diet (12.4%; Figure 2). Pairwise comparison of means showed that dietary starch supplementation reduced faecal recovery ($P<0.05$) from 23.9% in diets without starch supplementation (P- and F-diets) to 13.2% in diets supplemented with starch (C- and M-diets). In contrast, dietary fat supplementation did not affect faecal recovery (Figure 2; $P>0.05$).

Table 2. Growth performance of African catfish, (n = 3), fed 4 different diets (D) at 2 feeding levels (FL) for 30 days

FL	P	C	F	M				
	"protein"	"protein"	"protein"	"protein"		*P* values		
		+Carb	+Fat	+Carb+Fat	SEM	D	FL	D x FL
Final BW								
Low	140.3^{c}	123.0^{d}	141.8^{c}	123.3^{d}	1.25	***	***	***
High	223.2^{a}	186.3^{b}	226.0^{a}	184.0^{b}				
Feed intake (g.fish^{-1}.d^{-1})*								
Low	1.48	1.55	1.48	1.53	0.006	---*	---*	---*
High	3.05	3.19	3.06	3.15				
Daily weight gain (g.kg$^{-0.8}$.d^{-1})								
Low	13.9^{c}	11.3^{d}	14.0^{c}	11.2^{d}	0.16	***	***	***
High	23.9^{a}	20.0^{b}	24.2^{a}	19.8^{b}				
FCR								
Low	0.65	0.89	0.64	0.89	0.009	***	***	Ns
High	0.60	0.83	0.60	0.84				
Survival (%)								
Low	95	95	92	95	1.7	ns	ns	Ns
High	93	95	94	94				

P, the diet with a high protein content; C, the P diet supplemented with maize starch; F, the P diet supplemented with fat blend; M, the P diet supplemented with maize starch and fat blend; Carb, Carbohydrates; Fat blend (rapeseed oil: soybean oil = 1:1); FL, feeding level; *P* values for effects of diet, feeding level or the interaction, respectively; BW, body weight; (*) No statistics was conducted because feed intake was controlled at 2 feeding levels; FCR, feed conversion rate.

***, $P<0.001$; *, $P<0.05$; ns, non-significantly different.

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

Table 3. Digestible nutrients intake (g.kg$^{-0.8}$.d^{-1}) and energy balance (kJ.kg$^{-0.8}$.d^{-1}) of African catfish, (n = 3), fed 4 different diets (D) at 2 feeding levels (FL) for 30 days

	FL	P	C	F	M		*P values*		
		"P"	"P"	"P"	"P"				
			+C	+F	+C+F	SEM	D	FL	D x FL
dCP	Low	4.0^{d}	2.8^{f}	3.3^{e}	2.4^{g}	4.02	***	***	***
	High	6.4^{a}	4.6^{c}	5.3^{b}	4.0^{d}				
dFat	Low	0.6^{f}	0.4^{g}	2.0^{c}	1.6^{d}	0.13	***	***	***
	High	0.9^{e}	0.7^{f}	3.3^{a}	2.6^{b}				
dCarb	Low	1.4^{c}	4.0^{b}	0.9^{c}	3.3^{b}	0.14	***	***	***
	High	1.5^{c}	5.8^{a}	1.5^{c}	5.1^{a}				
GEI	Low	175^{g}	182^{f}	208^{e}	204^{e}	1.0	***	***	***
	High	281^{d}	301^{c}	335^{b}	343^{a}				
DEI	Low	140^{e}	150^{e}	173^{d}	174^{d}	3.6	***	***	***
	High	207^{c}	231^{b}	281^{a}	281^{a}				
BUE	Low	6	4	4	3	0.3	***	***	ns
Losses	High	9	6	6	4				
MEI	Low	134^{e}	146^{e}	169^{d}	171^{d}	3.5	***	***	***
	High	198^{c}	225^{b}	275^{a}	277^{a}				
HP	Low	50	63	52	59	4.3	***	***	ns
	High	47	81	61	76				
RE	Low	84^{e}	82^{e}	118^{d}	112^{d}	2.7	***	***	***
	High	151^{c}	144^{c}	214^{a}	201^{b}				
RE_{prot}	Low	58^{e}	43^{f}	52^{e}	38^{f}	1.2	***	***	***
	High	99^{a}	77^{c}	90^{b}	69^{d}				
RE_{fat}	Low	26^{e}	40^{d}	66^{b}	74^{b}	2.4	***	***	***
	High	52^{c}	67^{b}	125^{a}	132^{a}				
$RE_{fat:prot}$	Low	0.4	0.9	1.3	1.9	0.04	***	ns	ns
(J.J^{-1})	High	0.5	0.9	1.4	1.9				
FG: PG	Low	0.3	0.6	0.8	1.1	0.03	***	ns	ns
(g.g^{-1})	High	0.3	0.5	0.8	1.1				
PE	Low	61	64	67	67	1.3	***	***	ns
	High	65	70	71	73				

P, the diet with a high protein content; C, the P diet supplemented with maize starch; F, the P diet supplemented with fat blend; M, the P diet supplemented with maize starch and fat blend; Carb, Carbohydrates; Fat blend (rapeseed oil: soybean oil = 1:1); GEI, gross energy intake; DEI, digestible energy intake; BUE, branchial urinary energy; MEI, metabolisable energy intake; HP, heat production; RE, retained energy; FG: PG, fat to protein gain ratio; PE, digestible protein efficiency (%); PE = body protein gain X 100/digestible protein intake. Acid-insoluble ash was used to estimate digestibility.
***, P<0.001; *, P<0.05; ns, non-significantly different.
^{abcde}For parameters with a significant interaction effect between diet and feeding level, means lacking a common superscript differ significantly (*P*<0.05).

The first aim of this study was to assess the effect of diet composition (*i.e.*, macro-nutrient content) on the relationship between DE intake and RE in African catfish. The estimated linear relationships between DE and RE for each diet are given in Figure 3. The slopes of the relationships between DE and RE (k_{gDE}) differed between the diets with a low (diets P and F) or high starch content (diets C and M; P<0.05). Dietary starch supplementation reduced k_{gDE} from 93.5% (mean diets P and F) to 78.5% (mean diets C and M). Dietary fat supplementation had no effect on k_{gDE} (P>0.1); being 87.1% for the low fat diets (P- and C-diet) and 85.0% for the fat supplemented diets (F- and M-diet; Figure 3).

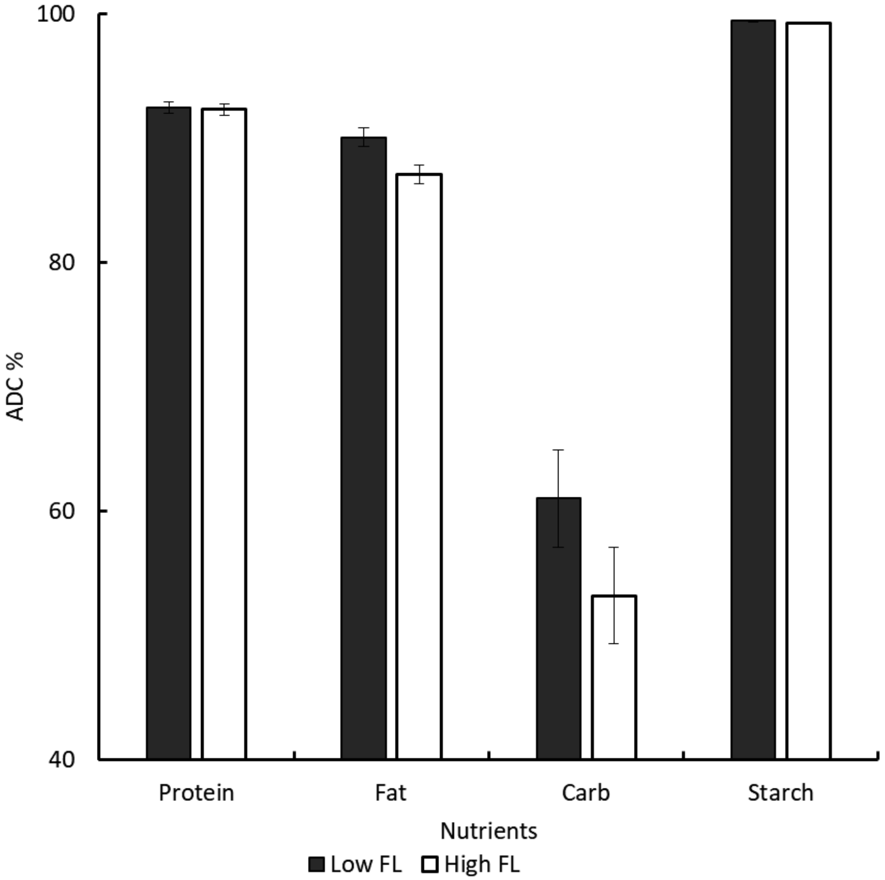

Figure 1. Mean ± SEM of protein, fat, carbohydrates (carb) and starch in African catfish averaged over 4 different diets: P, the diet with a high protein content; C, the P diet supplemented with maize starch; F, the P diet supplemented with fat blend; M, the P diet supplemented with maize starch and fat blend at 2 feeding levels (FL). Feeding level has significant effects on ADC of fat, carbohydrate and starch (P<0.05)

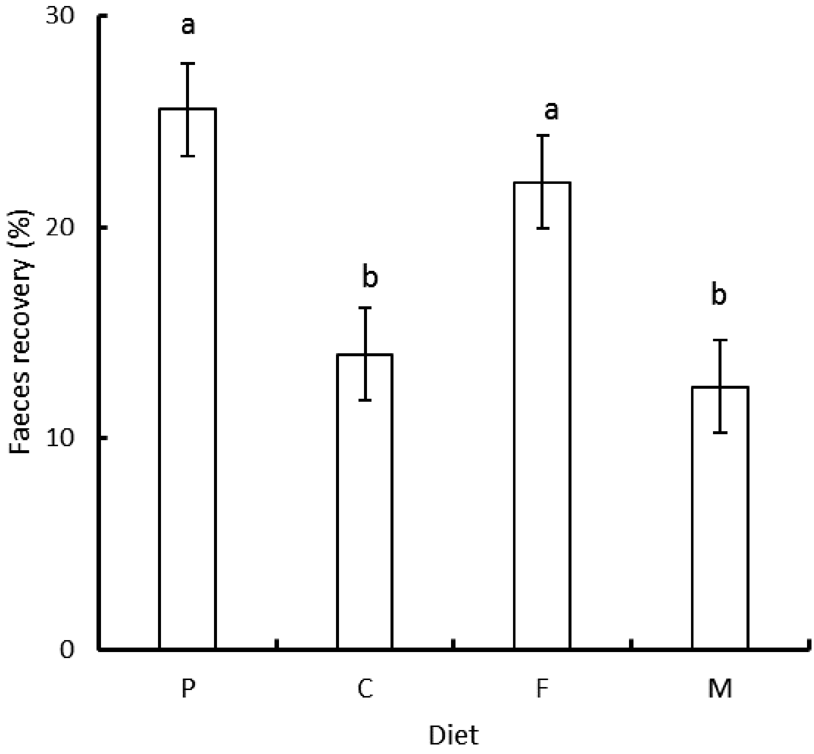

Figure 2. Mean ± SEM of faeces recovery in African catfish fed one of four experimental diets: P, the diet with a high protein content; C, the P diet supplemented with maize starch; F, the P diet supplemented with fat blend; M, the P diet supplemented with maize starch and fat blend averaged over feeding level. The significant effect was Diet ($P < 0.001$).

This study also aimed to quantify the energy utilization efficiencies of digested protein, fat and carbohydrates for growth (*i.e.*, estimating the NE equation for African catfish). Therefore, the energy and nitrogen balances as well as the digested nutrient intakes (dCP, dFat and dCarb) were measured (Table 3). The experimental design led as intended to a wide range in digestible nutrient intakes and a large variability in RE between the 8 experimental treatments. RE ranged from 82 to 214 $kJ.kg^{-0.8}.d^{-1}$ (Table 3). By conducting multiple linear regression between RE (in $kJ.kg^{-0.8}.d^{-1}$) and dCP, dFat and dCarb (in $g.kg^{-0.8}.d^{-1}$), the following NE equation was estimated:

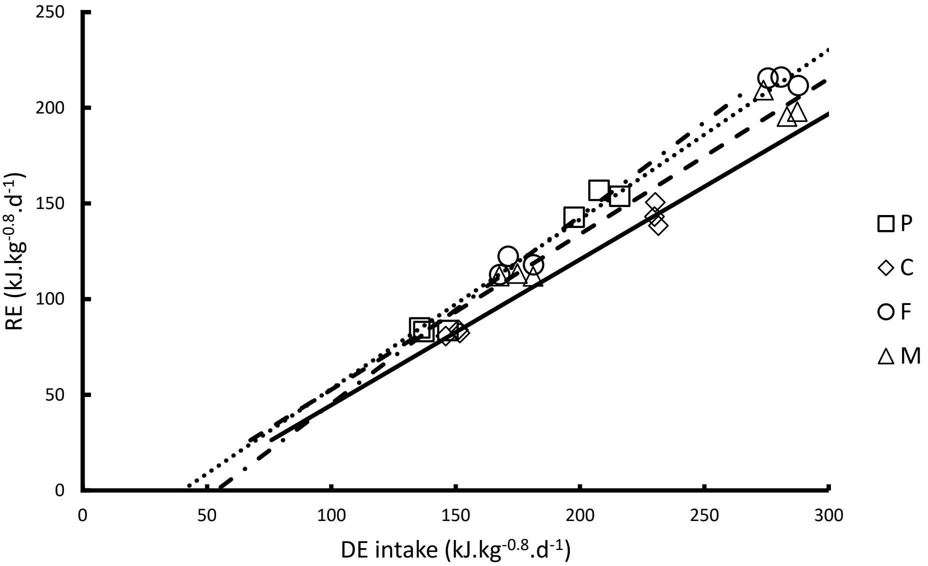

Figure 3. Relationship between retained energy (RE) and digestible energy intake (DE) for African catfish fed one of four experimental diets: P, diet with a high protein content; C, the P diet supplemented with maize starch; F, the P diet supplemented with fat blend; M, the P diet supplemented with maize starch and fat blend with ADC of energy measured using AIA as inert marker (□ Diet P: RE= - 53 (SE 13) + 0.98 (SE 0.073) DE (R^2=0.98), ◊ Diet C: RE= - 31 (SE 9.3) + 0.76 (SE 0.048) DE (R^2= 0.98), ○ Diet F: RE= - 36 (SE 12.6) + 0.89 (SE 0.054) DE (R^2= 0.99), Δ Diet M: RE= - 29 (SE 17.5) + 0.81 (SE 0.075) DE (R^2=0.97)) on African catfish.

NE = RE + 32.6 (se 5.45)

= 20.4 (se 1.00) dCP + 37.6 (se 1.26) dFat + 10.1 (se 0.71) dCarb $R^2 = 0.99$ (Equation 3)

The relationships between NE and respectively dCP, dFat and dCarb are depicted in Figure 4. Figure 4 and Eq. 3 show that the NE value per gram digested nutrient was highest for fat (37.6 kJ.g^{-1}), intermediate for protein (20.4 kJ.g^{-1}) and lowest for carbohydrates (10.1 kJ.g^{-1}), *i.e.* the slopes of the depicted lines in Figure 4. By dividing these coefficients of dCP, dFat and dCarb in Equation 3 by the gross energy values of these macronutrients (23.6 kJ.g^{-1}, 39.5 kJ.g^{-1} and 17.2 kJ.g^{-1} for CP, fat and carbohydrates, respectively), the energy utilisation efficiencies or $k_{g,NE}$ values of dCP, dFat and dCarb were estimated as 86%, 95% and 59%, respectively. All digestible nutrients intake (dCP, dFat and dCarb) were linearly related to RE. No polynomial effects were present (P>0.05).

3.4. Discussion

Faecal recovery of African catfish in this study (~18%) is low compared to values reported for other fish species; striped catfish (~40%) (Tran-Tu et al., 2018), tilapia (~70%) (Amirkolaie et al., 2005), common carp (~76) (Prabhu et al., 2019) and trout (~80%) (Meriac et al., 2014). This low faecal recovery in African catfish may be due to the lack of a mucus envelope around the faecal pellets which was also observed in striped catfish (Tran-Tu et al., 2018). Such a mucus envelope like present in tilapia might make faecal pellets or strings relatively more water stable and thereby easier to collect. This might explain high faecal recovery in tilapia, common carp and trout. African catfish faecal recovery is more comparable to striped catfish, which has poor faecal stability coinciding with a low faecal recovery and also lacks a mucus envelope around the faecal matter (Tran-Tu et al., 2018). Next to species difference, faecal recovery is dependent among others on tank design (Amirkolaie, 2013), length of the adaptation period in digestion studies (i.e., time after shifting diets) (Amirkolaie & Schrama, 2015) and especially dietary ingredient and/or macronutrient composition (Amirkolaie et al., 2005; Brinker & Friedrich, 2012; Meriac et al., 2014a; Prabhu et al., 2019).

Also in African catfish, faecal recovery is influenced by diet composition (Figure 2). Especially dietary supplementation of gelatinized maize starch had a negative impact on faecal recovery. Why faecal recovery in African catfish declines with starch supplementation is not clear. Especially the high ADC of starch (Figure 1) would suggest no direct effect on the faecal pellets. Indirectly starch fermentation in the intestine may have played a role. A decrease in faecal stability and faecal recovery was observed in Nile tilapia when intestinal fermentation was stimulated by inclusion of native starch in steam pelleted diets (Amirkolaie *et al.*, 2006). However, the role of fermentation is not likely since starch ADC was above 99%, gelatinized maize starch was used and diets were produced by extrusion. But intestinal fermentation can have been triggered by the presence of resistant starch. During extrusion under hydrothermal conditions may increase the resistant starch level (Sievert & Pomeranz, 1989), which can stimulate intestinal fermentation by bacteria (Bird *et al.*, 2007). However, effects of resistant starch on intestinal fermentation, nutrient digestion and the faecal recovery has not been assessed in fish. The current study confirms such possibilities shown in other fish species, that diet composition can affect the faecal recovery in fish.

The low faecal recovery might have affected the quality of the ADC measurements in the present study. ADC values estimated by using yttrium were much lower than the values estimated by using AIA, especially at the starch supplemented diets (Supplementary table S3a and S3b). Additionally, k_{gDE} values were quite high (>100%), which suggested an underestimation of ADCs using yttrium as marker. There is still the risk that the ADC values using AIA as marker are underestimated. However, mean protein ADC across treatments in the present study (92.4%) is higher than study means in literature for African catfish [(79.5%, (Leenhouwers *et al.*, 2007); 81.2%, (Weerd, 1999); 83.5%, (Leenhouwers *et al.*, 2006); 88.1%, (Elesho *et al.*, 2020)]. Similarly, mean energy ADC is higher in the present study (80.8%) compared to values reported in literature [56.1%, (Weerd, 1999); 67.8%, (Leenhouwers *et al.*, 2006); 80.1%, (Elesho *et al.*, 2020)]. Regarding fat digestibility, mean fat ADC (88.6%) is lower in the present study than those reported in literature (98.8%, Leenhouwers *et al*., 2007; 94.4%, Elesho *et al.*, 2020). Factors contributing to difference in ADC between studies are differences in markers, type, ingredient/nutrient composition of the diets, quality of ingredients used in those diets and faecal collection methods used between studies. The physical characteristics of markers can influence their passage rate, distribution and loss through the fish's digestive tract. Specifically, markers may, apart from diet components, distribute or concentrate, not uniformly in digesta, different intestine sections or faeces (Vandenberg & De La Noüe, 2001). The loss of markers (*i.e.*, chromic oxide) in the intestine or even through branchial particle elimination is observed in tilapia (Bowen, 1978). This loss of markers reduces the faecal marker concentration, which in turn leads to an underestimation of faecal recovery rates and ADC values. Inert markers like yttrium and chromium oxide have been shown to be acceptable in salmonids (Austreng *et al.*, 2000). However, the value of these markers in fish species, which void faeces free of mucus envelope needs further investigation.

In the current study, ADC of fat, starch and carbohydrates decreased with feeding level (Figure 1). (Henken *et al.*, 1985) also found an effect of feeding level on protein and energy ADC in African catfish. Similarly, ADC of protein, fat, energy, starch and carbohydrates in common carp were affected by feeding level (Phan *et al.*, 2019; Ufodike & Matty, 1983). A high feeding level may increase the digesta passage rate, reducing the residence time of feed in the digestive tract (Henken *et al*., 1985) and thereby decrease in the digestibility of fat, starch and carbohydrates. The negative effect of feeding level on fat digestion was also observed in rainbow trout, which was related to a higher faecal bile acid loss (Staessen *et al.*, 2020). Increased bile acid losses might explain the lower fat ADC at the high feeding level in the current study since feeding level did not affect protein ADC (Figure 1). However, the absence an effect of feeding level on protein digestion might relate to a high priority for protein digestion in African catfish. In fact, carnivorous and omnivorous fish possess a higher protease activity (*i.e.*, pepsin, trypsin) than herbivorous fish (Hidalgo *et al.*, 1999; Kuzmina *et al.*, 1996).

One aim of this study was to assess if diet composition affects the energy utilisation efficiency of DE for growth ($k_{\mathrm{g,DE}}$) in African catfish. The estimated $k_{\mathrm{g,DE}}$ in the current study are relatively high compared to most reported values across fish species (Schrama *et al*., 2012). But the range of the $k_{\mathrm{g,DE}}$ (76 to 98%) found for African catfish in this study is still overlapping with the range of values found for European seabass, 64 to 82% (Lupatsch *et al.*, 2001, 2003; Lupatsch *et al.*, 2010; Peres & Oliva-Teles, 2005). The high values of $k_{\mathrm{g,DE}}$ in the present study might be due to an underestimation of the ADC values. An underestimated ADC values result in an underestimation of the digested energy intake and consequently a overestimation of the energy utilisation efficiency when using the DE approach. The high $k_{\mathrm{g,DE}}$ values found for all diets might also be a reflection of a good ability of catfish to utilize starch as energy source for ATP production and fat synthesis. However, the high $k_{\mathrm{g,DE}}$ value in African catfish can also be related to a relatively large portion of energy being stored as fat instead of protein. Energy gain as fat is more efficient than energy gain as protein (Bureau *et al.*, 2006).

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 conducted as follows: the measured retained energy for each data point in the data set was added with the estimated fasting heat production (intercept) to get the NE value, which was then corrected towards zero dFat and dCarb in order to see only the effect of dCP on NE in panel (A); zero dCP and dCarb in order to see only the effect of dFat on NE in panel (B); and zero dCP and dFat in order to see only the effect of dCarb on NE in panel (C). This was conducted using Equation (2).

In line with observations in tilapia (Schrama *et al*., 2012), barramundi (Glencross *et al*., 2017), rainbow trout (Rodehutscord and Pfeffer, 1999) and snakehead (Phan *et al*., 2021), dietary macronutrient composition affects $k_{\mathrm{g,DE}}$ in African catfish (Figure 3). More specifically dietary starch supplementation reduced $k_{\mathrm{g,DE}}$ whereas fat supplementation had no impact. The implication of this effect of dietary macronutrient composition on $k_{\mathrm{g,DE}}$ is that feed evaluation/formulation using a DE approach can be biased. For this reason, in pig nutrition NE equations were developed (CVB, 1993, Noblet *et al*., 1994). With this as an example, an NE equation was estimated for African fish (Equation 3) in this study relating RE to the digested protein (dCP), fat (dFat) and carbohydrates intakes (dCarb). This approach was earlier done for the following fish species: tilapia and trout (Schrama *et al.,* 2018), common carp and barramundi (Phan *et al.*, 2019) and snakehead (Phan *et al.*, 2021).

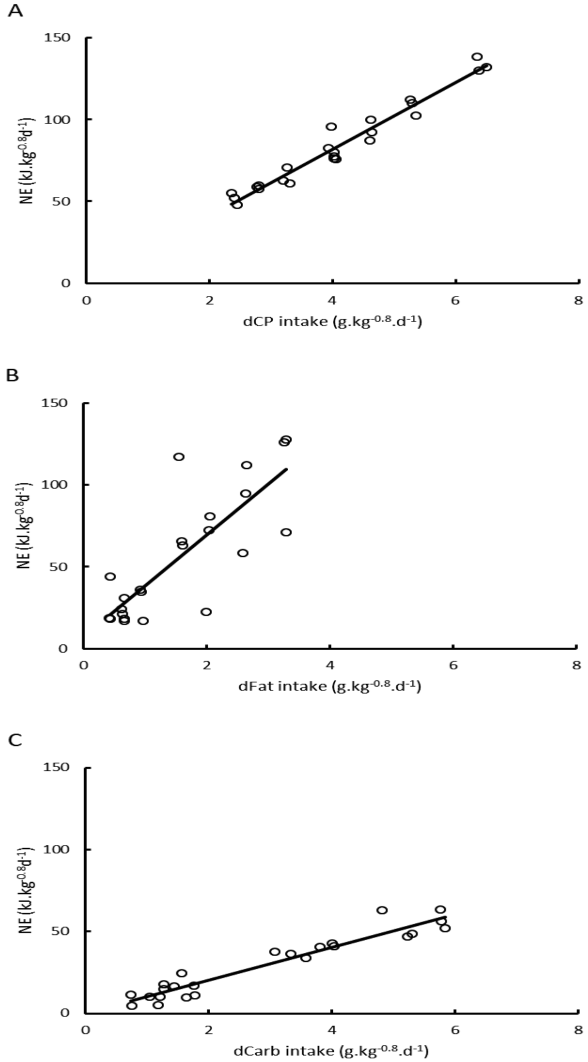

Figure 4. Relationship between net energy (NE) and digestible protein (dCP) intake (A), NE and digestible fat (dFat) intake (B), NE and digestible carbohydrates (dCarb) intake (C) in African catfish.

In Figure 5 the estimates of energy utilisation efficiencies of dCP, dFat and dCarb are compared between these fish species. The estimated energy utilisation efficiency of dCP ($k_{NE;dCP}$) for African catfish was 20.4 kJ.g^{-1} (*i.e.*, 86%), which is higher compared to estimates for all other fish species (Figure 5). This high $k_{NE;dCP}$ value can be caused by an underestimation of the protein ADC in the present study, which would result in an underestimation of dCP and thereby in an overestimation of $k_{NE;dCP}$. However, the protein retention efficiency (retained protein as percentage of digestible protein; Table 3) in the current study ranged from 61 to 73%. These values are comparable to the value of 63% found for African catfish in a recent study (Elesho *et al.*, 2020). This suggests that African catfish can use protein more efficiently than snakehead (54%) (Phan *et al.*, 2021), barramundi

(59%) (Glencross *et al.*, 2017), common carp (46%) (Phan *et al.*, 2019) and Nile tilapia (53%) (Haidar *et al.*, 2018). This indicates that African catfish is able to adapt to high protein intake (Supplementary table S4).

Compared to other other fish species (Figure 5), also the energy utilization efficiency of digestible fat ($k_{NE;dFat}$) for African catfish of 37.6 kJ.g^{-1} is highest, but very similar to the $k_{NE;dFat}$ value of barramundi (37.1 kJ.g^{-1}) (Phan *et al.*, 2019; Schrama *et al.*, 2018). This high $k_{NE;dFat}$ value is most likely related to the high body fat content of African catfish. As a result of the high fat gain relative to protein gain on an energy basis (Table 3), a larger proportion of the digested fat is directly stored in adipose tissue, especially since the utilization efficiency of digestible carbohydrates ($k_{NE;dCarb}$) was good (59%) for African catfish. In other words, a large part of dCarb could contribute to the energy retention and especially ATP production thereby sparing both dCP and dFat for ATP production resulting thereby in the high $k_{NE;dCP}$ and $k_{NE;dFat}$.

The estimated energy utilization efficiency of dCarb ($k_{NE;dCarb}$) in African catfish was 10.1 kJ.g^{-1} (*i.e.*, 59%), which implies that African catfish can metabolise dCarb. The $k_{NE;dCarb}$ value of African catfish is comparable to values found in tilapia, common carp and rainbow trout, but much higher than the value for barramundi (18%) and snakehead (5%) (Figure 5). RE was linearly related to dCarb (*i.e.*, no polynomial effect). This implies that African catfish is well able to metabolise carbohydrates efficiently even at high dCarb intakes.

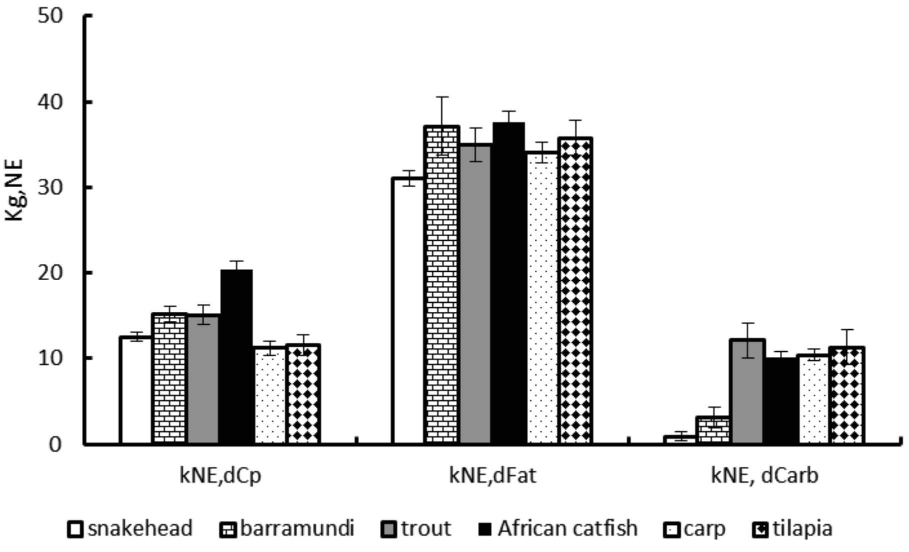

Figure 5. Energy utilisation efficiencies ($k_{g,NE}$) of digested protein (dCP) , fat (dFat) and carbohydrates (dCarb) in trout, tilapia (Schrama *et al.*, 2018), barramundi, carp (Phan *et al.*, 2019), snakehead (submitted) and African catfish (present study) using linear relationship between retained energy and intake of digested protein, fat and carbohydrates.

The current study demonstrates that faecal recovery is low in African catfish. Starch supplementation reduced the faecal recovery (*i.e.*, faecal stability) in African catfish. As shown in other species, the relationship between DE and RE is affected by dietary macronutrient composition in African catfish. Starch supplementation reduced the $k_{g,DE}$ value, while fat supplementation did not. The implication of this effect of dietary macronutrient composition on $k_{g,DE}$ is that feed evaluation/formulation using a DE approach can be biased. Applying an NE approach for energy evaluation of feed/ingredients is therefore also for African catfish a logic step forward. Our efforts to use AIA versus yttrium greatly improved the accuracy of ADC values, and thus the NE equation. However, , the current estimated NE equation needs future validation due to the potential under estimation of digestible nutrient intakes related to the low faecal recovery.

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Supplementary data

Supplementary Table S1. Amino acid composition of diets for African catfish, fed 4 different diets at 2 feeding levels (FL) for 30 days

	P	C	F	M
	“protein”	“protein”	“protein”	“protein”
		+Carb	+ Fat	+Carb + Fat
g.kg $^{-1}$ Crude protein				
Lys	62.6	61.6	62.2	62.2
Met	27.6	27.3	27.7	27.8
Met+Cys	42.0	41.3	42.2	42.6
Cys	14.4	14.0	14.5	14.7
Thr	42.0	41.0	41.9	42.0
Arg	61.5	60.1	61.8	61.6
Ile	40.9	40.5	40.7	41.0
Leu	73.1	71.6	72.6	73.2
Val	45.5	45.3	45.7	46.0
His	22.7	22.3	22.9	22.9
Phe	47.9	47.1	47.8	48.3
Gly	44.3	43.5	44.5	44.5
Ser	46.9	45.1	46.4	46.8
Pro	67.1	62.5	64.1	65.0
Ala	42.0	41.3	41.9	42.2
Asp	84.4	82.6	83.7	84.2
Glu	209.7	205.3	209.5	211.8

P, the diet with a high protein content; C, the P diet supplemented with maize starch; F, the P diet supplemented with fat blend; M, the P diet supplemented with maize starch and fat blend; Carb, Carbohydrates; Fat blend (rapeseed oil: soybean oil = 1:1).

Supplementary table S2. Initial and final body composition of African catfish (n=3), fed 4 diets (D) at 2 feeding levels (FL) for 30 days

	FL	Initial	P "protein"	C "protein" +Carb	F "protein" +Fat	M "protein" +Carb+Fat	SEM	*P* values D	 FL	 D x FL
DM		244								
	Low		251^{f}	263^{de}	276^{c}	287^{b}	2.0	***	***	***
	High		254^{ef}	269^{cd}	294^{b}	307^{a}				
Energy		6.2								
	Low		6.1^{f}	6.6^{de}	7.3^{c}	7.8^{b}	0.08	***	***	***
	High		6.3^{ef}	6.8^{d}	8.0^{b}	8.6^{a}				
Protein		159								
	Low		167	159	158	153	1.3	***	Ns	ns
	High		169	160	157	152				
Fat		64								
	Low		58^{e}	75^{d}	91^{c}	106^{b}	2.1	***	***	***
	High		60^{e}	79^{d}	110^{b}	126^{a}				
Ash		26.5								
	Low		29.4	29.2	28.3	28.2	0.79	ns	Ns	ns
	High		28.1	29.1	28.4	28.3				

P, the diet with a high protein content; C, the P diet supplemented with maize starch; F, the P diet supplemented with fat blend; M, the P diet supplemented with maize starch and fat blend; Carb, Carbohydrates; Fat blend (rapeseed oil: soybean oil = 1:1);FL, feeding level. Acid-insoluble ash was used as inert marker to estimate digestibility.

***, P<0.001; *, P<0.05; ns, non-significantly different

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

Supplementary table S3a. Apparent digestibility coefficient (ADC) (%) of dietary nutrients in African catfish (n=3) fed 4 diets (D) at 2 feeding levels (FL) for 30 days using acid insoluble ash as inert marker to estimate ADC

	FL	P	C	F	M				
		"protein"	"protein"	"protein"	"protein"		*P* values		
			+Carb	+Fat	+Carb+Fat	SEM	D	FL	D x FL
ADC Protein	Low	93.8	91.6	92.9	91.6	0.49	***	Ns	ns
	High	93.2	91.3	94.0	90.8				
ADC Fat	Low	87.4^{b}	83.4^{c}	94.9^{a}	94.5^{a}	0.77	***	***	***
	High	83.6^{c}	76.6^{d}	95.3^{a}	92.9^{a}				
ADC Carb	Low	51.1	77.0	40.7	75.2	3.89	***	*	ns
	High	33.4	68.2	42.3	68.8				
ADC DM	Low	76.0	81.4	77.2	82.6	1.68	***	***	ns
	High	69.0	76.0	78.2	79.0				
ADC energy	Low	80.1	82.4	83.3	85.3	1.38	***	***	ns
	High	73.6	76.7	84.0	82.0				
ADC starch	Low	99.3^{b}	99.7^{a}	99.1^{b}	99.7^{a}	0.08	***	*	*
	High	99.2^{b}	99.3^{ab}	99.2^{b}	99.5^{ab}				
ADC NSP	Low	-4.3	-8.4	-28.0	-41.1	11.11	*	***	ns
	High	-42.3	-49.0	-24.6	-77.2				
ADC ash	Low	55.6	64.6	58.5	61.5	3.22	*	Ns	ns
	High	48.1	63.6	59.0	60.5				
ADC P	Low	62	76	69	77	2.7	***	*	ns
	High	54	74	66	74				

P, the diet with a high protein content; C, the P diet supplemented with maize starch; F, the P diet supplemented with fat blend; M, the P diet supplemented with maize starch and fat blend; Carb, Carbohydrates; Fat blend (rapeseed oil: soybean oil = 1:1); FL, feeding level. DM, dry matter; NSP, non-starch polysaccharides.

***, $P<0.001$; *, $P<0.05$; ns, non-significantly different

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

Supplementary Table S3b. Apparent digestibility coefficient (ADC) (%) of dietary nutrients in African catfish (n=3) fed 4 diets (D) at 2 feeding levels (FL) for 30 days using yttrium as inert marker to estimate ADC

	FL	P	C	F	M				
		"protein"	"protein"	"protein"	"protein"		*P* values		
			+Carb	+Fat	+Carb+Fat	SEM	D	FL	D x FL
Protein	Low	93.3^{a}	89.6^{b}	91.9^{a}	89.2^{b}	0.35	***	***	***
	High	93.6^{a}	84.3^{c}	93.5^{a}	84.9^{c}				
Fat	Low	86.7^{bc}	79.6^{d}	94.2^{a}	93.0^{a}	0.47	***	***	***
	High	84.6^{c}	57.9^{e}	95.0^{a}	88.3^{b}				
Carb	Low	47.7^{b}	71.6^{a}	32.6^{c}	68.2^{a}	2.73	***	***	***
	High	37.6^{bc}	42.7^{bc}	38.3^{bc}	48.7^{b}				
DM	Low	74.3^{ab}	77.0^{a}	74.1^{ab}	77.7^{a}	1.12	***	***	***
	High	70.9^{bc}	56.7^{d}	76.7^{a}	65.5^{c}				
Energy	Low	78.7^{ab}	78.2^{ab}	81.0^{a}	81.1^{a}	1.03	***	***	***
	High	75.3^{bc}	57.9^{d}	82.9^{a}	70.4^{c}				
Starch	Low	99.2^{ab}	99.6^{a}	98.9^{ab}	99.6^{a}	0.16	ns	ns	*
	High	99.3^{ab}	98.8^{b}	99.1^{ab}	99.2^{ab}				
NSP	Low	-11.5^{a}	-33.9^{a}	-45.3^{ab}	-81.4^{b}	9.31	***	***	***
	High	-33.3^{a}	-168.6^{c}	-33.2^{a}	-191.6^{c}				
Ash	Low	52.6^{a}	56.3^{a}	52.8^{a}	50.5^{a}	1.6	***	***	***
	High	51.3^{a}	34.6^{b}	56.2^{a}	35.3^{b}				
Phosphorus	Low	59.6^{cd}	70.5^{a}	65.6^{ab}	70.5^{a}	1.15	***	***	***
	High	56.6^{de}	53.9^{e}	63.9^{bc}	57.2^{de}				

P, diet with a high protein content; C, the P diet supplemented with maize starch; F, the P diet supplemented with fat blend; M, the P diet supplemented with maize starch and fat; Carb, Carbohydrates; Fat blend (Rapeseed oil: soybean oil = 1:1); DM, dry matter; NSP, non-starch polysaccharides.

***, $P<0.001$; *, $P<0.05$; ns, non-significantly different

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

Supplementary table S4. Nitrogen ($mg.kg^{-0.8}.day^{-1}$) balance analysis of African catfish (n = 3), fed 4 different diets (D) at 2 feeding levels (FL) for 30 days

	FL	P	C	F	M		*P* values		
		"protein"	"protein"	"protein"	"protein"				
			+Carb	+Fat	+Carb+Fat	SEM	D	FL	D x FL
GNI	Low	684^{e}	490^{g}	562^{f}	421^{h}	3.1	***	***	***
	High	1102^{a}	811^{c}	904^{b}	707^{d}				
DNI	Low	641^{d}	449^{f}	522^{e}	386^{g}	4.5	***	***	***
	High	1027^{a}	740^{c}	849^{b}	642^{d}				
FN	Low	43^{cd}	41^{d}	40^{d}	35^{d}	2.7	***	***	*
	High	75^{a}	71^{a}	55^{bc}	65^{ab}				
BUN	Low	251	161	171	128	11.1	***	***	ns
	High	361	224	245	176				
RN	Low	391^{e}	288^{f}	352^{e}	258^{f}	8.1	***	***	***
	High	666^{a}	517^{c}	605^{b}	467^{d}				

P, the diet with a high protein content; C, the P diet supplemented with maize starch; F, the P diet supplemented with fat blend; M, the P diet supplemented with maize starch and fat blend; Carb, Carbohydrates; Fat blend (rapeseed oil: soybean oil = 1:1); FL, feeding level; *P* values for effects of diet, feeding level or the interaction, respectively; GNI, gross nitrogen intake; DNI, digestible nitrogen intake; FN, faecal nitrogen; RN, retained nitrogen; BUN, branchial and urinary nitrogen. ***, $P<0.001$; *, $P<0.05$; ns, non-significantly different

^{abcefgh}If interaction effect is significant, means lacking a common superscript differ significantly ($P<0.05$).

4

Energy utilisation efficiencies of digested protein, fat and carbohydrates in striped catfish (*Pangasius hypophthalmus*) for whole body and fillet growth

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Abstract

This study aimed to assess the effect of dietary macronutrient composition on the relationship between RE and DE intake (*i.e.*, the maintenance energy requirements and the slope [k_{gDE}]); to quantify the energy utilisation efficiencies of digested protein, fat and carbohydrates for whole body growth as well as fillet growth in striped catfish (*Pangasius hypoththalmus*). To achieve these aims, a 63-day experiment was conducted on striped catfish (29.1 g). A total of 4 diets were studied at 2 feeding levels, low vs. high (12 vs. 22 $g.kg^{-0.8}.d^{-1}$, respectively), which resulted in a 4×2 factorial design. The four diets had contrasting inclusion levels of protein, fat and carbohydrates. Striped catfish digested part of the non-starch polysaccharides (33.6 -71.0%) while starch is almost completely digested (> 94%). By conducting the regression between RE and DE intake over diets, the energy utilisation efficiency for striped catfish was estimated at 71% through the equation: RE = - 42 (se 9.2) + 0.71 (se 0.049) DE intake, ($R^2 = 0.95$). Diet composition did not affect the relationship between RE and DE intake. Multiple regression of RE as a function of digested protein, fat and carbohydrates intake (in $g.kg^{-0.8}.d^{-1}$) was also conducted to estimate the energy utilization efficiency of digested protein, fat and carbohydrates. The estimated energy efficiencies of digested protein, fat and carbohydrates for energy retention at the whole fish level were 64%, 80% and 58%, respectively. The energetic values of dCP, dFat and dCarb for whole body growth differ from the energetic values for fillet production. For fillet growth, digested protein had a higher potential compared to digested fat and carbohydrates, however this needs to be used in a balanced ratio with digested fat and carbohydrates.

Key words: Energy evaluation; Energy metabolism; Fillet growth; Bioenergetics; Net energy; Energy efficiency; Digestible nutrients; *Pangasius hypophthalmus*.

4.1. Introduction

Protein, fat and carbohydrates can provide the essential energy needs of fish for maintenance and growth. Protein is the key nutrient for new tissue accretion. Dietary protein is preferred to be used for growth instead of providing energy, because protein is often costly. In addition, the use of protein for energy causes NH_4^+ excretion, which burdens the culture environment. Fat and carbohydrates are preferably used for energy supply, either directly for ATP production or indirectly in the form of fat storage for future energy needs, in order to spare protein. The success of culturing striped catfish depends on the efficient conversion of protein, fat and carbohydrates into growth. To achieve an efficient feed conversion, formulating balanced diets requires information on the amount of nutrients needed for maintenance and for growth. For many, especially newly cultured fish species, such nutritional information is often lacking. Striped catfish is one of the major fish species cultured worldwide (FAO, 2018). The annual production of striped catfish has strongly increased over the past two decades. Currently, the annual production of striped catfish was over 1.1 million tons globally (FishstatJ, 2020). Although striped catfish has been already cultured at a large scale for years, information regarding their nutritional requirements is still limited.

For many fish species, the optimal dietary energy content is calculated by using the factorial approach (Glencross *et al.*, 2011; Glencross, 2008). In this approach the energy requirements for maintenance and for growth are calculated from an estimated relationship between the digestible energy intake (DE) and the retained energy (RE). This relationship (i.e., RE = intercept + slope x DE intake) is normally derived from experiments using only one diet given at various rations. As for instance was done for striped catfish (Glencross *et al.*, 2011), European seabass (Lupatsch *et al.*, 2010) and barramundi (Glencross, 2008). In this factorial approach of calculating the total DE requirements of a fish species, the slope of the linear relationship or the energy utilization efficiency ($k_{g,DE}$) is assumed to be constant and unaffected by the dietary composition. However, for various fish species it has been shown that the $k_{g,DE}$ is influenced by the dietary macronutrient composition; e.g. for barramundi (*Lates calcarifer*) (Glencross *et al.*, 2017), carp (Phan *et al.*, 2019) and Nile tilapia (*Oreochromis niloticus*) (Schrama *et al.*, 2012a). For striped catfish the information is insufficient to evaluate if the relationship between RE and DE is affected by the type of diet. In other words, it is unclear if the dietary macronutrient composition alters the energy requirements for maintenance and/or the energy utilisation efficiency ($k_{g,DE}$) in striped catfish.

The impact of the dietary macronutrient composition on the relationship between DE and RE has been the reason in pig nutrition to move from an energy evaluation system on a DE basis towards a system on a net energy (NE) basis already more than fifty years ago (CVB, 1993; Noblet *et al.*, 1994). In a NE approach, the DE is differentiated into digestible energy originated from protein, fat and carbohydrates with each having its own energy utilisation efficiency ($k_{g,NE}$). The advantage of a NE evaluation system is the ability to quantify and make a distinction between the energy utilisation efficiencies of digested protein ($k_{NE,dCP}$), fat ($k_{NE,dFat}$) and carbohydrates ($k_{NE,dCarb}$). In fish, the energy utilisation efficiency of digested protein, fat and carbohydrates have been quantified for tilapia and trout (Schrama *et al.*, 2018), barramundi and carp (Phan *et al.*, 2019), and snakehead (Phan *et al.*, 2021b). However, the energy utilisation efficiency of digested protein, fat and carbohydrates for striped catfish are still unknown.

Of the total global seafood production, fish fillets are the main part used for human consumption, while filleting waste *e.g.* liver, viscera, head, bone, skin and scales are commonly used as by-products for animal feed. Insights into the potential of digested macronutrients to attribute to specifically the fillet growth and the growth of possible other defined body compartments (*i.e.*, liver, viscera, and the rest fraction) might reduce the filleting waste. More importantly, it can attribute to the development of an alternative feed evaluation system considering the economic priority of the fish fillet. Currently, feed formulation has been mainly focused on getting the optimal macronutrient composition for the growth of fish at the whole body level. An alternative feed evaluation system, which would focus on the growth or the energy utilisation efficiency at the body compartment level could be a tool to make feed formulations more tailor-made and efficient. However, such an approach of relating fillet growth to the intake of digested macronutrients on the compartment level has, to the best of our knowledge, not been attempted earlier for any fish species.

To fulfil the above described knowledge gaps for striped catfish, this study aims to: 1. assess the effect of dietary macronutrient composition on the relationship between RE and DE intake; 2. quantify the energy utilisation efficiencies of digested protein, fat and carbohydrates for whole body growth on striped catfish to be able to use a NE evaluation system for striped catfish; 3. quantify the energy potential of digested protein, fat and carbohydrates to contribute to fillet growth in contrast to the growth of the other defined body compartments .

4.2. Materials and methods

Experimental diets. A total of four diets were formulated with different dietary inclusion levels of crude protein (243 – 380 g.kg^{-1}), crude fat (44 -236 g.kg^{-1}) and carbohydrates (352 – 601 g.kg^{-1}) using the triangle approach (Raubenheimer, 2011) to create a wide contrast between macronutrients (*i.e.,* crude protein, fat and total carbohydrates) (Table 1). The variability in the dietary macronutrient composition was created by varying the inclusion level of cassava (a high starch ingredient) and soya oil (Table 1).

The high protein diet (P-diet) was formulated by using protein sources like fish meal, soybean meal and rapeseed meal. This P-diet was mixed with cassava (30%) to create high starch diet (C-diet), with soya oil (12.5%) to create a high fat diet (F-diet), or with both cassava and soya oil to create a diet high in fat and starch (M-diet). All diets were studied at 2 feeding levels, low vs. high, which resulted in a 4×2 factorial design with a total of 8 treatments. This design aimed to create large contrasts between the digestible macronutrient intake among the 4 different diets to be able to conduct the multiple regression analysis of energy retention (*i.e.*, growth response) as a function of digestible protein (dCP), digestible fat (dFat) and digestible carbohydrates intake (dCarb). Due to this large range in macronutrients, diets were formulated to have a constant ratio between protein and premix content. Diets were formulated using the protein requirements averaged over freshwater teleost fish (NRC, 2011).

Diets were produced by De Heus (Vinh Long, Vietnam). All ingredients except soy oil in the F- and M-diet and premix were hammer-milled through a 0.9 mm screen at 1470 rpm and mixed in a 60-L batch mixer for 240 sec. Prior to extrusion, these mixtures were conditioned for 10 sec at a temperature between 85-100°C. Diets were extruded on a twin-screw extruder with a capacity of 150 kg/h using a 2 mm die at 95-110°C. This produced 3mm floating pellets, which were dried at 95°C for 10 min. Thereafter pellets of the F- and M-diet were vacuum coated with soy oil. After coating, pellets were cooled at 30-33°C for 10 min. Pellets were screened through a 2 mm mesh-sized basket to remove fines before feeding to fish.

Table 1. Formulation and composition of four experimental diets fed to striped catfish

	P	C	F	M
	"protein"	"protein"	"protein"	"protein"
		+Carb	+Fat	+Carb+Fat
Diet composition (g.100 g^{-1}, as-is)				
Cassava	0.0	34.3	0.0	30.0
Soy bean oil	0.0	0.0	17.9	12.5
Fishmeal	15.7	10.3	12.8	9.0
Rapeseed meal	7.0	4.6	5.7	4.0
Soybean meal	17.4	11.4	14.3	10.0
Feather meal	7.0	4.6	5.7	4.0
Methionine	0.5	0.3	0.4	0.3
Lysine	0.7	0.5	0.6	0.4
Tryptophan	0.2	0.1	0.1	0.1
Wheat	12.2	8.0	10.0	7.0
Rice bran full fat	17.4	11.4	14.3	10.0
Wheat flour	13.9	9.1	11.4	8.0
Mono calcium phosphate	4.2	2.8	3.5	2.4
Premix$^{\#}$	4.0	2.6	3.3	2.3
Chemical composition (g.kg^{-1}, DM)				
DM	961	949	950	953
Crude protein	380	260	300	243
Total fat	62	44	236	165
Total carbohydrate	430	601	352	505
Starch	209	375	187	334
NSP	221	226	164	171
Crude ash	132	99	117	91
Yttrium	0.35	0.23	0.28	0.19
Gross energy (kJ.g^{-1}, DM)	18.3	17.9	21.8	20.7
CP/GE	20.8	14.5	13.8	11.7
DP/DE at low feeding level	21.9	14.4	14.3	11.6
DP/DE at high feeding level	22.1	14.4	14.8	11.7

P, diet with a high protein content; C, the P diet supplemented with starch; F, the P diet supplemented with fat; M, the P diet supplemented with fat and starch; Carb, Carbohydrate; DM, dry matter; CP, crude protein, GE, gross energy, DP, digestible protein; DE, digestible energy; $^{\#}$De Heus Animal nutrition B.V. closed premix formula for vitamins and trace minerals to meet the requirements of fresh water fish (NRC, 2011).

Fish handling. The study (project number: 2018.W-0021.001) was evaluated by the Ethical Committee of Animal Experiments of Wageningen University, The Netherlands and carried out at the research and development centre of De Heus (Vinh Long, Vietnam) in compliance with Vietnamese law.

A total of 2980 striped catfish (*P. hypothalamus)*, with a mean body weight of 29.1 g (SE 0.05) were obtained from Vinh Long, Viet Nam. The experiment lasted 63 day. At the start of the experiment, groups of 120 fish were batch-weighed and randomly assigned to one of the twenty four tanks, giving 3 replicates for each of the 8 treatments (2 feeding levels x 4 diets). At the end of the experiment, fish in each tank were batch-weighed and counted to calculate the average final body weight. The growth performance was calculated based on the difference between the average initial and average final body weight of the fish. The experiment was conducted using 500-L tanks, integrated in a RAS system. The water flow per tank was 30 L/min. The measured water quality parameters during the experiment for temperature, oxygen, pH, conductivity, NH_4-N, NO_2-N and NO_3-N were 28.6 ± 0.49°C, 5.0 ± 1.04 mg/L, 7.2 ± 0.22, 2.8 ± 0.59 mS/m, <0.5 mg/L, < 0.5 mg/L, and < 50 mg/L, respectively.

Striped catfish were hand-fed twice a day from 09:00 to 10:00 hours and from 16:00 to 17:00 hours. Fish were fed restrictively one of two feeding levels based on metabolic body weight. The planned feeding levels were 12 vs. 22 $g.kg^{-0.8}.d^{-1}$. The daily feed amount was calculated based on the mean initial body increased with the expected growth which was derived from the realized feed intake and using an assumed FCR of 1.1. The first 2 weeks of the experiment were planned to gradually increase the feed intake from 10% to 100% of the planned feeding level. However, the first week after the start of the experiment the response of the fish was minimal. Therefore the adaptation period was extended to 3 weeks. The last two weeks of the experiment, fish fed Diet M were not able to finish all feed due to the rainy weather. Therefore the feeding level at all treatments were reduced.

Sampling. At the start of the experiment, 100 fish from the initial population were euthanized by an overdose of Aqui-S (Aqui-S New Zealand Ltd., Lower Hutt, New Zealand). Of these, 50 were used for the analysis of the initial whole body composition and the other for initial composition of body compartments. At the end of the trial, forty fish from each tank were euthanized similarly to determine the final whole body composition (n=20) as well as final composition of body compartments (n=20). To prepare for the chemical analyses of the final composition of body compartments, whole fish were dissected and separated into four compartments: 1) liver without bile bladder, 2) viscera, which including bile bladder, pancreas, stomach, intestine and gonad glands, 3) fillet, and 4) the rest fraction, which comprised of head, bones, skin and air bladder. Compartment samples were pooled per tank (being the experimental unit) and stored at -20°C.

Chemical analysis. After sampling the fish and fish compartments were pooled per tank (experimental unit) and stored at -20°C. Sample preparation and chemical analysis for protein, fat, energy, dry matter, ash and phosphorus were executed as described by Saravanan *et al.* (2012b). Starch was analysed as described in Maas *et al.* (2019). In feed and faeces, carbohydrates ($g.kg^{-1}$) on a dry matter basis was calculated by deducting protein, fat and ash from 1000. The total amount of NSP ($g.kg^{-1}$) was calculated by deducting starch from carbohydrates.

Nutrient digestibility estimates. Yttrium oxide was used as a marker (Table 1). Protein, fat, starch, dry matter and ash were analysed in feed and faeces. Feed was sampled every week to have a representative sample of the feed given to fish. Each tank was connected to a separate settling unit to collect faeces. Each settling unit was equipped with an ice-cooled glass bottle at the bottom to prevent bacterial degradation of the faecal nutrients during collection. Faeces settled overnight were collected daily prior to the morning feeding from week 4 to week 9 of the experiment. The procedure of faeces collection was identical as described by Meriac *et al.* (2014a)

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

$$ADC_{nutrient} = (1- (marker_{diet}/\ marker_{faeces}) \times (Nutrient_{faeces}/Nutrient_{diet})) \times 100\%,$$

where $marker_{diet}$ and $marker_{faeces}$ is the yttrium concentration of the diet and faeces, and the $Nutrient_{diet}$ and $Nutrient_{faeces}$ are the dry matter (DM), protein, fat, carbohydrates or energy content of the diet and faeces, respectively.

Nutrient balance calculations. Feed intake was the average of the daily feed intake. The average daily feed intake was calculated using the daily consumed amount of feed (in g) per tank divided by the number of fish per tank. To standardise for differences in body weight and digestible macronutrient intake, nitrogen and energy balance parameters were expressed per unit of mean metabolic body weight. Metabolic body weight was calculated as $BW^{0.8}$ with BW expressed in kg. The mean metabolic body weight was calculated as the average of the initial and final metabolic body weight. The calculation of the energy and nitrogen balances were based on those described by Saravanan *et al.* (2012b). The intake of each macronutrient on a gross basis was determined by multiplying the averaged feed intake for each treatment by the macronutrient content in the diet. The digestible macronutrient intake was determined by multiplying the gross nutrient intake with the diet-specific apparent digestibility coefficient (ADC) for each macronutrient. The energy and nutrient retention rates were determined from the gain of energy, protein, fat and carbohydrates, calculated by the difference between the initial and the final whole-body macronutrient composition. The branchial and urinary N losses (BUN) were calculated using the difference between digestible N, N intake and N retention. The branchial and urinary energy (BUE) was estimated by multiplying BUN by 24.85, which is the energy content (in kJ) of 1 g excreted nitrogen with the assumption that NH_3-N is the only form of N excreted (Bureau *et al.*, 2003). The metabolisable energy intake was determined by the difference between the digestible energy intake and the BUE. The heat production was measured by deducting the ME from the RE.

Retained energy in body compartments. The retained energy in each compartment was determined from the gain of energy, calculated by the difference between the initial and the final compartment energy composition. The retained energy in each compartment was also expressed per unit of metabolic body weight.

Statistics. Data was analysed by using the 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 the apparent digestibility coefficients, growth performance, nitrogen and energy balance data.

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

$$RE_i = \mu + \beta \text{ x } DE_i + e_i \qquad \text{(Equation 1)},$$

where μ is the intercept, β is the energy utilisation efficiency; e_i is error term and i =1,..., n with n = 6 per diet. The difference in the slopes of the regression lines between the different diets was tested using a general linear model with RE as dependent variable, DE as covariate and diet as a fixed factor. If the interaction effect diet x DE is significant ($P<0.05$), the slopes are different across diets.

Multiple regression of retained energy (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 model:

$$RE_i = \mu + \beta_1 \times dCP_i + \beta_2 \times dFat_i + \beta_3 \times dCarb_i + e_i \qquad \text{(Equation 2)},$$

where μ is the intercept, being an estimate for fasting heat production (FHP); β_1, β_2, β_3 the energy utilisation efficiency of dCP ($k_{NE;dCP}$), dFat ($k_{NE;dFat}$) and dCarb ($k_{NE;dCarb}$), respectively; e_i is the error term and i =1,..., 24. The linearity and curve-linearity were checked in the relationship of RE with dCP, dFat and dCarb. The similar procedure of multiple regression of RE (in $kJ.kg^{-0.8}.d^{-1}$) was applied for each body compartment. Significance was set at $P<0.05$.

4.3. Results

Striped catfish had a daily weight gain ranging from 5.9 to 12.6 $g.kg^{-0.8}.d^{-1}$ for the low vs. high feeding level, respectively (Table 2). At the end of the experiment, the final body weight almost doubled at the low feeding level and quadrupled at the high feeding level. Final body weight was affected by feeding level, diet and the interaction between the two ($P<0.01$; Table 2).

The ADCs of the macronutrients are given in Table 3. There was an interaction effect between diet type and feeding level for the ADC of protein (P<0.05), while there was a tendency of an interaction effect between diet type and feeding level for the ADCs of energy, fat and carbohydrates. A higher feeding level lowered the ADCs of most nutrients (P<0.05), except starch. Feeding level, starch and fat supplementation affected the digestibility of the non-starch polysaccharides (NSP) in striped catfish (P<0.01). Starch supplementation increased the ADC of NSP from 51% to 62%, averaged over the low starch diets (diet P and F) and the high starch diets (diet C and M) (P<0.01), while fat supplementation decreased the ADC of NSP from 61% to 50% averaged over the fish fed the low fat diets (diet P and C) and the fish fed the high fat diets (diet F and M) (P<0.01) (Figure 1). Increasing the feeding level decreased the ADC of NSP from 62% at the low feeding level to 49% at the high feeding level averaged over diets (P<0.01). Increasing the dietary starch inclusion level increased the ADC of starch from 96% to 99% averaged over the fish fed the low starch diets and the fish fed the high starch diets (P=0.03) (Figure 1).

Data on the initial and final body composition of striped catfish are presented in supplementary table S1. At the start of the experiment, the body fat content of striped catfish was 50 $g.kg^{-1}$ (on a wet weight basis) and at the end on averaged 119 $g.kg^{-1}$, ranging from 61 to 171 $g.kg^{-1}$. The final body fat content was affected by diet and feeding level (P<0.05). This was also reflected in the energy retention (RE) as fat (Table 4), being affected by feeding level and by diet (P<0.001). Averaged over all treatments, RE as fat was 58 $kJ.kg^{-0.8}.d^{-1}$ and RE as protein was 31 $kJ.kg^{-0.8}.d^{-1}$ (Table 4). On energy basis, the ratios between fat and protein gain was unaffected by feeding level (P>0.1), but differed between diets (P<0.001; Table 4). Dietary supplementation of starch as well as fat increased the proportion of RE retained as fat compared to RE as protein (Table 4). The final body protein content was neither affected by diet nor by feeding level (P>0.05), but the protein efficiency (i.e., retained N as percentage of digested N) was influenced by diet and feeding level (P<0.01; Table 4). Dietary supplementation of fat and starch increased protein efficiency. At the high feeding level, protein efficiency was 42% at diet P, 50% at diet C, 53% at diet F and 56% at diet M. The complete N balances of striped catfish is presented in Supplementary table S2.

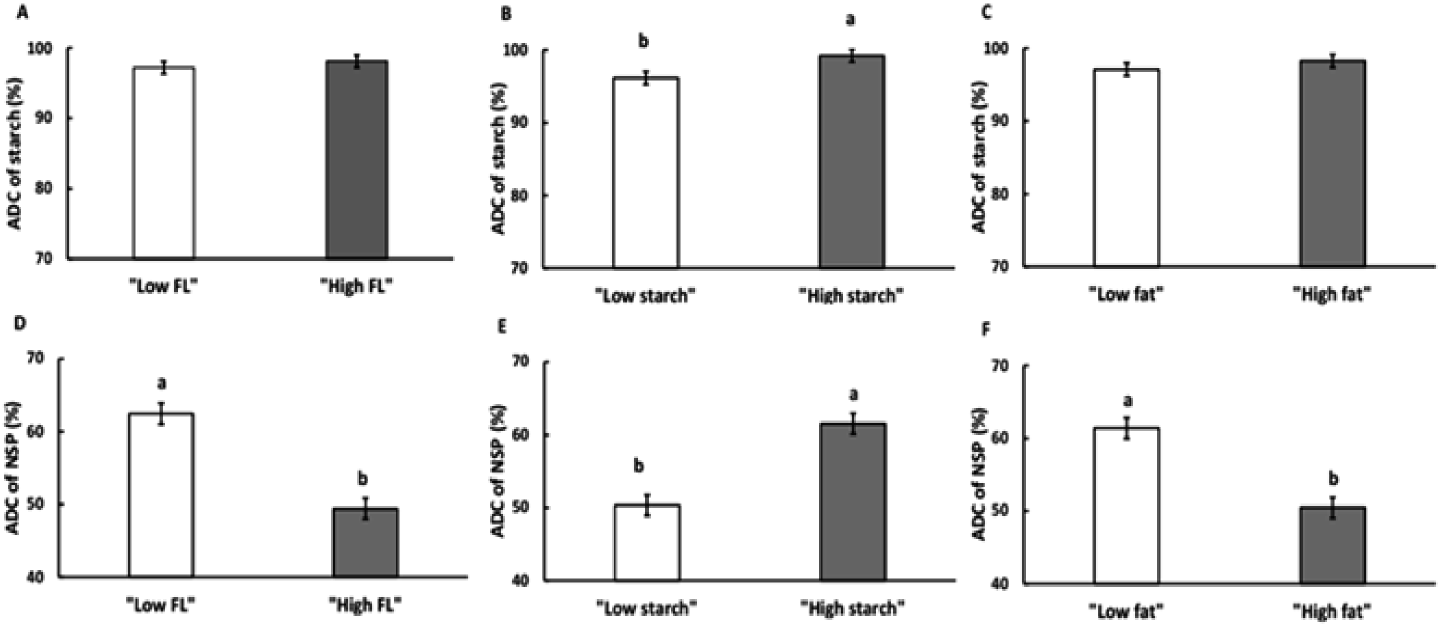

Figure 1. The effect of feeding level (FL), starch supplementation and fat supplementation on the apparent digestibility (ADC) of starch (Panel A, B, C) and on ADC of non-starch polysaccharides (NSP) (Panel D, E, F) in striped catfish. These main effects were analysed by three-way ANOVA (2 starch levels x 2 fat levels x 2 feeding levels). Values of “Low FL” and “High FL” are means values over all diets (panel A & D). Values of “Low starch” is the mean of Diet-P and Diet-F across both FL and “High starch” is the mean of Diet-C and Diet-M across both FL (Panel B & E). Values of “Low fat” is the mean of Diet-P and Diet-C across both FL and “High fat” is the mean of Diet-F and Diet-M across both FL (Panel C & F). Bars within panels having a different letter are different ($P<0.05$).

Table 2. Growth performance of striped catfish, (n = 3), fed 4 different diets at 2 feeding levels (FL) for 63 days

		P	C	F	M				
FL		protein	Protein	protein	protein		*P* values		
			+Carb	+Fat	+Carb+Fat	SEM	D	FL	D x FL
Final BW (g)									
	Low	72^{c}	59^{d}	69^{cd}	63^{cd}	2.4	***	***	***
	High	127^{a}	101^{b}	130^{a}	108^{b}				
Feed intake (g.d^{-1})									
	Low	0.8	0.8	0.7	0.7	---*	---*	---*	---*
	High	1.7	1.6	1.7	1.6				
Feed intake (g.kg$^{-0.8}$.d^{-1})									
	Low	8.4	9.2	8.5	8.9	---*	---*	---*	---*
	High	13.6	14.6	13.3	14.2				
Daily weight gain (g.kg$^{-0..8}$.d^{-1})									
	Low	7.4	5.9	7.2	6.4	0.19	***	***	ns
	High	12.3	10.4	12.6	11.0				
FCR									
	Low	1.13^{z}	1.57^{x}	1.17^{z}	1.40^{y}	0.030	***	***	ns
	High	1.10^{Z}	1.40^{X}	1.06^{Z}	1.29^{Y}				
Survival (%)									
	Low	100.0	99.7	100.0	100.0	0.14	ns	ns	ns
	High	100.0	100.0	99.7	100.0				

P, diet with a high protein content; C, the P diet supplemented with starch; F, the P diet supplemented with fat; M, the P diet supplemented with fat and starch; Carb, Carbohydrates; FL, feeding level; *P* values for effects of diet, feeding level or the interaction, respectively; BW, body weight; *No statistical analysis was conducted on feed intake because feed intake was controlled at 2 feeding levels; FCR, feed conversion rate.

***, *P*<0.01; *, *P*<0.05; ns, non-significantly different

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

$^{xyz\ \&\ XYZ}$ For parameters with a significant effect of diet, diets with a lacking a common letter in the superscript differ (*P*<0.05) and for parameters with a significant feeding level effect, means having a different case letter in the superscript differ between feeding level (*P*<0.05).

Table 3. Apparent digestibility coefficient (ADC) (%) of dietary nutrients in striped catfish (n=3) fed 4 diets at 2 feeding levels (FL) for 63 days

	FL	P	C	F	M		*P* values		
		"P"	"P"	"P"	"P"				
			+C	+F	+C+F	SEM	D	FL	D x FL
Dry matter	Low	77.7^{z}	83.4^{xy}	79.6^{yz}	84.6^{x}	0.64	***	***	ns
	High	71.5^{Z}	79.5^{XY}	75.6^{YZ}	82.4^{X}				
Energy	Low	85.9^{x}	88.2^{xy}	88.1^{xy}	90.2^{x}	0.67	***	***	ns
	High	80.1^{X}	84.8^{XY}	85.1^{XY}	88.5^{X}				
Protein	Low	90.6^{a}	87.9^{abc}	91.2^{a}	89.3^{a}	0.75	***	***	***
	High	85.2^{bc}	84.5^{c}	91.2^{a}	88.3^{ab}				
Fat	Low	88.2^{y}	88.0^{y}	94.6^{x}	95.6^{x}	0.73	***	***	ns
	High	84.0^{Y}	87.0^{Y}	92.4^{X}	95.5^{X}				
Carb	Low	78.9^{y}	88.5^{x}	75.7^{y}	87.0^{x}	0.76	***	***	ns
	High	72.5^{Y}	84.4^{X}	68.6^{Y}	83.6^{X}				
Starch	Low	95.5	99.0	95.1	99.3	1.77	ns	ns	ns
	High	94.7	99.1	99.3	99.4				
NSP	Low	63.1^{xy}	71.0^{x}	53.1^{y}	62.6^{x}	2.88	***	***	ns
	High	51.5^{XY}	60.0^{X}	33.6^{Y}	52.6^{X}				
Ash	Low	31.7^{c}	39.0^{a}	31.1^{c}	39.0^{a}	0.99	***	***	*
	High	23.7^{d}	34.0^{bc}	23.5^{d}	36.8^{ab}				
Phosphorus	Low	32.2^{y}	40.9^{x}	34.4^{y}	42.9^{x}	1.19	***	***	ns
	High	25.8^{Y}	34.5^{X}	23.9^{Y}	39.4^{X}				

P, diet with a high protein (P) content; C, the P diet supplemented with starch; F, the P diet supplemented with fat (F); M, the P diet supplemented with fat and starch ; Carb, Carbohydrates (C); DM, dry matter; NSP, non-starch polysaccharides.

***, *P*<0.01; *, *P*<0.05; ns, non-significantly different

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

$^{xyz\ \&\ XYZ}$ For parameters with a significant effect of diet, diets with a lacking a common letter in the superscript differ (*P*<0.05) and for parameters with a significant feeding level effect, means having a different case letter in the superscript differ between feeding level (*P*<0.05).

Table 4. Energy balance (kJ.kg$^{-0.8}$.d^{-1}) of striped catfish, (n = 3), fed 4 different diets at 2 feeding levels (FL) for 63 days

	FL	P	C	F	M				
		"protein"	"protein"	"protein"	"protein"		*P* values		
			+Carb	+Fat	+Carb+Fat	SEM	D	FL	D x FL
GE	Low	147	157	175	175	3.1	***	***	ns
	High	239	248	275	279				
DE	Low	127^{f}	139^{ef}	154^{de}	158^{d}	3.2	***	***	***
	High	191^{c}	210^{b}	234^{a}	247^{a}				
BUE	Low	7	5	5	4	0.3	***	***	ns
	High	10	6	6	5				
ME	Low	120^{f}	134^{ef}	149^{de}	154^{d}	3.2	***	***	***
	High	182^{c}	204^{b}	228^{a}	242^{a}				
HP	Low	74	85	72	76	5.1	*	***	ns
	High	88	102	91	113				
RE	Low	46	49	77	78	3.9	***	***	ns
	High	93	102	137	129				
REprot	Low	26	18	25	21	2.1	***	***	ns
	High	42	36	44	38				
REfat	Low	20^{y}	30^{xy}	52^{x}	57^{x}	3.7	***	***	ns
	High	51^{Y}	66^{XY}	93^{X}	90^{X}				
$RE_{fat:prot}$	Low	0.8^{z}	1.7^{y}	2.1^{xy}	2.8^{x}	0.18	***	Ns	ns
	High	1.2^{z}	1.9^{y}	2.2^{xy}	2.4^{x}				
PE^{*}	Low	39^{z}	39^{y}	48^{y}	48^{x}	3.1	***	***	ns
	High	42^{z}	50^{y}	53^{y}	56^{x}				

P, diet with a high protein content; C, the P diet supplemented with starch; F, the P diet supplemented with fat; M, the P diet supplemented with fat and starch; Carb, Carbohydrates; GE, gross energy; DE, digestible energy; BUE, branchial urinary energy; ME, metabolisable energy, HP, heat production; RE, retained energy; REprot, RE as protein; REfat, RE as fat; $REf_{at:prot}$, RE as fat : RE as protein. *PE, protein efficiency is retained protein divided by digestible protein intake (%).

***, *P*<0.01; *, *P*<0.05; ns, non-significantly different

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

$^{xyz\ \&\ XYZ}$ For parameters with a significant effect of diet, diets with a lacking a common letter in the superscript differ (*P*<0.05) and for parameters with a significant feeding level effect, means having a different case letter in the superscript differ between feeding level (*P*<0.05).

The first research aim was to assess the effect of diet composition (*i.e.*, macro-nutrient content) on the relationship between RE and DE intake for striped catfish. The estimated linear relationships between RE and DE for each diet are given in Figure 2. For striped catfish, the slopes of the relationships or k_{gDE} values were not affected by dietary composition (P>0.05). Because the slopes or the k_{gDE} values were similar between diets, all data were pooled to generate a general relationship between RE and DE intake over diets. By conducting the regression between RE and DE intake over diets, the energy utilisation efficiency for striped catfish was estimated at 71% through the equation: RE = - 42 (se 9.2) + 0.71 (se 0.049) DE intake, (R^2 = 0.95). From this equation the energy requirements for maintenance were estimated at 50 kJ.kg$^{-0.8}$.d^{-1}.

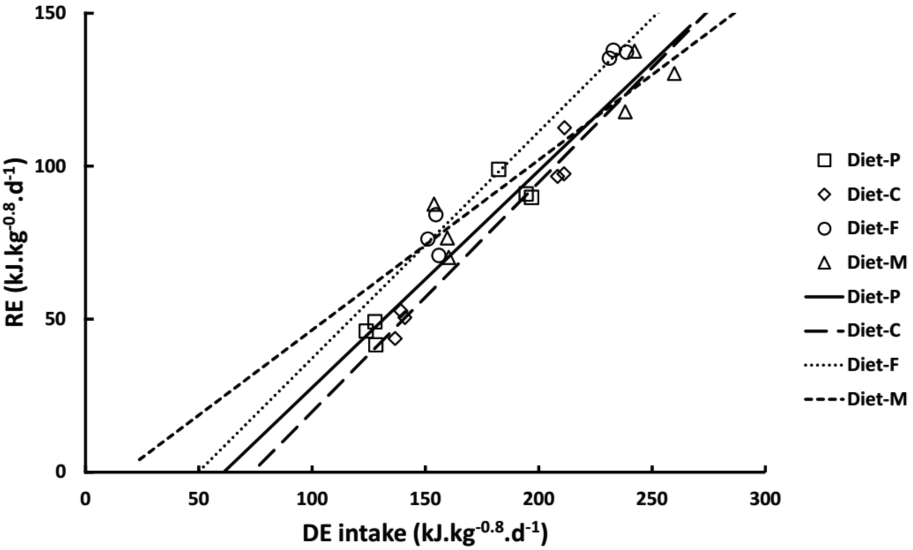

Figure 2. Relationship between retained energy (RE) and digestible energy intake (DE) for striped catfish fed one of four experimental diets: P, diet with a high protein content; C, the P diet supplemented with starch; F, the P diet supplemented with fat; M, the P diet supplemented with fat and starch (□ Diet P: RE= -43 (SE 16.5) + 0.71 (SE 0.102) DE (R^2=0.92), ◊ Diet C: RE= -55 (SE 13.0) + 0.75 (SE 0.073) DE (R^2= 0.96), ○ Diet F: RE= -37 (SE 10.9) + 0.74 (SE 0.055) DE (R^2= 0.98), Δ Diet M: RE= -9 (SE 19.5) + 0.56 (SE 0.094) DE (R^2=0.90)) on striped catfish. Digestible energy demand for maintenance is 61, 74, 50 and 17 kJ.kg$^{-0.8}$.d^{-1} for diet P, C, F and M, respectively.

The second aim was to quantify the energy utilization efficiencies of digested protein, fat and carbohydrates for growth (*i.e.*, estimating the NE equation for striped catfish). Therefore multiple linear regression between RE (in kJ.kg$^{-0.8}$.d^{-1}) and dCP, dFat and dCarb (in g.kg$^{-0.8}$.d^{-1}) was conducted and resulted in following estimated relationship with an R^2 of 0.95:

RE = - 33.7 (se 7.93) + 15.1 (se 2.24) dCP + 31.5 (se 2.04) dFat + 9.9 (se 1.14) dCarb (Equation 3)

By dividing the coefficients of dCP, dFat and dCarb of 15.1, 31.5, 9.9 kJ.g^{-1}, respectively in Equation 3 by the energetic value of these macronutrients (23.6 kJ.g^{-1}, 39.5 kJ.g^{-1} and 17.2 kJ.g^{-1} for CP, fat and carbohydrates, respectively), the energy utilisation efficiency of dCP, dFat and dCarb ($k_{NE;dCP}$, $k_{NE;dFat}$, and $k_{NE;dCarb}$) were determined as 64%, 80% and 58% for striped catfish, respectively. The intake of dCP, dFat and dCab were all linearly related to RE (*i.e.*, no polynomial effect was present, *P*>0.05).

The third aim was to quantify the energy utilization efficiencies of digested protein, fat and carbohydrates for growth of the different body compartments. RE in four different body compartments (fillet, liver, viscera and rest fraction) were measured at all treatments (Table 5). By summation of the energy retention of these four body compartments an alternative total energy retention ($RE_{\sum comp}$) was calculated. The estimated values of $RE_{\sum comp}$ and also treatment effects on this parameter (Table 5), match very well with that of RE measured by homogenizing whole fish (Table 4). Also the estimated relationship between RE and dCP, dFat and dCarb using both types of RE gave similar equations (Equation 3 versus Equation 4; Table 6).

Table 5. Retained energy ($kJ.kg^{-0.8}.d^{-1}$) in compartments of striped catfish, (n = 3), fed 4 different diets at 2 feeding levels (FL) for 63 days

	FL	P	C	F	M				
		"P"	"P"	"P"	"P"		*P* values		
			+C	+F	+C+F	SEM	D	FL	D x FL
Liver RE	Low	0.7	1.0	0.8	1.1	0.08	*	***	ns
	High	1.4	1.6	1.4	1.5				
Viscera RE	Low	4^{y}	6^{xy}	13^{xy}	14^{x}	2.8	***	***	ns
	High	10^{Y}	13^{XY}	18^{XY}	22^{X}				
Fillet RE	Low	12	11	14	12	2.0	ns	***	ns
	High	25	23	30	31				
Rest RE	Low	34	38	50	54	3.6	***	***	ns
	High	62	58	82	81				
RE total	Low	50	56	77	81	3.8	***	***	ns
	High	98	96	132	135				

P, diet with a high protein (P) content; C, the P diet supplemented with starch; F, the P diet supplemented with fat (F); M, the P diet supplemented with fat and starch; Carb, Carbohydrate (C); RE, retained energy; RE total, the total of RE in compartments (RE total = Liver RE + viscera RE+ fillet RE + the rest fraction RE).

***, $P<0.01$; *, $P<0.05$; ns, non-significantly different

$^{xyz\ \&\ XYZ}$ For parameters with a significant effect of diet, diets with a lacking a common letter in the superscript differ ($P<0.05$) and for parameters with a significant feeding level effect, means having a different case letter in the superscript differ between feeding level ($P<0.05$).

The estimated relationships between RE and digestible nutrient intake for each of the four compartment are given in Table 6. The ratio between the regression coefficients of dCP, dFat and dCarb varied between the different compartments. In the viscera the major contribution to RE came from dFat

and dCarb, while the energetic contribution of dCP was minor to viscera gain (Equation 6; Table 6). In contrast, fillet energy gain was strongly derived from dCP and less from dFat and dCarb compared to other compartments (Equation 7; Table 6). In fillet, the energy utilisation efficiency of dCP (5.9/23.6x100) was 25% and higher than the energy utilisation efficiency of dFat and dCarb with values of 14.9 % and 14.5 %, respectively which were similar.
By dividing the coefficients of dCP in liver (0.2), viscera (0.1), fillet (5.9) and the rest fraction (7.9) by the total of these values, the energy distribution of digested protein intake in liver, viscera, fillet and the rest fraction was determined (Figure 3). The majority of the energy from the digested protein was allocated to the fillet (42%). The energy distribution values of digested fat and carbohydrates in fillet were 20% and 26%, respectively (Figure 3).

Table 6. The net energy equations of the four different compartments, their sum and the whole body of striped catfish

	Equation	**R^2**	
Whole body homogenised	$NE = RE + 33.7 = 15.1\ dCP + 31.5\ dFat + 9.9\ dCarb$	0.95	(3)
Whole body Σ compartments	$NE = RE + 25.0 = 14.1\ dCP + 29.7\ dFat + 9.4\ dCarb$	0.95	(4)
Liver	$NE = RE + 0.1 = 0.2\ dCP + 0.1\ dFat + 0.2\ dCarb$	0.84	(5)
Viscera	$NE = RE + 4.2 = 0.1\ dCP + 6.6\ dFat + 2.0\ dCarb$	0.65	(6)
Fillet	$NE = RE + 14.4 = 5.9\ dCP + 5.9\ dFat + 2.5\ dCarb$	0.85	(7)
Rest fraction	$NE = RE + 6.2 = 7.9\ dCP + 17.1\ dFat + 4.8\ dCarb$	0.89	(8)

NE, net energy; RE, retained energy; dCP, digestible protein; dFat, digestible fat; dCarb, digestible carbohydrates (comprising of starch, sugars and non-starch polysacchrides)
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}.

Whole body homogenised, the equation was created with RE calculated based on the whole body energy composition data. Whole body Σ compartments , the equation was created with RE calculated based on the sum of RE in the four defined compartments.

4.4. Discussion

Carbohydrates is an important energy source for non-carnivorous fish species like tilapia, carp and catfish. In this study on striped catfish, between 15.2% to 42.3% of the total DE intake originated from digested starch, depending on the dietary composition. This large contribution of starch to DE is partly due to the high digestibility of starch observed for striped catfish in this study. The digestibility of starch was larger than 95% (Table 3), which is comparable to the ADC values reported for starch in rainbow trout (Burel *et al.*, 2000), common carp (Phan *et al.*, 2019), African catfish (Leenhouwers *et al.*, 2006) and Nile tilapia (Amirkolaie *et al.*, 2006). The current ADC values of starch for striped catfish are higher than the values reported for barramundi (88%) (Glencross *et al.*, 2017) and turbot (82%) (Burel *et al.*, 2000). The variability in starch digestibility between studies might relate to differences in the degree of gelatinization of the starch. Gelatinization of (native) starch has been proven to enhance its digestibility in a wide range of fish species, especially in carnivorous fish (Krogdahl *et al.*, 2005; Peres & Oliva-Teles, 2002). But also for non-carnivorous fish (*i.e.*, tilapia), extruded feeds generally have a higher starch digestibility compared to steam pelleted feeds (Maas *et al.*, 2020). In the current study the striped catfish diets were produced by extrusion and therefore the starch present in the diets was most likely well gelatinized, which contributed to the high ADC of starch. In various fish species (often carnivores) the digestibility of starch decreases at increasing starch inclusion levels; *e.g.* in barramundi (Glencross *et al.*, 2012; Glencross *et al.*, 2017), snakehead (Phan *et al.*, 2021b) and rainbow trout (Meriac *et al.*, 2014a). Opposite to this, striped catfish in the current study showed an increased ADC of starch when cassava was included into the diets. Even at a starch inclusion level of 375 g.kg^{-1} DM, the digestion of starch was not hampered in striped catfish. These findings indicate that striped catfish is well able to digest starch. Therefore, starch can be an important source providing DE in practical diets for striped catfish.

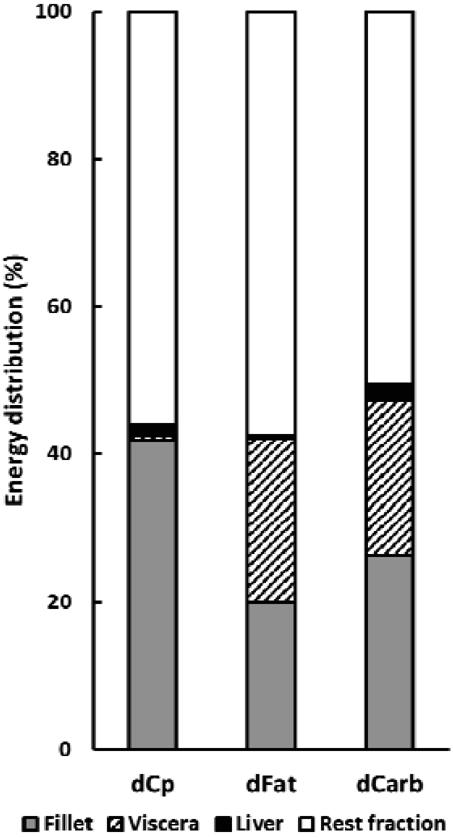

Figure 3. Energy distribution of digested protein (dCP), digested fat (dFat) and digested carbohydrate (dCarb) over different body compartments (liver, viscera, fillet and the rest fraction) in striped catfish. Distribution of digested protein (dCP) in liver, viscera, fillet and the rest fraction was calculated by dividing the coefficients of dCP in in liver (Equation 5, Table 6), viscera (Equation 6, Table 6), fillet (Equation 7, Table 6) and the rest fraction (Equation 8, Table 6) by the coefficients of dCP in Equation 4 (Table 6). Similarly, energy distribution of digested fat (dFat) and digested carbohydrate (dCarb) in different body compartments (Eq 5, 6, 7, 8, Table 6) were calculated by dividing the coefficients of dFat and dCarb in compartments by the coefficients of dFat and dCarb in Equation 4, Table 6, respectively.

It is often suggested that NSP have no nutritional value for fish, because they are not digested and or fermented in the fish intestine. However, the current study on striped catfish shows that between 4.7 to 17.8% of total the DE originated from digested NSP. The lowest ADC of NSP in the current study was 33.6% and indicated that NSP are digested and/or fermented in striped catfish. Similarly, positive ADCs for NSP have been reported for Nile tilapia (Maas *et al.*, 2019) and African catfish (Leenhouwers *et al.*, 2007). Comparable to Nile tilapia (Maas *et al*., 2020), there was a large variability in the ADC of NSP between treatments in the current study. In Nile tilapia, the ADC of NSP depends on the type of NSP, with soluble NSP being better digestible than in-soluble NSP and pectins better than cellulose (Maas *et al.*, 2019). The (high) digestibility of NSP in striped catfish and tilapia can be due to the activity of exogenous enzymes and or fermentation in the intestine. Depending on the type of NSP, NSP are digested by enzymes like xylanase, β-glucanase, β-mananase and or (enzymes from) bacteria in the intestine (Romano *et al.*, 2018). In the present study, fish fed the low feeding level had a higher ADC of NSP than fish fed the high feeding level. This indicates that the digestibility of NSP is dependent on feeding level, *i.e*. the amount of NSP intake, which was also found in Nile tilapia (Haidar *et al.*, 2016). The more NSP being consumed, the lower its ADC. Hydrolysis or fermentation of NSP takes time and requires interaction between NSP and enzymes and or bacteria. A higher NSP intake possibly increased the throughput and consequently decreased the time for the NSP and the bacteria or enzymes to interact. Additionally, fat supplementation in the current study decreased the ADC of NSP, possibly by hampering a proper contact between NSP, enzymes as well as bacteria. An adverse effect of fat on the abundance of the microbial population by disrupting their membrane integrity, impairing the uptake of nutrients, and inhibiting energy production results in cell death with the surfactant properties of fat (Desbois & Smith, 2010). The present study demonstrates that NSP are not inert for striped catfish. In other words, NSP can be digested and contribute to the digested energy. However, understanding the factors affecting the ADC’s of NSP’s in striped catfish , like NSP intake as well as dietary fat content requires further assessment.

Feed evaluation systems are often based on digestible nutrients, *i.e.* the DE approach. These evaluation systems assume that the ADC values of ingredients are additive when formulating diets. In the current study, diet type significantly affected the ADC of protein. Diluting the high protein diet with cassava starch and oil, which do not contain protein, changed the ADC of protein. This indicates that the ADC of protein is not additive as it is dependent on the ingredients included in the diet. This compiles with earlier findings for barramundi (Glencross *et al.*, 2017) and snakehead (Phan *et al.*, 2021b). This indicates that the assumption of the additivity of ingredients in the current feed evaluation systems is not always valid. Another assumption in feed evaluations systems based on digestible nutrients is that the ADC values of diets or dietary macro-nutrients are independent of the context, *e.g*. being not affected by feeding level, salinity or temperature. In the present study, the interaction between feeding level and diets affected the protein ADC and tended to affect ADC of energy, carbohydrates and fat. Such an interaction effect was also found in carp (Phan *et al.*, 2019) and snakehead (Phan *et al.*, 2021b). This suggests that the feeding level should be considered in digestibility trials, which are used to obtain data for practical diet formulation. The effect of feeding level and its interaction with diet on ADC of macronutrient implies that the nutritional value of an ingredient and/or diet is dependent on the feeding level. The practical implication of this is, that digestibility trails, which are done to determine the ADC of ingredients for formulating balance diets, should be done at feeding levels that are equal/representative for the practical conditions during the commercial culture of fish.

Averaged over the four diets, the digestible energy demand for maintenance for striped catfish was determined at 50 kJ.kg$^{-0.8}$.d^{-1}. This value is comparable to the value found for striped catfish of 40 kJ.kg$^{-0.8}$.d^{-1} by Glencross *et al.* (2011) and other fish species like: rainbow trout (*Oncorhynchus mykiss)* (38 kJ.kg$^{-0.8}$.d^{-1}) (Glencross, 2009), barramundi (*Lates calcarifer*) (43 kJ.kg$^{-0.8}$.d^{-1}) (Glencross, 2006b, 2008), European sea bass (*Dicentrarchus labrax*) (45 kJ.kg$^{-0.8}$.d^{-1}) and gilthead seabream (*Sparus auratus*) (48 kJ.kg$^{-0.8}$.d^{-1}) (Glencross, 2008; Glencross & Bermudes, 2012; Lupatsch *et al.*, 2003; Williams *et al.*, 2006; Williams *et al.*, 2003). It is also comparable to the minimal values reported for Nile tilapia (Meyer-Burgdorff *et al.*, 1989), but the current value in striped catfish is only half of the maximal value found for Nile tilapia (110 kJ.kg$^{-0.8}$.d^{-1}) (Haidar *et al.*, 2016). This example of Nile tilapia, shows that a wide range in the digestible energy demand for maintenance (53-110 kJ.kg$^{-0.8}$.d^{-1}) can exist within the same species (Haidar *et al.*, 2016; Schrama *et al.*, 2012a). Within a species, factors like stocking density (Lupatsch *et al.*, 2010), temperature (Glencross & Bermudes, 2010) or body size (Glencross, 2008) can affect the energy demand for maintenance. Differences in maintenance requirements between species may also be species-related (*i.e*., feeding habit, living habitat). Yet, irrespective of the type of energy evaluations system used, variation in the digestible energy for maintenance needs to be considered in the determination of the optimal dietary energy content of the diet.

When combining the data of all diets tested in this study, the estimated energy utilisation efficiency for striped catfish was 71%. This value is higher than the k_{gDE} value of 51% for striped catfish estimated by Glencross *et al.* (2011). However, the energy utilisation efficiency in the current study is in line with the range of $k_{g,DE}$ values estimated of 55 to 79% for barramundi, (Glencross, 2006b, 2008); 49 to 66% for common carp (Phan *et al.*, 2019); 62 to 74% for rainbow trout (Glencross, 2009) and 64 to 82% for European seabass (Lupatsch *et al.*, 2001, 2003; Lupatsch *et al.*, 2010; Peres & Oliva-Teles, 2005).

It was expected on forehand that the striped catfish used in the present study would have a lower energy utilisation efficiency than those used by Glencross *et al*. (2011), as the fish in this study were smaller than the fish used by Glencross *et al.* (2011). Generally, within a species, smaller fish show a lower energy utilisation efficiency than larger fish (Glencross, 2008). This contradiction could be because the body size factor in the present study was less influential than the nutrient composition of the experimental diets. In fact, the inclusion level of dietary fat is higher in the present study than in the study of Glencross *et al.* (2011). The energy utilisation efficiencies of the high fat diets were higher than the ones of the low fat diets (Glencross *et al.*, 2017; Phan *et al.*, 2019). This suggests that diet composition might have played a role in the differences of the energy utilisation efficiency found for striped catfish between the present study and the study of Glencross *et al.* (2011).

Although the dietary macronutrient composition tended to result in small numerically differences in the energy utilisation efficiency ($k_{g,DE}$), the dietary macronutrient composition did not significantly affected the relationship between RE and DE intake. This finding is in contradiction with the results found for carp (Phan *et al.*, 2019), barramundi (Glencross *et al.*, 2017), tilapia (Schrama *et al.*, 2012a), rainbow trout (Rodehutscord & Pfeffer, 1999; Schrama *et al.*, 2018) and snakehead (Phan *et al.*, 2021b), where dietary macronutrients composition affected $k_{g,DE}$. The absence of a diet effect on the energy utilisation efficiency for striped catfish in the current study may be because the contrast in the dietary composition between treatments is not large enough to create a significant effect. However, the contrast applied in the current study was similar to those in earlier studies. Another reason for the absence of an effect of dietary macronutrient composition on $k_{g,DE}$ might be that in striped catfish the energy utilisation efficiencies of digested protein, fat and carbohydrates (64%, 80% and 58%) are relatively similar compared to other fish species.

The estimated energy utilisation efficiency of digested protein ($k_{NE;dCP}$) for striped catfish was 15.1 $kJ.g^{-1}$. The value of $k_{NE;dCP}$ estimated for striped catfish is in the range of the estimates of $k_{NE;dCP}$ for carp 11.2 $kJ.g^{-1}$ (Phan *et al.*, 2019), tilapia 11.5 $kJ.g^{-1}$ (Schrama *et al.*, 2018), snakehead 12.5 $kJ.g^{-1}$ (Phan *et al.*, 2021b), barramundi 15.2 $kJ.g^{-1}$ (Phan *et al.*, 2019) and trout 15.1 $kJ.g^{-1}$ (Schrama *et al.*, 2018) (Figure 3). In addition, the digestible protein retention efficiency (DPE), or the retained protein as percentage of digestible protein for striped catfish was 56% (Table 4) and is comparable to the values found for Nile tilapia (53%) (Haidar *et al.*, 2018) and snakehead (54%) (Phan *et al.*, 2021b).

The energy utilization efficiency of digestible fat ($k_{NE;dFat}$) for striped catfish was 31.5 $kJ.g^{-1}$ and is comparable to the $k_{NE;dFat}$ values found for carp (34.1 $kJ.g^{-1}$), tilapia (35.8 $kJ.g^{-1}$) and snakehead (31.0 $kJ.g^{-1}$) based on linear relationships (Phan *et al.*, 2019; Schrama *et al.*, 2018). This similarity indicates that the ability to utilise digested fat for growth is comparable between the species mentioned.

The estimated energy utilization efficiency of dCarb ($k_{NE;dCarb}$) for striped catfish was 9.9 $kJ.g^{-1}$. This reflects that 58% of the digested carbohydrates were retained as energy in the body, which implies that striped catfish can metabolise dCarb. The $k_{NE;dCarb}$ value for striped catfish is comparable to the value found for tilapia (Schrama *et al.*, 2018), common carp (Phan *et al.*, 2019; Schrama *et al.*, 2018) and rainbow trout (Schrama *et al.*, 2018), but much higher than the value found for barramundi (18%) (Phan *et al.*, 2019) and snakehead (5%) (Phan *et al.*, 2021b) (Figure 3). In addition, the linearity in the NE and dCarb relationship in the current study indicates that striped catfish can deal with high intake levels of dCarb. This indicates that digested carbohydrates can be absorbed, liberated to ATP for daily activities or converted to adipose tissue through lipogenesis in an efficient way.

The present study found similarities in the energy utilisation efficiencies of digested protein, fat and carbohydrates between striped catfish, tilapia, trout (Schrama *et al.*, 2018) and common carp (Phan *et al.*, 2020), but it was different for barramundi (Phan *et al.*, 2020) and snakehead (Phan *et al.*, 2021). However, within a species, it is unknown whether environmental conditions, *i.e.*, temperature, salinity, dissolved oxygen can affect the energy utilisation efficiencies of digested protein, fat and carbohydrates. Furthermore, it can be hypothesised that with age of the fish, these utilization efficiencies alter due to changes in the ratio between protein and fat deposition. These topics require further assessment.

Currently, NE equations developed for fish feed are based on the whole body level. The potential to use a NE equation for fillet growth has as far as we know not yet been investigated. The advantage of a NE equation for fillet growth is to predict the energy potential of digested protein, fat and carbohydrates of a diet formulation for fillet growth specifically. In the present study, the energy potential of digested protein for fillet growth is 42% and twice the amount of that for digested fat (20%) and 1.6 times the amount for carbohydrates (26%) (Figure 3). Because digested protein is the most valuable macronutrient for fillet energy gain, the optimal dietary protein to energy ratio may be determined at a higher level in the diet tailor-made for fillet growth compared to the optimal dietary protein to energy ratio for growth based on whole body level.

As stated above the energy potential of digested carbohydrates for fillet growth is only 6% higher than that of digested fat for sparing protein in fillet (Figure 3). However, this still implies that for fillet production, carbohydrates are a better energy source than fat. Yet, when using carbohydrates as an energy source this can also increase the amount of faecal waste depending on the type of carbohydrates used (starch vs. NSP). The ADC of starch is higher than that of NSP (Maas *et al.*, 2020). Formulating practical diets with only protein is not feasible because carbohydrates is required to provide energy and necessary for the matrix of the pellet. Fat can also provide energy and is required for the essential fatty acids and fat soluble vitamins. If protein is used as a main energy source, this will increase the total ammonium nitrate (TAN) excretion and hamper the environment. A formulation with only protein is likely not economically viable making the inclusion of carbohydrates or fat as an energy source an economic necessity.

4.5. Conclusions

Starch is almost completely digested by striped catfish and non-starch polysaccharides are partly digested. The dietary macronutrient composition did not affect the energy utilisation efficiency in striped catfish. This might be due the relative small differences in the energy utilisation efficiencies of dCP, dFat and dCarb, which were 64%, 80% and 58%, respectively, in striped catfish. Digested starch was utilised efficiently in stripe catfish. The energetic values of dCP, dFat and dCarb for whole body growth differ from the energetic values for fillet production. For fillet growth, digested protein has a higher potential compared to digested fat and carbohydrates, however this needs to be used in a balanced ratio with digested fat and carbohydrates.

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Supplementary data

Supplementary table S1.

Initial and final body composition (g.kg^{-1}, wet basis) of striped catfish, (n = 3), fed 4 different diets at 2 feeding levels (FL) for 63 days

			P	C	F	M				
			"protein"	"protein"	"protein"	"protein"		*P* values		
	FL	Initial		+Carb	+Fat	+Carb+Fat	SEM	D	FL	D x FL
DM		236								
	Low		245^{y}	267^{y}	310^{x}	325^{x}	6.8	***	***	ns
	High		271^{Y}	305^{Y}	345^{X}	363^{X}				
Energy		5.6								
	Low		5.9^{z}	7.0^{y}	8.6^{x}	9.2^{x}	0.24	***	***	ns
	High		7.1^{Z}	8.6^{Y}	9.7^{X}	10.1^{X}				
Protein		147								
	Low		146	139	147	141	5.4	ns	ns	ns
	High		145	146	146	147				
Fat		50								
	Low		61^{z}	96^{y}	126^{y}	146^{x}	6.0	***	***	ns
	High		89^{Z}	132^{Y}	129^{Y}	171^{X}				
Ash		31								
	Low		30	31	30	30	1.8	ns	ns	ns
	High		30	27	33	28				

P, diet with a high protein content; C, the P diet supplemented with starch; F, the P diet supplemented with fat; M, the P diet supplemented with fat and starch; Carb, Carbohydrates; D, diet; FL, feeding level; DM, dry matter.

***, *P*<0.01; *, *P*<0.05; ns, non-significantly different

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

Supplementary table S2. Nitrogen (mg.kg$^{-0.8}$.day^{-1}) balance analysis of striped catfish (n = 3), fed 4 different diets at 2 feeding levels (FL) over 63 days

	FL	P	C	F	M				
		"protein"	"protein"	"protein"	"protein"		*P* values		
			+Carb	+Fat	+Carb+Fat	SEM	D	FL	DxFL
GNI	Low	490^{e}	365^{f}	385^{f}	329^{g}	6.3	***	***	***
	High	793^{a}	575^{c}	607^{b}	524^{d}				
DNI	Low	444^{d}	321^{ef}	351^{e}	294^{f}	7.4	***	***	***
	High	676^{a}	486^{c}	554^{b}	463^{cd}				
FN	Low	46^{cd}	44^{cd}	34^{d}	35^{cd}	5.5	***	***	***
	High	117^{a}	89^{b}	53^{cd}	61^{c}				
BUN	Low	271^{x}	196^{y}	183^{y}	154^{y}	13.3	***	***	ns
	High	389^{X}	243^{Y}	259^{Y}	204^{Y}				
RN	Low	173	125	169	140	14.2	***	***	ns
	High	286	243	294	259				

P, diet with a high protein content; C, the P diet supplemented with starch; F, the P diet supplemented with fat; M, the P diet supplemented with fat and starch; Carb, Carbohydrates; FL, feeding level; *P* values for effects of diet, feeding level or the interaction, respectively; GNI, gross nitrogen intake; DNI, digestible nitrogen intake; FN, faecal nitrogen; RN, retained nitrogen; BUN, branchial and urinary nitrogen.

***, *P*<0.01; *, *P*<0.05; ns, non-significantly different

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

Supplementary table S3.

The net energy equations of the four different compartments, their sum and the whole body of striped catfish

	Equations		**R^2**	
Whole body Σ compartments	NE	= RE + 25.0 (se 7.19)	0.95	(3)
		= 14.1 (se 2.03) dCP + 29.7 (se 1.85) dFat + 9.4 (se 1.03) dCarb		
Liver	NE_{liver}	= RE_{liver} + 0.1 (se 0.14)	0.84	(5)
		= 0.2 (se 0.04) dCP + 0.1 (se 0.04) dFat + 0.2 (se 0.02) dCarb		
Viscera	$NE_{viscera}$	= $RE_{viscera}$ + 4.2 (se 4.41)	0.65	(6)
		= 0.1 (se 1.24) dCP + 6.6 (se 1.13) dFat + 2.0 (se 0.63) dCarb		
Fillet	NE_{fillet}	= RE_{fillet} + 14.4 (se 3.48)	0.85	(7)
		= 5.9 (se 0.98) dCP + 5.9 (se 0.89) dFat + 2.5 (se 0.50) dCarb		
Rest fraction	$NE_{rest\ fraction}$	= $RE_{the\ rest\ fraction}$ + 6.2 (SE 6.29)	0.89	(8)
		= 7.9 (se 1.78) dCP + 17.1 (se 1.62) dFat + 4.8 (se 0.90) dCarb		

NE, net energy; RE, retained energy; dCP, digestible protein; dFat, digestible fat; dCarb, digestible carbohydrates (comprising of starch, sugars and 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}.

Whole body homogenised , the equation was created with RE calculated based on the whole body energy composition data. Whole body Σ compartments , the equation was created with RE calculated based on the sum of RE in the four defined compartments

5

Differences in energy utilisation 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 utilisation 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 4x2 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 $g.kg^{-0.8}.d^{-1}$) 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, $k_{NE;dCP}$, $k_{NE;dFat}$, and $k_{NE;dCarb}$) 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.

Key words: Energy evaluation; Energy metabolism; Bioenergetics: Net energy; Energy efficiency; Digestible nutrients; *Channa striata*.

5.1. 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 & Abdul Manan, 2012). It uses the foraging strategy of hiding and suddenly attacking its preys (i.e., small fish, frogs, birds) (Liu *et al.*, 2000; Shafri & Abdul Manan, 2012). Its rapid growth and high foraging activity 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 utilisation efficiencies of these macronutrients. The ingredients used for snakehead feed appear to be increasingly diversified (Aliyu-Paiko & Hashim, 2012; Hien *et al.*, 2017; Zehra & Khan, 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 (Glencross, 2008; Glencross & Bermudes, 2012; Lupatsch *et al.*, 2003; Williams *et al.*, 2006; Williams *et al.*, 2003). In such an approach, the digestible energy utilisation 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.*, 2012b) and rainbow trout (*Oncorhynchus mykiss*) (Rodehutscord & 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 alternative fish feed energy evaluation systems has been suggested in various studies (Azevedo *et al.*, 2005; Glencross *et al.*, 2014; Hua *et al.*, 2010). 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 utilisation 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 utilisation 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 utilisation 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 utilisation efficiency of digested protein, fat and carbohydrates by multiple regression on energy retention (NE approach).

5.2. Materials and methods

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 though a 0.9 mm screen at 1470 rpm. All ingredients, except soy oil in the LIPID- and MIX-diet, were mixed in a 60-L batch mixer for 240 sec. Prior to extrusion, these mixtures were conditioned for 10 sec at a temperature between 85-100°C. Diets were processed by 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 3mm floating pellets, which were dried at 95°C for 10min. 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.

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 hours and from 16:00 to 17:00 hours.

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.

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.* (2012b).

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.

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:

$$ADC_{nutrient} = (1- (Y_{diet}/Y_{faeces}) \times (Nutrient_{faeces}/Nutrient_{diet})) \times 100\%,$$

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

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.* (2012b). 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-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.

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_{gDE}) of each diet using the following model:

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

where μ is the intercept, β is the energy utilisation efficiency; e_i is error term and i =1,..., 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:

$$RE_i = \mu + \beta_1 \times dCP_i + \beta_2 \times dFat_i + \beta_3 \times dCarb_i + e_i \qquad \text{(Equation 1)},$$

where μ is the intercept, being an estimate for fasting heat production (FHP); β_1, β_2, β_3 are the energy utilisation efficiency of dCP ($k_{NE;dCP}$), dFat ($k_{NE;dFat}$) and dCarb ($k_{NE;dCarb}$); e_i is error term and i =1,..., n with n = 24.

5.3. 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 feed 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 (Figure 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 (Figure 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 at these diets remained similar to the initial body fat content (32 g.kg^{-1} on wet basis).

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

		PROT	CARB	LIPID	MIX				
		"protein"	"protein"	"protein"	"protein"		*P*-value		
	FL		+Carb	+Fat	+Carb+Fat	SEM	D	FL	D x FL
Final BW (g)									
	Low	62^{de}	56^{f}	66^{d}	58^{ef}	0.93	***	***	***
	High	109^{a}	95^{b}	99^{b}	88^{c}				
Feed intake (g.d^{-1})									
	Low	0.74	0.74	0.74	0.74	----*	---*	---*	---*
	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	***	***	***
	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	***	***	***
	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	ns	ns	Ns
	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 fat; MIX, the PROT diet supplemented with starch and fat; carb, carbohydrates; FL, feeding level; *P* values for effects of diet, feeding level or the interaction, respectively; BW, body weight; FCR, feed conversion rate.

*No statistical analysis was conducted on feed intake, because it was controlled at two levels.

***, P<0.01; *, P<0.05; ns, non-significantly different

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

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.

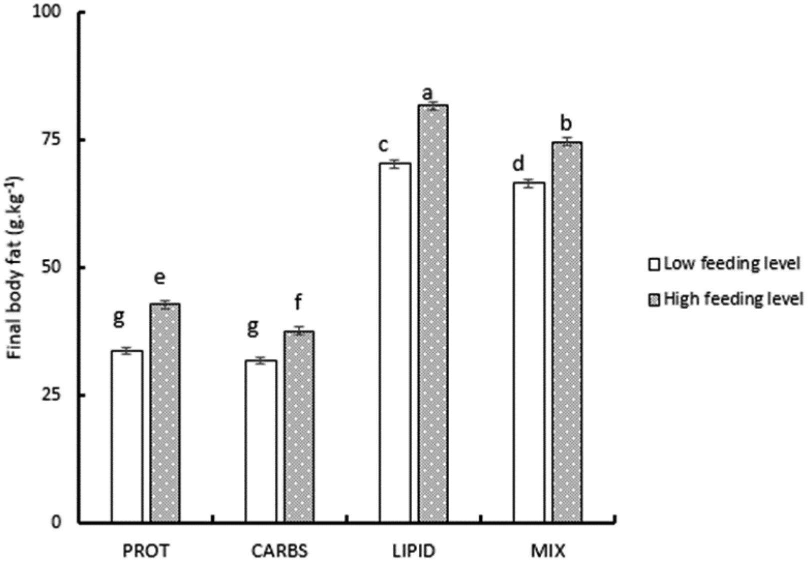

Figure 1. Final body fat content (g.kg^{-1}, wet basis) of snakehead (*C. striata*) as affected by diet and feed level (n=3): Diet effect, $P< 0.001$; Feeding level effect, $P< 0.001$; Diet x Feeding level interaction effect, $P = 0.012$. PROT, diet with a high protein content; CARB, the PROT diet supplemented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

Within all experimental treatments, starch had the highest ADC 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 (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 (Figure 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 utilisation 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 (Figure 2).

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

		PROT	CARB	LIPID	MIX		*P*-values		
		"protein"	"protein"	"protein"	"protein"				
Nutrient	FL		+Carb	+Fat	+Carb+Fat	SEM	D	FL	D x FL
Energy									
	Low	86.7^{cd}	87.6^{bc}	89.0^{a}	88.0^{ab}	0.24	***	***	*
	High	84.4^{fg}	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	***	***	***
	High	90.3^{b}	89.5^{bc}	90.1^{b}	89.0^{c}				
Fat									
	Low	93.0^{c}	89.3^{e}	95.9^{a}	94.3^{b}	0.25	***	***	***
	High	90.4^{e}	84.7^{f}	91.7^{d}	92.1^{cd}				
Carbohyd									
	Low	63.2^{g}	81.5^{a}	67.0^{e}	79.1^{b}	0.34	***	***	***
	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	***	***	***
	High	99.2^{a}	95.2^{e}	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 fat; MIX, the PROT diet supplemented with starch and fat; carb, carbohydrates; DM, dry matter; NSP, non-starch polysaccharides; D, diet; FL, feeding level.

***, P<0.01; *, P<0.05; ns, non-significantly different

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

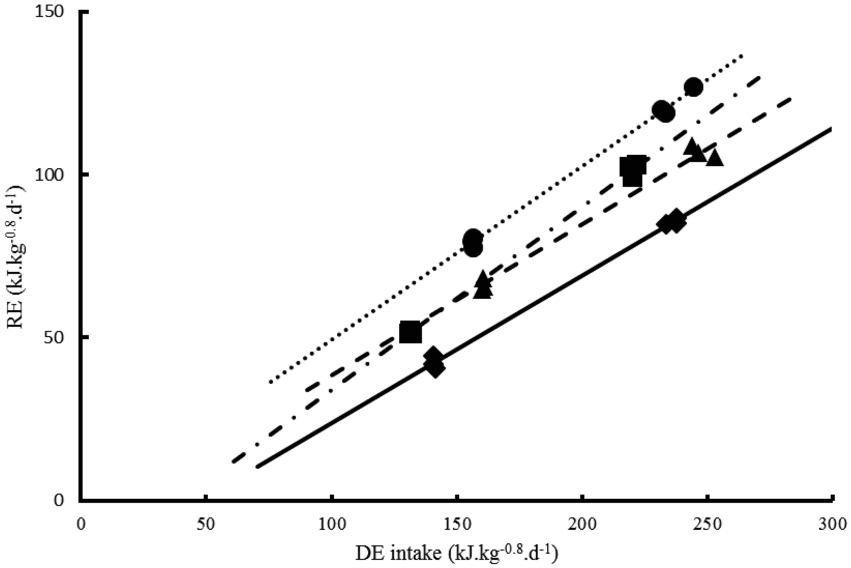

Figure 2. Relationship between retained energy (RE) and digestible energy intake (DE) for snakehead fed one of four experimental diets: PROT, diet with a high protein content; CARB, the PROT diet supplemented with starch; LIPID, the PROT diet supplemented with fat; MIX, the PROT diet supplemented with starch and fat. (■ 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 kJ.kg$^{-0.8}$.d^{-1} at PROT, CARB, LIPID and MIX diet, respectively.

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 kJ.kg$^{-0.8}$.d^{-1} (Table 4).

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

RE = -14.2 (SE 2.46) + 12.5 (SE 0.46) dCP + 31.0 (SE 0.85) dFat + 0.9 (SE 0.51) dCarb

R^2 =0. 99 (Equation2)

By dividing the coefficients of dCP, dFat and dCarb (respectively, 12.5, 31.0 and 0.9 kJ.g^{-1}) in Equation 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 utilisation efficiency of dCP, dFat and dCarb (respectively, $k_{NE;dCP}$, $k_{NE;dFat}$, and $k_{NE;dCarb}$) were determined as 53%, 79% and 5%, respectively. In Figure. 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 (Figure 3c) only marginally increased NE compared to increasing dFat (Figure 3b) and increasing dCP (Figure 3a).

Table 4. Energy balance parameters (kJ. $kg^{-0.8}.d^{-1}$) and protein efficiency (%) in snakehead (*C. striata*), means per experimental diet and feeding level (FL) during the 42-d experimental period (n = 3).

		PROT	CARB	LIPID	MIX				
		“protein”	“protein”	“protein”	“protein”		*P*-value		
	FL		+Carb	+Fat	+Carb+Fat	SEM	D	FL	D x FL
GE intake	Low	151^{e}	161^{e}	175^{d}	182^{d}	2.1	***	***	***
	High	261^{c}	282^{ab}	275^{b}	290^{a}				
DE intake	Low	131^{f}	141^{e}	156^{d}	161^{d}	1.8	***	***	***
	High	220^{c}	236^{b}	236^{b}	248^{a}				
BUE losses	Low	9^{c}	7^{e}	6^{f}	5^{g}	0.1	***	***	***
	High	14^{a}	12^{b}	9^{c}	8^{d}				
ME intake	Low	122^{f}	133^{e}	150^{d}	155^{d}	1.7	***	***	***
	High	206^{c}	225^{b}	227^{b}	240^{a}				
HP	Low	70^{d}	91^{c}	71^{d}	89^{c}	1.6	***	***	***
	High	104^{b}	139^{a}	105^{b}	133^{a}				
RE	Low	52^{f}	42^{g}	79^{d}	66^{e}	1.2	***	***	*
	High	102^{b}	86^{c}	122^{a}	107^{b}				
REprot	Low	41^{d}	35^{e}	41^{d}	35^{e}	0.5	***	***	***
	High	76^{a}	67^{b}	65^{b}	57^{c}				
REfat	Low	10^{g}	7^{g}	38^{c}	31^{d}	1.0	***	***	***
	High	26^{e}	19^{f}	57^{a}	50^{b}				
FG: PG	Low	0.19^{de}	0.17^{e}	0.59^{a}	0.59^{a}	0.007	***	*	***
(g/g)	High	0.24^{c}	0.21^{cd}	0.58^{ab}	0.55^{b}				
REfat:prot	Low	0.25^{bc}	0.21^{c}	0.92^{a}	0.90^{a}	0.019	***	ns	***
(J/J)	High	0.34^{b}	0.28^{bc}	0.89^{a}	0.88^{a}				
PE*	Low	43^{c}	44^{c}	53^{a}	52^{a}	0.5	***	***	***
	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 fat; MIX, the PROT diet supplemented with starch and fat; carb, carbohydrates; GE, gross energy; DE, digestible energy; BUE, branchial urine energy; ME, metabolisable energy, RE, retained energy; REprot, retained energy as protein; REfat, retained energy as fat; FG: PG, fat gain: protein gain; D, diet; FL, feeding level

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

***, P<0.01; *, P<0.05; ns, non-significantly different

[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).

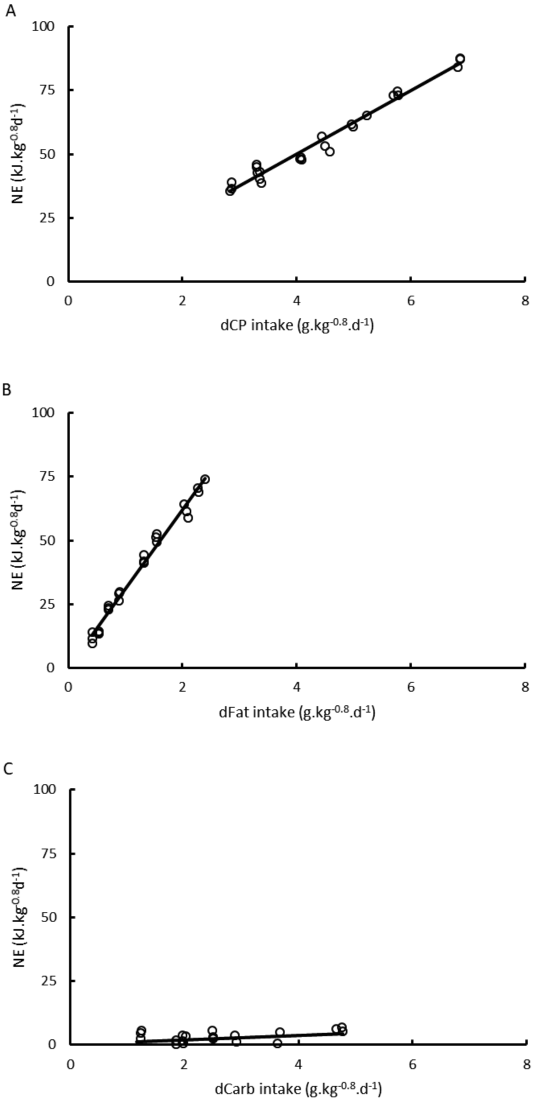

Figure 3. Relationship between net energy (NE) and digestible protein (dCP) intake (A), NE and digestible fat (dFat) intake (B), NE and digestible carbohydrates (dCarb) intake (C). The NE values are corrected for variation in dFat and dCarb intake in panel (A); for variation in dCP and dCarb intake in 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 Equation (2) given in Table 5

5.4. 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; Figure 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 & Hashim, 2012; Arockiaraj *et al.*, 1999; Dayal *et al.*, 2012; Mohanty & Samantaray, 1996; Wee & 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 (Azevedo *et al.*, 1998; Bureau *et al.*, 2006; Glencross, 2009; Glencross *et al.*, 2007; Glencross *et al.*, 2008), Nile tilapia (Schrama *et al.*, 2012) and common carp (Phan *et al.*, 2019). Comparison between species studied can be affected by differences in dietary fat content. Therefore, in Figure 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^{-1}. 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}) (Nwanna *et al.*, 2007; Phan *et al.*, 2019) and rainbow trout (0.8 to 1.1 g.g^{-1}) (Azevedo *et al.*, 1998; Bureau *et al.*, 2006; Glencross, 2009; Glencross *et al.*, 2007; Glencross *et al.*, 2008). 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 (Figure 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 utilisation 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) utilisation 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 observations in European sea bass (98%) (Peres & 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 be shown to improve starch digestibility in a variety of fish species (Krogdahl *et al.*, 2005; Peres & 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 supressed starch ADC at high dietary starch levels in carnivorous fish like barramundi (Glencross *et al.*, 2012; Glencross *et al.*, 2017) and rainbow trout (Meriac *et al.*, 2014).

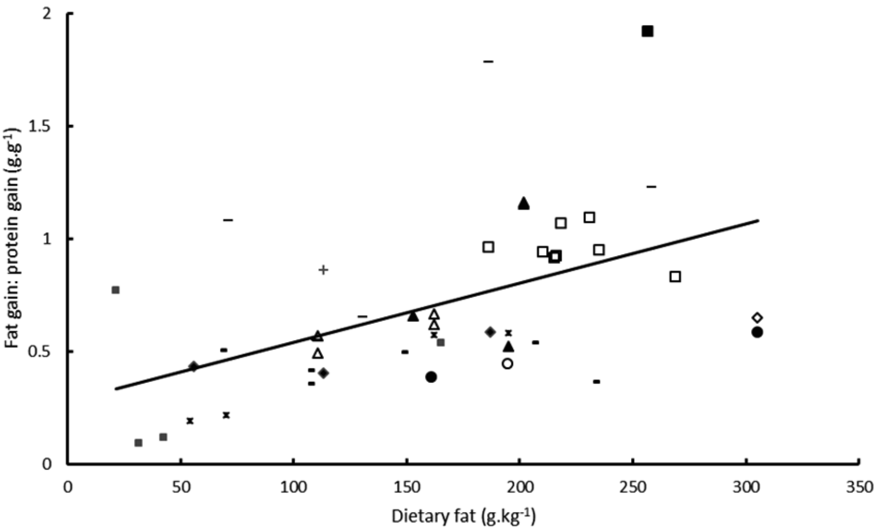

Figure 4. Relationship between fat gain-to-protein gain ratio and dietary fat content. Data are derived from Table 5 in study of Schrama *et al. (*2012) and supplemented with the data of Glencross *et al. (*2017b), Phan *et al. (*2019) and the present study (○ *Argyrosomus japonicus,* ◇ *Salmo salar,*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

For diet formulation on digestible energy (DE) the energy utilization efficiency for growth of DE (k_{gDE}, the slope of the lines in Figure 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, 2006b, 2008); common carp, 49 to 66% (Phan *et al.*, 2019) ; rainbow trout, 62 to 74% (Glencross, 2009); European seabass (64 – 82%) (Lupatsch *et al.*, 2001, 2003; Lupatsch *et al.*, 2010; Peres & 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; Figure 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.*, 2012b) , barramundi (Glencross *et al.*, 2017) and rainbow trout (Rodehutscord & Pfeffer, 1999; Schrama *et al.*, 2018). The relatively low k_{gDE} for all diets might be an indication of a poor ability to utilize 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 (Figure 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 – 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.$^{-1}$ based on linear relations ships; 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 appear 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 & Houlihan, 2001). However, it maintains to be clarified whether snakehead uses fat as the major energy source for protein synthesis.

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

Species	Equation*	R^2	
Snakehead	NE = 12.5 dCP + 31.0 dFat + 0.9 dCarb	0.99	(2)
Carp	NE = 11.2 dCP + 34.1 dFat + 10.4 dCarb	0.99	(3)
Barramundi	NE = 15.2 dCP + 37.1 dFat + 3.1 dCarb	0.99	(4)
Barramundi	NE = 15.9 dCP + 35.2 dFat + 9.4 dCarb – 1.9 $(dCarb)^2$	0.99	(5)
Trout	NE = 15.1 dCP + 35.0 dFat + 12.1 dCarb	0.91	(6)
Trout	NE = 13.5 dCP + 33.0 dFat + 34.0 dCarb – 3.64 $(dCarb)^2$	0.92	(7)
Tilapia	NE = 11.5 dCP + 35.8 dFat + 11.3 dCarb	0.99	(8)
Pig	NE = 11.3 dCp + 35.0 dFat + 14.4 ST + 12.1 dRest		(9)
Pig	NE = 10.8 dCp + 36.1 dFat + 13.5 dST_e + 9.5 dST_f + 9.5 dNSP		(10)

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) (Phan *et al.*, 2019; Schrama *et al.*, 2018) 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.

5.5. 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 utilized 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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Supplementary data

Supplementary Table S1. Amino acid composition of the four diets fed to snakehead (*C. striata*)

	PROT	CARB	LIPID	MIX
	“protein”	“protein”	“protein”	“protein”
		+Carb	+Lipid	+Carb+Lipid
On dry matter basis (%):				
Lys	4.3	3.0	3.5	2.8
Met	1.4	1.0	1.2	1.0
Met+Cys	2.0	1.5	1.6	1.4
Cys	0.6	0.5	0.5	0.4
Thr	2.2	1.6	1.8	1.5
Arg	3.1	2.3	2.6	2.0
Ile	2.0	1.6	1.7	1.4
Leu	3.5	2.7	2.9	2.4
Val	2.3	1.8	1.9	1.6
His	1.0	0.8	0.9	0.7
Phe	2.1	1.7	1.7	1.5
Gly	3.5	2.6	2.9	2.2
Ser	2.1	1.7	1.8	1.5
Pro	2.8	2.5	2.3	2.1
Ala	2.8	2.1	2.3	1.8
Asp	4.5	3.3	3.7	2.9
Glu	7.5	6.8	6.3	6.1
On crude protein basis (g.kg $^{-1}$):				
Lys	79.4	71.6	78.2	74.0
Met	26.1	24.4	25.7	25.4
Met+Cys	36.5	36.3	36.4	37.1
Cys	10.4	11.9	10.7	11.7
Thr	40.9	38.5	40.4	38.9
Arg	56.2	54.5	57.3	53.8
Ile	36.9	37.0	37.1	36.7
Leu	63.9	64.7	63.3	63.3
Val	42.6	42.8	42.5	41.6
His	19.1	19.4	19.1	19.4
Phe	37.9	39.4	38.2	39.0
Gly	63.4	60.4	65.1	57.4
Ser	39.2	40.1	40.0	39.0
Pro	51.1	59.3	51.7	55.8
Ala	51.7	49.3	52.1	48.1
Asp	83.1	78.2	82.5	77.3
Glu	137.2	161.5	138.8	160.0

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.

Supplementary table S2. Initial and final body composition of snakehead (*C. striata*) (n=3), fed one of four diets (D) each at two feeding levels (FL) during the 42-day experimental period.

			PROT	CARB	LIPID	MIX				
			"protein"	"protein"	"protein"	"protein"			*P*-value	
	FL	Initial		+Carb	+Lipid	+Carb+Lipid	SEM	D	FL	D x FL
DM		261								
	Low		276^{d}	274^{d}	303^{b}	299^{b}	1.8	***	***	ns
	High		286^{c}	279^{cd}	313^{a}	306^{ab}				
Energy		5.5								
	Low		5.5^{ef}	5.4^{f}	6.8^{bc}	6.6^{c}	0.05	***	***	ns
	High		5.9^{d}	5.7^{e}	7.2^{a}	7.0^{ab}				
Protein		176								
	Low		181^{bc}	181^{bc}	174^{d}	174^{d}	1.1	***	***	ns
	High		189^{a}	185^{ab}	176^{cd}	175^{d}				
Fat		32								
	Low		34^{g}	32^{g}	70^{c}	66^{d}	0.7	***	***	*
	High		43^{e}	38^{f}	82^{a}	75^{b}				
Ash		45								
	Low		57^{ab}	57^{a}	54^{b}	54^{b}	0.6	***	***	*
	High		50^{c}	51^{c}	50^{c}	50^{c}				

PROT, diet with a high protein content; CARB, the PROT diet supplemented with starch diet; LIPID, the PROT diet supplemented with lipid; MIX, the PROT diet supplemented with starch and lipid; carb, carbohydrates; carb, carbohydrate, FL, feeding level.

***, P<0.01; *, P<0.05; ns, non-significantly different

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

Supplementary Table S3. Mean digestible nutrient intake and apparent nutrient digestibility coefficient (ADC) in snakehead (*C. striata*) per experimental diet (D) and feeding levels (FL) during the 42-day experimental period (n = 3).

		PROT	CARB	LIPID	MIX			*P*-value	
		"P"	"P"	"P"	"P"				
	FL		+C	+L	+C+L	SEM	D	FL	D x FL
Digestible nutrient intake (g.kg$^{-0.8}$.d^{-1})									
dCP									
	Low	4.1^{e}	3.4^{f}	3.3^{f}	2.9^{g}	0.04	***	**	***
	High	6.8^{a}	5.7^{b}	5.1^{c}	4.5^{d}				
dFat									
	Low	0.5^{g}	0.4^{h}	1.5^{c}	1.3^{d}	0.02	***	**	***
	High	0.9^{e}	0.7^{f}	2.3^{a}	2.1^{b}				
dCarb									
	Low	1.2^{f}	2.9^{c}	1.2^{f}	2.5^{d}	0.03	***	**	***
	High	2.0^{e}	4.7^{a}	1.9^{e}	3.7^{b}				
dstarch									
	Low	1.2^{g}	3.0^{c}	1.1^{g}	2.5^{d}	0.02	***	**	***
	High	2.1^{e}	5.0^{a}	1.8^{f}	3.8^{b}				
ADC (%)									
DM									
	Low	76.0^{d}	81.1^{a}	80.5^{ab}	81.3^{a}	0.22	***	**	***
	High	74.2^{e}	77.2^{c}	78.0^{c}	79.6^{b}				
NSP									
	Low	-5.8	-9.7	14.7	1.8	1.47	***	**	***
	High	-7.5	-33.7	11.6	-12.8				
Ash									
	Low	34.3^{de}	39.0^{bcd}	45.5^{a}	40.1^{bc}	1.06	***	ns	***
	High	32.8^{e}	35.9^{cde}	43.3^{ab}	48.4^{a}				
Phos									
	Low	46.5^{ef}	54.7^{cd}	59.2^{abc}	61.0^{ab}	1.28	***	*	*
	High	43.0^{f}	49.5^{de}	56.0^{bc}	63.8^{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; phos, phosphorus.

***, P<0.01; *, P<0.05; ns, non-significantly different

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

Supplementary table S4. Means nitrogen balance parameters (mg.kg$^{-0.8}$.d^{-1}) in snakehead (*C. striata*) per experimental diet (D) and feeding levels (FL) during the 42-day experimental period (n = 3).

	FL	PROT	CARB	LIPID	MIX	SEM			
		"protein"	"protein"	"protein"	"protein"		*P*-value		
			+Carb	+Lipid	+Carb+Lipid		D	FL	D x FL
GNI									
	Low	704^{e}	586^{f}	574^{f}	508^{g}	6.6	***	***	***
	High	1214^{a}	1028^{b}	899^{c}	809^{d}				
DNI									
	Low	651^{e}	537^{f}	528^{f}	456^{g}	5.8	***	***	***
	High	1096^{a}	919^{b}	810^{c}	720^{d}				
FN									
	Low	53^{d}	48^{d}	45^{d}	52^{d}	1.7	***	***	***
	High	118^{a}	108^{b}	89^{c}	89^{c}				
BUN									
	Low	372^{c}	301^{e}	249^{f}	221^{g}	4.6	***	***	***
	High	582^{a}	470^{b}	374^{c}	335^{d}				
RN									
	Low	279^{d}	236^{e}	279^{d}	236^{e}	3.4	***	***	***
	High	513^{a}	449^{b}	436^{b}	385^{c}				

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; carb, carbohydrates; FL, feeding level; *P* values for effects of diet, feeding level or the interaction, respectively; GNI, gross nitrogen intake; DNI, digestible nitrogen intake; FN, faecal nitrogen; RN, retained nitrogen; BUN, branchial and urinary nitrogen loss.

***, P<0.01; *, P<0.05; ns, non-significantly different

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

6

Effect of dietary starch levels on plasma glucose and triglycerides levels in striped catfish and snakehead

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Submitted

Abstract

This study aimed to assess the effect of dietary starch content (low vs. high) on postprandial plasma glucose and triglycerides levels in snakehead and striped catfish, fed with the same diets. This study had a 2x2x3 factorial design: factor 1, dietary starch content (low vs. high); factor 2, feeding regime (fed vs unfed on the day of blood sampling); and factor 3, the postprandial moment of blood sampling (3h, 7h or 24h). This experimental design was identical for both fish species. Both snakehead and striped catfish were fed restrictively at a level of 20 $g.kg^{-0.8}.d^{-1}$. This feeding level is close to apparent satiation for these fish. Snakehead and striped catfish were fed twice daily at 9.00 am and 3.00 pm for 28 days and 38 days, respectively. The plasma glucose was higher in snakehead (12 $mmol.L^{-1}$) than in striped catfish (5.4 $mmol.L^{-1}$) but the plasma triglycerides level was higher in striped catfish (7.4 $mmol.L^{-1}$) than in snakehead (1.2 $mmol.L^{-1}$). Increasing dietary starch increased the plasma glucose levels in snakehead and triglycerides levels in striped catfish. Striped catfish can maintain the plasma glucose homeostasis better than snakehead. The ability to convert dietary starch, i.e., glucose into triglyceride is larger in striped catfish than in snakehead. Overall, striped catfish has a better ability to utilize dietary carbohydrates than snakehead.

Key words: snakehead, striped catfish, dietary starch, plasma glucose, plasma triglycerides.

6.1. Introduction

Glucose is an important energy source originated from dietary carbohydrates which can fuel part of the daily activities of fish. The main dietary source of glucose in formulated feeds for fish includes ingredients with the high carbohydrate content (e.g., wheat, wheat flour, cassava, rice by-products, etc.). Carbohydrates are preferred as energy source because carbohydrates rich ingredients are generally cheaper than protein rich and oil sources. The dietary inclusion level of carbohydrates can be up to 0.45 for rohu (*Labeo robita*) (Mohapatra *et al.*, 2003), Asian catfish (*Pangasius bocourti*) and striped catfish (*Pangasius hyphophthalmus*) (Hung *et al.*, 2003). However, on weight basis carbohydrates have a lower energy content (17.2 kJ.g^{-1}) compared to protein (23.6 kJ.g^{-1}) and fat (39.5 kJ.g^{-1}) (NRC, 2011).

The energy potential of digested carbohydrate (i.e., starch) for growth is comparable between tilapia, common carp and pig (Phan *et al.*, 2019; Schrama *et al.*, 2018). However, the energy utilisation efficiency of digested carbohydrates is only 18% in barramundi (*Lates calcarifer*) (Phan *et al.,* 2019) and 5% in snakehead (*Channa striata*)(Phan *et al*., 2021b), while the values are higher in tilapia (66%; Schrama *et al*., 2018), carp (64%; Phan *et al.*, 2019) and striped catfish (58%; Phan *et al.*, 2021a). The restricted utilisation of digested carbohydrate in snakehead and barramundi may relate to the glucose intolerance of carnivores (Moon, 2001; Panserat *et al.*, 2001b; Panserat *et al.*, 2002). The large difference in the energy utilisation efficiency of digested carbohydrates between omnivorous fish and carnivorous fish raised a question about the postprandial absorption of digested starch (*i.e.*, glucose) from the digestive tract into the blood stream and the metabolism of the absorbed glucose. In addition, the excessive glucose can be stored in the form of fat (*i.e.*, triglycerides) (Kamalam *et al.*, 2017). Assessing postprandial levels of plasma glucose and triglycerides in both snakehead and striped catfish may help to explain the difference in energy utilization of digested carbohydrates between these species. Therefore, the effect of dietary starch content (low vs. high) on postprandial plasma glucose and triglycerides levels was assessed in snakehead and striped catfish, fed with the same diets.

6.2. Materials and methods

This study was evaluated and approved by the Ethical Committee of Animal Experiments of Wageningen University, The Netherlands and carried out at the research and development centre of De Heus (Vinh Long, Vietnam) in compliance with Vietnamese law.

6.2.1 Experimental design and diets

This study had a 2x2x3 factorial design: factor 1, dietary starch content (low vs. high); factor 2, feeding regime (fed vs unfed on the day of blood sampling); and factor 3, the postprandial moment of blood sampling (3h, 7h or 24h). The unfed regime on the blood sampling day was for the control purpose which reflected the effect of possible stressors, *i.e.*, chasing or netting or the daily flux of plasma glucose and triglycerides. This experimental design was identical for both fish species.

First, the low starch diet was formulated. For this diet fish meal, poultry meal and soy protein concentrate were used as protein source. The high starch diet was made by diluting the low starch diet with wheat flour (350 g.kg^{-1}) (Table 1). This approach resulted in a contrast in carbohydrate of 168 g.kg^{-1} (on DM basis) between both diets.

Diets were produced by De Heus (Vinh Long, Vietnam). All ingredients, except squid liver oil and premix, were hammer-milled through a 0.9 mm screen at 1470 rpm. All ingredients were mixed in a 60-L batch mixer for 240 sec. Before being extruded, these mixtures were conditioned for 10 sec at a temperature between 85-100°C. Diets were extruded using a twin-screw extruder with a capacity of 150 kg.h^{-1} using a 2 mm die at 95-110°C. This produced 3mm floating pellets, which were dried at 95°C for 10min. Pellets were cooled at 30-33°C for 10 min before packaging.

6.2.2 Experimental fish

Snakehead, 151 g (SE 0.3) were obtained from a local hatchery (Vinh Long, Viet Nam) and striped catfish, 258 g (SE 0.8) were obtained at the research & development farm of De Heus Viet Nam (Vinh Long, Viet Nam). Prior to blood sampling, 320 snakehead were housed in 4 tanks (500 L.tank^{-1}; n=80 fish.tank^{-1}) for 28 days and 240 striped catfish in 4 tanks (2000 L.tank^{-1}; n=60 fish.tank^{-1}) for 38 days. Both snakehead and striped catfish were fed restrictively at a level of 20 g.kg$^{-0.8}$.d^{-1} for 28-d and 38-d experimental periods. This feeding level is close to apparent satiation for these fish. Fish were fed twice a day at 9.00 am and 3.00 pm with the daily meal (20 g.kg$^{-0.8}$.d^{-1}) equally split between the two feedings. Each experimental diet was fed to 2 tanks per fish species (Table 1).

For snakehead, the mean temperature, oxygen, pH, conductivity, NH_4-N, NO_2-N and NO_3-N during the experiment were 28.2 ± 0.34°C, 5.5 ± 1.06 mg.L^{-1}, 7.4 ± 0.24, 2.7 ± 0.62 mS.m^{-1}, <0.5 mg.L^{-1}, < 0.5 mg.L^{-1}, and < 50 mg.L^{-1}, respectively. For striped catfish, the mean temperature, oxygen, pH, conductivity, NH_4-N, NO_2-N and NO_3-N were 28.4 ± 0.45°C, 5.8 ± 0.53 mg.L^{-1}, 7.3 ± 0.32, 3.0 ± 0.70 mS.m^{-1}, <0.5 mg.L^{-1}, < 0.5 mg.L^{-1}, and < 50 mg.L^{-1}, respectively.

On the day of blood sampling (time point zero), only one tank per fish species and diet was fed half the daily portion (10 g.kg$^{-0.8}$; the normal morning portion) in the morning at 9.00 am. The other tanks were not fed and fish in these tanks received their last meal 18 h prior to time point zero. At each postprandial point (3h, 7h and 24h), 6 fish from each tank were euthanized by using an overdose of Aqui-S (Aqui-S New Zealand Ltd., Lower Hutt, New Zealand) and sampled for blood. Blood was taken from the veins using a syringe coated with heparin (LEO pharma B.V., the Netherlands). After centrifuging at 4000 rpm for 10 min (Rotofix, 32 A, Hettich corp., Germany), plasma samples were directly analyzed on the same day.

6.2.3 Chemical analysis

The proximate composition (dry matter, crude protein, fat, energy, starch and energy) of experimental diets was analysed following the methods described by AOAC, 1995. The plasma glucose and triglycerides levels were measured using the Beckman coulter Au640 (Mishima Olympus co., Japan) at 505 nm. To measure the plasma glucose, the glucose reagent was added automatically to the plasma samples. The glucose reagent (Erba Mannhein, Germany) included glucose oxidase (20000 IU.L^{-1}), peroxidase (3250 IU.L^{-1}), 4-Aminoantipyrine (0.52 mmol.L^{-1}), 4-Hydroxybenzoic acid (10 mmol.L^{-1}), phosphate buffer (110 mmol.L^{-1}). To measure the plasma triglycerides, the triglycerides reagent was added automatically to the plasma samples. The triglycerides reagent (Erba Mannhein, Germany) included Good's buffer (pH 7.2) (50 mmol.L^{-1}), 4-Chlorophenol (4 mmol.L^{-1}), Mg^{2+} 15 mmol.L^{-1}, ATP 2 mmol.L^{-1}, Glycerolkinase (0.4 KU.L^{-1}), peroxidase (2.0 KU.L^{-1}), lipoproteinlipase (2.0 azKU.L^{-1}), glycerol-3-phosphate-oxidase (0.5 KU.L^{-1}), 4-Aminoantipyrine (0.5 mmol.L^{-1}).

Table 1. Formulation and chemical composition of the two diets fed to striped catfish and snakehead

	Low starch	High starch
Diet composition (g.100 g^{-1}, as-is)		
Wheat flour	5.2	37.7
Wheat	17.4	11.4
Poultry meal	13.9	9.1
Fishmeal	34.8	22.9
Soy protein concentrate	20.9	13.7
Mono calcium phosphate	2.6	1.7
Methionine	0.5	0.3
Lysine	1.6	1.0
Threonine	0.4	0.2
Squid liver oil	0.9	0.6
Premix	2.0	1.3
Chemical composition of diets (g.kg^{-1}, DM)		
Dry matter	914	948
Crude protein	512	414
Fat	71	50
Carbohydrates	268	436
Starch	139	292
NSP	129	144
Ash	149	101
Energy (MJ.kg^{-1}. DM)	19.0	18.9

NSP, non-starch polysaccharides.

6.2.4 Data analysis

Data analysis was conducted using statistical analysis systems (SAS Institute) statistical software package version 9.1. Three-way ANOVA was used to investigate the effect of diet type, feeding regime, postprandial time and their interaction on the plasma glucose and triglycerides levels. Significance was set at $P<0.05$.

6.3. Results

The first aim of this study was to assess the effect of dietary starch content on the plasma glucose level in snakehead and striped catfish. Averaged over treatments, the plasma glucose was higher in snakehead (12 mmol.L^{-1}) than in striped catfish (5.4 mmol.L^{-1}) (Figure 1, Supplementary Table A). In snakehead, the fish fed the high starch diet had higher plasma glucose levels than the low starch diet ($P<0.05$) while this was not the case for striped catfish. In contrast, stripped catfish showed no significant difference in glucose levels between fish fed high and low starch diets. The unfed snakehead had lower plasma glucose levels than the fed fish ($P<0.05$). The plasma glucose level of snakehead maintained high within 7h postprandial and decreased by 6.5 mmol.L^{-1} at 24h postprandial averaged over both feeding regime and diets. There was an effect of interaction between the feeding regime and diet type on the plasma glucose levels in striped catfish ($P<0.05$;

Figure 1). In striped catfish, the average plasma glucose level increased by 2.1 mmol.L^{-1} at 7h postprandial and the level maintained stable until 24h postprandial averaged over feeding regime and diets.

The second aim of this study was to assess the effect of dietary starch content on the plasma triglycerides level in snakehead and striped catfish. The plasma triglycerides level was higher in striped catfish (7.4 mmol.L^{-1}) than in snakehead (1.2 mmol.L^{-1}) averaged over treatments. In both species, the level of plasma triglycerides was higher in fed fish than in unfed fish. However, an effect of feeding regime (fed vs. unfed on the day of blood sampling) was observed at 3h and 7h postprandial in snakehead. There was an effect of interaction between the feed regime on the day of blood sampling and the postprandial time of blood sampling on the average plasma triglycerides level in snakehead ($P<0.05$). At 3h and 7h postprandial, the average plasma triglycerides level was more than twice as high in fed snakehead compared to unfed snakehead ($P<0.05$). In striped catfish, fish fed the high starch diet had a higher plasma triglycerides level than the fish fed the low starch diet ($P<0.05$), however this was not the case in snakehead.

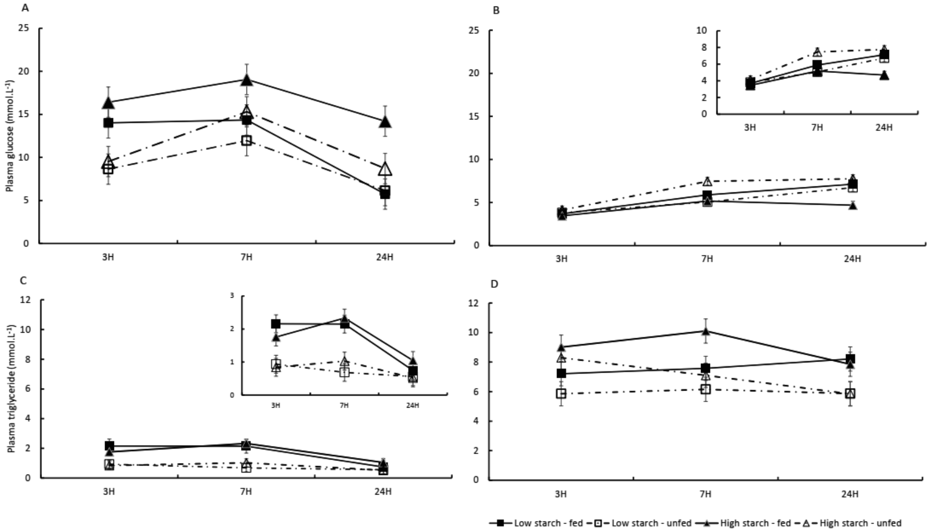

Figure 1. The plasma glucose (A,B) and triglyceride (C,D) levels of snakehead (A, C) and striped catfish (B, D) under one of the two feeding regimes on the day of blood sampling (fed vs. unfed) and one of the two diets (low vs. high starch) after 3h, 7h and 24h post-prandial

6.4. Discussion

In the present study, the range of the plasma glucose levels was higher in snakehead (12 mmol.L^{-1}) than in striped catfish (5.4 mmol.L^{-1}). The high level of blood plasma glucose in snakehead is comparable to the plasma glucose levels found in carnivorous fish like barramundi (Wade *et al.*, 2020) and trout (Figueiredo-Silva *et al.*, 2013). The low levels of plasma glucose in striped catfish are comparable to the plasma glucose levels found in omnivorous and herbivorous fish like grass carp (Su *et al.*, 2020) and tilapia (Chen *et al.*, 2020; Figueiredo-Silva *et al.*, 2013). The difference in the average plasma glucose level between snakehead and striped catfish indicates the limited capacity to regulate the glucose level in snakehead. This is similar to the previous findings on the limited ability of carnivorous fish in regulating plasma glucose levels (Moon, 2001; Panserat *et al.*, 2001b; Panserat *et al.*, 2002).

The effect of dietary starch supplementation on the average plasma glucose level is higher in snakehead than in striped catfish. The difference in the average plasma glucose level between the fish fed the low and high starch diet observed in snakehead (3.8 $mmol.L^{-1}$) was larger compared to striped catfish (0.05 $mmol.L^{-1}$). Similarly, feeding on the day of blood sampling increased the average plasma glucose level by 4 $mmol.L^{-1}$ (40%) in snakehead, but by only 0.8 $mmol.L^{-1}$ (16%) in striped catfish. These findings indicate that the ability to maintain the plasma glucose level at the homeostasis is better in striped catfish than in snakehead. In addition, between 7h and 24h postprandial, a large decrease of 43% in the plasma glucose level was found in snakehead, while the plasma glucose level maintained stable in striped catfish. In addition, plasma triglyceride levels did not increase in snakehead. This seems to indicate that for snakehead plasma glucose is not converted to plasma triglycerides. The slow decline in blood glucose in snakehead might suggest that glucose is only gradually disappearing due to the direct ATP production. Another explanation for the decline in plasma glucose levels in snakehead postprandial is excreted. Whether this is active or passive is not clear as well as the route of excretion via urine and/or gills. The plasma glucose in humans can be excreted via urine (Sha *et al.*, 2011), which might also the case in snakehead. Another way of losing glucose in blood could be through osmoregulation via the gills (Thompson *et al.*, 1989). It needs further study to conclude the dietary glucose fate in snakehead.

The unfed snakehead and striped catfish fed the high starch diet had a higher average plasma glucose level than the fish fed the low starch diet. This could be due to the effect of the last meal which was high in starch. It was observed that in various unfed fish digesta was present in the intestine after 24 hours in snakehead and even after 48 hours in striped catfish. The fact that the intestines were not fully empty in unfed fish may have caused the relatively high plasma glucose levels. Another reason could be the handling stress (chasing and netting) interfering with the effect of dietary starch on the plasma glucose levels. The intensity of the chasing stressor exposed to fish in the current study was lower at 3h than at 7h and 24h postprandial. At each time point, a total of 6 fish per tank were caught after chasing all fish in the tank. At 7h and 24h postprandial, all fish were chased twice and thrice, as a sequence to the previous sampling, respectively. Similarly, the effect of handling stress might have biased the values found in grass carp (Su *et al.*, 2020) because of the similarity in the plasma glucose levels between snakehead in this present study and grass carp at 3h after starch ingestion (Su *et al.*, 2020). It is expected that grass carp, as an omnivore, would have an average lower plasma glucose level than snakehead as a carnivore. In fact, grass carp in (Su *et al.*, 2020) was forced to ingest starch through a tube (oral starch administration). This might have created an effect of the handling stress on the blood glucose levels analyzed. Stressors, i.e., chasing, netting or the lack of oxygen, can also increase blood glucose levels in fish (Davis *et al.*, 2002; Evans *et al.*, 2003; Lee *et al.*, 2019). This indicates that the study method may cause the difference in the blood glucose level between fish species in literature.

The average plasma triglycerides level was higher in striped catfish (7.4 $mmol.L^{-1}$) than in snakehead (1.2 $mmol.L^{-1}$). The contrast in the average plasma triglycerides levels between striped catfish and snakehead in the present study is comparable to the contrast observed in tilapia – a herbivore

(Figueiredo-Silva *et al.*, 2013) and Chinese long snout catfish – a carnivore (Su *et al.*, 2020). Yet, for snakehead and striped catfish, the higher plasma triglycerides levels in fed fish compared to unfed fish may indicate that the amount of plasma triglycerides converted from the plasma glucose sourced from the dietary starch is higher in fed fish than in unfed fish. It could be arguable that the amount of plasma triglycerides originated from the dietary fat. However, this seems unlikely because of the comparable amount of fat between the experimental diets. Moreover, the difference in the plasma triglycerides level between fed and unfed fish was larger in striped catfish (1.8 mmol.L^{-1}) than in snakehead (0.9 mmol.L^{-1}). Considering the similar amount of starch ingested between snakehead and striped catfish, this indicates that digested starch (i.e., glucose) might be converted to triglycerides in striped catfish at a higher rate. The excessive amount of blood glucose can be stored in the form of fat (i.e., triglycerides) (Kamalam *et al.*, 2017). The increase of the average plasma triglycerides was also observed after ingestion of high amounts of glucose in tilapia (Chen *et al.*, 2018), grass carp and Chinese long snout catfish (Su *et al.*, 2020). These findings confirm a stronger conversion rate for plasma glucose to triglycerides in striped catfish than in snakehead when a similar amount of starch is ingested. The limited ability to convert plasma glucose into triglycerides in snakehead may relate to the limited ability of using carbohydrates for growth in snakehead (Phan *et al*., 2021b). In fact, protein efficiency did not differ between the carb vs. protein diet in snakehead (49% vs. 47%; Phan *et al*., 2021b) but improved in striped catfish when carbohydrates were supplemented to the diet (50% vs. 42%) (Phan *et al.*, 2021a). Results of our study would have implications in using carbohydrate as an energy source to spare dietary protein levels in the commercial feed production of snakehead and striped catfish.

6.5. Conclusion

Increasing dietary starch increased the plasma glucose levels in snakehead and triglycerides levels striped catfish. Striped catfish can maintain the plasma glucose homeostasis better than snakehead. The ability to convert dietary starch, i.e., glucose into triglyceride is larger in striped catfish than in snakehead. Overall, striped catfish has a better ability to utilize dietary carbohydrates than snakehead.

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Supplementary Table A. Plasma glucose and triglyceride mmol.L^{-1} levels of snakehead and striped catfish fed one of the two diets

	Low starch		High starch			*P* values						
	Fed	Unfed	Fed	Unfed	SEM	D	F	DxF	T	DxT	FxT	DxFxT
Snakehead												
Glucose												
3H	14.0	8.6	16.4	9.5								
7H	14.3	12.0	19.1	15.3	1.76	***	***	ns	***	ns	ns	ns
24H	5.7	6.1	14.2	8.7								
Triglyceride												
3H	2.2	0.9	1.8	0.8								
7H	2.1	0.7	2.3	1.0	0.27	ns	***	ns	***	ns	*	ns
24H	0.7	0.6	1.0	0.5								
Striped catfish												
Glucose												
3H	3.7	3.8	3.5	4.1								
7H	5.9	5.1	5.2	7.5	0.46	ns	*	***	***	ns	ns	ns
24H	7.1	6.7	4.7	7.8								
Triglyceride												
3H	7.2	5.9	9.0	8.3								
7H	7.6	6.2	10.1	7.1	0.82	*	***	ns	ns	ns	ns	ns
24H	8.2	5.9	7.9	5.8								

D, Diet type (low starch vs. high starch); F, feeding regime on the day of blood sampling (Fed vs. Unfed); T, postprandial time (3H, 7H vs. 24H).***, *P*<0.01; *, *P*<0.05; ns, non-significantly different.

7

Effect of dietary carbohydrates and fat supplementation on the yield and chemical composition of fillet and the location of fat deposition in striped catfish, African catfish and snakehead

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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 2x2 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).

Key words: dietary carbohydrates supplementation; dietary fat supplementation; striped catfish; African catfish; snakehead; fillet yield; fillet fat; fillet protein; the location of fat deposition; fillet; liver; viscera; rest fraction.

7.1. 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.*, 2012b), barramundi (*Lates calcarifer*) (Glencross *et al.*, 2017), snakehead (*Channa striata*) (Phan *et al.*, 2021b) 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, *e.g*. being around 30% in striped catfish (*Pangasius hypophthalmus*) (Asemani *et al.*, 2019; Da *et al.*, 2012), African catfish (*Clarias gariepinus*) (Jantrarotai *et al.*, 1998) and snakehead (*C. striata*) (Tan & 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 increased 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 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 & 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 & 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 (African catfish – *C. gariepinus*, striped catfish - *P. hypophthalmus*, Phan *et al.,* 2021b and 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 2x2 factorial design: with or without extra carbohydrates supplementation; and with or without extra fat supplementation; and fed at two different feeding levels. The data used in the current study are from fish sampled at the highest feeding level.

7.2. Materials and methods

7.2.1 Experimental diets. This study had a 3x2x2 factorial design with the following factors: species including striped catfish (*P. hypophthalmus*), African catfish (*C. gariepinus*) and snakehead (*C. striata*); dietary carbohydrate level (low versus high); and dietary fat level (low versus high). For all fish species, first 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 catfish (*C. gariepinus*).

Wheat flour was used as a 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 3mm 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 daily 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

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), on a dry matter basis. Starch, protein, fat and ash were determined based on chemical analysis.

the diets, but the supplementation of carbohydrate and fat sources for the three species were comparable (Figure 1).

7.2.2 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 (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*) study was 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.

7.2.3 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) and integrated into a recirculating aquaculture system (RAS) with a water flow per tank of 30 L.min^{-1} while the experiment on African catfish (*C. gariepinus*) was conducted in 70-L rectangular glass tanks (70 x 35 x 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 and snakehead experiments, respectively 120, 35 and 100 fish were stocked per tank and the experimental duration 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).

7.2.4 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

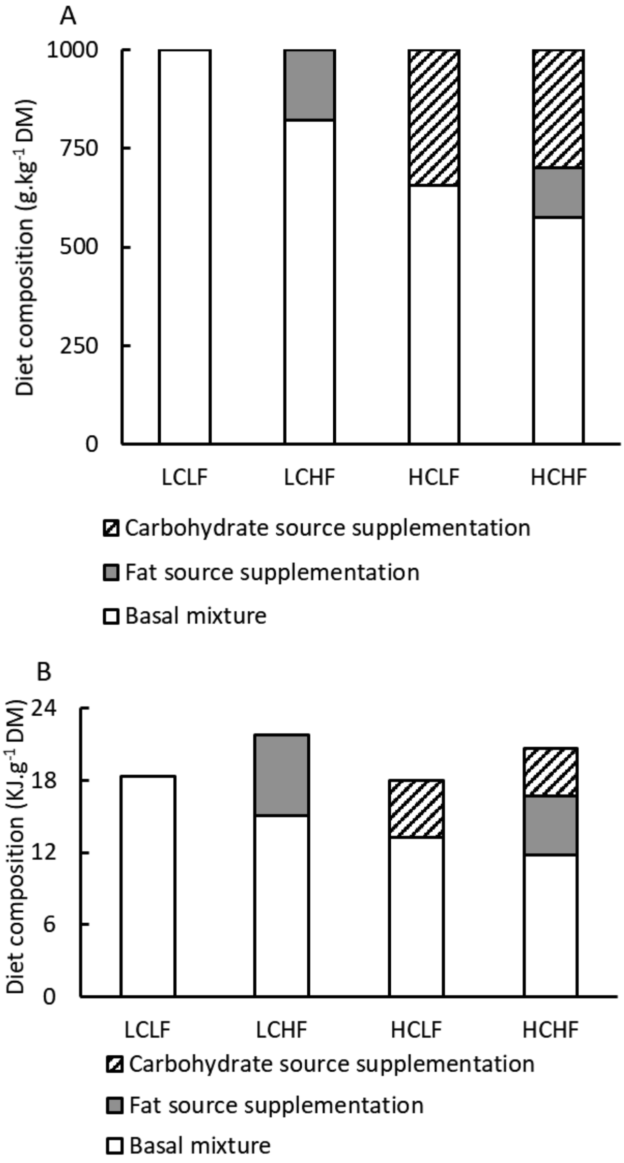

Figure 1. The composition of the four diets: low carbohydrates low fat (LCLF), low carbohydrates high fat (LCHF), high carbohydrates low fat (HCLF), high carbohydrates high fat (HCHF) fed to striped catfish, African catfish and snakehead on a weight basis (A) and on an energy basis (B).

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.* (2012b).

Before chemical analysis, fish compartment samples were thawed and minced to ensure the 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 9831,1998).

7.2.5 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.

7.2.6 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 $P<0.05$.

7.3. Results

7.3.1 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.

7.3.2 *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).

Only carbohydrates supplementation showed an interaction effect on the hepato-somatic index (HSI) between species ($P<0.05$) (Figure 2). Except for the HSI, the responses of the other somatic indices to differences in the dietary macronutrient content were similar for the studied species. Details about the effect of species, carbohydrates and fat supplementation on the organ somatic indices are presented in Table 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).

7.3.3 *Chemical composition of compartments*

There was interaction between species and carbohydrates on fillet protein and fat content (Figure 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 (Figure 5 and 6), viscera and the rest fraction are given in supplementary table S3, S4 and S5, respectively.

7.3.4 *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 (6.6%) while the deposition of fat in viscera (17.5%) and fillet (13.5%) was 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 (Figure 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*) ($P<0.05$) (Figure 7).

In fillet, the deposition of fat was comparable between striped catfish (*P. hypophthalmus*) and African catfish (*C. gariepinus*), which was almost 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 ($P<0.05$) (Figure 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% (Figure 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).

7.4. 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 (*C. striata*) (Tan & 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 in another study (Orban *et al.*, 2008). The effect of dietary carbohydrates supplementation on the fillet fat content in snakehead (*C. striata*) was 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*) (Figure 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 (Figure 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 & Gatlin III, 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 & Oliva-Teles, 1999) and hybrid striped bass (Gaylord & Gatlin Iii, 2000) were unaffected by increasing dietary fat levels. The differences between species might be due to the

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				
	Striped catfish	**African catfish**	**Snakehead**	**SEM**	***P* values**
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.

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.

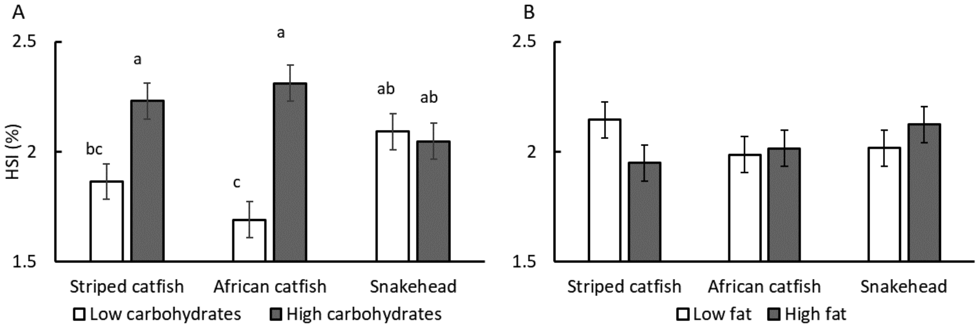

Figure 2. Interaction effect between fish species (striped catfish, African catfish and snakehead) and carbohydrates supplementation (low vs. high) (A), and between fish species and fat supplementation (low vs. high) (B) on the hepato-somatic index (HSI). In the case of a significant interaction effect, means lacking a common superscript within a panel differ (P<0.05).

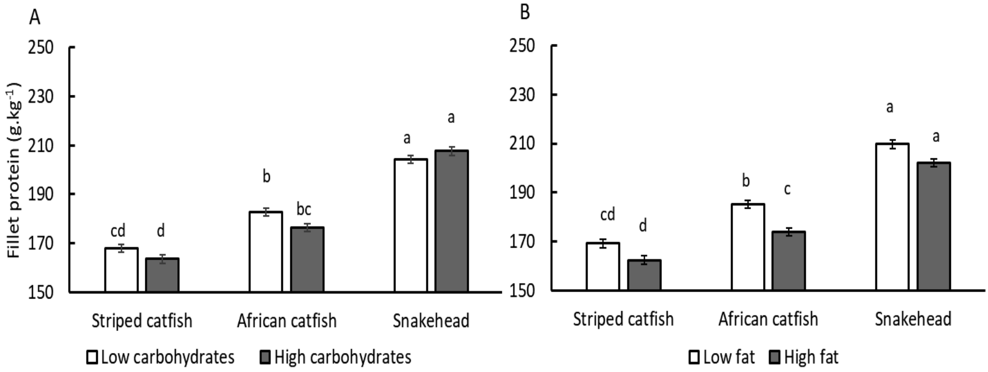

Figure 3. Interaction effect between fish species (striped catfish, African catfish and snakehead) and carbohydrates supplementation (low vs high) (A), and between fish species and fat supplementation (low vs high) (B) on fillet protein content. In the case of a significant interaction effect, means lacking a common superscript within a panel differ (P<0.05).

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.

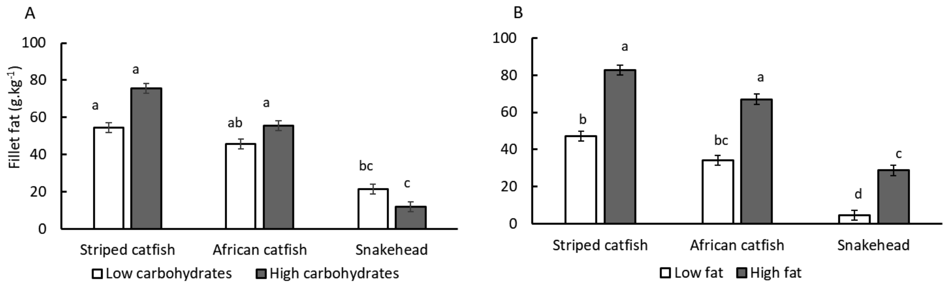

Figure 4. Interaction effect between fish species (striped catfish, African catfish and snakehead) and carbohydrates supplementation (low vs. high) (A), and between fish species and fat supplementation (low vs. high) (B) on fillet fat content. In the case of a significant interaction effect, means lacking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

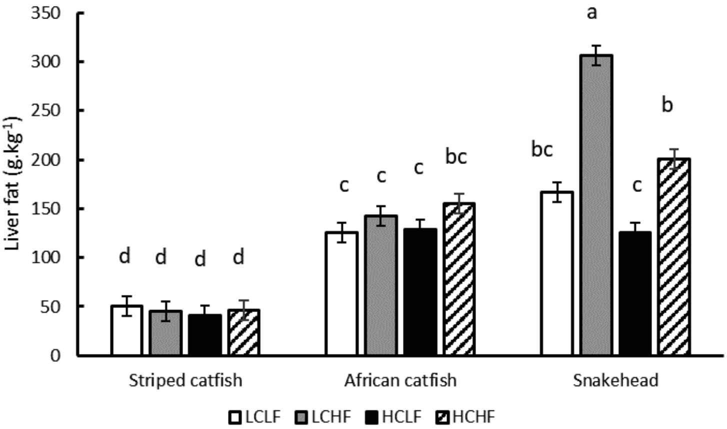

Figure 5. Interaction effect between species (striped catfish, African catfish and snakehead), carbohydrates supplementation (low vs. high) and fat supplementation (low vs. high) ($P<0.05$) on liver fat content of studied fish fed one of the four diets: low carbohydrates low fat (LCLF), low carbohydrates 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 ($P<0.05$).

applied contrast in the dietary fat. However, this explanation seems unlikely since the contrast in dietary fat level in European seabass study (Peres & Oliva-Teles, 1999) and hybrid striped bass study (Gaylord & 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 are species-dependent.

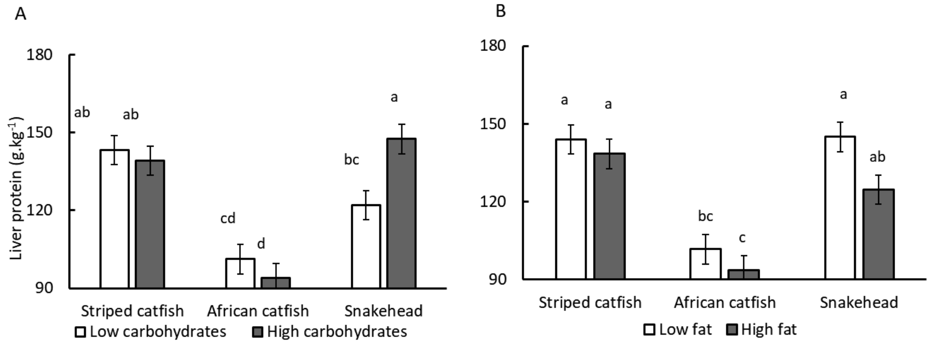

Figure 6. Effect of interaction between species (striped catfish, African catfish and snakehead) and carbohydrates supplementation (low vs. high) (A), and between species and fat supplementation (low vs. high) (B) on liver protein content. In the case of a significant interaction effect, means lacking a common superscript within a panel differ (*P*<0.05).

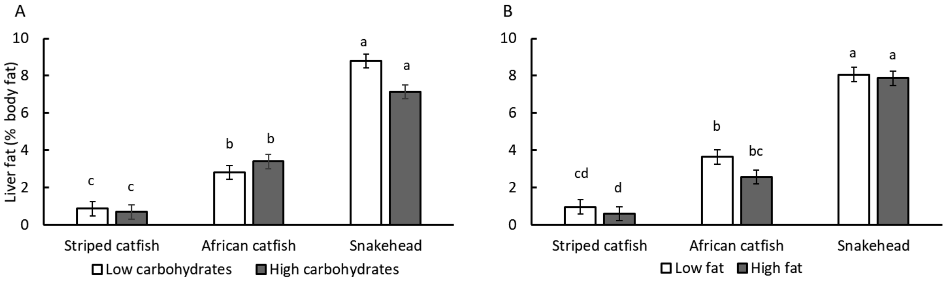

Figure 7. Interaction effect between fish species (striped catfish, African catfish and snakehead) and carbohydrates supplementation (low vs high) (A), and between fish species and fat supplementation (low vs high) (B) on the amount of fat deposition in the liver as percentage of total body fat. In the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

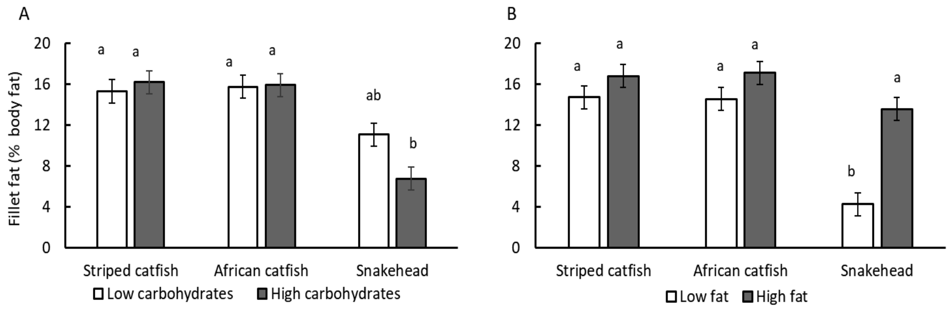

Figure 8. Interaction effect between fish species (striped catfish, African catfish and snakehead) and carbohydrates supplementation (low vs high) (A), and between fish species and fat supplementation (low vs high) (B) on the amount of fat deposition in the fillet as percentage of total body fat. In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

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 & 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 & 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 (Figure 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, Figure 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, different 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*) (Figure 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 only from 3.1 to 17.3% of the total fish body fat even when supplementing large amounts of either carbohydrates or fat to fish diets. 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.

7.5. Conclusion

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).

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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.

Supplementary data

Supplementary table S1. Body weight and somatic indices of body compartments in striped catfish, African catfish and snakehead fed one of four diets

	Low carbohydrate		High carbohydrate			*P* values						
	Low fat	High fat	Low fat	High fat	SEM	Sp	C	SpxC	F	SpxF	CxF	SpxCxF
HSI (%)												
Striped catfish	1.9	1.8	2.4	2.1	0.11	Ns	***	***	ns	ns	ns	ns
African catfish	1.7	1.7	2.3	2.3								
Snakehead	2.0	2.2	2.1	2.0								
VSI (%)												
Striped catfish	5.0	6.4	6.1	7.5	0.33	***	***	ns	***	ns	ns	ns
African catfish	4.0	5.3	4.3	6.2								
Snakehead	3.6	3.7	3.3	4.2								
Fillet yield (%)												
Striped catfish	33.5	30.4	32.2	31.0	1.54	*	ns	ns	ns	ns	ns	ns
African catfish	30.2	30.3	29.2	29.4								
Snakehead	34.8	33.4	33.3	31.7								
The rest fraction (%)												
Striped catfish	55.6	57.3	55.2	55.0	1.25	***	ns	ns	ns	ns	ns	ns
African catfish	58.9	56.1	58.5	55.3								
Snakehead	54.7	54.9	52.6	53.5								
Final body weight (g)												
Striped catfish	132.0	137.7	106.4	114.7	6.8	***	***	ns	ns	*	ns	ns
African catfish	222.0	229.8	189.0	207.2								
Snakehead	121.7	109.0	108.6	98.0								

HSI, hepato-somatic index; VSI, viscera-somatic index; Sp, is effect of fish species; C, is effect of carbohydrate supplementation; F, is effect of fat supplementation. ***, P<0.01; *, P<0.05; ns, not significant.

Supplementary table S2. Fillet composition (g.kg^{-1} on fresh basis) of striped catfish, African catfish and snakehead fed one of four diets

	Low carbohydrate		High carbohydrate			*P* values						
	Low fat	High fat	Low fat	High fat	SEM	Sp	C	SpxC	F	SpxF	CxF	SpxCxF
Dry matter												
Striped catfish	218	250	232	267	4.4	ns	ns	*	***	*	ns	ns
African catfish	225	247	229	253								
Snakehead	236	255	235	250								
Ash												
Striped catfish	13	14	13	12	0.5	***	ns	ns	ns	ns	ns	ns
African catfish	12	12	12	11								
Snakehead	12	11	12	12								
Protein												
Striped catfish	171	165	167	160	2.3	***	ns	*	***	ns	ns	ns
African catfish	189	177	181	172								
Snakehead	209	199	210	205								
Energy (MJ.kg^{-1})												
Striped catfish	5.4	6.9	6.0	7.6	0.2	***	*	***	***	*	ns	ns
African catfish	5.6	6.6	5.8	7.0								
Snakehead	5.5	6.5	5.5	6.2								
Fat												
Striped catfish	38	71	57	94	3.8	***	***	***	***	ns	ns	ns
African catfish	30	62	39	72								
Snakehead	6	37	3	21								

Sp, is effect of fish species; C, is effect of carbohydrate supplementation; F, is effect of fat supplementation. ***, P<0.01; *, P<0.05; ns, not significant.

Supplementary table S3. Liver composition (g.kg^{-1} on fresh basis) of striped catfish, African catfish and snakehead fed one of four diets

	Low carbohydrate		High carbohydrate			*P* values						
	Low fat	High fat	Low fat	High fat	SEM	Sp	C	SpxC	F	SpxF	CxF	SpxCxF
Dry matter												
Striped catfish	228	228	227	231	6.16	***	ns	ns	*	ns	ns	ns
African catfish	281	296	296	318								
Snakehead$^{\$}$	-	-	-	-								
Ash												
Striped catfish	10	10	9	9	1.46	***	ns	ns	*	*	ns	ns
African catfish	14	17	14	21								
Snakehead$^{\$}$	-	-	-	-								
Protein												
Striped catfish	147	139	141	137	8.0	***	ns	*	*	ns	ns	ns
African catfish	106	96	97	91								
Snakehead	134	111	156	139								
Energy (MJ.kg^{-1})												
Striped catfish	5.6	5.7	5.6	5.7	0.43	***	*	***	***	***	ns	ns
African catfish	8.6	9.0	8.7	9.7								
Snakehead	9.7	14.7	8.7	11.2								
Fat												
Striped catfish	51	46	41	46	10.1	***	***	***	***	***	ns	*
African catfish	126	142	129	155								
Snakehead	167	306	126	201								

$^{\$}$Missing data because of insufficient amount of samples.
Sp, is effect of fish species; C, is effect of carbohydrate supplementation; F, is effect of fat supplementation.
***, P<0.01; *, P<0.05; ns, not significant.

Supplementary table S4. Viscera composition (g.kg^{-1} on fresh basis) of striped catfish, African catfish and snakehead fed one of four diets

	Low carbohydrate		High carbohydrate			*P* values						
	Low fat	High fat	Low fat	High fat	SEM	Sp	C	SpxC	F	SpxF	CxF	SpxCxF
Dry matter												
Striped catfish	410	537	437	608	38.2	***	ns	ns	***	ns	ns	ns
African catfish	382	511	389	570								
Snakehead	370	470	319	445								
Ash												
Striped catfish	12	34	10	6	8.4	ns	ns	ns	ns	ns	ns	ns
African catfish	10	8	9	8								
Snakehead	13	10	14	10								
Protein												
Striped catfish	94	68	78	84	10.6	***	ns	ns	***	ns	ns	ns
African catfish	120	91	106	78								
Snakehead	143	140	165	141								
Energy (MJ.kg^{-1})												
Striped catfish	13.8	18.0	14.9	20.6	1.47	***	ns	ns	***	ns	ns	ns
African catfish	12.1	17.9	12.8	20.0								
Snakehead	10.8	14.8	9.1	13.7								
Fat												
Striped catfish	288	440	327	522	40.8	***	ns	ns	***	ns	ns	ns
African catfish	230	380	257	455								
Snakehead	194	275	129	263								

Sp, is effect of fish species; C, is effect of carbohydrate supplementation; F, is effect of fat supplementation. ***, P<0.01; *, P<0.05; ns, not significant.

Supplementary table S5. The rest fraction composition (g.kg^{-1} on fresh basis) of striped catfish, African catfish and snakehead fed one of four diets

	Low carbohydrate		High carbohydrate			*P* values						
	Low fat	High fat	Low fat	High fat	SEM	Sp	C	SpxC	F	SpxF	CxF	SpxCxF
Dry matter												
Striped catfish	312	376	327	399								
African catfish	286	323	297	333	6.4	***	*	ns	***	***	ns	ns
Snakehead	327	356	328	348								
Ash												
Striped catfish	42	45	45	41								
African catfish	43	41	41	40	3.2	***	ns	ns	ns	ns	ns	ns
Snakehead	82	80	78	72								
Protein												
Striped catfish	152	141	141	137								
African catfish	165	159	166	153	3.5	***	ns	ns	***	ns	ns	ns
Snakehead	192	188	196	189								
Energy (MJ.kg^{-1})												
Striped catfish	8.1	10.0	8.7	11.2								
African catfish	6.7	8.3	7.2	8.8	0.22	***	***	*	***	*	ns	ns
Snakehead	6.4	7.6	6.3	7.6								
Fat												
Striped catfish	115	135	141	195								
African catfish	72	120	87	130	3.7	***	***	***	***	*	*	***
Snakehead	51	79	47	79								

Sp, is effect of fish species; C, is effect of carbohydrate supplementation; F, is effect of fat supplementation. ***, P<0.01; *, P<0.05; ns, not significant.

Supplementary table S6. Deposition of protein (% total body protein) in different body compartments of striped catfish, African catfish and snakehead fed one of four diets

	Low carbohydrate		High carbohydrate			*P* values						
	Low fat	High fat	Low fat	High fat	SEM	Sp	C	SpxC	F	SpxF	CxF	SpxCxF
Liver												
Striped catfish	1.9	1.8	2.4	2.1								
African catfish	1.1	1.1	1.4	1.5	0.12	***	***	ns	ns	ns	ns	ns
Snakehead	1.4	1.4	1.8	1.6								
Viscera												
Striped catfish	3.1	3.1	3.4	4.7								
African catfish	3.0	3.2	2.9	3.4	0.39	ns	ns	ns	ns	ns	ns	ns
Snakehead	2.8	2.9	3.0	3.4								
Fillet												
Striped catfish	38.4	36.4	38.6	37.1								
African catfish	35.5	35.8	33.7	35.5	1.38	*	ns	ns	ns	ns	ns	ns
Snakehead	39.3	37.6	38.4	37.2								
The rest fraction												
Striped catfish	56.6	58.7	55.7	56.1								
African catfish	60.4	59.9	62.0	59.6	1.59	***	ns	ns	ns	ns	ns	ns
Snakehead	56.5	58.1	56.8	57.8								

Sp, is effect of fish species; C, is effect of carbohydrate supplementation; F, is effect of fat supplementation. ***, P<0.01; *, P<0.05; ns, not significant.

Supplementary table S7. Deposition of fat (% total body fat) in different body compartments of striped catfish, African catfish and snakehead fed one of four diets

	Low carbohydrate		High carbohydrate			*P* values						
	Low fat	High fat	Low fat	High fat	SEM	Sp	C	SpxC	F	SpxF	CxF	SpxCxF
Liver												
Striped catfish	1.1	0.6	0.8	0.5	0.54	***	ns	*	ns	ns	ns	ns
African catfish	3.4	2.2	3.9	2.9								
Snakehead	8.2	9.4	7.9	6.4								
Viscera												
Striped catfish	15.6	22.3	17.2	22.1	2.66	ns	ns	ns	*	ns	ns	ns
African catfish	14.6	18.4	14.5	22.6								
Snakehead	17.6	14.1	13.2	17.5								
Fillet												
Striped catfish	13.7	17.0	15.8	16.7	1.59	***	ns	ns	***	***	ns	ns
African catfish	14.3	17.3	14.9	17.0								
Snakehead	5.4	16.7	3.1	10.4								
The rest fraction												
Striped catfish	69.7	60.2	66.2	60.7	2.58	ns	ns	*	***	ns	ns	ns
African catfish	67.7	62.1	66.8	57.5								
Snakehead	68.8	59.8	75.8	65.7								

Sp, is effect of fish species; C, is effect of carbohydrates supplementation; F, is effect of fat supplementation. ***, P<0.01; *, P<0.05; ns, not significant.

8

General discussion

8.1. Introduction

The major aim of this thesis was to assess differences in energy utilisation between fish species. These differences can be observed, according to the fish species- and diet, in the relationships between retained energy (RE) and digestible energy (DE) intake, using the DE approach; and between RE and digested protein (dCp), fat (dFat) and carbohydrates (dCarb), using the NE approach, for growth on an energy basis. This thesis also validated whether the net energy equations are similar across fish species studied, thus whether the net energy system can be applied to all fish. To fulfill this validation, the energy utilisation efficiencies of digested protein, fat and carbohydrates were quantified and compared across the fish species studied. Most striking was the large difference in the energy utilisation efficiency of digested carbohydrates between some species, which is discussed in relation to trout, barramundi and snakehead. To explain differences in the relationship between NE and digested carbohydrates between snakehead and striped catfish, chapter 8 discussed the post-prandial plasma glucose and triglyceride levels in these two fish species, when fed a similar amount of starch. Insights and ideas for future research are suggested, including the potential practical implications for the fish farming and fish feed production industry.

8.2. The impact of digestibility of nutrients on the energy evaluation

The nutrient digestibility of ingredients/feeds is an important step in most feed evaluation systems and thus also in DE and NE evaluation systems (Figure 1). The DE value of a diet/ingredient is dependent on its gross energy content (GE) and on its apparent digestibility coefficient (ADC) of energy. The GE content is composed of energy from protein, fat and carbohydrates. Similarly, the ADCs of protein, fat and carbohydrates determine the DE value of a feed/ingredient. The NE value of a diet/ingredient is also dependent on the ADC of protein, fat and carbohydrates. In the NE approach, as used in this thesis, the NE content of a diet/ingredient is calculated from its digestible protein, fat and carbohydrate content (respectively, dCP, dFat and dCarb) (Figure 4, **Chapter 1;** Figure 1). Thus, both the DE and NE value of fish feed/ingredients are dependent on the ADC of protein, fat and carbohydrates (Figure 1).

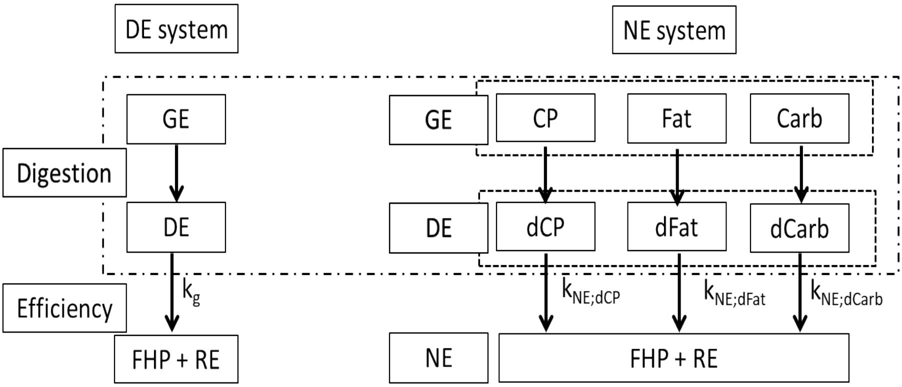

Figure 1. The digestible energy (DE) system and the net energy (NE) system.

GE, gross energy; DE, digestible energy; FHP, fasting heat production; RE, retained energy; $k_{g,DE}$, energy utilization efficiency, NE, net energy; CP, crude protein; Carb, carbohydrates; dCP, digestible protein intake; dFat, digestible fat intake; dCarb, digestible carbohydrates intake; $k_{NE;dCP}$ energy utilisation efficiency of dCP, $\mathrm{k}_{NE;dFat}$ energy utilisation efficiency of dFat, $k_{NE;dCarb}$ energy utilisation efficiency of dCarb.

Table 1. Apparent digestibility coefficient (ADC, %) of energy, protein, fat, carbohydrates (Carb), starch and non-starch polysaccharides (NSP) averaged over diets and feeding levels

Species	Energy	Protein	Fat	Carb	Starch	NSP
African catfish	80.9	92.4	88.6	57.1	99.4	-34.4
Barramundi	81.0	91.0	97.0	27.0	88.0	-4.4
Carp	90.4	95.3	91.4	81.3	99.1	49.6
Striped catfish	86.4	88.5	90.6	79.9	97.7	55.9
Snakehead	86.4	90.6	91.4	71.0	97.3	-5.2

In this thesis, NE equations were estimated for different fish species (Chapter 2 to 5). This required a large contrast in dCP, dFat and dCarb between the dietary treatments. A similar approach was taken for all fish species studied, by formulating four contrasting diets in terms of protein, fat and carbohydrate content. In all studies, this was done by diluting a protein rich diet, containing high quality protein sources, with a pure starch source, an oil source or both. This similar approach, together with the high quality ingredients in the diet formulation, ensured that the average measured nutrient ADC values, over all four diets per study, were indicative for species-related differences in digestive capacity. The average nutrient ADC values are given in Table 1. The variability in ADC between species/studies was relatively small for protein (ranging from 89 to 95%) and fat (ranging from 89 to 97%), compared to carbohydrate (ranging from 27 to 81%; Table 1). Most likely, this narrow range in protein and fat ADC is due to the high quality of protein and fat sources used in the current studies. This parallels the finding of Glencross (2008) and Haidar *et al*. (2018). The larger variability in carbohydrate ADC between species/studies indicates that, next to the macronutrient content, carbohydrate digestibility is an important factor in determining the DE as well as the NE value of ingredients/diets.

Carbohydrate is a collective name for numerous types of compounds, which can be classified in various ways, *e.g.*, enzymatically digestible (free sugars, starch etc.), versus fermentable carbohydrates (pectin, hemicellulose, etc.). In Chapters 2 to 5, in addition to carbohydrate ADC, the ADC of starch (including free sugars) and non-starch polysaccharides (NSP) was measured. Starch ADC was high (>88%) in all fish species studied (Table 1). The high starch ADC in this thesis might be due to factors like the production of the diets by extrusion, the ingredients being well grinded, and the inclusion of pre-gelatinised wheat/maize starch in the test diets. Particle size of ingredients (granule size) has previously been found to determine nutrient ADC in striped catfish (Tu-Tran *et al.*, 2019). Various studies have shown that increasing the degree of starch gelatinisation by the feed production process, including pre-conditioning and extruding conditions (*i.e.* steaming, heating), enhances starch ADC in fish (Krogdahl *et al.*, 2005, Peres and Oliva-Teles, 2002). Consequently, (well) gelatinised starch results in high starch ADC values in most fish species (European sea bass, 98%

Peres and Oliva-Teles, 2002; rainbow trout, 98%, Burel *et al.*, 2000; barramundi, 88%, Glencross *et al.*, 2017; and turbot, 82%, Burel *et al.*, 2000). The high starch ADC values, as well as the low variability in starch ADC between the fish species studied, is an indication that between-species variations in (gelatinised) starch ADC are small. Furthermore, it also implies that the observed large variability in carbohydrate ADC (Table 1) is not related to starch digestion.

Despite the high starch ADC, in combination with a low variability in starch ADC for the fish species studied in this thesis, the ADC of the total carbohydrates was much lower and showed considerable variability (Table 1). This indicates that the remaining part of the carbohydrates, *i.e.* the non-starch polysaccharides (NSP), were responsible for this. In fact, the variability in carbohydrate ADC is an outcome of 1) NSP ADC and 2) NSP content of the diet. Historically, dietary NSP has often been assumed to be inert (indigestible) in all fish species. However, recently there is evidence that in some fish species NSP can partially be digested, like in Nile tilapia (Maas *et al*., 2020) and trout (Staessen *et al*., 2019). This thesis showed that NSP is also digested by common carp and striped catfish (Table 1). In contrast, the NSP was inert for barramundi, snakehead and African catfish. In these species, the estimated NSP digestibility was even negative (Table 1). These negative NSP ADC values can be due to, among others: 1) a low NSP content of diets, which causes the magnification of small measurement errors; and 2) the use of a fixed coefficient of 6.25 for converting the nitrogen content into crude protein content. In fact, this coefficient differs between types of proteins and thus also between ingredients (NRC, 2011). The ADC of NSP was higher in striped catfish (56%), compared to carp (50%), when averaged over diets and feeding levels (Table 1). These NSP digestibilities in striped catfish and carp are comparable to the values found for tilapia (Haidar *et al*., 2016), but higher than the values found for trout (Staessen *et al*., 2019). The between species/study variability in NSP digestibility might relate to: the fish's feeding habit; the presence of bacteria or exogenous enzymes, *i.e.* phytase or xylanase (Romano *et al.*, 2018); the type of NSP (cellulose, hemicellulose, pectin, gums, etc.); or the amount of NSP consumed (Maas *et al.*, 2020). The higher digestibility of NSP in striped catfish is most likely caused by factors other than the level of NSP consumed. In fact, the inclusion level of NSP in the striped catfish diets tested (42% of carbohydrates) was even higher than in the carp diets tested (34% of carbohydrates). As a result, the NSP intake was higher in striped catfish (0.24 $g.kg^{-0.8}.d^{-1}$) than in carp (0.16 $g.kg^{-0.8}.d^{-1}$), when averaged over diets and feeding levels. It is expected that NSP would be more inert in striped catfish than in carp, due to the higher NSP intake in striped catfish. However, this was not the case in our studies. This thesis confirms that in some fish species (carp and striped catfish) NSP are digested, whereas for other fish (barramundi, snakehead and African catfish), NSP are inert.

For all feed evaluations systems that are fully or partially based on digestible nutrients, an important assumption is that the digestible nutrient content from the different ingredients in a feed mixture is additive. For all fish species studied in this thesis, the ADC of one or more macronutrients were affected by feeding level. In all species studied, the ADC of energy declined with feeding. This implies that the DE value, and also the NE value, of an ingredient/diet is dependent on the feeding level that is applied under practical conditions. A decline in ADC of various macronutrients and energy with increasing feeding level has been observed recently in, for example, Nile tilapia (Haidar *et al*., 2016), trout (Staessen *et al*., 2019) and African catfish (Elesho *et al*., 2021). A decline in ADC for various nutrients has recently been more frequently reported in the literature, which is most likely related to the current application and evaluation of low quality ingredients. Haidar *et al*. (2016) showed that the decline in ADC with feeding level was greater for a diet with a high NSP content, compared to a diet with a low NSP content. In figure 2, the impact of feeding level on total carbohydrate ADC is given. It shows that there are large differences in the impact of feeding level on carbohydrate ADC between studies/fish species. Next to the principal effect of feeding level, there was an interaction effect between diet and feeding level for many macronutrient's ADCs, for most of the fish species studied in this thesis. This implies that the difference in ADC between diets, and thus the difference in nutritional value between diets, altered with feeding level. Thus the assumption that the ADCs of ingredients in a feed formulation are additive is not valid. However, this poses a problem not only for the application of a NE system, but also for a DE system. To summarise, the nutritional value of diets and/or ingredients depends on the feeding level. The nutritional characterisation (*e.g.* digestibility) of feed ingredients should be assessed at feeding levels that are representative of practical culture conditions for the species involved.

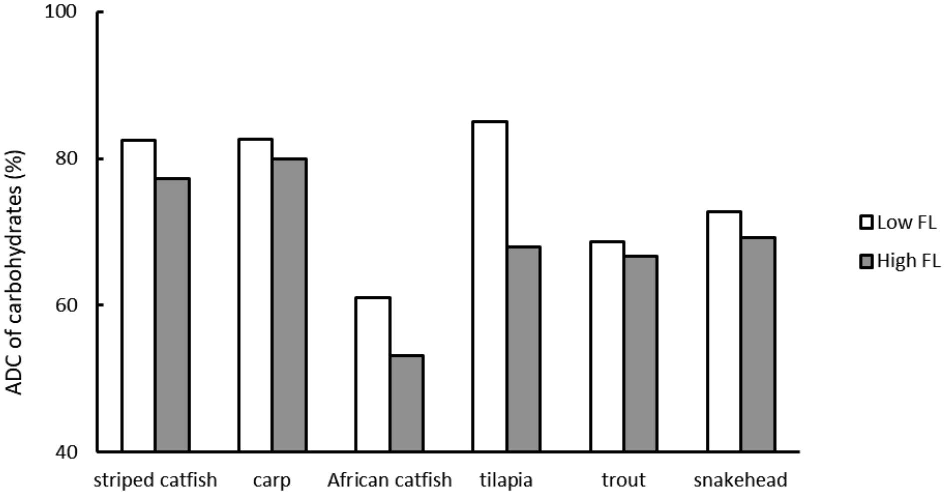

Figure 2. Digestibility of carbohydrates in striped catfish, carp, African catfish, snakehead (this thesis), tilapia (Haidar *et al*., 2016) and trout (Staessen *et al*., 2019) at low versus high feeding levels (FL).

8.3. Motivations to move from a digestible energy system to a net energy system

For many years, pig feed formulation has been based on a NE evaluation system. In contrast, fish feed formulation is still mostly based on a DE evaluation system. Diet formulation based on DE is effective only if there is low variability in the constituent ingredients, and if dietary macronutrient composition has no or minimal impact on the relationship between retained energy (RE) and DE intake. However, both currently and in the future, fish feed formulation will face increasing variability in its constituent ingredients. Therefore, the first major aim of this thesis was to investigate the effect of dietary macronutrient composition on the relationship between RE and DE in different fish species. This relationship can be altered both by changes in the DE requirement for maintenance (DE_m, the intercept with the x-axis) and in the DE used for growth ($k_{g,DE}$, the slope; Figure 3). In this thesis, the relationship between DE and RE was estimated for four diets, differing in macronutrient content, in five fish species. The mean, and range, of the estimated DE_m and $k_{g,DE}$ values are given in Table 2.

The DE_m, averaged over the four experimental diets, ranged from 28 to 50 kJ. $kg^{-0.8}.d^{-1}$ for the five fish species studied in this thesis (Table 2). The current estimates of DE_m fit well with the reported range of 18 to 88 kJ. $kg^{-0.8}.d^{-1}$ across different fish species, as reviewed by Schrama *et al*. (2012). Within the same fish species, large variability in DE_m is still observed, which is related to the fact that sub-optimal environmental conditions increase DE_m. The most well studied factor is the effect of temperature. An increase in temperature causes an increases in DE_m (Glencross, 2008; Glencross & Bermudes, 2010). Also, stocking density can alter DE_m (Lupatsch *et al.*, 2010; Santos *et al.*, 2010);in this study DE_m declined with increasing stocking density, which was hypothesised to be due to reduced swimming activity (Santos *et al*., 2010). Glencross (2009) found in rainbow trout that DE_m declined when fish were exposed to hypoxia. Even when expressed per unit of metabolic body weight (kJ. $kg^{-0.8}.d^{-1}$), differences in DE_m are present between fish with different body weights. The current thesis also demonstrated that dietary macronutrient composition can cause differences in DE_m. As shown in Table 2, a wide range in DE_m was observed within the same species, between the four extreme diets studied. This is the first time that a dietary effect on DE_m has been reported for fish. Previous studies have not shown this, for example, in Nile tilapia, replacing starch with fat (Schrama *et al*. 2012) or with NSP (Haidar *et al*., 2016) did not result in differences in DE_m. This might be due to smaller contrasts in dietary macronutrient content. As shown in Figure 3, the difference in DE_m might be the result of the observed effects of dietary macronutrients on $k_{g,DE}$, but not on the fasting heat production.

Table 2. Digestible energy demand for maintenance (DE_m) (kJ.$kg^{-0.8}.d^{-1}$) and digestible energy utilisation efficiency for growth ($k_{g,DE}$) (%) of various fish species

Species	DE_m		$k_{g,DE}$	
	Mean	Range	Mean	Range
Snakehead(1)	28	7 – 48	50	45 – 56
African catfish(1)	43	36 – 54	86	81 – 98
Common carp(1)	35	10 – 50	62	49 – 66
Striped catfish(1)	50	16 – 73	69	56 -75
Barramundi(2)	27	14 - 41	64	55 – 79
Rainbow trout(3)	38	-	-	62 – 74
European seabass(4)	45	-	-	64 – 82
Tilapia(5)	-	53 - 110	-	58 - 65

(1) In this thesis, (2)Glencross et al., 2017, (3)Glencross, 2009, (4)Lupatsch *et al.*, 2010, Lupatsch *et al.*, 2001, Lupatsch *et al.*, 2003, Peres and Oliva-Teles, 2005, (5) Schrama *et al*., 2012; Haidar *et al*., 2016.

Starch, as well as fat, supplementation affected DE_m within the five fish species studied. However, the effect of starch supplementation showed a high degree of variability between fish species. Increasing the level of dietary starch increased DE_m in carp and snakehead by 34 and 40%, respectively, but it decreased DE_m by 19% in both African catfish and striped catfish. This suggests that starch plays a more important role in energy use for African catfish and striped catfish. This is because the supplementation of energy from starch can lower $DE_{m,}$ consequently sparing more energy for growth. The effect of dietary fat supplementation on DE_m showed a greater similarity between fish species, compared to dietary starch supplementation. In all fish species, fat supplementation decreased DE_m; by 20, 40, 51 and 72% in African catfish, carp, striped catfish and snakehead, respectively. Mathematically, a higher inclusion level of dietary fat rotates the regression line resulting in a decreasing energy demand for maintenance (**Figure 3**). Biologically, this indicates that fat is used more efficiently for energy than starch. In the herbivorous and omnivorous fish species studied, *i.e.*, African catfish, carp and striped catfish, fat supplementation decreased the energy demand for maintenance by 20– 51% and even up to 72% in snakehead. This observation indicates that increasing dietary fat leaves more energy for growth, due to a lower demand for energy for maintenance. Especially in snakehead, fat seems to be a more important source of energy, compared to African catfish, carp and striped catfish.

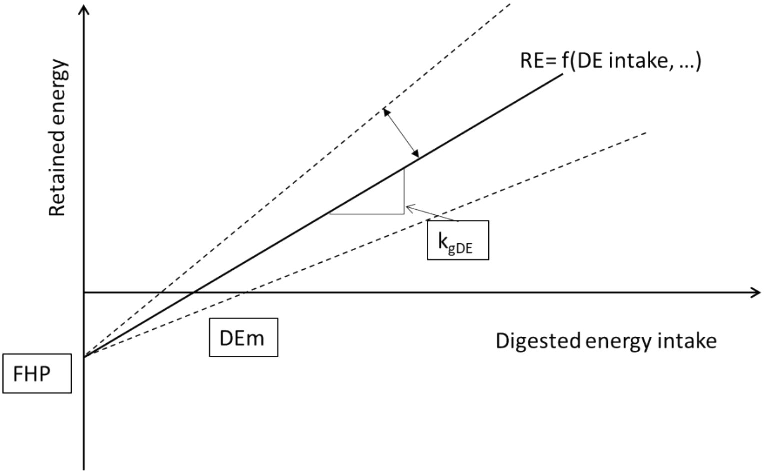

Figure 3. The conceptual variability in the relationship between retained energy (RE) and digested energy (DE) intake, *i.e.* energy utilisation efficiency ($k_{g,DE}$), energy demand for maintenance (DE_m) and fasting heat production (FHP).

The $k_{g,DE}$ averaged over the four experimental diets, ranged from 50 to 86% for the five fish species studied in this thesis (Table 2). The current estimates of $k_{g,DE}$ are generally in line with the reported range of 31 to 82% across different fish species, as reviewed by Schrama *et al.* (2012). Only the $k_{g,DE}$ value of 86% for African catfish is high compared to values found for other fish species. As discussed in Chapter 3, this might be related to an underestimation of the ADC of energy. In line with earlier studies on tilapia (Schrama *et al.*, 2012), barramundi (Glencross *et al.*, 2017) and rainbow trout (Rodehutscord and Pfeffer, 1999), this thesis demonstrated a wide variation in $k_{g,DE}$ between diets differing in macronutrient composition (Table 2). Except for striped catfish, in all the fish species studied, $k_{g,DE}$ was significantly affected by diet. However, even for striped catfish there was a large numerical variability in $k_{g,DE}$ between the diets (Table 2).

Even though, in all fish species studied, $k_{g,DE}$ was influenced by dietary macronutrient composition, the response to either starch or fat supplementation differed between species. In carp, both starch and fat supplementation increased $k_{g,DE}$ by 5 and 3%, respectively. While in African catfish, snakehead and striped catfish, both starch and fat supplementation decreased $k_{g,DE}$. Dietary starch supplementation decreased $k_{g,DE}$ by 16, 10 and 17% in African catfish, snakehead and striped catfish, respectively. The impact of dietary fat supplementation on $k_{g,DE}$ was smaller compared to starch supplementation. Fat supplementation resulted in a 2, 11 and 2% reduction in $k_{g,DE}$ in African catfish, snakehead and striped catfish, respectively. This thesis demonstrates that the relationship between RE and DE intake is affected by dietary macronutrient composition. This implies that the increasing diversification of ingredients used in fish feeds, accompanied by an increase in dietary macronutrient content variability, results in a biased prediction of energy gain (RE) when using an energy evaluation system that is based on DE.

This potential bias of a DE energy evaluation approach, due to changes in $k_{g,DE,}$ was the reason why NE equations were estimated for the five different fish species in this thesis. The different estimated NE equations are summarised in Table 3, including earlier estimates for the fish species Nile tilapia and rainbow trout (Schrama *et al*., 2018), and pigs (Noblet *et al*., 1994; CVB, 1993). In line with Schrama *et al*. (2018), NE equations for the five fish species were made by relating RE to dCP, dFat and dCarb. These equations (Table 3) can be used to calculate the NE value of formulated diets and/or single ingredients. An important question that needs to be addressed when moving from one energy evaluation system to another is whether it results in improvements. In order to address this, data from a study on striped catfish by Tu-Tran (2019) , on the ADC of energy and macronutrients in 12 different ingredients, was used. In Table 4, the measured DE values of these ingredients are compared to the calculated NE value, using the NE equation for striped catfish. Additionally this was also done using the NE equation for snakehead, on the assumption that the ADCs of the different macronutrients were equal to that of striped catfish.

In Table 4, the ingredients were ranked according their DE value. This was also done for the NE value in striped catfish, as well as in snakehead. This shows that the ranking of ingredients on a DE basis is different from that on a NE basis, for both fish species. Using the DE system, rice bran, fishmeal and full fat soybean meal are the three most energy-rich ingredients studied for both fish species (Table 4). When using the NE system, full fat soybean meal, soybean meal and fishmeal are the three most energy-dense ingredients studied for striped catfish; with fishmeal, full fat soyabean and defatted soybean meal being the equivalents for snakehead (Table 4). This comparison on ranking already shows that the energetic value of ingredients is different when using DE, compared to NE, in both fish species. The changes in ranking when moving from a DE to a NE seemed to be larger in striped catfish. This is also reflected when the DE of the 12 ingredients is related to their NE value, as shown in Figure 4. The R^2 of the relationship between DE and NE of the set of ingredients is 78% in striped catfish. This means that 78% of the variation in NE between the ingredients is explained by differences in DE. In snakehead this is much lower. In snakehead only 53% of the variability in NE is related to differences between DE. This demonstrates that moving from a DE to a NE system to improve the prediction of the energy potential is more relevant for snakehead than striped catfish.

This is due to the limited ability of snakehead to utilise carbohydrates for retained energy, even though digestibility was relatively high. The energy utilisation efficiency of carbohydrate for growth ($k_{NE;dCarb}$) is minimal, at only 5% for snakehead (**Chapter 5**).

Table 3. Estimated net energy equations in different fish species and in pigs

Species	Equations
Pig⁽¹⁾	$NE = 11.3\ dCP + 35.0\ dFat + 14.4\ ST + 12.1\ dRest$
Pig⁽²⁾	$NE = 10.8\ dCP + 36.1\ dFat + 13.5\ dST_e + 9.5\ dST_f + 9.5\ dNSP$
Linear*	
Trout⁽³⁾	$NE = 15.1\ dCP + 35.0\ dFat + 12.1\ dCarb$
Tilapia	$NE = 11.5\ dCP + 35.8\ dFat + 11.3\ dCarb$
Carp⁽⁴⁾	$NE = 11.2\ dCP + 34.1\ dFat + 10.4\ dCarb$
Barramundi⁽⁴⁾	$NE = 15.2\ dCP + 37.1\ dFat + 3.1\ dCarb$
African catfish⁽⁴⁾	$NE = 20.4\ dCP + 37.6\ dFat + 10.1\ dCarb$
Striped catfish⁽⁴⁾	$NE = 15.1\ dCP + 31.5\ dFat + 9.9\ dCarb$
Snakehead⁽⁴⁾	$NE = 12.5\ dCP + 31.0\ dFat + 0.9\ dCarb$
Quadratic for dCarb**	
Trout⁽³⁾	$NE = 13.5\ dCP + 33.0\ dFat + 34.0\ dCarb - 3.6\ (dCarb)^2$
Barramundi⁽⁴⁾	$NE = 15.9\ dCP + 35.2\ dFat + 9.4\ dCarb - 1.9\ (dCarb)^2$

(1) Noblet *et al*., 1994, (2) CVB, 1993, (3) Schrama *et al*., 2018, (4) in this thesis. 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, dgestible NSP.
In the NE equations for pigs, NE is expressed in $MJ.kg^{-1}$ feed and digestible nutrients in $g.kg^{-1}$ feed

*In the linear equations for fish, NE is expressed in $kJ.kg^{-0.8}.d^{-1}$, $kJ.g^{-1}$ and $g.d^{-1}$ and digestible nutrient intakes (dCP, dFat and dCarb) in $g.kg^{-0.8}.d^{-1}$, $kJ.g^{-1}$ and $g.d^{-1}$.

** In the equations for fish quadratic for dCarb, NE is expressed in $kJ.kg^{-0.8}.d^{-1}$and digestible nutrient intakes dCP and dFat in $kJ.kg^{-0.8}.d^{-1}$ and $(dCarb)^2$ in $g^2.kg^{-0.8x2}.d^{-2}$, and the coefficient of $(dCarb)^2$ in $kJ.g^{-1}.kg^{-0.8}.d^{-1}$

This is also reflected in Figure 5, where the NE value of the 12 ingredients is expressed as a percentage of the NE content of fishmeal, when using the NE equation for snakehead and striped catfish. The largest differences in NE value between snakehead and striped catfish were for carbohydrate-rich ingredients (Figure 5). This implies that the energetic value of carbohydrate-rich ingredients is low in snakehead.

Table 4. The estimated energy value (kJ.g^{-1}) and ranking of ingredients using the digestible energy (DE) and net energy (NE) systems for striped catfish (SC) and snakehead (SN), assuming equal digestibility values of macronutrients for the two fish species

	DE	NE		DE rank	NE rank	
		SC	SN		SC	SN
Fish meal (FM)	17.5	11.3	9.0	2	2	1
Full fat soybean (FFSB)	17.4	11.9	8.8	3	1	2
Defatted soybean meal (SBM)	16.2	10.1	6.2	4	3	3
Dried distillers grains with soluble (DDGS)	15.2	7.9	4.8	6	6	4
Rapeseed meal (RM)	14.8	9.1	4.6	7	5	5
Palm kernel meal (PKM)	14.4	7.8	4.5	8	8	6
Rice bran (RB)	18.4	9.8	3.5	1	4	7
Wheat bran (WHE)	13.9	7.8	2.6	10	7	8
Defatted rice bran (DFRB)	15.5	7.7	2.5	5	9	9
Wheat bran (WB)	14.4	7.0	2.2	9	11	10
Broken rice (BR)	13.9	7.5	1.4	11	10	11
Cassava (CAS)	10.7	5.0	0.2	12	12	12

The digestible energy (DE) content of ingredients was calculated by using the equation DE (kJ.g^{-1}) = ADC_{energy} x energy content (kJ.g^{-1}), where ADC_{energy} is the digestibility of energy of ingredients.
The net energy (NE) values of ingredients were calculated by using the equation: NE (kJ.g^{-1}) = β_1 x $ADC_{protein}$ (%) x protein (g.kg^{-1}) + β_2 x ADC_{Fat} (%) x fat (g.kg^{-1}) + β_3 x $ADC_{Carbohydrates}$ (%) x carbohydrates content (g.kg^{-1}) (Equation A), where NE is the net energy value of ingredients, $ADC_{protein}$, ADC_{Fat} and $ADC_{Carbohydrates}$ are the digestibility values of protein, fat and carbohydrates of the ingredients. By using the data on $ADC_{protein}$, ADC_{Fat} and $ADC_{Carbohydrates}$, and the macronutrient content of the ingredients (Tu-Tran, 2019), the net energy values of ingredients were quantified for striped catfish and snakehead, by using equation A and β_1, β_2, β_3 estimated for whole body growth of striped catfish in **Chapter 4,** and snakehead in **Chapter 5**, respectively.

In conclusion, the results of this thesis indicate two important reasons to move from the DE system to the NE system for the energy evaluation of ingredients/diets for fish: 1. When using the DE system, the energy utilisation efficiency and the energy demand for maintenance do vary depending on the dietary macronutrient composition. This affects the estimate of the digestible energy value of an ingredient or fish feed; and 2. The energy value and the energy ranking of ingredients differ between the DE vs. NE system. The value based on the NE system is more accurate than the value based on the DE system because the DE system only considers the loss of faecal energy, while the NE system considers the loss of energy through faeces, gills and urine and also via heat production.

8.4. The consistency of the net energy equations for fish species studied

To apply the net energy system for fish species, the net energy equations, *i.e.*, β_1, β_2, β_3, must be similar for all fish species. The similarities in values of β_1, β_2, β_3 between the different species can indicate a consistent ability to use protein, fat and carbohydrates. This thesis found that digested fat is the most efficiently used macronutrient. Averaged over fish species, about 87.6% of digested fat can be converted to retained energy (Figure 6). The energy utilisation efficiency of digested fat, varied from 78.5 – 95.2%, and is comparable between the fish species studied.

More variable between the fish species studied is the energy utilisation efficiency of digested protein (47.5 – 86.4%). African catfish has an outlier value for energy utilisation efficiency of digested protein. This is due to the underestimated ADC, as discussed in Chapter 3. The energy utilisation efficiency of digested protein is similar in carp, tilapia and snakehead. Barramundi, trout and striped catfish also use digested protein with a similar efficiency, but more efficiently than carp, tilapia and snakehead.

Most variable between the fish species studied is the energy utilisation efficiency of digested carbohydrates (5.2 – 70.3%). However, when leaving out barramundi and snakehead, the energy utilisation efficiency of digested carbohydrates (57.6 – 70.3%), determined by using the linear relationship between RE and digested carbohydrates, is comparable for African catfish, carp, striped catfish, trout and tilapia. The large variation in the energy utilisation efficiency of digested carbohydrates originates from the deviating values for snakehead (5.2%) (**Chapter 5**) and barramundi (18%) (**Chapter 2**). In addition, a quadratic relationship between NE and dCarb was found for barramundi (Table 3) and trout (Schrama *et al.*, 2018). These findings indicate that, for trout and barramundi, the energetic value of digested carbohydrates diminishes at higher intake levels. In contrast, the linearity in the relationship between RE and dCP, dFat and dCarb in a wide range of digested carbohydrates shows the high capacity to use carbohydrate for energy retention in African catfish, carp and striped catfish over a wide range of digested carbohydrate intakes.

This difference can originate from the limited capacity of snakehead to convert plasma glucose to plasma triglycerides, compared to striped catfish (**Chapter 6**). The plasma glucose and triglyceride levels can be regulated differently depending on fish species, *i.e*. trout, tilapia (Figueiredo-Silva *et al.*, 2013), grass carp and Chinese long snout catfish (Su *et al.*, 2020), even though similar amounts of dietary starch and fat were supplied. The plasma lipogenic enzyme activities in the high starch diet, *i.e.*, the synthesis of de novo fat from dietary starch, were higher in tilapia than in trout (Figueiredo-Silva *et al.*, 2013). It is questionable whether the plasma glucose level can indicate the feeding habits of fish, *i.e.* carnivore or omnivore. In this thesis and the study of Figueiredo-Silva *et al.* (2013), the plasma glucose level is higher in carnivorous fish, *i.e.* snakehead and trout, than in omnivorous fish, *i.e.* striped catfish and tilapia. In the study of Su *et al.* (2020), the opposite was observed with the higher plasma glucose level in omnivorous fish, *i.e.* carp. This indicates that the ability to use the absorbed glucose, *i.e.* dietary carbohydrates, for retained energy is species-related. The implication of the quadratic relationship between NE and dCarb is that the net energy equations can only be applied to trout and barramundi within a smaller range of carbohydrate intake, compared to that of African catfish, carp and striped catfish.

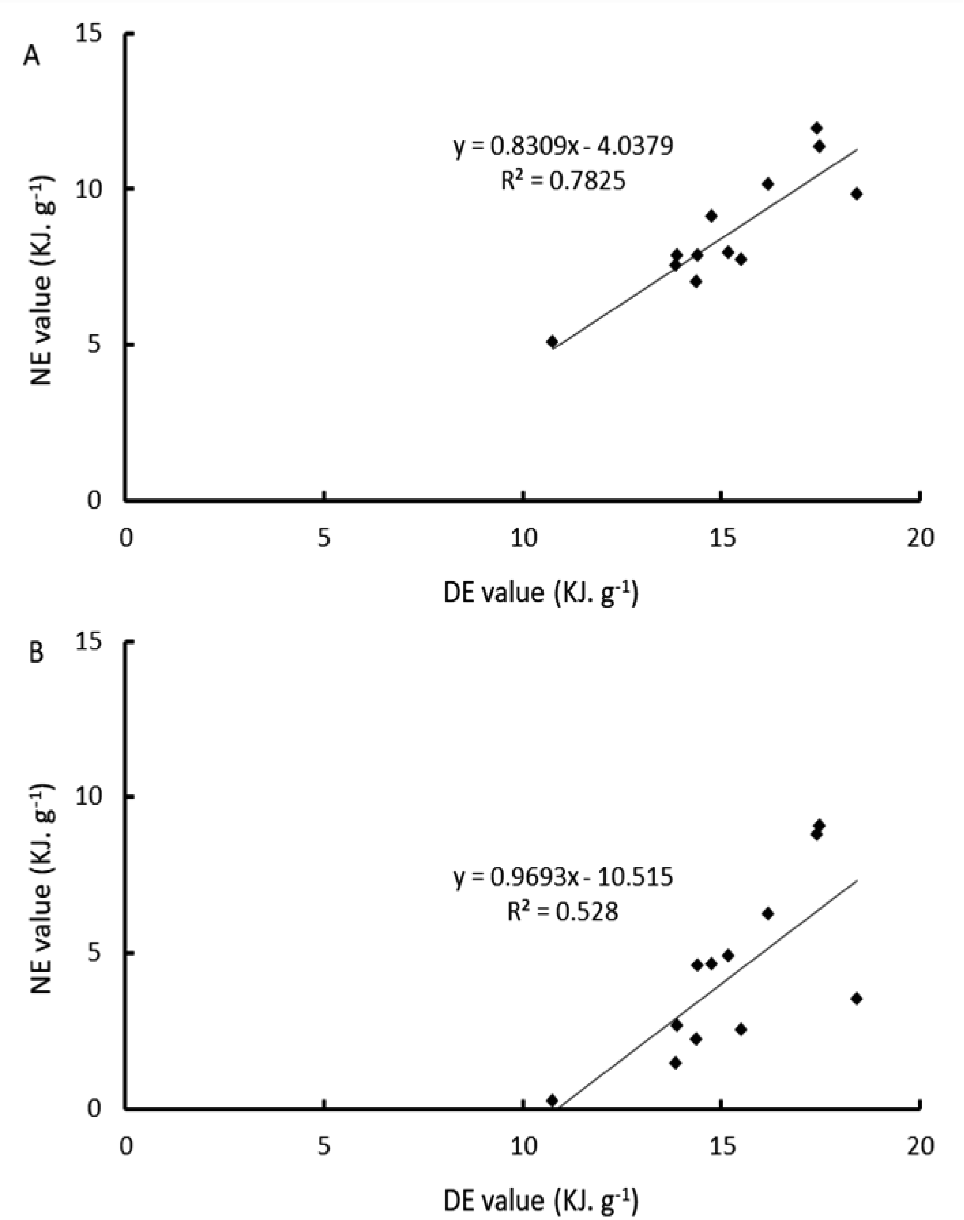

Figure 4. The relationship between net energy (NE) and digestible energy (DE) values for striped catfish (A) and snakehead (B). Data were presented in Table 4.

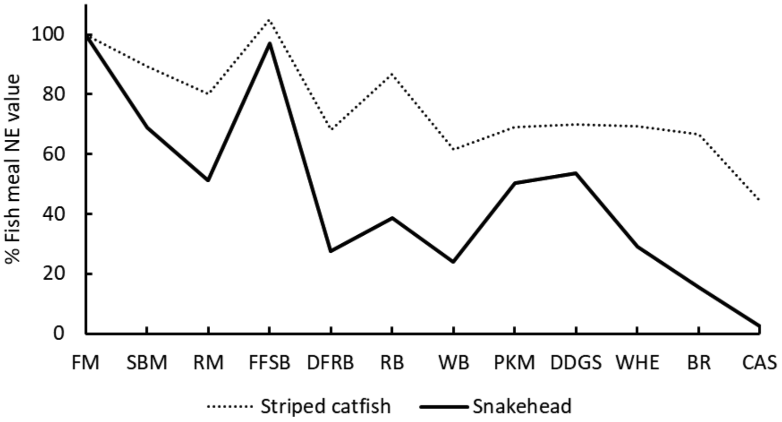

Figure 5. Variation in the estimated net energy (NE) value of ingredients relative to that of fish meal for striped catfish and snakehead, assuming ADC values are equal between the two fish species. Abbreviations are defined in Table 4.

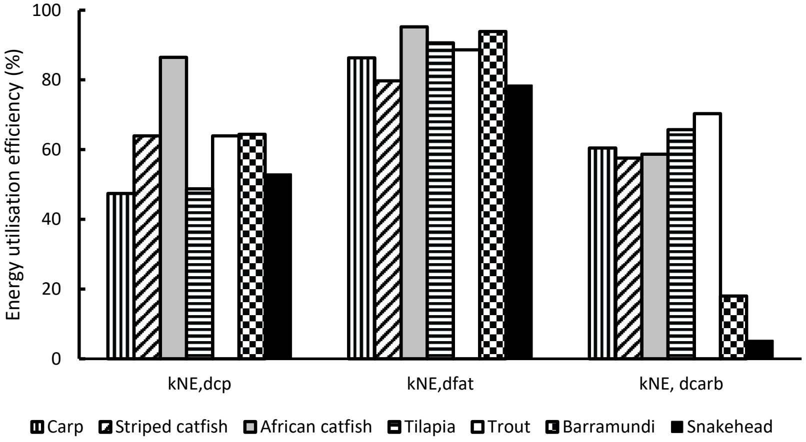

Figure 6. The energy utilisation efficiencies of digested protein ($k_{NE,dCP}$), fat ($k_{NE,dFat}$) and carbohydrates ($k_{NE,dCarb}$) for trout, tilapia (Schrama *et al.*, 2018), carp, striped catfish, African catfish, barramundi and snakehead (this thesis).

The net energy equation includes three coefficients of digested protein, digested fat and digested carbohydrate, which indicates the contribution of each digested macronutrient to the net energy of fish feed. Except for African catfish, the coefficient of digested protein is comparable for fish species studied. The coefficient of digested fat is also comparable for all fish species studied. In contrast, the coefficient of digested carbohydrate is different for barramundi and snakehead, compared to the other fish species. Therefore, net energy equations are not uniform across all fish species, due to their different abilities to utilise carbohydrates. This means that two types of net energy equation can be applied to fish species, according to whether they have: 1. a high carbohydrate utilisation efficiency; or 2. a low carbohydrate utilisation efficiency.

8.5. Next steps for energy evaluation of fish feed

8.5.1. The energy utilisation efficiency of non-starch polysaccharides

Despite non-starch polysaccharides (NSP) appearing to be difficult to digest by many fish species, they are not inert in tilapia (Haidar *et al*., 2016), carp (**Chapter 2**) or striped catfish (**Chapter 4**). Thus digested NSP can be an energy source for these fish. It is essential to estimate the energy utilisation efficiency of digested NSP. The capacity for NSP digestion in tilapia, carp and striped catfish is comparable to that of pigs. Fermentation by bacteria contribute to the NSP digestion process (Romano *et al.*, 2018) and most likely lead to the formation of volatile fatty acids (like in pigs), which might also be available as an energy source in fish. Part of the energy is however used by the gut microbiota. Therefore, it can be hypothesised that the net energy value of digested NSP is lower than enzymatically digested carbohydrate in fish. In the net energy equations for pigs, the total carbohydrate fraction is therefore split into various components (enzymatically digested versus fermented carbohydrates) (Equation 2, Table 3). Despite the same gross energy content, the NE value of "digested" NSP is lower than that of digested starch (CVB, 1993). However, the number of publications on the benefit of NSP for fish growth is limited. NSP can be digested by tilapia (Haidar *et al*., 2016), carp and striped catfish (this thesis). As these fish species partially "digest" NSP a further development of their NE equations could be to split the digested carbohydrate fraction into more fractions (e.g., starch vs. NSP). This requires separate estimations of the energy utilisation efficiencies for digested starch and "digested" NSPs for these fish species. In this thesis, although time and effort was spent quantifying the contribution of digested NSP, the coefficient of NSP in the net energy equations could not be quantified for carp and striped catfish because of the correlation between the digested NSP intake and the intake of digested protein, fat and starch. This correlation makes it impossible to conduct the multiple regression between RE and digested protein, fat, starch and NSP. Therefore, it is necessary to quantify the coefficient of NSP in the net energy equations for tilapia, carp and striped catfish, as well as for other fish species with a high ability to digest NSP. This quantification will clarify the benefits of digested NSP in those fish species potentially able to use it, *i.e*. tilapia, carp and striped catfish. To achieve this, there are three key approaches: 1. increase the number of diets, with more levels of NSP increased stepwise (minimum 4 levels of NSP), 2. increase the number of feeding levels (minimum 2 feeding levels), and 3. combine approaches 1 and 2.

8.5.2. The optimal balance between DP and NE for fillet and whole body growth

In this thesis a net energy equation was estimated for fillet growth (**Chapter 5**) and evaluated the effect of non-protein energy (*i.e.* carbohydrate and fat) supplementation on the characteristics of body compartments, *i.e.* the chemical composition and location of deposited fat (**Chapter 7**). These results indicate that the potential of protein is greater than that of fat and carbohydrate in supporting fillet growth. Additionally, non-protein energy supplementation increased the fillet fat content in African catfish and striped catfish (**Chapter 7**). Increasing the dietary non-protein energy, *i.e.* carbohydrates or fat, can conserve dietary protein for somatic protein growth (Glencross, 2008; Haidar *et al*., 2018). However, the optimal balance between protein and non-protein energy for fillet growth has not been studied in fish. The findings regarding net energy for fillet growth could facilitate future studies on the optimal ratios between DP and NE for fillet growth. In addition, the optimal ratio between protein and energy has been widely expressed as the ratio between digested protein and digested energy for fish (NRC, 2011). Because of the similarities in the ADCs of protein and energy (Glencross, 2008; Haidar *et al*., 2018, Phan *et al*., 2019, Phan *et al*., 2021a,b), the variation between ratios of protein to energy, on both a gross and digestible basis, is normally relatively small. Using this way of expressing the ratio between protein and energy, the variation between species with similar abilities to use carbohydrates is also small, because of the comparable ADCs of protein and energy between species (Glencross, 2008; Haidar *et al*., 2018, Phan *et al*., 2019, Phan *et al*., 2021a,b). However, expressing the protein to energy ratio as DP: NE increases the variation, due to the large differences in the net energy contribution of carbohydrates between fish species that have different abilities to use carbohydrates. The largest variation in the contribution of carbohydrates to the feed net energy budget exists between two fish groups studied: 1. barramundi and snakehead; and 2. the other fish species in this thesis, based on the linear relationship between NE and digested protein, fat and carbohydrate intake (Figure 6). This causes the variation in the net energy values of ingredients, using striped catfish and snakehead as examples (Figure 4). Therefore, the optimal ratios of protein to net energy for these fish species needs to be validated. To expand the application of the net energy equations to whole body and fillet growth, it is more accurate to express the optimal balance between protein and energy as the ratio of digested protein (DP) and net energy (NE). This expression can indicate the species-specific ability of using carbohydrates for growth. This is because the lower variability in NE is related to differences between DE values of ingredients for snakehead, when compared to striped catfish (the lower R^2 of the relationship between NE and DE values of different ingredients) (Figure 4). Therefore, it is crucial to recalculate the optimal balance between protein and energy as DP: NE for the two fish groups having low and high carbohydrate utilisation capacities.

8.5.3. Net energy equations for different production systems

The net energy equations presented in this thesis were quantified by experiments with fish in recirculating aquaculture systems (RAS). Currently, only a minority of cultured fish are kept in RAS. The majority of fish (~70%) are cultured in ponds and the remainder in sea/loch cages and raceways. In systems without a food web, the diet is the main/sole source of ingested macronutrients, *i.e*. protein, fat and carbohydrate. The waste produced by the fish needs to be removed from the culture system as far as possible. When using ponds as the culture system, the waste excreted by the fish can be reused by the surrounding fauna and flora, thus providing an extra source of macronutrients. Similarly, bioflocs can trap organic particles and solids (Ekasari *et al*., 2014). These extra sources of macronutrients can be ingested by the fish. Up to 50% of protein gain in tilapia cultured in ponds originates from natural food consumption (Kabir, 2019). This may change the energy utilisation efficiencies of digested protein, fat and carbohydrates, as extra energy for growth comes from the natural food web. The current estimated net energy equations from studies in RAS are applicable to culture systems without a food web. However, for pond systems, the interaction between feed and food web should be considered in the energy evaluation of pond feeds.

Conclusions

In this thesis, the energy utilisation efficiencies were studied in African catfish, barramundi, carp, snakehead and striped catfish. Additionally, the postprandial plasma glucose and triglycerides were studied in striped catfish and snakehead. Moreover, effects of fat and carbohydrate supplementation on body compartment composition and gain were assessed. The main conclusions of this thesis are:

- The digestibility of starch is high in the fish species studied.
- NSP is not inert in striped catfish and carp.
- The dietary macronutrient composition affects the relationship between retained energy and digestible energy intake in the fish species studied, except for striped catfish.
- The energy utilisation efficiencies of digested protein and fat are similar across the fish species studied.
- The energy utilisation efficiency of digested carbohydrates is different across the fish species studied.
- Net energy equations are different between the fish species studied.
- The dietary net energy value depends on the contribution of carbohydrates, in the fish species studied.
- Striped catfish is better able to use digested carbohydrates than snakehead.
- Fat supplementation increased the fat content of liver, viscera, fillet and the rest fraction in striped catfish, African catfish and snakehead.

Summary

To facilitate the expansion of global aqua-feed production, whilst sources of conventional (wild-caught) marine protein and fat remain equal, the use of alternative ingredients has increased. These are mainly of animal (*e.g.* processing by-products, offal, trimmings) and terrestrial plant origin, which have now become major constituents of aqua-feeds. This change in the feed ingredients has resulted in a variable quantity and quality of dietary macronutrients. The increasing use of plant protein sources in herbivorous and omnivorous fish diets has caused a rise in their carbohydrate content. Many plant-based ingredients have lower protein and higher carbohydrates levels, compared to animal-based ingredients. Consequently, the inclusion levels of other dietary macronutrients, *i.e*. protein or fat, have to be adapted, as the level of either protein or energy is constant in a species specific commercial diet, *i.e.* following species-specific requirements. For carnivorous fish, the trend is to use higher levels of fat in the diet, as this can reduce the cost of feeds by partially sparing protein for growth.

The retained energy, *i.e.* fish growth on an energy basis, can be predicted from the intake of digestible energy sourced from protein, fat and carbohydrates. Therefore, retained energy can be predicted from the amount of digestible macronutrients, *i.e.*, protein, fat and carbohydrates. The estimate of the retained energy between different fish species, fed similar amounts of digested protein, fat and carbohydrates, can differ according to the energy metabolism of the species. This can affect the energy utilisation efficiency of digested protein, fat and carbohydrates. Consequently, the increasing non-protein energy in fish diets, *i.e.* fat and/or carbohydrates, can affect the estimated values of retained energy, due to differences in the energy utilisation efficiencies of dietary macronutrients. It is known that the ability to regulate and use plasma glucose and triglycerides differs between fish species, despite similar amounts of dietary starch and triglycerides being given to the fish. The levels of plasma glucose and triglycerides can explain the variability between fish species in using carbohydrates for growth.

Data on the potential of digested macronutrients to contribute to fillet growth, fillet quality and/or composition, and the growth of other defined body compartments (*i.e*. liver, viscera, and the rest fraction) could contribute to the improvement of the quantity and quality of fillets. This knowledge could also mitigate filleting waste. More importantly, it could facilitate the development of an alternative feed evaluation system, which considers the economic priority of fillet production. To date, fish feed formulations have mainly focused on obtaining the optimal macronutrient inclusion level for whole body growth. An alternative feed evaluation system, which would focus on growth, or energy utilisation efficiency, at the fillet level, could constitute a tool for tailoring feed formulations to meet the expectations of different market sectors, *e.g.* low fat vs. high fat fillets. However, an approach linking fillet growth to the intake of digested macronutrients on the compartment level has, to the best of our knowledge, not yet been published for any fish species. In addition, insights into the effects of dietary macronutrient composition on fillet yield and chemical composition, and the location of fat deposits, are important factors in achieving the most efficient use of macronutrients.
Therefore, the major aims of this thesis were:

1. To investigate the effect of dietary macronutrient composition on relationships between retained energy (RE) and digested energy (DE) for different fish species. The following species were selected for this: African catfish, barramundi, common carp, snakehead and striped catfish.

2. To quantify the net energy equations for the selected fish species.

3. To investigate whether the net energy equations are similar across the fish species studied.

4. To assess the impact of dietary macronutrient composition on the chemical composition and location of fat deposits in the different body compartments of African catfish, striped catfish and snakehead.

The minor aims of this thesis were:

1. To investigate differences in the digestibility of carbohydrates between fish species

2. To study the effect of dietary starch levels on plasma glucose and triglycerides in snakehead and striped catfish.

Carbohydrates are a cheap energy source and therefore increasingly used in fish feed formulations, especially for herbivorous and omnivorous fish. Carbohydrates include starch and non-starch polysaccharides. One aim of this thesis was to investigate the difference in the digestibility of carbohydrates between carp, African catfish, striped catfish and snakehead. This thesis observed a high digestibility of starch in carp (99.1%) (**Chapter 2**), African catfish (99.4%) (**Chapter 3**), striped catfish (97.7%) (**Chapter 4**) and snakehead (97.3%) (**Chapter 5**). The high digestibility of gelatinised starch is mainly due to the high degree of gelatinisation achieved during the feed production process, *i.e.* steaming, heating and extruding. Because of the high ADCs of starch, which is consistent between fish species studied, the variation in the digestion of carbohydrates depends on the digestion of non-starch polysaccharides. In this thesis, the digestibility of NSP was only positive in carp (**Chapter 2**) and striped catfish (**Chapter 4**). The NSP digestibility was higher in striped catfish (63.1%) compared to carp (49.6%), when averaged over diets and feeding levels. This thesis shows that the digestibility of NSP is species-related. Within species, which are able to digest NSP, feeding level affected the digestibility of NSP, and thus the digestibility of the total carbohydrates. In this thesis, for both carp and striped catfish, an increase in feeding level decreased the digestibility of NSP from 45.3% and 62.5% to 29.3% and 49.4%, respectively.

Another aim of this thesis was to investigate the effect of dietary macronutrient composition on the relationship between retained energy and digestible energy intake. The energy utilisation efficiencies were established for African catfish (76 – 98%), carp (59 – 66%), striped catfish (56 -75%), snakehead (45 – 56%) and barramundi (51 – 73%). Dietary macronutrient composition affected the energy utilisation efficiency, except for striped catfish.

A major aim of this thesis was to quantify the net energy equation for African catfish, barramundi, common carp, snakehead and striped catfish. To facilitate this aim, equations making retained energy (RE) a function of digested intakes of protein (dCP), fat (dFat) and carbohydrates (dCarb), *i.e.*, RE=f(dCP, dFat, dCarb, …) was developed for carp (Chapter 2), African catfish (Chapter 3), striped catfish (Chapter 4) and snakehead (Chapter 5). Net energy equations were developed for these fish species and compared. The energy utilisation efficiency of digested carbohydrates (5.2 – 70.3%) was found to be the most variable between fish species studied; whilst the energy utilisation efficiency of digested fat (78.5 – 95.2%) and protein (47.5 – 86.4%) was the most consistent. These findings indicate that the ability to use fat and protein is similar between the fish species studied, while the ability to use carbohydrates is largely variable.

The energy utilisation efficiency of digested carbohydrates was largely different between snakehead (**Chapter 4**) and striped catfish (**Chapter 5**). The origin of this difference could not be explained by a difference in digestion of carbohydrates, i.e. starch, but must be related to differences in glucose metabolism. To compare the ability to use carbohydrates between fish species, the postprandial plasma glucose and triglyceride levels were studied. In terms of the difference in the use of carbohydrates between striped catfish and snakehead, the levels of plasma glucose were higher in snakehead than in striped catfish, even with similar dietary starch levels (**Chapter 6**). In contrast, the levels of plasma triglycerides were higher in striped catfish than in snakehead (**Chapter 6**). This may suggest that striped catfish have a better ability to convert plasma glucose to plasma triglycerides, and retained energy from plasma glucose, than snakehead.

Dietary protein, fat and carbohydrates can be converted to somatic fat and stored at different locations in the body, for example in the liver, viscera, fillet and the rest fraction (head, skin, subcutaneous fat, scales, bones and air bladders). Species with different abilities to use carbohydrates may vary in their strategy to convert these macronutrients to somatic fat. Three different fish species, striped catfish, African catfish and snakehead, were compared with regards to the effect of dietary macronutrient composition on: 1. the fillet yield and the fillet chemical composition; and 2. the location of fat deposits in the body (fillet, liver, viscera or the rest fraction). Fillet fat and protein content changed with a change in the dietary macronutrient composition (**Chapter 7**). In all compartments (liver, viscera, fillet and the rest fraction), both an increase in dietary fat and dietary carbohydrate level increased the fat content. In snakehead, fillet fat content decreased with an increase in dietary carbohydrates; the opposite was observed in both species of catfish studied. The total body fat deposition over the different compartments was not influenced by the level of dietary carbohydrate, but was affected by dietary fat level. Dietary fat supplementation resulted in relatively more fat in viscera and fillet, but less fat in the rest fraction. In striped catfish, African catfish and snakehead, most of the body fat is deposited in the rest fraction (head, skin, subcutaneous fat, scales, bones and air bladders).

In this thesis, energy utilisation efficiencies were studied in African catfish, barramundi, carp, snakehead and striped catfish. Additionally, the postprandial plasma glucose and triglycerides were studied in striped catfish and snakehead. Moreover, the effects of fat and carbohydrate supplementation on body compartment composition and gain were assessed. The main conclusions of this thesis are:

- The digestibility of starch is high in the fish species studied.
- NSP is not inert in striped catfish and carp.
- The dietary macronutrient composition affects the relationship between retained energy and digestible energy intake in the fish species studied, except for striped catfish.
- The energy utilisation efficiencies of digested protein and fat are similar across the fish species studied.
- The energy utilisation efficiency of digested carbohydrates is different across the fish species studied.
- Net energy equations are different between the fish species studied.

- The dietary net energy value depends on the contribution of carbohydrates in fish species studied.
- Striped catfish has a better ability to use digested carbohydrates than snakehead.
- Fat supplementation increased the fat content of liver, viscera, fillet and the rest fraction in striped catfish, African catfish and snakehead.

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About the author

Phan Le Thien Thuat was born on 26th Dec 1989 in Ho Chi Minh city, Vietnam and he was raised up in a small peaceful town called Vinh Long. He loved to raise ornamental fish (seamese fish), reproduced the fish in different ways and certainly failed many times. He had years spent on serial failures which inspired him to pursue a bachelor degree in aquaculture at Can Tho university, Can Tho city. He spent three years after graduation playing with a game called hatching barramundi and nursing striped catfish. Expectedly, this could be more successful than the previous practice but he faced more severe failures regarding feeding and water management of the production system. These failures motivated him to look for solutions by learning about the technologically advanced production systems. The strong curiosities about these systems and aspirations to learn about this inspired him to pursue a master course in Marine environment with a focus on aquaculture at University of Tasmania (Utas). This course funded by the Australian government allows him to enjoy practicums at various fish farms on abalone, salmon and barramundi in Melbourne, Launceston (Tasmania), the exploration of the multicultural environment and the insightful knowledge gain about aquaculture and fish nutrition. Fortunately, after 2 years spent in Tasmania, he found a great opportunity funded by De Heus nutrition B.V. and TKI to pursue his Phd in fish nutrition at Aquaculture and Fisheries group, Wageningen university and research, the Netherlands. In this study, he can explore the diversified ways of using energy sourced from digested protein, fat and carbohydrates for growth between studied fish species. Also, the two- year stay in Wageningen, the Netherlands was worthy, lovely and enjoyable in spite of some barriers to get fully understood between different cultural backgrounds. Though coding is challenging, distressing and frustrating sometimes, he launched into Matlab and SAS. He was playing with Matlab to visualize the algal blooms in the coastal area of Tasmania in a summer internship at UTas. SAS did support and save time for his data analysis, tabulation and visualization. He has combined broken fragments of English picked from different destinations from the walking streets for tourists in Cantho city to distant islands named Tasmania, Australia and Wageningen, the Netherlands to build this work. Going back to his home country, he expects to have positive impacts on aquaculture through the proactive involvement in fish feed industry. At some points of his life, he would prefer to combine traveling and working in different parts of the world to explore and enjoy the diverse wonders of life.

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List of publications

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

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Completed training and supervision plan

The Basic Package	**3 ETC**
WIAS Introduction Day (mandatory)	2017
Course on philosophy of science and/or ethics (mandatory)	2019
Course on essential skills (Frank Little) (recommended)	2019

Disciplinary Competences	**14 ETC**
Advanced statistics course Design of experiments	2017
Species specific course	2017
Introduction to R for statistical analysis	2018
Lab animal science	2018
RAS course (aqua-excel)	2019
China PhD knowledge exchange program	2019
Proposal writing	2019

Professional Competences	**8.8 ETC**
Research data management	2017
Techniques for scientific writing and presenting	2018
Supervising BSc and MSc thesis students	2019
Writing grant proposal	2019
Brain training	2019
Organisation committee for China PhD knowledge exchange program	2019
Efficient writing strategies	2021
Career assessment	2021
Working on your phd research in times of crisis	2021

Presentation Skills (maximum 4 credits)	**4 ETC**
WIAS science days poster	2017
Aquaculture Europe (Berlin, 07/10 - 11/10/2019, oral)	2019
Shanghai symposium (Poster and oral presentation)	2019
WIAS science days oral presentation 2021	2021

Teaching competences (max 6 credits)	**6 ETC**
Teaching assistant	2017
Minor thesis (Marjanne Verhoeven) at AFI, WUR, the Netherlands	2018
Internship (Marjanne Verhoeven) at De Heus R&D, Vietnam	2018
Bsc thesis (Samantha Popma) at AFI, WUR, the Netherlands	2019
Bsc thesis (Le Quoc Khanh) at De Heus R&D, Viestnam	2020
Bsc thesis (La Nguyen The Hien) at De Heus R&D, Vietnam	2020

Education and Training Total (minimum 30 credits)*	**35.8 ETC**

Completion of the training activities in fulfilment of the requirements for the education certificate of the Graduate School of the Wageningen Institute of Animal Sciences (WIAS). One ETC equals a study load of 28 hours.

Colophon

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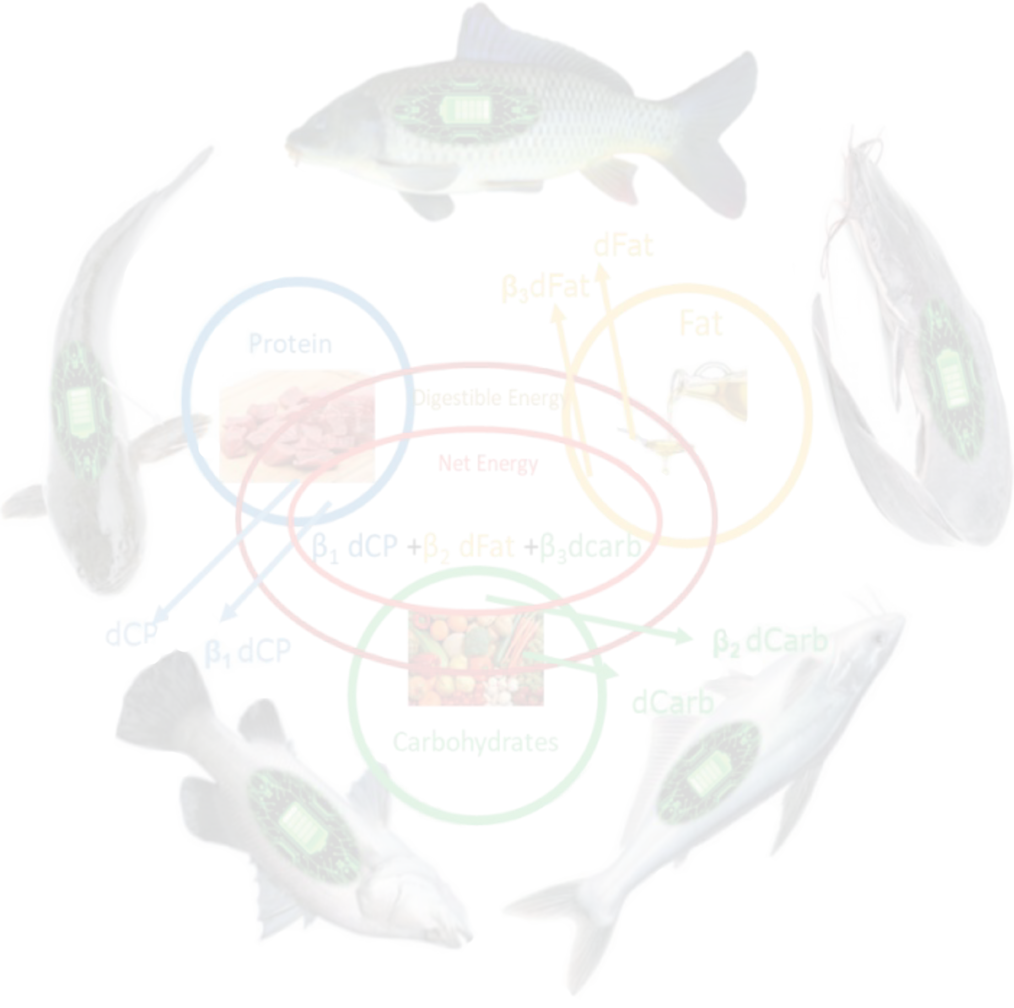
dFat
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Carbohydrates