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Apparent Digestibility of key feed ingredients by largemouth bass (*Micropterus salmoides*)

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Abstract

The aim of this project was to determine apparent digestibility coefficients (ADC) of macronutrients, amino acids (AA), phosphorus (P) and energy (EN) in protein-rich ingredients in extruded diets for largemouth bass (*Micropterus salmoides*). Digestibilities were assessed by the ingredient replacement method. Yttrium oxide was used as inert marker and faeces was collected by manual stripping. Peruvian anchovy fishmeal (PAF) was the sole protein source in the reference diet. Each ingredient was examined in triplicate with 30% replacement.

Animal protein ingredients included four fishmeals (Peruvian anchovy fishmeal PAF, three Chinese fishmeals made from sardine CSF, anchovy CAF or mackerel CMF), four sources of poultry by-products (poultry by-product meal PBM, poultry carcass meal PCM, hydrolysed feather meal HFM, spray-dried granulated inedible egg product SGE) and two potential alternative animal protein ingredients (defatted *Tenebrio molitor* beetle larvae meal DTM, and hydrolysates of stickwater and soybean HSS). The results showed that the apparent digestibility coefficients (ADC) of dry matter (DM, ranging from 67.0 to 96.4%), EN (75.6-98.6%) and crude protein (CP, 72.0-95.3%) for animal protein ingredients varied considerably. The ADC of SGE was significantly higher than others, while the ADCs of HFM and DTM were lowest. ADCs of DM (77.4-86.2%), EN (81.8-94.4%) and CP (84.7-88.9%) for other ingredients were quite close. ADCs of proteins in animal ingredients reflected the ADCs of total amino acids. The ADC of P showed no significant difference except for CMF (37.2%) and PAF (61.7%). All values for ADC of P were low.

Plant protein ingredients included soybean products (soybean meal SBM, soy protein concentrate SPC, two fermented soybean meals TB FSM-TB and YH FSM-YH), and cottonseed products (cottonseed meal CSM, degossypolled cottonseed protein TY DCP-TY, degossypolled cottonseed protein JL DCP-JL). The results showed that ADCs of DM (55.0-78.0%) and EN (67.0-84.8%) in the plant ingredients varied greatly. Compared with PAF, DM and EN digestibility of SPC and SBM showed no significant difference, while that of other raw materials were significantly lower ($P < 0.05$). ADCs of DM was highly correlated (r = 0.95) with ADC of energy. ADC of CP for all plant ingredients were high and only varied moderately (83.1-96.5%). ADC of CP of the soy products was significantly ($P < 0.05$) higher than that of the fish meal. No significant difference was seen among the cottonseed products. ADCs of total amino acids (AA) was highly correlated (r = 0.93) with the ADCs of CP.

Overall, ADCs of DM, CP, AA and EN of SGE were highest in animal protein ingredients, followed by PBM and PCM. ADCs of four sources of fish meal and HSS were slightly lower (67.0-84.8%) than those of animal products. The ADCs in HFM and DTM were significantly lower than that of any of the other ingredients.

The results showed that all animal protein ingredients except HFM and DTM can be used as main protein sources for largemouth bass. HFM and DTM should only added as a part of a mix of protein sources in the diet, and essential amino acids must be added to achieve balanced composition. The requirement of phosphorus in fish must be noted, in view of the low values for ADC of P in the tested ingredients. In plant protein ingredients, the ADC of P was highly variable. ADC of P of PAF, SPC and SBM were not significantly different, while ADCs of FSM-TB, FSM-YH, CSM, DCP-TY and DCP-JL were significantly lower. Therefore, SPC and SBM can replace fish meal as the main protein source in largemouth bass diets, while the usage of other protein-rich ingredients should be limited.

The absence significant difference of PAF in the reference diet tested in two experiments. This indicates that slightly different rearing conditions did not significantly affect the results, and that the results obtained in these experiments were repeatable and reliable.

Keywords:

Largemouth bass, Ingredient replacement method, Protein ingredients, Extruded pellets, Apparent digestibility

Sammendrag

Målet med dette prosjektet var å bestemme fordøyelighet av makro-næringsstoff, aminosyrer (AA), fosfor (P) og energi (EN) i ekstruderte fôr til «largemouth bass» (*Micropterus salmoides*). Fordøyeligheter ble bestemt ved hjelp av utbyttingsmetoden. Yttriumoksyd ble benyttet som inert markør, og faeces ble samlet inn ved å stryke fisken. Peruansk fiskemel av ansjos (PAF) var eneste proteinkilde i referansefôret. Hver ingrediens ble bestemt med 3 gjentak med 30% utbytting.

Animalske proteinkilder omfattet fire fiskemel (PAF og tre kinesiske fiskemel fra sardin CSF, ansjos CAF eller makrell CMF), fire biprodukter fra fjørfe (Biproduktmel av fjørfe PBM, mel laget av fjørfeskrotter PCM, hydrolysert fjørmel HFM, spraytørret granulert produkt av vraket egg SGE), og to potensielt alternative proteinkilder fra andre dyr (mel av avfettede larver av biller *Tenebrio molitor* DTM, og hydrolysat av limvann og soyabønner HSS. Resultatene viste at estimatene av apparent fordøyelighet (ADCs) var høyst forskjellige i ulike råvarer, og dekket området 67.0-94.4% for tørrstoff (TS), 75.6-98.6% for energi (EN) og 72.0-95.3% for råprotein (CP). ADC-verdiene for SGE var signifikant høyere enn hva som var tilfelle for de andre råvarene. ADC for DM, EN og CP for andre råvarer var nærmere hverandre. ADC for HFM og DTM var lavest. Verdiene for ADC av DM (77.4-86.2%), EN (81.8-94.4%) og CP (84.7-88.9% CP) i de andre ingrediensene var rimelig like hverandre. AD av CP i animalske ingredienser reflekterte verdiene for total AA. ADC for P var generelt lave, og det framkom bare signifikante forskjeller mellom CMF og PAF.

Råvarer fra planteriket omfattet soyaprodukter (soyamel SBM, soya protein konsentrat SPC, to fermenterte soyamel FSM-TB og FSM-YH) og bomullsfrø (bomullsfrø CSM, 2 typer bomullsprotein uten gossypol DCP-TY og DCP-JL). Resultatene viste at ADC av DM (55.0 - 78.0%) og EN (67.0 - 84.8%) i planteingredienser varierte mye. ADC av DM og EN i SBM of SPC var ikke signifikant forskjellig fra verdiene i PAF, mens verdiene for de andre plantebaserte råvarene var lavere ($P<0.05$). ADC av DM og energi var høyt korrelert ($r = 0.95$). ADC av CP for alle planteråvarer var høy og rangerte seg forholdsvis tett (83.1-96.5%). ADC av CP i soyaproduktene var signifikant ($P<0.05$) høyere enn hva som var tilfelle med fiskemelene. Ingen signifikante forskjeller ble funnet mellom produktene fra bomullsfrø. ADC av total AA var sterkt ($r=0,93$) korrelert med ADC av CP. Generelt var ADC av DM, CP, AA

og EN i SGE høyest i animalske ingredienser, etterfulgt av PBM og PCM. ADC i de fire fiskemelene og HSS var noe lavere enn hva som var tilfelle med animalske råvarer.

Resultatene viste at alle de animalske proteinkildene, unntatt HFM og DTM kan benyttes som hovedproteinkilde i for til «largemouth bass». HFM og DTM kun bør brukes i fôret som en av flere proteinkilder. Essensielle AA må tilføres for å få balansert sammensetning. Behovet for P i fôret til fisk må tas tillegges vekt. ADC av P varierte mye mellom ulike plantebaserte fôr. ADC av P I PAF, SPC og SBM var på same nivå, mens verdiene for FSM-TB, FSM-YH, CSM, DCP-TY og DCP-JL var signifikant lavere. Derfor kan SPC og SBM erstatte fiskemel som hovedkilde til protein hos «largemouth bass». Bruk av andre proteinkilder bør begrenses.

Mangelen på signifikante forskjeller ved bruk av PAF i referansefôr i to forsøk, tyder på at små forskjeller i oppdrettsmiljøet ikke signifikant påvirket resultatene, og at resultatene i disse forsøkene var reproduserbare og pålitelige.

Contents

List of figures

List of tables

Abbreviations

- AFM — Anchovy fish meal
- CAF — Chinese anchovy fishmeal
- CMF — Chinese mackerel fishmeal
- CSF — Chinese sardine fishmeal
- CSM — Cottonseed meal
- DTM — Defatted *Tenebrio molitor* larvae meal
- DCP-JL — Degossypolled cottonseed protein JL
- DCP-TY — Degossypolled cottonseed protein TY
- EXFM — Extruded feather meal
- FSM-TB — Fermented soybean meal TB
- FSM-YH — Fermented soybean meal YH
- HSS — Hydrolysates of stickwater and soybean
- HFM — Hydrolysed feather meal
- PAF — Peruvian anchovy fishmeal
- PFM — Pollock fish meal
- PBM — Poultry by-product meal
- PCM — Poultry carcasses meal
- SFM — Sardine fishmeal
- SPC — Soya protein concentrate
- SBM — Soybean meal
- SGE — Spray-dried granulated inedible egg product
- SFM — Steam dried anchovy fish meal
- TFM — Tuna fish meal
- RAS — Recirculating aquaculture systems

1 Introduction

Largemouth bass (*Micropterus salmoides*) is one of the most widely distributed and popular sport fish in the world. It was introduced into China in the 1970s, and has become a major freshwater fish for aquaculture mainly because of its wide adaptability and disease resistance. As a typical carnivorous fish, largemouth bass has a short digestive tract which is only 0.6 times the length of its body and food passes through its digestive tract quickly. Therefore, it is crucial that its feed is efficiently digested. One of the critical steps in diet development for farmed fish is the determination of apparent digestibility coefficients (ADC)s for a range of feed ingredients. Nowadays, pellets of largemouth bass are mainly produced by extrusion. However, most digestibility data of different ingredients is based on steam pelleted feeds. It is necessary to evaluate relevant ADCs of different raw materials in feed for largemouth bass based on relevant processing to provide a basis for optimization ratios between digestible protein and energy.

Animal protein ingredients are the important components in diets of carnivorous fish species. Therefore, the digestibility of fish meal and poultry by-product meal were evaluated in this study with largemouth bass. In addition, potentially alternative animal protein ingredients, such as defatted *Tenebrio molitor* larvae meal as well as hydrolysate of stickwater and soybean, were also tested. Plant ingredients usually have higher fiber content, unbalanced essential amino acids profile as well as antinutrients which affects the digestibility of protein and energy. However, the moderate price and high yield of plant raw materials are useful to support the further production and processing, so as to improve their digestibility in fish. Many studies have reported that fish meal can be replaced by plant ingredients after further processing. In practice, a large proportion protein in diets of Atlantic salmon in Norway are provided by soya protein concentrate. It indicates that plant protein ingredients have great potential. In this study, the digestibility of high yield plant ingredients, soybean products as well as cottonseed products, were evaluated.

Thus, this study aims at determining the digestibility nutrients in animal and plant protein ingredients for largemouth bass. The results will provide the Chinese fish feed and aquaculture industries with data which documents the ADC of dry matter, crude protein, gross energy, individual amino acids and phosphor in key animal and plant protein ingredients. This wide

span of relevant feed ingredients represents the starting point of a feed optimization tool which will be made available to the Chinese ingredient and feed industries, as well as the aquaculture industries.

2 Literature background

This literature background mainly gives an introduction to apparent digestibility and the digestibility of animal and plant protein ingredients in fish.

2.1 Apparent digestibility

The approaches of assessing digestibility mainly have direct assessment method and indirect assessment method. In direct assessment method, digestibility of ingredients is determined by gross feed intake minus total feces excreted. However, it is impossible to get accurate data especially for fish because of the leaching of nutrients as well as the dispersing of feces in the water. In indirect method, digestibility can be measured by collecting representative samples of feces with the using of marker in the feed and the ratios between marker and nutrients both in the feed and feces were calculated as digestibility of ingredients. Compared with direct assessment method, indirect method is more reliable and time saving, therefore it is widely used in most fish species and the digestibility measured by this method is also known as apparent digestibility.

Apparent digestibility may underestimate the true digestibility because collected fecal samples also contain not only undigested material, but also includes the endogenous loss from the digestive tract. However, it was also noted that the effect of endogenous nitrogen loss in total nitrogen content of feces was actually very little when fish were fed full and the difference between true and apparent digestibility can be neglected in this case (NRC, 2011).

With the development of global aquaculture industry, it is increasingly urgent to reduce the dependency on fish meal and fish oil especially for carnivorous fish species, which have a natural rely on aquatic or marine sources to meet the needs for energy and protein. Therefore, it is essential to find alternative raw materials for feed ingredients of aquatic or marine origin. The evaluation of nutrients and energy digestibility for raw materials is an important effort on the way towards defining the nutritional value of feed ingredients to aquatic animals. When formulations for research or commercial use are based on digestible nutrient and energy balances, the risk of error is limited. Efficient feed formulations are also beneficial to the environment, due to reduced pollution caused by undigested nutrients and catabolism. This will

contribute to the fulfilment of sustainable development goals of aquaculture. Likewise, a wide span of digestibility data ensures the stability of commercial feed quality whose formula is always adjusted based on the least cost principle.

2.1.1 Methods measuring apparent digestibility of raw materials

2.1.1.1 Formulation

Few raw materials can be used as single feed ingredient to assess the digestibility of ingredients because carnivorous fish may refuse to eat this. Therefore, apparent digestibility of raw materials usually determined by the substitution method, which means the test ingredients will replace a part of the components in the reference diet. There are two ways of using substitution method, one is diet replacement method, another is ingredient replacement method (Aksnes et al., 1996). In the diet replacement method, a part of the reference diet is replaced by a test ingredient. While in ingredient replacement method, a single component in the reference diet is replaced by a test ingredient. Aksnes et al. (1996) reported that there was no significant difference in the digestibility obtained by these two methods in rainbow trout under different substitution levels. Repeatability of the results, however, was higher when using the ingredient replacement method. Glencross et al. (2007) indicated that using a reference ingredient as one of the test ingredients in diet replacement method can effectively combine the advantages of the two methods.

Nowadays, the digestibility formulation proposed by Cho et al. (1982), 30% reference diet is replaced by test ingredients, has been widely used. Mo et al. (2019), however, concluded that different substitution ratios (15%, 30%) significantly affect digestibility of raw materials. Besides, Dam et al. (2019) used only 15% blood meal to replace the reference diet when studying the digestibility of blood meal in yellowtail kingfish. Dam et al. believed that 30% substitution would significantly lower the palatability of the diet and reduce the reliability of the digestibility data. To some extent, the higher the proportion of test ingredients in feed, the more reliable the test results. However, the proportion of test materials is actually limited by the feed intake of aquatic animals.

2.1.1.2 Usage of inert markers and calculation of ingredient digestibility

Inert markers are widely used in most digestibility experiments to assess the ADCs of nutrients and energy in fish. During the experiment, the representative fecal samples are taken at intervals after continuous and regular feeding. This indirect assessment method not only greatly improves the quality of fecal samples, but also reduces the stress to the animals.

The ideal inert markers should be uniform and easy to analysis when added to diet at low concentrations. They must be inert and cannot be toxic or allergenic. They can't be absorbed or interfere with the digestion and metabolism of animals or intestinal flora. They also should have the same intestinal transit rate as the dietary nutrients (Austreng et al., 2000). Cr_2O_3 was the most commonly used inert marker to estimate the nutrient digestibility previously (Davies & Gouveia, 2006). However, it did not fully satisfy the criteria above all. Austreng et al. (2000) reported that dietary Cr_2O_3 was not completely recovered in the feces. Therefore, high concentrations of Cr_2O_3 (5-10 g kg^{-1}) should be incorporated into the diet in order to ensure the accuracy of the results. Studies have shown that high levels of Cr_2O_3 not only lower lipid levels in feces and affect intestinal flora (Ringø, 1993), but also chromium may be toxic even at low concentrations and cause allergies (Austreng et al., 2000). Therefore, a variety of other trivalent metal oxides were tested as candidates to replace Cr_2O_3 and estimated the digestibility of dietary nutrients at lower concentrations (Austreng et al., 2000). The results showed La_2O_3, Y_2O_3 and Yb_2O_3 could substitute Cr_2O_3 and more accurate even in lower concentrations. Alan Ward et al. (2005) studied the recovery rate of different doses of Y_2O_3 and reported that the dose with highest recovery rate was 1g kg^{-1}.

After feeding, some nutrients and energy in feed are digested but others are undigested, and excreted in feces. Thus, the composition of nutrients and energy in digesta are gradually changing on their passage through the digestive system. Inert marker is not digested or absorbed by animals. Thus, the apparent digestibility of energy and nutrients in diets can be calculated by comparing the proportion of nutrients or energy both in diet and feces through inert marker. The ADCs of nutrients and energy in diets was calculated using the following formula:

$$ADC_{Diet} = \left(1 - \frac{Y_2O_{3\,Diet} \times N_{feces}}{Y_2O_{3\,feces} \times N_{Diet}}\right) \times 100\%$$

where $Y_2O_{3\ diet}$ and $Y_2O_{3\ feces}$ represent the yttrium oxide content of diet and feces respectively. N_{diet} and N_{feces} represent the nutrients and energy in diet and feces. The ADCs of nutrients and energy in ingredients are calculated using the following formula:

$$N.ADC_{ingredient} = \frac{\left(ADC_{test} \times N_{test} - ADC_{reference} \times N_{reference} \times P_{reference}\right)}{P_{ingredient} \times N_{ingredient}}$$

where N. $ADC_{ingredient}$ refers to the apparent digestibility of nutrients and energy in ingredients. ADC_{test} and $ADC_{reference}$ represent the apparent digestibility of test diet and reference diet. N_{test}, $N_{reference}$ and $N_{ingredient}$ are the nutrient or energy concentration of the test diet, reference diet and ingredients, respectively. $P_{reference}$ and $P_{ingredient}$ are the proportion of reference diet and test ingredients.

2.1.1.3 Methods of feces collection

Austreng (1978) used two manual stripping methods to determine the digestibility of diet by rainbow trout (*Oncorhynchus mykiss*). In method I, fish were stripped from the middle of the pectoral and ventral fins to the anus. While in method II, feces were stripped from ventral fins to the anus. The results showed that the digestibility with method II was more accurate and recommended. Windell et al. (1978) used anal suction and intestinal dissection to collect feces from the hindgut. A small tube was used to absorb feces in anus by applying a certain vacuum pressure. While intestinal dissection is different. Intestine was taken out after fish was killed and feces were dissected from the hindgut (Choubert et al., 1982). Hemre et al. (2003) found that it was easier to get the feces after freezing the intestine when using intestinal dissection. Spyridakis et al. (1989) also used immediate pipetting, continuous filtration and decantation to collect feces. Cho et al. (1982) designed the Guelph system and used the settlement tank to collect feces. By adjusting the water velocity, feces were rushed into a vertical settlement column after discharge. The flow velocity of the settlement column was very slow, and the feces particles could settle smoothly, while the wastewater could flow out slowly from the top of the column. Choubert et al. (1982) created an automatic collection device which separated the drainage water and feces through metallic screens when they move linearly then the feces were injected into a refrigerated pan which can be automatically frozen with an abrupt stop of the screens. In recent years, Shomorin et al. (2019) used wedge wire screen used to collect

feces. The design of this wire screen ensures efficient drainage and the research showed that the ADCs of diets estimated by this method had low random variation.

The manual stripping, anal suction and intestinal dissection are active feces collection methods, where the fish are forced to defecate. Therefore, they not only contain undigested feed, but the samples also may be contaminated with mucus and urine. This may result in underestimation of the ADCs. The immediate pipetting, continuous filtration and decantation are passive feces collection methods which collecting the fecal material naturally excreted by the fish. The longer the feces in the water, the more nutrients will leach then it results in overestimation of the digestibility. Spyridakis et al. (1989) investigated the protein and lipid digestibility in European sea bass (*Dicentrarchus labrax*) by using dissection, stripping, anal suction, immediate pipetting, continuous filtration and decantation. The results showed that the digestibility obtained by active collection method was significantly lower than that by passive collection method and this is also consistent with others (Storebakken et al., 1998; Vandenberg & De La Noüe, 2001).

2.1.2 Factors affect the assessment of digestibility

2.1.2.1 Characteristic of ingredients and processing

Nutritional value varies in different raw materials. Even the same ingredient may differ greatly due to climate and origin (Glencross et al., 2007). Glencross et al. (2003) investigated the chemical composition and digestibility of different samples of lupin kernel meals when fed to rainbow trout. It was found that the chemical composition of five lupin kernel meals varied greatly, and the protein, fat, and energy ranged from 35.9% to 48.2%, 5.4% to 6.6%, 20.22 to 21.14 MJ kg^{-1}, respectively. The ADCs of five lupin kernel meals by adult rainbow trout also differed and the ADCs of dry matter, protein, energy and phosphorus ranged from 0.210 to 0.555, 0.799 to 0.896, 0.383 to 0.654 and 0.497 to 0.729, respectively.

Different processing may also affect the ADCs for raw materials. Liu et al. (2020) evaluated the ADCs of different animal protein ingredients under extrusion and steam pelleting processing. The results showed that ADCs of these ingredients were totally different. The ADCs of extruded ingredients except for poultry by-product meal was significantly higher than

that of steam pelleting processing since protein of these ingredients was moderately modified, thereby improving the digestibility of raw materials.

2.1.2.2 Fish species, size and genetic background

Absorption and utilization of nutrients and energy varies in different fish species. Glencross et al. (2004) studied the ADCs of three lupin protein products and three soybean protein products by Atlantic salmon (*Salmo salar*) and rainbow trout. The results showed that Atlantic salmon appeared to be more efficient in digesting ingredients that did not contain non-starch polysaccharides than what was found for rainbow trout.

Absorption and utilization of energy and nutrients of different fish size varies. Liu et al. (2017) substituted fish meal by soybean meal on different sizes of gibel carp (*Carassius auratus gibelio*) (0.8g, 5.0g, 62.7g, 135.6g) and found that the ADCs of diet by juvenile fish (5.0g) was lower than that of adult fish (62.7g, 135.6g). Although soybean meal had negative effects on survival rate, growth and feed utilization to gibel carp, the adult fish had higher tolerance than that of juvenile fish.

In addition, genetic backgrounds of fish also have a great impact on the determination of digestibility. Dvergedal et al. (2019) estimated the genetic variance and heritability of nitrogen and carbon digestibility in Atlantic salmon and their genetic and phenotypic correlations with growth. The results showed that the heritability of nitrogen and carbon digestibility were 0.39 ± 0.17 and 0.51 ± 0.18, respectively. The digestibility and growth rate had negative genetic correlations. A possible explanation is that high growth rates were associated with higher feed intake and increased intestinal transit rates, thereby reducing nutrient digestibility.

2.1.2.3 Water temperature

Temperature is one of the key factors affecting the metabolism and physiology of fish (Bowyer et al., 2014). Due to the season and climate change, temperature of aquaculture ranges widely. Besides, with the development of aquaculture, fish is reared in different areas where the temperature may not be optimal, such as salmon project in Ningbo, China. Therefore, it is necessary to know the impact of water temperature on digestibility.

The research of temperature effects on digestibility mainly focused on Atlantic salmon and rainbow trout. Although some researchers concluded that water temperature had minor effects on digestibility (Glencross et al., 2007; Ng et al., 2004), Huguet et al. (2015) reported that the digestibility of lipid, energy and nitrogen free extracts increased significantly with increased temperature from 10 to 20 ºC in Atlantic salmon. Ng et al. (2003) formulated four isolipidic diets with 0, 5, 10 and 20% crude palm oil then fed rainbow trout at water temperatures of 7, 10 or 15 ºC to evaluate the interactive effects of crude palm oil concentration and water temperature on lipid and fatty acids digestibility. The results showed that lowering water temperature significantly reduced the digestibility of saturated fatty acids, but it was not related to the crude palm oil concentration. Hua and Bureau (2009) analyzed the data from 16 studies with rainbow trout and Atlantic salmon by meta-analysis to explore the relationship between the digestible lipid content and fatty acids under different water temperature. This analysis showed that the digestibility of saturated fatty acids was affected by water temperature, but the digestibility of monounsaturated or polyunsaturated fatty acids was not affected.

2.2 Characteristic and digestibility of animal and plant protein ingredients

2.2.1 Animal protein ingredients

Animal protein ingredients are important components in diets for carnivorous fish species. These fishes naturally rely on aquatic and marine animals to satisfy its protein and energy demands. Most research indicate that ADC of raw materials is affected by both the composition and processing technology (Drew et al., 2007; Glencross et al., 2003). Therefore, it's highly necessary to know the characteristic and digestibility of feed ingredients.

2.2.1.1 Fishmeal

Fishmeal is considered an ideal protein source in the diets of aquatic animals. The protein content of fish meal is high which varies from 60 to 72%. It normally has high energy content, is rich in nutrients, is palatable and highly digestibility (Rahman et al., 2016).

The most common fishmeal is produced from fatty species such as herring, anchovy, mackerel, sardine and tuna. Co-products fishmeal from cod has also been reported. Dam et al. (2019) and

Rahman et al. (2016) indicated that the nutritional value of fish meal in different fish species or even in same species varies considerably. Feng et al. (2014) investigated the chemical composition of fish meal produced by fish co-products. The results showed that fishmeal made from head, viscera and skin were low in crude protein, methionine and lysine. Fishmeal made from steak has high calcium, phosphorus and crude protein, but the content of vitamins was low. Therefore, fish species and raw materials are the key factors affecting the chemical composition of fish meal. In addition, processing technology also affects fishmeal quality. According to the intensity of heating, fishmeal can be divided into Fair average quality (FAQ), Steam dried (SD), Hot air dried (HAD), and Low temperature dried (LT). FAQ fishmeal is usually drum dried at 500–600 °C with hot air. Although this method works efficiently, it brings strong smokes and the nutrients in fishmeal are easily damaged (Hertrampf & Piedad-Pascual, 2012). Processing temperature of steam dried fishmeal is lower than 90°C which improves the digestibility of fishmeal. However, it consumes more energy, and the production efficiency of fishmeal is low. For LT fishmeal, the processing temperature is 70-80°C and the fishmeal quality is higher than SD fishmeal. But this fishmeal is expensive. While HAD fishmeal combines all advantages of processing technology. Li et al. (2017) indicated that hot air drying could also improve the freshness of fishmeal, by running off volatile components such as ammonia.

Some research has been done to determine the digestibility of fishmeal produced from different fish species. Rahman et al. (2016) studied ADCs of fishmeal from herring, anchovy, mackerel, two meals from sardine, tuna and two cod, and fed these to olive flounder (*Paralichthys olivaceus*) (Table 2-1). The results showed that ADCs of the fishmeals from mackerel, sardine, tuna and one meal from cod fishmeal were very high (Table 2-2). The ADCs of tuna fishmeal and the other of the meals from cod were significantly lower than that of other ingredients. This difference may be caused by the chemical composition of fish species. Tuna fishmeal and cod fishmeal have higher ash content than other fishmeal which affects the digestibility of nutrients. Yu et al. (2013) also studied the ADCs of Peruvian fishmeal, poultry by-product meal, meat and bone meal, spray dried blood meal, hydrolyzed feather meal, corn gluten meal, soybean meal, cottonseed meal and rapeseed meal by juvenile snakehead (*Ophiocephalus argus*). The results showed that the digestibility of Peruvian fishmeal was higher than that of other ingredients. Similar results also found in Zhou et al. (2012) who found that digestibility of Peruvian fishmeal and Chinese fishmeal was significantly higher than other animal and plant protein ingredients in hybrid tilapia (*Oreochromis niloticus* x *Oreochromis aureus*).

These studies prove that many of the fishmeals are high-quality protein source. With the rapid growth in aquaculture, the amount of fishmeal is not sufficient to meet these demands. The price of fishmeal is also rising. Moreover, it is reported that fishmeal also contains organic chlorine compounds such as PCBs, dioxins and flame retardants which are highly toxic and will accumulate in the aquatic and marine food chains (Hites et al., 2004). EU is tightening its legislation on these contaminants, and an increasing proportion of fish are being deemed useless for fish meal or oil. Alternatively, fish oil and meal from these raw materials must be industrially de-contaminated (Oterhals, 2011). This is a strongly cost driving factor. Therefore, alternative protein ingredients are increasingly used to replace fishmeal in commercial diets for the further development of aquaculture.

2.2.1.2 Poultry by-product meal

Poultry by-product meal (PBM) is produced by by-products from broilers or laying hens. It can be divided into pet-food grade and feed-food grade poultry by-product meal. The PBM-pet does not contain low-quality components such as feathers and heads, and the processing standards of PBM-pet are stricter than these of PBM-feed (Dozier et al., 2003). Dozier et al. collected 26 PBM-feed samples and 10 PBM-feed samples from southeast of the United States to compare their chemical compositions. It was found that the protein content of PBM-pet was higher and essential amino acids were more balanced than that of PBM-feed (NRC, 2011) (Table 2-3). While crude lipid and ash were higher in PBM-feed. When compared with fishmeal, the essential amino acids of both poultry by-product meal were lower, especially for isoleucine, methionine and lysine. In general, the quality of PBM-pet is higher than PBM-feed.

Many studies have been done on ADCs of poultry by-product meal. Dong et al. (1993) collected poultry by-product meal from six major producers in North America and evaluated the digestibility of them both *in vitro* and *in vivo* for salmonids. The results showed that chemical compositions and protein digestibility of them varied. Rawles et al. (2010) measured ADCs of PBM-feed and PBM-pet by sunshine bass (*Morone chrysops* × *M. saxatilis*). It showed that protein and amino acids digestibility of PBM-feed were significantly lower than that of PBM-pet (Table 2-4). Similar results were also found in Dozier et al. (2003). Therefore, digestibility of PBM is largely affected by the sources of raw materials and processing. Besides, fish species may also an important factor affect the determination of digestibility. Dam et al.

(2019) measured the digestibility of two sources of PBM by yellowtail kingfish (*Seriola lalandi*). Although, protein content of two PBM ranged widely (69.7% to 84.5%), no significant difference was found on the ADC of crude protein, which was 66.5% and 71.3%, respectively. Digestibility of crude protein for PBM by largemouth bass was 81.5%, while that of crude protein was 57.8% (Portz and Cyrino, 2004).

2.2.1.3 Feather meal

Feather meal is made from the feathers of poultry. The protein content of feather meal is usually over 80%, but most of it is keratin with strong disulfide bonds. Digestibility of fresh, untreated feathers may be lower than 5%. It is difficult to hydrolyse feathers into polypeptides and free amino acids by peptidase so that the amino acids become available in feed for animals (Hertrampf & Piedad-Pascual, 2012). Moreover, the amino acids of feather meal are unbalanced, and especially low in methionine, lysine and histidine (Bandara, 2018). However, the yield, price and protein content of feathers are superior to some other animal rendering raw materials, and recycling of feathers was environmentally friendly. Therefore, current research pay attention to developing improved methods for improving digestion of feathers.

Hydrolysed feathers meal (HFM) is the most commonly used feather product. It is made by breaking the spatial structure of keratin with high temperature and high pressure. The digestibility of protein and essential amino acids of HFM is higher through hydrolysis, but the quality of it is greatly affected by the degree of hydrolysis. High pressure or improperly long reaction time will significantly reduce the protein quality especially for methionine and lysine (Hertrampf & Piedad-Pascual, 2012; Tiwary & Gupta, 2012). Extruded feather meal (EXFM) is made through extrusion. High temperature, high pressure and shearing effect in the extruder increased the temperature of feathers to 160°C instantly. The spatial structure of keratin is destroyed, thereby improving the digestibility of feather meal (Zhang et al., 2014). Enzymatic feather meal (EFM) is produced by single enzyme or compound enzymes. Fermented feather meal (FFM) is fermented by bacteria such as *Vibrio* sp. strain kr2, *Bacillus licheniformis*, *Streptomyces fradiae* and *Kocuria rosea* (Bertsch & Coello, 2005). By comparing the data from Chi et al. (2017) and Liang (2011) (Table 2-3), it can be concluded that EFM in the current study had lower protein content and higher fat content than other feather meals and amino acids content in HFM and FFM were higher than EFM and EXFM.

Few studies have been done to determine the digestibility of different feather meal products in fish. Chi et al. (2017) reported that there was no significant difference between ADCs of protein, amino acids and energy for HFM and FFM by cobia (*Rachycentron canadum*). The lipid digestibility of FFM was significantly higher than that of HFM. Liang (2011) indicated that the digestibility of EFM was lower than that of EXFM. Digestibility of HFM has been done on different fish species. The digestibility of protein, lipid and energy in HFM by juvenile mulloway (*Argyrosomus japonicus*) was 57.3%, 60.5% and 61.2%, respectively (Booth et al., 2013). The digestibility of HFM in cobia were much higher (protein: 77.0%, lipid: 71.1%, energy:77.5%) (Chi et al., 2017). HFM was produced from different manufacturers and different raw materials, thus the digestibility of it was highly variable (Booth et al., 2013). In general, ADCs for HFM is low which may be caused by overheating of raw materials or incomplete hydrolysis (Tibbetts et al., 2006; Tiwary & Gupta, 2012).

2.2.1.4 Spray-dried granulated inedible egg product

Spray-dried granulated inedible egg product (SGE) is the granular powder product processed by pasteurization and spray drying technology with grade B eggs. Spray drying dries the liquid in seconds or milliseconds so the exposure time of objects in thermal environment is relatively short. Therefore, it ensures the integrity and availability of thermal sensitive substances. Meanwhile, *Salmonella* and other pathogens were also completely killed after pasteurization. Thus, SGE has high nutritional values without significant safety risk (Hertrampf & Piedad-Pascual, 2012). SGE has more balanced amino acids and high amino acids content than HFM, especially for methionine and lysine when compared to fishmeal (Table 2-3) though the protein content of SGE is lower. Ash content in SGE is also low, about one third of that in fish meal. In addition, the quality of lipid in SGE is high. Phospholipids account for 12% of dry matter in SGE and the cholesterol reaches 16 g kg^{-1}. Except for used as energy sources, phospholipids and cholesterol also play important roles in fish physiology. Phospholipids in feeds could improve the survival rate, stress resistance and reduce the deformity rate of juvenile fish. Cholesterol is one of the major components of cell membranes which reduces the fluidity and permeability of cell membranes. Also, it is the precursors of many functional substances, such as sex hormone and steroid hormone. It was indicated that cholesterol was an irreplaceable nutrient for growth, metamorphosis and survival for larval Kuruma prawn (*Macrobrachium japonicus*) (NRC, 2011). Besides, the contents of choline, vitamin B2 and vitamin B12 in SGE are also high.

SGE has been used in food for a long history. The essential amino acids in SGE are considered as the ideal nutrients for all species, but there are few reports on the research of SGE in aquaculture nutrition. Theoretically, SGE is especially suitable for fish with high protein and energy requirements, such as salmon and trout. The price of SGE is high, which reduces the use of aquaculture, but as a perfect protein and energy component, these factors are worth reconsidering.

2.2.1.5 Defatted *Tenebrio molitor* larvae meal

Tenebrio molitor larvae meal is an insect protein raw material obtained by *Tenebrio molitor* after starvation, cleaning, boiling, low temperature storage and drying. The chemical compositions of *Tenebrio molitor* varies greatly during different life stages. The protein content of *Tenebrio molitor* usually decreases continuously with growing, while lipid increases. When the larvae become an imago, the changes in protein and lipid are the opposite. In general, the lipid content of *Tenebrio molitor* is around 10%-30% and it changes with life stage and food sources. Since full-fat *Tenebrio molitor* larvae meal are rich in unsaturated fatty acids which is easily oxidized, defatted *Tenebrio molitor* larvae meal (DTM) is mostly used. The protein, lipid and ash content of defatted *Tenebrio molitor* meal are similar to that of fishmeal (Table 2-3), while amino acids profile is unbalanced, especially for histidine, isoleucine, lysine, methionine, arginine and leucine. Meanwhile, DTM contains chitin which will affect the digestibility of other nutrients (Bandara, 2018; Hua et al., 2019).

Few studies have been done on the digestibility of DTM. Cheng et al. (2021) determined the ADCs of DTM, hydrolyzed feather meal, degossypolled cottonseed meal, blood meal, poultry by-product meal, isolated soy protein and soy protein concentrate by Japanese seabass (*Lateolabrax japonicus*). The results showed that the digestibility of DTM was significantly lower than other ingredients except for hydrolyzed feather meal and degossypolled cottonseed meal. ADCs of dry matter, protein and essential amino acids for DTM were 68.7%, 74.5% and around 40 to 50%, respectively. However, Liu et al. (2020) indicated that digestibility of DTM by yellow catfish (*Pelteobagrus fulvidraco*) was high. ADCs of dry matter, protein and lipid of DTM were 84.9%, 87.7% and 77.7%, respectively. This was not significantly different from what was found in Chinese fishmeal. Similar results were also found by Fontes et al. (2019), which indicates the dry matter, protein, energy digestibility of DTM by juvenile Nile tilapia

(*Oreochromis niloticus*) were 95.8%, 85.4% and 82.1%, respectively. Likewise, Fontes et al. (2019) also found that the digestibility of dry matter and protein decreased when chitin increased. These results showed that chitin lowers the digestibility of DTM, but the tolerance in different fish species varies.

DTM has been identified as a sustainable component for aquaculture (Hua et al., 2019). The production of *Tenebrio molitor* not only provides protein efficiently in a small land area, but also discharge lower levels of greenhouse gas than other ingredients which keeps in line with the principles of sustainable development. Therefore, it is believed to be the main protein ingredient for aquaculture in the future (Sogari et al., 2019; Sørensen et al., 2011).

2.2.1.6 Hydrolysates of fish protein

Hydrolysates of fish protein uses whole fish or by-products as raw materials which are hydrolyzed into free amino acids or small peptides. This promotes efficient protein digestion. Short peptides can also be directly absorbed and utilized in intestinal villi and can be distributed in the body through blood circulation (Tang, 2008). Martínez-Alvarez et al. (2015) also found that hydrolysates of fish protein improve the feed utilization and improve the immunity of aquatic animals.

Studies of hydrolysates of fish protein mainly focused on protein substitution and physiology rather than digestibility. Silva et al. (2017) studied the digestibility of enzymatic stickwater by Nile tilapia (*Oreochromis niloticus*), which showed that the digestibility of dry matter, protein and energy for enzymatic stickwater were 98.29%, 99.28% and 99.13%, respectively. Similar results were also found *in vitro* digestibility experiments (Foh et al., 2011; Hevrøy et al., 2005).

2.2.2 Plant protein ingredients

Plant ingredients usually have low protein content, unbalanced amino acids, poor palatability, antinutrients, fiber and starch. This may lead to many drawbacks in the substitution of fish meal (Daniel, 2018). However, the cost and yield of plant ingredients are superior to fish meal and these advantages allow further processing of plant ingredients, thereby increasing the digestibility and utilization in fish (Drew et al., 2007).

2.2.2.1 Soy protein products

Soybean meal (SBM) is one of the most widely used plant raw materials in pigs, livestock and aquatic feed. SBM is produced from flaked, defatted soybean that has water solubles removed by extraction with hot water or a mixture of water and alcohol. The chemical composition and nutrition value of soybean meal varies (Table 2-5). High protein types are obtained from dehulled seeds (48% protein) and protein content in SBM with hulls is 44% (Gatlin Iii et al., 2007). Although soybean meal is regarded as economical and nutritious ingredients with high crude protein and amino acids, the presence of antinutrients (Table 2-6) and some limiting essential amino acids, especially methionine, should be discussed. Antinutrients can be grouped into heat liable (protease inhibitors and lectins) and heat stable compounds (saponins, phytic acid, tannin, oligosaccharides, non-starch polysaccharides) (Bandara, 2018). By heat processing, heat sensitive antinutrients will be eliminated but most heat stable compounds still remained in soybean meal (Drew et al., 2007). SBM causes enteritis in the distal intestine fish. It was first detected in Atlantic salmon and rainbow trout but has later become recognized in several other fish species. In order to improve the utilization of soybean, researchers used water and solvent extraction, microbial fermentation, and enzymes to remove heat-sensitive antinutrients and therefore produced other soy products.

Soya protein concentrate (SPC) is a high protein product obtained by low temperature heating and water & ethanol extraction of soy flakes. The factors causing enteritis are removed, palatability is better, the protein content is higher, and amino acid profile is more balanced than that of SBM. Meanwhile, the essential amino acids of SPC except for methionine were equivalent or even higher than those of fish meal. Therefore, SPC improves growth performance of fish better than SBM. The main challenge with SPC in coldwater fish diets, is phytic acid, since phytase as feed enzymes is limited by temperature.

Fermented soybean meal (FSM) is a product produced by specific microorganisms and protease which are able to degrade non-starch polysaccharides and antinutrients in soybean into high content of polypeptides, free amino acids and bioactive components (He, 2020). Although many bacteria have been studied in FSM, including lactic acid bacteria, *Aspergillus oryzae* and yeast, lactic acid bacteria is most widely recommended since it not only effectively improve the palatability of soybean meal but also the nutritional value and digestibility (Liu,

2012). As for the protease, papain and bromelain have been used for production in industry. In general, FSM have more balanced amino acids profile than SBM.

Many studies have been done to determine the digestibility of SBM, SPC and FSM (Table 2-7). Dong et al. (2010) studied the digestibility of these ingredients in hybrid tilapia and the results showed that no significant difference was found on lipid and dry matter digestibility, but energy digestibility of SPC was significantly higher than other soy products and protein ADC of FSM was significantly higher than SPC and SBM. ADC of these soy ingredients were lower than of fish meal. Zhou et al. (2012) also studied the ADCs of SBM, FSM and fish meal by hybrid tilapia. The study indicated that there was no significant difference in digestibility of SBM and FSM, but the digestibility of soy products was lower than fish meal. In general, the research above indicated that the digestibility of soy products varied but all lower than fish meal. However, Tibbetts et al. (2006) found that ADC of protein and energy in SPC was significantly higher than fish meal and SBM in Atlantic cod (*Gadus morhua*). Liang (2011) also indicated that dry matter, energy and protein digestibility of SBM was significantly higher than fish meal in Jian carp (*Cpyrinus carpio* var. Jian). The reason for the difference may be that the processing of soy products was lenient, or that smaller fish were used to assess digestibility of the fish meal.

2.2.2.2 Cottonseed meal

Cottonseed meal (CSM) is processed by dehulling, rolling, roasting, oil extraction and drying (Figure 2-1). In general, the crude protein of CSM is around 41% and that of cottonseed protein concentrate is over 60%. Protein and amino acids concentration of CSM are rather high. Especially arginine and phenylalanine are abundant in the protein. CSM contains antinutrients such as phytic acid and gossypol. Phytic acid is the main form of phosphorus in cottonseed meal, accounting for 71 % of total phosphorus. Phytic acid binds divalent and trivalent metal ions (such as Zn^{2+}, Mg^{2+}, Cu^{2+}, Fe^{3+}) and reduce the utilization of minerals of fish. It also damages the pyloric ceca, and cause enteritis (Liang, 2011). Free gossypol is toxic and will reduce the growth rate of fish and may even cause death. Thus, researchers have developed methods for degossypolling cottonseed meal to lower the gossypol content at low temperature. The processing of DCM is shown in Figure 2-2. In general, protein and amino acids contents of degossypolled cottonseed meal are higher than that of regular cottonseed meal. Simultaneously, free gossypol is decreased from 1200 to 400 mg kg^{-1} after processing.

Zhou and Yue (2012) have studied the apparent digestibility of cottonseed meal and degossypolled cottonseed meal in hybrid tilapia. The results showed that there was no significant difference between two products with respect to digestibility although free gossypol varied. The reason may be related to the non-starch polysaccharides both in CSM and DCP which increased the viscosity of feed and reduced the digestion time, thereby reducing the effect of gossypol. Another reason may be related to the imbalanced amino acids in two products. Similar results also found for the digestibility of cottonseed meal and degossypolled cottonseed meal (Zhou et al., 2008) (Table 2-7). Cheng et al. (2021) studied the apparent digestibility of hydrolyzed feather meal, spray-dried blood meal, poultry by-product meal, yellow worm meal, soy protein concentrate and degossypolled cottonseed protein by Japanese seabass. The results showed that the ADC of degossypolled cottonseed meal was significantly lower than that of other ingredients except for hydrolyzed feather meal and yellow worm meal, and the digestibility of dry matter and crude protein were only 69.2% and 74.4%, respectively. Lee (2002) measured the apparent digestibility of fishmeal, soybean meal and cottonseed meal on adult and juvenile rock fish (*Sebastes schlegeli*). The results showed that the ADC of dry matter in cottonseed meal was 34 % and 46 %, which were significantly lower than that of fishmeal and soybean meal. This result was consistent with Zhou and Yue (2012). In general, the apparent digestibility of cottonseed meal and degossypolled cottonseed meal in fish were rather low. However, the yield of cottonseed is high. If the nutritional value is further improved through processing, cottonseed may make a huge contribution to the world feed protein supply.

Table 2-1 Chemical compositions and amino acids of reported fish meal, dry matter basis.

	Tuna fishmeal (Dam et al., 2019)	Tuna fishmeal (Rahman et al., 2016)	Herring fishmeal (Rahman et al., 2016)	Anchovy fishmeal (Rahman et al., 2016)	Mackerel fishmeal (Rahman et al., 2016)	Sardine fishmeal-A (Rahman et al., 2016)	Sardine fishmeal-B (Rahman et al., 2016)	Pollock fishmeal-A (Rahman et al., 2016)	Pollock fishmeal-B (Rahman et al., 2016)
Chemical composition, g kg^{-1}									
Crude protein	682.2	627	734	673	766	715	710	747	633
Lipid	115.5	106	104	86	68	100	102	59	54
Ash	198.0	201	166	197	167	160	146	157	264
Energy, MJ kg^{-1}	20.8	18.0	20.5	18.8	19.2	19.7	20.1	19.7	16.3
Essential amino acid composition, g $16gN^{-1}$									
Arg	4.9	6.4	6.4	6	6.5	7.1	6.4	7.1	7.0
His	2.7	3.3	2.8	2.0	4.5	2.5	3.0	2.5	2.5
Ile	-	4.2	4.4	4.0	4.5	4.1	4.7	3.9	4.2
Leu	7.2	7.6	8.0	6.6	7.9	7.8	8.3	8.0	8.0
Lys	6.9	9.3	8.4	7.2	8.6	5.8	8.9	5.7	5.3
Met	2.7	-	-	-	-	-	-	-	-
Phe	4.1	-	-	-	-	-	-	-	-
Thr	4.5	4.8	4.8	4.8	4.7	4.2	4.9	4.9	4.3
Val	5.0	5.6	5.9	4.9	5.0	4.4	5.3	4.3	4.7
Cys	0.7	-	-	-	-	-	-	-	-

Table 2-2 ADCs of different reported fish meal.

	Herring fishmeal (Rahman et al., 2016)	Anchovy fishmeal (Rahman et al., 2016)	Mackerel fishmeal (Rahman et al., 2016)	Sardine fishmeal-A (Rahman et al., 2016)	Sardine fishmeal-B (Rahman et al., 2016)	Tuna fishmeal (Rahman et al., 2016)	Pollock fishmeal-A (Rahman et al., 2016)	Pollock fishmeal-B (Rahman et al., 2016)
Species	Oliver flounder, *Paralichthys olivaceus*							
Methods	Filtration							
ADCs of nutrients and energy, %								
Dry matter	81.5±1.47	80.7±1.71	83.6±0.74	84.4±0.51	83.5±0.06	77.5±1.04	87.0±0.45	69.2±2.97
Crude protein	93.2±0.31	91.6±1.47	95.3±0.16	95.1±0.18	90.8±0.08	87.2±0.70	95.4±0.20	87.2±1.29
Crude lipid	90.5±1.24	94.6±0.71	94.7±0.91	95.9±0.06	93.1±0.46	92.4±1.59	93.6±1.24	83.0±1.82
Energy	90.7±0.65	90.3±0.24	93.5±0.49	93.0±0.07	89.3±0.11	86.2±0.40	93.9±0.39	83.5±0.98
ADCs of amino acids, %								
Arg	94.3±0.39	93.1±1.46	98.1±0.03	96.6±0.19	92.9±0.27	89.8±0.65	96.1±0.14	92.0±0.39
His	93.2±0.41	90.8±1.77	98.1±0.13	96.1±0.06	92.1±0.15	90.3±0.62	95.0±0.28	89.8±0.75
Ile	92.7±0.60	90.5±2.23	97.0±0.13	94.9±0.10	90.6±0.28	87.6±0.86	94.8±0.32	89.0±0.48
Leu	93.1±0.48	91.3±2.04	97.3±0.11	95.3±0.14	91.0±0.21	88.0±0.79	95.0±0.19	89.3±0.43
Lys	84.5±0.62	92.3±2.08	97.8±0.11	96.4±0.04	91.3±0.12	88.6±0.93	95.3±0.21	89.5±0.50
Met+Cys	96.1±0.26	94.5±1.23	98.5±0.02	97.3±0.01	94.0±0.11	88.5±0.36	97.1±0.09	91.1±0.37
Phe+Tyr	92.2±0.67	90.2±1.89	96.9±0.10	94.8±0.21	90.2±0.22	88.5±0.71	94.6±0.35	88.7±0.42
Thr	92.0±0.42	90.1±1.83	96.7±0.12	94.4±0.24	90.1±0.12	86.6±0.66	94.7±0.34	87.7±0.48
Val	89.9±0.36	86.5±1.94	95.7±0.16	92.7±0.24	88.6±0.20	83.9±0.81	93.4±0.76	86.3±0.51

Table 2-3 Chemical compositions and amino acids of animal protein ingredients, dry matter basis.

	Peruvian steam fishmeal (Glencross, 2020)	Poultry by-product meal-feed (Dozier et al., 2003)	Poultry by-product meal-pet (Dozier et al., 2003)	Hydrolyzed feather meal (Chi et al., 2017)	Extruded feather meal (Liang, 2011)	Enzymatic feather meal (Liang, 2011)）	Fermented feather meal (Chi et al., 2017)	Egg meal	Defatted *Tenebrio molitor* larvae meal (Cheng et al., 2021)	Fish protein hydrosate (Zhou, 2019)
Chemical composition, %										
Crude protein	67.0	58.1	66.1	89.2	93.6	82.6	87.9	47.2	67.3	69.7
Lipid	10.9	14.4	12.6	2.35	2.4	5.1	1.93	41.1	7.1	13.7
Ash	13.9	17.1	15.1	-	2.4	10.5	-	3.6	8.4	13.0
Energy, MJ kg^{-1}	20.1	-	-	25.6	23.4	21.5	23.8	22.2	-	-
Essential amino acid, %										
Arg	3.9	3.63	3.49	6.20	4.60	4.40	6.05	6.02	4.15	2.89
His	2.5	1.65	1.62	0.68	0.61	0.42	0.67	2.37	1.11	4.69
Ile	3.0	1.36	1.30	4.15	3.22	3.07	3.96	5.47	2.09	1.93
Leu	5.0	3.16	3.00	6.71	5.91	5.16	6.34	8.58	5.98	3.99
Lys	5.3	2.75	2.92	1.86	1.39	0.95	1.87	7.20	4.18	5.51
Met	2.0	0.77	0.84	0.68	0.28	0.17	0.71	3.14	1.28	1.47
Phe	2.7	1.57	1.52	3.90	3.33	3.10	3.78	5.34	2.65	1.78
Thr	2.5	1.85	1.82	3.94	3.46	3.05	3.88	4.81	2.39	2.29
Val	3.7	1.86	1.74	5.33	5.18	4.90	5.07	5.47	3.20	2.65

Table 2-4 ADCs of animal protein ingredients in carnivorous fish species.

	Mackerel fishmeal (Rawles et al., 2010)	Poultry by-product meal-pet (Rawles et al., 2010)	Poultry by-product meal-feed (Rawles et al., 2010)	Hydrolyzed feather meal (Chi et al., 2017)	Fermented feather meal (Chi et al., 2017)	Extruded feather meal (Liang, 2011)	Enzymatic feather meal (Liang, 2011)	Defatted *Tenebrio molitor* larvae meal (Liu et al., 2020)	Fish protein hydrosate (Silva et al., 2017)
Species	Sunshine bass *Morone chrysops* x *M. saxatilis*			Cobia *Rachycentron canadum*		Jian carp *Cpyrinus carpio* var. Jian		Yellow catfish *Pelteobagrus fulvidraco*	Nile tilapia *Oreochromis niloticus*
Methods	Stripping			Stripping		Net collection		Siphoning	Filtration
ADCs of nutrients and energy, %									
Dry matter	-	-	-	53.5	55.6	64.3	76.3	84.9	98.29
Crude protein	99	84	80	77.0	79.9	66.5	75.6	87.7	99.28
Crude lipid	-	-	-	71.1	82.2	66.5	67.9	77.7	-
Energy	-	-	-	77.5	79.0	58.7	78.0	-	99.13
ADCs of amino acids, %									
Arg	100	86	89	91.0	95.7	73.6	80.0	91.4	-
His	-	-	-	81.7	87.0	62.5	63.0	95.4	-
Ile	103	91	91	85.8	91.6	70.9	78.5	94.0	-
Leu	101	84	85	87.0	93.4	71.4	76.2	89.5	-
Lys	-	-	-	77.1	87.7	64.0	59.4	64.1	-
Met	-	-	-	78.2	89.1	79.8	70.3	89.0	-
Phe	101	83	43	84.7	90.2	71.7	77.1	58.0	-
Thr	103	91	90	83.3	86.5	64.5	74.0	93.1	-
Val	102	82	38	84.2	87.3	67.6	76.0	93.7	-

Table 2-5 Chemical compositions and amino acids of plant protein ingredients, dry matter basis.

	Soybean meal (NRC, 2011)	Dehulled soybean meal (NRC, 2011)	SPC (NRC, 2011)	Fermented soybean meal (Zhou & Yue, 2012)	Cotton seed meal (NRC, 2011)	CSM (TYCOON Group Co., Ltd., China)	DCP-TY (TYCOON Group Co., Ltd., China)
Chemical composition, %							
Dry matter	89	90	92	92.5	92	91.6	93.2
Crude protein	44.0	48.5	63.6	48.2	41.7	66.7	64.2
Lipid	1.5	0.9	0.5	1.3	1.8	0.7	0.1
Crude fibre	7.3	3.4	4.5	-	11.3	-	-
Ash	6.3	5.8	-	-	6.4	7.3	7.8
Energy, MJ kg^{-1}	-	-	-	20.5	-	19.4	19.1
Essential amino acids, g $16gN^{-1}$							
Arg	3.23	3.60	4.64	4.56	4.18	12.00	11.89
His	1.17	1.30	1.58	1.45	1.07	2.62	2.61
Ile	1.99	2.60	2.94	2.49	1.45	2.55	2.67
Leu	3.42	3.80	4.92	4.36	2.32	5.30	5.40
Lys	2.83	2.24	3.93	5.39	1.60	4.18	4.24
Met	0.61	0.70	0.81	1.45	0.58	1.07	1.22
Phe	2.18	2.70	3.28	3.11	2.18	5.47	5.31
Thr	1.73	2.00	2.47	2.90	1.34	3.11	3.16
Val	2.40	2.70	3.06	2.90	1.90	3.72	3.87
Trp	0.61	0.70	0.84	-	0.53	-	-
Cys	0.70	0.71	0.89	-	0.73	-	-

Table 2-6 Antinutrients in plant protein ingredients (source from Bandara, 2018).

	Antinutrients	Features	Effects on fish
Heat labile	Protease inhibitor	Kunitz: inhibit trypsin Bowman-Birk: inhibit trypsin and chymotrypsin Making stable complex with protease and reduce the activity of protease	Reduce the apparent digestibility of protein and lipid
	Lectins	Specifically combining with carbohydrate of sugar-protein on the cells	Reduce the apparent digestibility of nutrients and cause enteritis
Heat stable	Saponins	Highly toxic to fish with the detergent action	Harm fish gill and cause enteritis on high level
	Phytic acid	Bind bivalent and trivalent metal ions	Reduce the digestibility and utilization of phosphorus and minerals
	Tannins	Combine with protein, carbohydrate and enzymes	Reduce the utilization of vitamin B2 and protein in fish
	Oligosaccharides	Cannot be digested in small intestine without α-galactosidase	Combine and affect the digestibility of other nutrients
	Non-starch polysaccharides	Soluble non-starch polysaccharides increase viscosity of feed and fiber decrease the digestion time	Reduce the absorption of nutrients, especially vitamin and lipid
	Gossypol	Bound gossypol: combine protein and amino acids Free gossypol: toxic with highly active aldehyde and hydroxyl group	Reduce the digestibility of protein and affect the health and growth of fish

Table 2-7 ADCs of reported plant protein ingredients.

	Dehulled soybean meal (Wang, 2012)	Fermented soybean meal (Wang, 2012)	SPC (Wang, 2012)	Soybean meal (Dong et al., 2010)	Fermented soybean meal (Dong et al., 2010)	SPC (Dong et al., 2010)	Cotton seed meal (Zhou & Yue, 2012)	Degossypolled cottonseed protein (Zhou & Yue, 2012)	Cotton seed meal (Zhou et al., 2008)	Degossypolled cottonseed protein (Zhou et al., 2008)
Species	Saddletail grouper, *Epinephelus coioides*			Hybrid tilapia, *Oreochromis niloticus*×*Oreochromis aureus*					Bluntnose black bream, *Megalobrama amblycephala*	
Methods	Siphoning			Settling					Filtering	
ADCs of nutrients and energy, %										
Dry matter	73.38	68.79	70.36	69.8	69.9	67.5	65.9	67.2	58.1	62.5
Crude protein	81.15	87.21	83.35	97.8	95.7	98.2	79.2	84.0	92.1	87.6
Crude lipid	79.23	91.84	83.01	93.0	92.5	93.5	92.6	97.2	-	-
Energy	76.43	63.71	88.27	77.2	76.6	80.4	78.9	79.5	65.9	69.9
ADCs of amino acids, %										
Arg	86.16	90.49	92.59	95.8	97.2	98.9	94.0	97.7	-	-
His	82.07	91.33	92.91	96.1	95.9	98.6	92.6	93.0	-	-
Ile	81.47	84.18	87.20	93.5	94.1	96.5	88.1	87.7	-	-
Leu	79.04	83.13	84.91	95.0	95.0	97.5	91.1	92.0	-	-
Lys	80.35	89.08	91.72	96.0	94.3	97.9	90.0	89.4	-	-
Met	73.30	74.73	75.22	100.0	100.0	100.0	73.3	77.0	-	-
Phe	82.86	82.15	88.20	95.8	97.0	98.6	87.8	93.2	-	-
Thr	74.43	73.99	76.48	94.3	95.4	96.2	79.4	85.9	-	-
Val	72.00	77.98	81.86	94.1	94.8	96.5	82.1	85.2	-	-

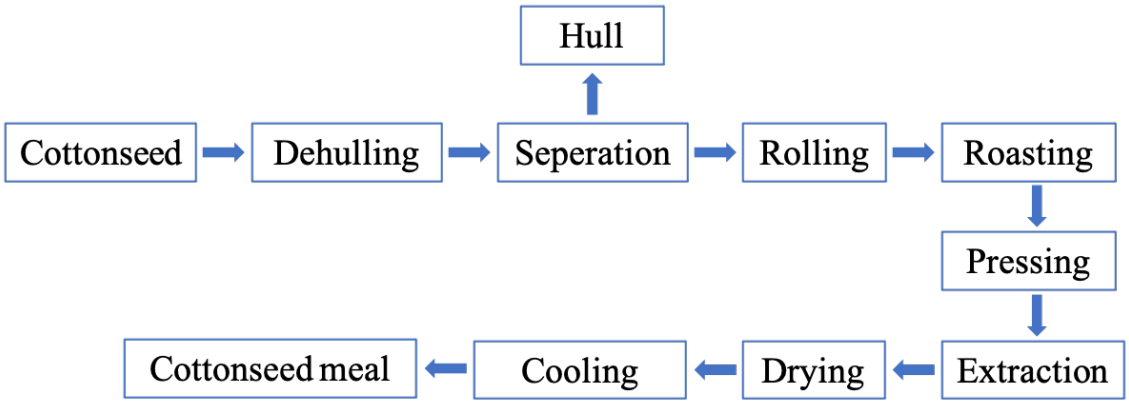

Figure 2-1 Processing of cottonseed meal (TYCOON Group Co., Ltd., Xinjiang, China).

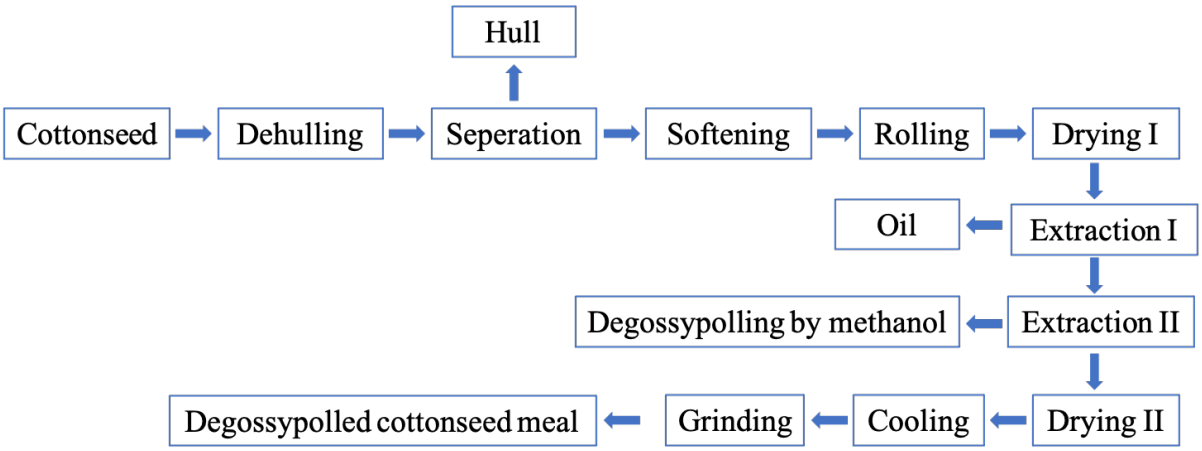

Figure 2-2 Processing of degossypolled cottonseed meal (TYCOON Group Co., Ltd., Xinjiang, China).

3 Materials and methods

3.1 Ingredients and formulation

Peruvian anchovy fishmeal (PAF) was used as the sole protein source in the basal diet. This diet was formulated and processed to satisfy the requirements of largemouth bass. Appropriate proportions of fish oil, soybean oil, tapioca and premix were added to formulate a control diet with 59.7% of crude protein, 13.5% of crude fat, 14.5% of ash and 20.6 MJ kg^{-1} of gross energy (Table 4-3). 30% PAF in reference diet was replaced by the test ingredients. Other components were consistent with these in reference diet. 0.05% Y_2O_3 was added as an inert marker in both reference and test diets. The formulas used for determining apparent digestibility coefficients (ADC) of various nutrients in the protein-rich feed ingredients are shown in Table 4-3 and Table 4-4.

Two experiments were conducted in order to determine ADCs of nutrients and energy in different protein-rich feed ingredients. Test ingredients in experiment I were of animal nature and included four sources of fishmeal (Peruvian anchovy PAF, Chinese sardine CSF, Chinese anchovy CAF and Chinese mackerel CMF), poultry by-products (poultry by-product meal PBM, poultry carcasses meal PCM, hydrolyzed feather meal HFM and spray-dried granulated inedible egg product SGE) and two potential alternative animal protein ingredients (defatted *Tenebrio molitor* larvae meal DTM and hydrolysates of stickwater and soybean HSS). Chemical compositions of these animal protein ingredients and diets are shown in Table 4-1 and Table 4-3.

Test ingredients in experiments II were from plants and included soy products (soybean meal SBM, soy protein concentrate SPC, fermented soybean meal TB FSM-TB, and fermented soybean meal YH FSM-YH) and cottonseed products (cottonseed meal CSM, degossypolled cottonseed proteins TY DCP-TY and JL DCP-JL). Chemical compositions of plant protein ingredients and diets are shown in Table 4-2 and Table 4-4.

3.2 Diet production

The experimental feeds were processed in Asia-Pacific Production and Research Center of Bühler (Changzhou, Jiangsu, China). In order to simulate the production of commercial feed and provide reliable data on digestibility, all pellets were produced by Bühler's process line. Before extrusion, raw materials were weighed by using an electronic scale (TCS-150, Mettler Toledo, OH, USA). After that, raw materials were ground (AHZC0655, Bühler, Uzwil, Switzerland). In order to improve the quality of diets, all ingredients (except for SGE) were re-ground by using micro-pulverizer (AHFL 110B, Bühler, Uzwil, Switzerland). Particle size of each ingredient is showed in Table 4-7 and Table 4-8. Then premix and ground materials were weighed by an electronic balance (TCS-3, Mettler Toledo, OH, USA) and mixed in a single-axis impeller mixer (AHML 1000, Bühler, Uzwil, Switzerland). Before extrusion, the materials were sent into the modulator (BCCC-22, Bühler, Uzwil, Switzerland) which ripened the dry material in a hot and humid environment. After that, a twin-screw extruder (BCCG-62, Bühler, Uzwil, Switzerland) was used for extrusion. The processing parameters of extrusion is showed in Table 4-9 and Table 4-10. Pellets were dried by a dual-temperature dryer (BDBD P2G0.5C, Bühler, Uzwil, Switzerland) for 30 minutes. A vacuum coater (ZJB-100, Xindeli Food Machine Co., Ltd., Zhucheng, Shandong, China) was used to add oil into pellets. Finally, all diets were stored in -10°C until fed to the fish.

3.3 Pellet quality

The indices of pellets quality include pellet durability index (PDI), pellet diameter and length (D & L), hardness (HD), bulk density (BD), and average weight of pellets (AWP).

Pellet durability index (PDI, %) means the ability of feed pellets to resist crushing during transportation and handling. It was measured in duplicates in pellet durability tester (ST-136, Shengtai Instrument Co., Ltd., Shandong, China). Each diet was examined in triplicate and samples were lightly sieved with 6 mm screen. Then 2 x 500 g samples were poured into two rotary boxes. The test procedure was set as 50 r min^{-1} for 10 min. After that, feed samples were re-sieved with 6 mm screen and weighed.

The diameter and length (D & L, mm) of pellets were measured with a vernier calliper with a precision of 0.01 mm. 50 pellets were randomly selected from each diet for measuring.

Hardness (HD, N) of pellets demonstrates the strength of feed pellets which was related to storage, water stability, digestion of fish (Han, 2016; Yang et al., 2000). It was measured by the hardness measurement instrument (ST-120B, Shengtai Instrument Co., Ltd., Shandong, China). Fifty pellets were randomly selected from each uncoated diet and the breaking value of each pellet was recorded.

Bulk density (BD, g L^{-1}) of pellets was measure in a 1000 ml beaker. Pellets are filled into the beaker as much as possible and then recorded weights. Each diet was examined in triplicate.

All indices of pellet quality are shown in Table 4-11 and Table 4-12.

3.4 Feeding trial and feces sampling

Two experiments were carried out in fish room I and II in Sino-European Aquatic Nutrition and Feed Resource Institute (SEA-NUTR) at Zhejiang Ocean University (ZJOU), Zhoushan, Zhejiang, China. The largemouth bass fingerlings were purchased from the fish farm in Xiaoshan, Zhejiang, China. The fingerlings were reared in a large cylindrical glass fiber tank temporarily, volume of 24 m^3. They were fed with a commercial feed from Biomar Group (Aarhus, Denmark). The digestibility experiment started when the fish weight reached about 350 g. To reduce stress, fish were starved for 24h before the experiment, and then anesthetized with MS-222. In experiment I, 1050 healthy largemouth bass with average weight of 370g were put into 30 cylindrical glass fiber tanks with a volume of 1000 L.

In experiment II, 840 healthy largemouth bass with average weight of 350g were selected and put into 30 cylindrical glass fiber tanks with a volume of 1000 L. Each diet was examined in triplicate.

The experimental fish were cultured in indoor recirculating aquaculture systems (RAS). In order to ensure the healthy conditions of fish, the whole system was aerated with air for 24 h. Water temperature was kept at 23 °C by the use of a heat exchanger (ZWH-KFX-BT2011,

Zhengxu Technology Co. Ltd., Guangdong, China). Dissolved oxygen and pH in water were above 5.0 mg L^{-1} and 7.0, respectively. Ammonia and nitrite were less than 0.2 mg L^{-1} and 0.1 mg L^{-1}, respectively. Fish were fed three times per day (8:00, 13:30, 19:30) with 15min intervals for each group (each group had 4 tanks). Feed intake of each diet was shown in Figure 4-1 and Figure 4-2. Feces was collected per 5 days after the feed intake of largemouth bass was stable by using manual stripping method (Austreng, 1978). Fish was anesthetized by MS-222 and the feces were stripped from ventral fins to the anus. Body fluid, urine and blood in the feces were slightly wiped. Faecal samples were immediately stored in a freezer at -80 °C, then dried by a vacuum freeze dryer (SJIA-10N-50A, Shuangjia Instrument Co. Ltd., Zhejiang, China).

3.5 Chemical analysis

The ingredients (except SGE) and dried feces were ground through a 60-mesh sieve before analysis, and the diets were ground through a 30-mesh sieve before analysis. The chemical indices of ingredients and diets were dry matter, ash, crude protein, amino acids, crude fat, total phosphorus, gross energy and trace elements. The chemical indexes for feces mainly included crude protein, amino acids, total phosphorus, gross energy and trace elements.

Dry matter was measured after drying at 105°C for 24h in a drying oven (DHG-9140A, Shanghai Jinghong Experimental Equipment Co., Ltd., Shanghai, China). Ash content was measured in a muffle furnace (SX2410A, Shangyu Daoxu Scientific Analysis Instrument Factory, Zhejiang, China). The procedure for ash measurement was carbonization at 220°C for 2h and then samples were heated and kept at 550°C for 36-48h. Crude protein content was determined by a Kjeldahl nitrogen analyzer (KD210, OPSIS, Sweden) after high temperature digestion with concentrated sulfuric acid. Crude fat was extracted and measured by ether for 4 h in an automatic fat extractor (SoxROC, OPSIS, Furulund, Sweden). The diet samples were hydrolyzed by 2 mol L^{-1} HCl for 2h in acid hydrolysis instrument (HydROC, OPSIS, Furulund, Sweden) before crude fat was determined. Gross energy was measured by recording the energy released from the combustion of samples with highly pure oxygen in an automatic oxygen bomb instrument (Parr 1271, Parr Instrument Company, IL, USA). Samples were hydrolyzed with 6 mol L^{-1} HCl in vacuum environment at 110°C for 22h (pretreatment with oxidation hydrolysis described in GB/T15399-2018 was necessary when measuring for methionine and

cysteine in samples). The amino acids in the hydrolysate of samples were determined by automatic amino acid analyzer (L-8900, Hitachi, Tokyo, Japan). The total phosphorus in the sample was determined by molybdenum yellow spectrophotometry. The accurately weighed sample was diluted to constant volume after microwave accelerated digestion (MARS5, CEM Corporation, NC, USA). A certain volume of diluted solution was added with ammonium vanadate molybdenum. The absorbance was measured at 405 nm after 1-2 h, and the real total phosphorus content was calculated from a standard curve drawn in advance. Trace elements were determined by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7900, Agilent, CA, USA). The samples were digested after microwave accelerated reaction system, and contents of various trace elements in the samples were determined by ICP-MS.

3.6 Calculation

Formula for apparent digestibility coefficient of nutrients in diets:

$$ADC_D, \% = \left(1 - \frac{F_N * D_Y}{F_y * D_N}\right) * 100\%$$

Where F_N and D_N represent concentration of nutrients in feces and diets, F_Y and D_Y represent concentration of yttrium in diets and feeds, ADC_D represents apparent digestibility coefficient of diet.

Formula for apparent digestibility coefficient of nutrients in ingredients:

$$ADC_I, \% = \frac{(ADC_D - P_c * ADC_c)}{P_I}$$

Where ADC_I, ADC_D and ADC_C represent apparent digestibility coefficient of nutrients in test ingredients, test diets, and reference ingredient (Peruvian anchovy fishmeal, PAF), P_C and P_I represent proportion of nutrients in test ingredients and PAF.

3.7 Data analysis

Statistical analysis, plots, and correlation (Spearmen's test) were carried out in R-studio (Boston, MA, USA). The Shapiro-Wilk normality test and homogeneity test of variances were carried out in advance. Significant differences between ingredients were tested by one-way analysis of variance (ANOVA), using a significance level (α) of 0.05. Duncan's test was used

for multiple comparisons (post hoc tests) when variances were equal, or the Kruskal-Wallis test and Nemenyi test were used.

3.8 Ethics statement

This study did not involve any endangered species. Largemouth bass (*Micropterus salmoides*) is not the protected species by Chinese law. It is a commercially harvested and farmed species in China. During the feeding period and sampling procedures, the experimental fish were maintained in compliance with the Laboratory Animal Welfare Guidelines of China (Decree No. 2 of Ministry of Science and Technology, issued in 1988).

4 Results

The ADCs of dry matter, protein, energy or phosphorus of animal protein and plant protein ingredients are listed in Table 4-13 and Table 4-14. The ADCs of amino acids of animal protein and plant protein ingredients are shown in Table 4-15 and Table 4-16.

4.1 Diet parameters and feed intake of ingredients by largemouth bass

4.1.1 Diet parameters and feed intake of animal protein ingredients

In order to simulate the production of commercial feed and provide reliable data on digestibility, all pellets were produced by Bühler's process line and raw materials were ground into micron except SGE. All diet parameters and physical characteristic are shown in Table 4-9 and Table 4-11. During the experiment, feed input and uneaten pellets were counted and removed every day. Feed intake of each diet of animal protein ingredients is shown in Figure 4-1 which indicated that the feed intake of each diet was high and had no significant difference. The first sampling time of feces was on 28-29, December in 2020 after the feed intake by largemouth bass became stable and later take samples on January 4-5, 11-12, 17-18 in 2021, respectively. Although feed intake of each diet fluctuated after sampling, the wave changed mildly. The stress caused by sampling did not seem to affect the feed intake of largemouth bass greatly.

4.1.2 Diet parameters and feed intake of plant protein ingredients

Plant protein ingredients were processed as the same as animal ingredients. All diet parameters and physical characteristic are shown in Table 4-10 and Table 4-12. During the experiment, feed intake of plant protein diets was assessed every day and the data are shown in Figure 4-2. The figures show that the feed intake of each diet was relatively high and uniform. In order to eliminate the effect of sudden drop of water temperature on digestibility, the first sampling time was on January 6 to 7, 2021 although the feed intake did not reach the peak. Then feces were collected on January 11-12, 16-17 and 20, respectively. In general, the feed intake of largemouth bass in each group was relatively uniform.

4.2 Apparent digestibility of dry matter

4.2.1 Apparent digestibility of dry matter of animal protein ingredients in experiment I

In animal protein ingredients, ADCs of dry matter of four fish meal ranged from 77.9% to 80.8% and did not reveal significant differences. ADCs of dry matter of four sources of poultry by-products were significantly different ($P < 0.05$). Digestibility of SGE was 96.4%, which was highest among four poultry by-products, followed by PBM, and ADCs of HFM was lowest (69.5%). In two potential alternative animal protein ingredients, dry matter digestibility of DTM was significantly lower than HSS, which were 67.0% and 77.4%, respectively. Generally, dry matter digestibility of ten animal protein ingredients ranged from 67.0% to 96.4%. ADCs of dry matter for SGE was significantly higher than that of other ingredients ($P < 0.05$), followed by PBM (84.2%), and PCM, HSS and four sources of fish meal were slightly lower than PBM but all of them had no significantly difference. ADCs of dry matter of HFM and DTM were significantly lower than that of other ingredients.

4.2.2 Apparent digestibility of dry matter of plant protein ingredients in experiment II

In four soybean products, ADCs of dry matter of SPC (71.3%) and SBM (78.0%) were significantly higher than that of FSM-TB (61.5%) and FSM-YH (63.4%). There was no significant difference among SPC and SBM, FSM-TB and FSM-YH, respectively. ADCs of dry matter of cottonseed products also had no significant difference but all of them were digested inefficiently, 55.0%-61.9%. In general, dry matter digestibility in plant protein ingredients varied greatly and SPC as well as SBM were significantly higher than those of the other plant ingredients. In addition, ADCs of dry matter of SPC and SBM had no significant difference with that of PAF (76.0%), while ADCs of dry matter of FSM-TB, FSM-YH and three cottonseed products were significantly lower than PAF.

4.3 Apparent digestibility of protein and amino acids

4.3.1 Apparent digestibility of protein and amino acids of animal protein ingredients in experiment I

In animal protein ingredients, ADCs of protein for four sources of fish meal were high (84.7%-87.0%). The only significant difference observed was for CAF which was significantly lower than other fish meals ($P < 0.05$). Protein ADCs of four poultry by-products varied greatly ($P < 0.05$). SGE was significantly higher than other ingredients, followed by PBM (88.4%) and PCM (88.6%). ADC of protein in HFM (75.1%) was significantly lower than that for other poultry by-products. For two potential alternative animal protein ingredients, protein digestibility of DTM (72.0%) was significantly lower than HSS (88.9%). In summary, a significant difference of protein digestibility was observed in the ten animal protein ingredients ($P < 0.05$), which ranged from 72.0% to 95.3%. ADCs of SGE was significantly higher than that of the other ingredients. ADCs of CMF, PBM, PCM and HSS were significantly higher than that of PAF, CSF and CAF but all of them were well digested by largemouth bass (84.7%-88.9%). The two potential protein ingredients had the lowest protein digestibility.

ADCs of amino acids in animal ingredients was similar with the ADCs of protein generally (Figure 4-3). In all animal ingredients, total amino acids digestibility of SGE by largemouth bass was the highest (94.9%), followed by PAF (90.6%), CSF (91.3%), CMF (89.8%), PBM (89.0%), and HSS (88.6%). ADCs of total amino acids of CAF (88.4%) and PCM (88.0%) were slightly lower and HFM (75.3%) and DTM (70.7%) were the lowest. On the other hand, the digestibility of essential amino acids in each ingredient were quite different. ADCs of nine essential amino acids in SGE (90.9%-98.8%) was significantly higher than that of other ingredients. ADC of amino acids in HFM and DTM were the lowest and large differences were observed, which ranged from 60.3% to 85.6% and 62.8% to 84.5%, respectively. The essential amino acids of other ingredients were well digested, and ADCs of each essential amino ranged closely. In addition, the digestibility of each ingredient also differed. ADCs of histidine (60.3%-92.2%), lysine (64.3%-96.6%) and isoleucine (69.3%-95.9%) in each ingredient ranged widely.

4.3.2 Apparent digestibility of protein and amino acids of animal protein ingredients in experiment II

The protein of the four soybean products was well digested by largemouth bass, and their ADC of protein ranged from 89.8% to 96.5%. The ADC of protein of SBM was significantly higher than that of other soybean products. There was no significant difference among ADC of protein in SPC, FSM-TB and FSM-YH. In the three cottonseed ingredients, ADCs of protein was also high, ranging from 83.1% to 85.3%, but no significant difference was observed. To sum up, ADCs of plant protein ingredients was high (83.1%-96.5%). Protein digestibility of SBM was significantly higher than that of other raw materials, followed by SPC, FSM-TB and FSM-YH. ADCs of protein of CSM, DCP-TY, DCP-JL and PAF were lowest.

ADCs of proteins in plant protein ingredients reflected the ADCs of total amino acids (Figure 4-4). The digestibility of total amino acids in SBM was still the highest (96.3%). There was no significant difference in ADCs of total amino acids of FSM-YH, SPC, FSM-TB and PAF. ADCs of total amino acids of CSM, DCP-TY and DCP-JL were the lowest but were still well digested by largemouth bass (83.9%-85.1%). The utilization of essential amino acids in each ingredient varied. ADCs of nine essential amino acids of SBM was higher than others (92.2%-99.0%). While that of CSM, DCP-TY and DCP-JL varied a lot, which were 69.3%-93.8%, 67.3%-93.8% and 71.9%-94.5%, respectively. As for the digestibility of each essential amino acid, arginine (92.9%-98.4%) and phenylalanine (88.4%-97.3%) were quite close, while those of lysine (67.3%-94.9%), methionine (69.3%-92.2%) and isoleucine (76.1%-96.7%) were highly variable.

4.4 Apparent digestibility of energy of raw materials

4.4.1 Apparent digestibility of energy of animal protein ingredients in experiment I

ADCs of energy of ten animal protein ingredients were quite different. SGE (98.6%) was almost completely digested, and the ADCs of energy was significantly higher than that in other protein ingredients of animal nature. That of CSF, CMF, PBM and PCM were slightly lower than SGE, but the values were still above 90%. ADCs of energy for PAF, CAF and HSS were around 81.8%-89.6% and that of HSS was significantly lower than those of PAF and CAF.

HFM and DTM had the significantly lowest energy ADCs, at only 77.6% and 75.6%, respectively.

4.4.2 Apparent digestibility of energy of plant protein ingredients in experiment II

Among eight protein ingredients, ADCs of energy of PAF (84.8%), SBM (82.7%) and SPC (81.4%) were significantly higher than other plant ingredients. ADCs of FSM-YH was 77.5% which was significantly higher than FSM-TB, CSM and DCP-JL and that of DCP-TY (67.0%) was lowest.

4.5 Apparent digestibility of phosphorus

4.5.1 Apparent digestibility of phosphorus of animal protein ingredients in experiment I

The phosphorus concentration in HFM, SGE, DTM and HSS were only one third of that of PAF. This made the ratio of phosphorus of these ingredients in diets only accounted for 10% and resulted in errors in calculation. Therefore, ADCs of HFM, SGE, DTM and HSS were not used for analysis. In the remaining six animal protein ingredients, only ADC of phosphorus in CMF (37.2%) and PAF (61.7%) were significantly different. ADCs of phosphorous in the six animal protein ingredients were low and those in CSF, CAF, PBM and PCM ranged from 40.2% to 51.5%.

4.5.2 Apparent digestibility of phosphorus of plant protein ingredients in experiment II

The phosphorus contents of all plant ingredients were low. This resulted in errors in calculation. Therefore, the data of ADCs in plant protein ingredients were not used for analysis.

4.6 Apparent digestibility of PAF in experiment I & II

The same reference diet was used in the two experiments. One way-ANOVA was used to test ADCs difference of PAF. The result was showed that there was no significant difference in ADC of dry matter, protein, energy and phosphorous for PAF used in two experiments (Figure 4-5).

Tables and figures:

Table 4-1 Chemical compositions and amino acids of experimental animal protein ingredients, dry matter basis.

Chemical compositions, g kg^{-1}	PAF	CSF	CAF	CMF	PBM	PCM	HFM	SGE	DTM	HSS
Dry matter	919.66	931.65	896.26	930.87	969.07	950.36	942.88	945.48	939.32	926.16
Crude protein	729.48	718.15	762.87	735.53	710.09	701.67	866.93	521.76	728.33	627.63
Crude fat	94.54	114.88	78.16	113.44	124.94	100.41	70.78	372.39	96.40	3.76
Crude ash	178.35	190.19	172.32	166.17	125.56	173.92	46.19	47.24	80.08	77.08
Phosphorus	26.21	27.84	29.45	26.19	23.48	32.54	7.37	7.87	5.61	6.34
Gross energy, MJ kg^{-1}	19.86	20.42	20.23	20.78	22.19	20.45	23.68	27.42	21.86	19.17
Amino acids, g $16gN^{-1}$										
Arg	5.47	5.48	5.40	5.59	6.42	6.41	6.60	5.89	6.17	5.51
His	3.21	2.08	2.57	2.68	1.95	2.11	1.15	2.10	1.26	2.12
Ile	3.47	3.49	3.74	3.45	3.12	3.24	4.08	4.47	2.69	3.24
Leu	6.91	6.89	7.19	6.81	6.28	6.63	7.97	8.22	6.75	6.32
Lys	7.55	7.48	7.56	7.45	6.05	6.67	2.63	6.97	6.45	5.95
Met	2.75	2.84	2.89	2.79	1.91	1.99	0.95	3.43	2.05	1.17
Phe	3.76	3.72	3.95	3.61	3.62	3.51	5.01	5.28	3.57	3.99
Thr	4.10	4.11	4.28	4.19	3.77	4.02	4.63	4.72	3.35	3.76
Val	4.27	4.21	4.50	4.16	3.88	3.82	6.03	5.67	4.07	3.72
Essential amino acids	41.50	40.31	42.10	40.74	37.00	38.40	39.05	46.75	36.37	35.77
Ala	6.00	5.94	5.95	5.95	6.32	6.31	5.08	5.66	5.82	5.62
Asp	8.63	8.73	8.97	8.66	7.73	8.11	6.79	9.76	6.47	9.73
Cys	1.67	1.61	1.70	1.75	1.95	1.80	9.36	4.14	3.54	1.97
Glu	13.64	14.52	14.23	14.45	14.10	14.71	11.91	14.31	13.75	17.78
Gly	5.70	5.85	5.39	6.09	8.91	7.96	7.59	3.21	9.56	8.15
Pro	3.55	3.92	3.62	3.80	5.82	5.60	9.51	3.86	6.75	6.21
Ser	3.88	3.75	3.99	3.97	4.01	4.04	10.69	7.40	5.80	4.51
Tyr	2.98	2.99	3.32	3.08	2.54	2.39	2.84	3.82	2.65	2.58
Total amino acids	87.54	87.61	89.26	88.48	88.37	89.32	102.82	98.90	90.71	92.32

Table 4-2 Chemical compositions and amino acids of experimental plant protein ingredients, dry matter basis.

Chemical compositions, g kg^{-1}	PAF	SPC	SBM	FSM-TB	FSM-YH	CSM	DCP-TY	DCP-JL
Dry matter	919.66	920.96	896.72	897.51	918.96	916.15	932.43	943.62
Crude protein	729.48	696.67	531.32	557.89	530.16	667.11	641.84	679.97
Crude fat	94.54	6.16	20.05	13.53	12.94	6.89	10.93	28.17
Crude ash	178.35	63.22	68.03	75.06	95.69	72.96	78.38	80.20
Phosphorus	26.21	8.15	6.68	6.84	7.16	14.35	15.18	15.82
Gross energy, MJ kg^{-1}	19.86	19.87	19.25	19.75	19.13	19.42	19.12	19.18
Amino acids, g $16gN^{-1}$								
Arg	5.47	6.97	6.90	6.65	6.68	12.00	11.89	12.23
His	3.21	2.40	2.38	2.33	2.32	2.62	2.61	2.59
Ile	3.47	3.84	3.87	3.76	3.99	2.55	2.67	2.62
Leu	6.91	7.43	7.37	7.29	7.36	5.30	5.40	5.39
Lys	7.55	6.24	6.06	5.71	5.54	4.18	4.24	4.18
Met	2.75	1.15	1.08	1.15	1.10	1.07	1.22	1.18
Phe	3.76	4.95	5.03	4.96	5.08	5.47	5.31	5.33
Thr	4.10	4.11	4.03	4.02	3.96	3.11	3.16	3.17
Val	4.27	4.11	4.12	3.95	4.15	3.72	3.87	3.83
Essential amino acids	41.50	41.21	40.83	39.82	40.17	40.01	40.37	40.53
Ala	6.00	4.00	3.99	4.26	4.14	3.46	3.46	3.37
Asp	8.63	11.26	11.22	11.35	11.17	8.84	8.85	8.96
Cys	1.67	2.64	2.63	2.73	2.72	2.80	3.23	3.26
Glu	13.64	20.36	20.38	20.32	20.25	22.30	22.19	22.39
Gly	5.70	3.97	4.01	4.00	4.06	3.77	3.81	3.80
Pro	3.55	5.00	5.08	5.00	5.04	3.65	3.57	3.53
Ser	3.88	5.43	5.35	5.28	5.17	4.32	4.35	4.40
Tyr	2.98	3.13	3.27	3.36	3.36	2.52	2.54	2.55
Total amino acids	87.54	97.00	96.75	96.12	96.06	91.67	92.38	92.78

Table 4-3 Formulation and chemical compositions of diets based on animal protein ingredients, dry matter basis.

Ingredients, g kg^{-1}	Experimental diets									
	CON	F1	F2	F3	C1	C2	C3	C4	YW	W1
Peru anchovy fishmeal[1]	836.65	585.66	585.66	585.66	585.66	585.66	585.66	585.66	585.66	585.66
China sardine fishmeal[2]		251.00								
China anchovy fishmeal[3]			251.00							
China Mackerel fishmeal[4]				251.00						
Poultry by-product meal[5]					251.00					
Poultry carcasses meal[6]						251.00				
Hydrolyzed feather meal[7]							251.00			
Spray-dried granulated inedible egg product[8]								251.00		
Defatted *Tenebrio molitor* larvae meal[9]									251.00	
Hydrolysates of stickwater and soybean[10]										251.00
Fish oil	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Soybean oil	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Tapioca	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Antioxidant	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Mold inhibitor	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Yttrium	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Premix[11]	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Choline chloride	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Total	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Chemical compositions, g kg^{-1}										
Dry matter	895.93	921.28	936.26	921.46	923.11	929.58	939.93	937.89	914.31	937.25
Crude protein	597.48	607.44	613.31	615.40	611.37	610.44	646.18	570.49	619.65	595.12
Crude fat	135.20	140.87	131.27	134.08	139.52	130.52	122.03	174.39	121.34	91.05
Crude ash	144.99	155.81	148.38	150.05	143.13	152.76	126.36	122.49	131.35	127.81
Phosphorus	20.80	22.46	22.11	21.97	22.10	24.69	19.15	18.34	18.15	16.95
Gross energy, MJ kg^{-1}	20.58	20.65	20.45	20.57	20.94	20.63	21.19	22.42	20.83	20.24

[1] Peru anchovy fishmeal, Exalmar S.A.A., Lima, Peru.

[2] China sardine fishmeal, Beihai Qunhua Industry and Trade Co., Ltd., Guangxi, China.
[3] China anchovy fishmeal, Wufeng Steamed Dry Fishmeal Factory, Zhejiang, China.
[4] China Mackerel fishmeal, Wufeng Steamed Dry Fishmeal Factory, Zhejiang, China.
[5] Poultry by-product meal, Fieldale Farms Corporation, GA, USA.
[6] Poultry carcasses meal, Guangzhou Lianmu Protein Biotechnology Co., Ltd., Guangdong, China.
[7] Hydrolyzed feather meal, Mountaire Farms, DE, USA.
[8] Spray-dried granulated inedible egg product, IsoNova Technologies LLC, MO, USA.
[9] Defatted Tenebrio molitor larvae meal, Shandong Lang's Insect Industry Co., Ltd., Shandong, China.
[10] Hydrolysates of stickwater and soybean，Guangdong VTR Bio-tech Co., Ltd., Guangdong, China.
[11] Premix contains (kg^{-1}): vitamin A, 2000000IU; vitamin D3, 100000IU; vitamin E, 20000IU; vitamin K3, 10000mg; vitamin B1, 3466mg; vitamin B2, 3734mg; vitamin B6, 2858mg; vitamin B12, 40mg; D-vitamin H, 114mg; D-vitamin B5, 12400mg; folic acid, 2000mg; nicotinamide, 20534mg; vitamin C, 20000mg; copper, 200mg; manganese, 1600mg; zinc, 10000mg; iodine, 240mg; ethoxyquin, 500mg; carrier, fine hull; Biomar Group, Aarhus, Denmark.

Table 4-4 Formulation and chemical compositions of diets based on plant protein ingredients, dry matter basis.

Ingredients	Experimental diets							
	CON	S1	S2	S3	S4	M1	M2	M3
Peru anchovy fishmeal[1]	836.65	585.66	585.66	585.66	585.66	585.66	585.66	585.66
Soy protein concentrate[2]		251.00						
Soybean meal[3]			251.00					
Fermented soybean meal-TB[4]				251.00				
Fermented soybean meal-YH[5]					251.00			
Cottonseed meal[6]						251.00		
Degossypolled cottonseed protein-TY[7]							251.00	
Degossypolled cottonseed protein-JL[8]								251.00
Fish oil	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Soybean oil	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Tapioca	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Antioxidant	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Mold inhibitor	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50

Yttrium	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Premix[9]	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Choline chloride	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Chemical compositions								
Dry matter	895.93	923.79	939.41	937.04	927.81	934.59	933.53	929.00
Crude protein	597.48	615.23	573.89	577.14	570.34	600.79	605.88	602.61
Crude fat	135.20	105.70	110.47	21.53	89.13	103.27	99.55	102.43
Crude ash	144.99	126.99	127.37	128.30	134.30	129.22	129.45	130.54
Phosphorus	20.80	18.41	18.63	18.13	17.82	18.08	19.26	18.82
Gross energy, MJ kg^{-1}	20.58	20.61	20.21	20.33	20.25	20.39	20.29	20.13

[1] Peru anchovy fishmeal, Exalmar S.A.A., Lima, Peru.
[2] Soy protein concentrate, Shandong Zhongyang Biotechnology Co. Ltd., Shandong, China.
[3] Soybean meal, Zhoushan Lianghai Grain and Oil Co. Ltd., Zhejiang, China.
[4] Fermented soybean meal-TB, Tech-Bank Food Co., LTD., Zhejiang, China.
[5] Fermented soybean meal-YH, Zhanjiang Yinheng Biological Technology Co., Ltd., Guangdong, China.
[6] Cottonseed meal, Tycoon Group Co., Ltd., Xinjiang, China.
[7] Degossypolled cottonseed protein-TY, Tycoon Group Co., Ltd., Xinjiang, China.
[8] Degossypolled cottonseed protein-JL, Xinjiang Jinlan Vegetable Protein Co., Ltd., Xinjiang, China.
[9] Premix contains (kg^{-1}): vitamin A, 2000000IU; vitamin D3, 100000IU; vitamin E, 20000IU; vitamin K3, 10000mg; vitamin B1, 3466mg; vitamin B2, 3734mg; vitamin B6, 2858mg; vitamin B12, 40mg; D-vitamin H, 114mg; D-vitamin B5, 12400mg; folic acid, 2000mg; nicotinamide, 20534mg; vitamin C, 20000mg; copper, 200mg; manganese, 1600mg; zinc, 10000mg; iodine, 240mg; ethoxyquin, 500mg; carrier, fine hull; Biomar Group, Aarhus, Denmark.

Table 4-5 Amino acid composition of diets based on animal protein ingredients, dry matter basis.

Amino acids, g $16gN^{-1}$	Experimental diets									
	CON	F1	F2	F3	C1	C2	C3	C4	YW	W1
Essential amino acids										
Arg	5.76	5.52	5.38	5.44	5.64	5.59	5.69	5.26	5.57	5.27
His	3.26	3.01	3.02	3.05	2.85	2.88	2.58	2.81	2.63	2.84
Ile	3.56	3.47	3.50	3.41	3.31	3.35	3.55	3.48	3.22	3.33
Leu	7.24	6.96	7.03	6.88	6.67	6.78	7.07	6.88	6.88	6.89
Lys	7.61	7.71	7.44	7.39	6.95	7.13	6.04	6.88	6.81	6.73
Met	2.56	2.68	2.70	2.62	2.38	2.40	2.08	2.65	2.33	2.17
Phe	4.00	4.02	3.86	3.71	3.61	3.69	4.12	3.89	3.71	3.76
Thr	4.30	4.18	4.13	4.15	4.02	4.09	4.22	4.09	3.97	4.00
Val	4.43	4.32	4.32	4.18	4.00	3.99	4.67	4.29	4.14	3.97
Essential amino acids	42.72	41.86	41.38	40.82	39.42	39.91	40.02	40.24	39.25	38.95
Non-essential amino acids										
Ala	6.15	6.03	5.91	5.96	6.14	6.12	5.78	5.75	5.82	5.77
Asp	9.08	8.79	8.70	8.64	8.33	8.46	8.00	8.49	7.97	8.68
Cys	1.94	1.65	1.80	1.64	1.80	1.74	3.56	2.26	1.76	1.74
Glu	14.77	14.18	13.96	13.97	14.03	14.15	13.30	13.47	13.67	15.00
Gly	5.90	5.77	5.49	5.70	6.53	6.17	6.27	4.97	6.62	5.97
Pro	4.05	3.93	3.77	3.77	4.33	4.29	5.89	3.69	4.57	4.37
Ser	4.26	3.93	3.94	3.95	3.95	3.95	5.65	4.51	4.64	4.09
Tyr	3.04	2.90	3.06	2.90	2.68	2.70	2.65	2.82	2.64	2.69
Total amino acids	91.90	89.03	88.00	87.36	87.20	87.49	91.12	86.21	86.95	87.27

Table 4-6 Amino acid composition of diets based on plant protein ingredients, dry matter basis.

Amino acids, g $16gN^{-1}$	Experimental diets							
	CON	S1	S2	S3	S4	M1	M2	M3
Essential amino acids								
Arg	5.76	5.72	5.67	5.74	5.61	7.29	7.14	7.26
His	3.26	2.89	2.97	3.05	2.97	3.06	2.97	3.08
Ile	3.56	3.44	3.55	3.69	3.55	3.30	3.28	3.32
Leu	7.24	6.86	7.01	7.07	6.88	6.49	6.48	6.59
Lys	7.61	6.92	7.05	7.21	6.98	6.62	6.55	6.73
Met	2.56	2.11	2.10	2.12	2.10	2.08	2.10	2.10
Phe	4.00	3.96	4.17	4.10	3.98	4.23	4.21	4.39
Thr	4.30	4.12	4.13	4.17	4.05	3.89	3.90	3.90
Val	4.43	4.15	4.10	4.22	4.09	4.14	4.15	4.14
Essential amino acids	42.72	40.18	40.75	41.39	40.20	41.10	40.78	41.52
Non-essential amino acids								
Ala	6.15	5.28	5.50	5.53	5.38	5.24	5.22	5.30
Asp	9.08	9.11	9.14	9.31	9.08	8.75	8.67	8.81
Cys	1.94	1.76	1.88	1.89	1.84	2.05	1.98	2.06
Glu	14.77	15.42	15.37	15.60	15.18	16.20	16.23	16.41
Gly	5.90	5.32	5.25	5.30	5.18	5.13	5.13	5.16
Pro	4.05	4.32	4.09	4.11	4.05	3.75	3.66	3.65
Ser	4.26	4.46	4.29	4.30	4.17	4.07	4.09	4.13
Tyr	3.04	2.71	2.86	2.89	2.81	2.71	2.64	2.74
Total amino acids	91.90	88.54	89.13	90.32	87.89	89.00	88.41	89.78

Table 4-7 Particle sizes of animal protein ingredients, μm.

Ingredients	Particle size，μm				
	Average size	Middle size	D90	D95	D97
PAF	51	40	95	127	151
CSF	56	40	116	160	190
CAF	54	38	109	151	182
CMF	58	40	124	171	203
PBM	57	41	113	157	189
PCM	57	40	123	166	195
HFM	52	37	104	145	175
DTM	62	44	130	177	208
HSS	65	48	130	180	214

Table 4-8 Particle sizes of plant protein ingredients, μm.

Ingredients	Particle size, μm				
	Average size	Middle size	D90	D95	D97
SPC	44	57	106	145	174
SBM	39	51	95	128	155
FSM-TB	43	57	107	147	177
FSM-YH	51	65	121	163	193
CSM	40	56	116	158	187
DCP-TY	41	57	114	158	188
DCP-JL	47	65	138	187	218

Table 4-9 Extrusion parameters for diets based on animal protein ingredients.

	Experimental diets									
	CON	F1	F2	F3	C1	C2	C3	C4	YW	W1
Extrusion rank	19	13	11	12	15	14	17	18	16	6
Feeder, kg h^{-1}	278.5	318.6	350.4	350.7	338.4	298.8	351.3	338.6	351.0	351.1
Water, kg h^{-1}	40.6	57.6	56.0	59.5	61.2	54.0	63.0	51.0	63.0	49.0
Water, % of mash	14.6	18.0	15.9	17.0	18.1	18.1	17.9	15.1	17.9	14.0
Steam in modulator, kg h^{-1}	34.8	36	37.2	35.1	35.0	36.3	34.4	28.5	34.1	29.8
Temperature in modulator, °C	102	93.1	88.5	91.0	92.4	92.6	96.3	93.8	96.4	97.8
Temperature of output, °C	100.1	92.0	81.0	86.0	87.0	90.0	92.0	90.0	92.0	95.0
Screw speed, rpm	343	396	386	386	396	396	396	291	396	368
Screw torque, Nm	242	268	288	272	276	274	264	213	237	272
SME, Wh kg^{-1}	31.3	34.9	32.8	31.4	33.8	38.0	31.1	19.2	28.0	29.8
Extrusion cavity 3, °C	99.9	102.7	98.8	104.3	105.3	108.6	107.9	98.3	103.7	107.6
Extrusion cavity 4, °C	111.0	113.3	108.2	111.3	114.0	116.3	115.6	109.4	115.9	115.2
Extrusion cavity 5, °C	104.4	109.3	108.9	109.6	108.6	112.9	112.0	102.8	111.2	111.6
Extrusion cavity 5, bar	4.6	8.8	8.2	8.7	8.2	7.5	8.3	9.4	6.4	8.1
Pressure in front of die, bar	8.8	12.1	11.9	12.0	11.9	12.0	11.6	11.3	9.4	11.2
Die temperature, °C	101	112	110	111	109	116	114	106	111	111
Knife speed, rpm	477	821	800	821	802	821	851	700	802	851
Bulk density, g L^{-1}	410	361	374	345	368	362	359	493	362	342

Table 4-10 Extrusion parameters for diets based on plant protein ingredients.

	Experimental diets						
	S1	S2	S3	S4	M1	M2	M3
Extrusion rank	5	2	3	4	9	8	7
Feeder, kg h^{-1}	298.8	249.2	249.1	249.1	351.2	351.2	351.1
Water, kg h^{-1}	57.0	47.5	47.5	50.0	63.0	63.0	63.0
Water, % of mash	19.1	19.1	19.1	20.1	17.9	17.9	17.9
Steam in modulator, kg h^{-1}	34.6	34.7	34.8	29.7	34.1	34.8	33.5
Temperature in modulator, °C	92.9	99.1	97.8	102.0	94.0	97.8	95.2
Temperature of output, °C	84.0	98.0	97.0	100.2	87.0	88.0	90.0
Screw speed, rpm	368	387	387	368	387	387	387
Screw torque, Nm	274	260	272	237	300	272	280
SME, Wh kg^{-1}	35.4	42.3	44.2	36.6	34.5	44.2	32.2
Extrusion cavity 3, °C	106.7	108.5	110.6	106.9	104.7	106.2	107.0
Extrusion cavity 4, °C	112.4	117.6	117.3	115.0	115.0	116.0	115.2
Extrusion cavity 5, °C	109.3	114.9	113.1	113.2	112.9	113.6	112.9
Extrusion cavity 5, bar	11.1	9.9	10.6	6.3	14.5	13	12.3
Pressure in front of die, bar	14.4	13.6	12.7	9.9	16	15.2	14.6
Die temperature, °C	108	115	113	115	116	116	116
Knife speed, rpm	700	750	750	803	802	902	851
Bulk density, g L^{-1}	376	340	345	376	368	335	346

Table 4-11 Pellet quality of animal protein diets.

Diets	Diameter, mm (n=50)	Length, mm (n=50)	Expansion, % (n=50)	Hardness, N (n=50)	PDI, % (n=2)	Bulk density before coating, g L^{-1} (n=3)	Bulk density after coating, g L^{-1} (n=3)
CON	6.39±0.06^{e}	5.92±0.09^{e}	21.35±0.78^{d}	14.98±0.49^{b}	2.67±0.01^{i}	434.81±0.93^{b}	460.95±0.59^{b}
F1	6.97±0.09^{c}	6.73±0.12^{a}	27.75±0.89^{b}	11.27±0.50^{c}	10.19±0.17^{b}	373.07±0.07^{g}	400.33±0.72^{g}
F2	6.44±0.07^{e}	6.39±0.09^{bc}	21.93±0.82^{d}	10.00±0.33^{cd}	9.95±0.02^{c}	399.20±0.94^{c}	428.45±1.05^{c}
F3	6.72±0.09^{d}	6.56±0.10^{ab}	24.96±0.96^{c}	9.29±0.31^{d}	10.91±0.05^{a}	370.29±1.00^{h}	407.70±0.80^{e}
C1	7.27±0.06^{b}	6.27±0.08^{cd}	30.99±0.51^{a}	14.06±0.38^{b}	7.90±0.05^{e}	356.31±0.69^{i}	384.80±0.88^{h}
C2	6.83±0.07^{cd}	6.04±0.07^{de}	26.42±0.69^{bc}	10.69±0.39^{cd}	6.31±0.04^{f}	389.40±0.18^{d}	414.97±0.62^{d}
C3	6.29±0.05^{e}	6.58±0.08^{ab}	20.22±0.64^{d}	10.37±0.35^{cd}	9.15±0.01^{d}	383.83±0.43^{e}	415.19±0.09^{d}
C4	6.02±0.05^{f}	6.07±0.08^{de}	16.56±0.75^{e}	14.32±0.43^{b}	3.33±0.04^{h}	532.16±0.23^{a}	572.62±0.80^{a}
YW	6.97±0.07^{c}	6.22±0.11^{cd}	27.90±0.73^{b}	18.06±0.58^{a}	4.44±0.06^{g}	377.55±1.22^{f}	403.69±0.40^{f}
W1	7.49±0.09^{a}	6.77±0.13^{a}	32.78±0.82^{a}	18.61±0.77^{a}	10.26±0.02^{b}	318.57±0.48^{j}	345.38±0.26^{i}

Data in the table are represented by means ± SE. In each row, data with different letter superscripts indicate significant differences (P<0.05).

Table 4-12 Pellet quality of plant protein diets.

Diets	Diameter, mm (n=50)	Length, mm (n=50)	Expansion, % (n=50)	Hardness, N (n=50)	PDI, % (n=2)	Bulk density before coating, g L^{-1} (n=3)	Bulk density after coating, g L^{-1} (n=3)
CON	6.39±0.06^{f}	5.92±0.09^{d}	21.35±0.78^{f}	14.98±0.49^{cd}	2.67±0.01^{h}	434.81±0.93^{a}	460.95±0.59^{b}
S1	6.56±0.06^{e}	6.62±0.08^{b}	23.48±0.68^{e}	13.64±0.36^{d}	7.34±0.00^{d}	384.34±0.56^{c}	412.84±0.43^{c}
S2	7.08±0.04^{b}	5.96±0.06^{d}	29.27±0.41^{b}	17.31±0.70^{ab}	6.09±0.02^{f}	376.90±0.64^{d}	398.37±0.73^{f}
S3	6.71±0.06^{d}	6.38±0.11^{c}	25.10±0.69^{d}	16.19±0.66^{bc}	6.36±0.11^{e}	384.60±0.48^{c}	403.69±0.43^{d}
S4	6.07±0.05^{g}	6.01±0.11^{d}	17.33±0.68^{g}	18.59±0.68^{a}	3.74±0.00^{g}	431.77±0.46^{b}	463.19±0.27^{a}
M1	6.87±0.04^{c}	6.26±0.09^{c}	27.14±0.42^{c}	16.36±0.46^{bc}	8.87±0.14^{b}	373.96±1.11^{e}	400.67±0.59^{e}
M2	7.70±0.05^{a}	6.90±0.07^{a}	34.91±0.40^{a}	14.90±0.45^{cd}	9.37±0.09^{a}	325.86±0.78^{g}	346.59±0.30^{h}
M3	7.57±0.05^{a}	6.87±0.07^{a}	33.78±0.46^{a}	14.27±0.41^{d}	8.06±0.09^{c}	330.22±0.66^{f}	350.85±0.35^{g}

Data in the table are represented by means ± SE. In each row, data with different letter superscripts indicate significant differences (P<0.05).

Table 4-13 ADCs for dry matter, crude protein, gross energy and phosphorus of animal protein ingredients in largemouth bass, %.

Ingredients	Dry matter	Crude Protein	Gross energy	Phosphorus
PAF	79.0±0.36^{c}	86.2±0.18^{cd}	86.9±0.30^{d}	61.7±1.10^{a}
CSF	80.8±0.66^{bc}	85.2±0.43^{cd}	94.0±0.90^{b}	46.0±4.57^{ab}
CAF	77.9±1.08^{c}	84.7±0.36^{d}	89.6±0.43^{c}	40.2±8.36^{ab}
CMF	78.0±0.62^{c}	87.0±0.35^{bc}	92.0±0.48^{b}	37.2±4.00^{b}
PBM	84.2±0.15^{b}	88.4±0.83^{b}	94.4±0.93^{b}	51.5±6.80^{ab}
PCM	78.2±0.62^{c}	88.6±0.91^{b}	93.2±1.08^{b}	44.9±4.64^{ab}
HFM	69.5±1.62^{d}	75.1±0.94^{e}	77.6±1.33^{f}	NA[1]
SGE	96.4±1.37^{a}	95.3±0.73^{a}	98.6±0.16^{a}	NA
DTM	67.0±2.94^{d}	72.0±0.01^{f}	75.6±0.43^{f}	NA
HSS	77.4±0.61^{c}	88.9±0.59^{b}	81.8±0.71^{e}	NA

Data in the table are represented by means ± SE. In each row, data with different letter superscripts indicate significant differences (P<0.05).

[1] NA: Not analyzed.

Table 4-14 ADCs of dry matter, crude protein, gross energy of plant-based ingredients for Largemouth bass, %.

Ingredients	Dry matter	Crude protein	Gross energy
PAF	76.0±0.81^{a}	84.1±0.35^{c}	84.8±0.52^{a}
SPC	71.3±5.72^{ab}	89.8±1.21^{b}	81.4±2.12^{ab}
SBM	78.0±3.62^{a}	96.5±0.36^{a}	82.7±1.51^{a}
FSM-TB	61.5±1.49^{c}	89.9±1.08^{b}	73.5±1.14^{cd}
FSM-YH	63.4±3.21^{bc}	92.0±1.07^{b}	77.5±1.73^{bc}
CSM	61.9±0.63^{c}	83.1±1.11^{c}	71.8±1.86^{d}
DCP-TY	55.0±0.92^{c}	83.5±0.96^{c}	67.0±1.44^{e}
DCP-JL	60.9±2.18^{c}	85.3±0.80^{c}	71.6±1.00^{d}

Data in the table are represented by means ± SE. In each row, data with different letter superscripts indicate significant differences (P<0.05).

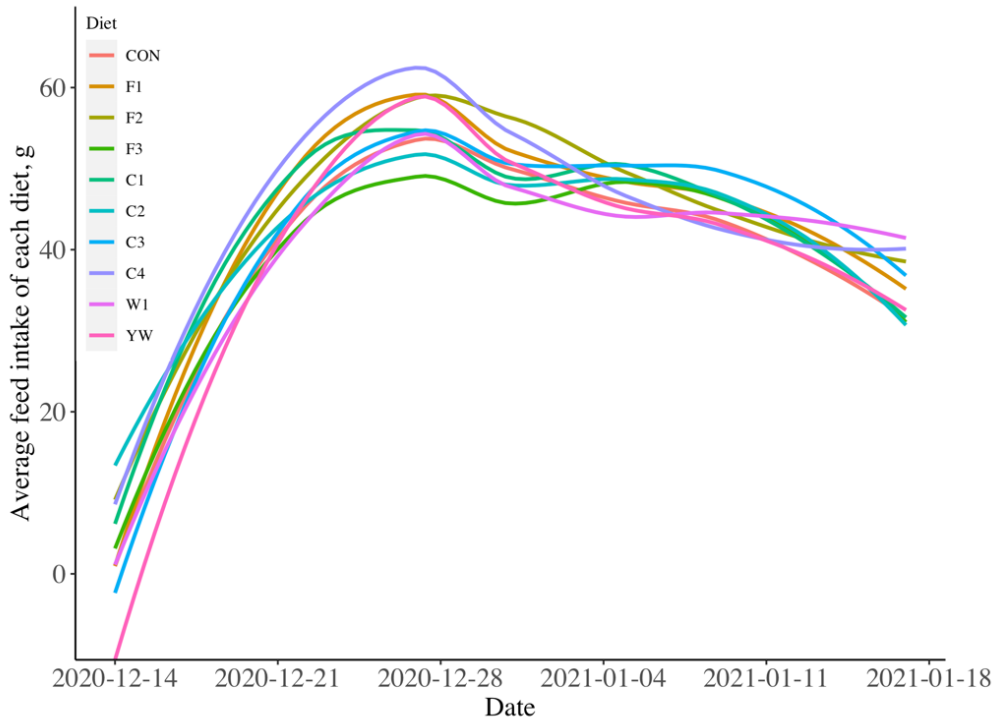

Figure 4-1 Feed intake of animal protein diets by largemouth bass.

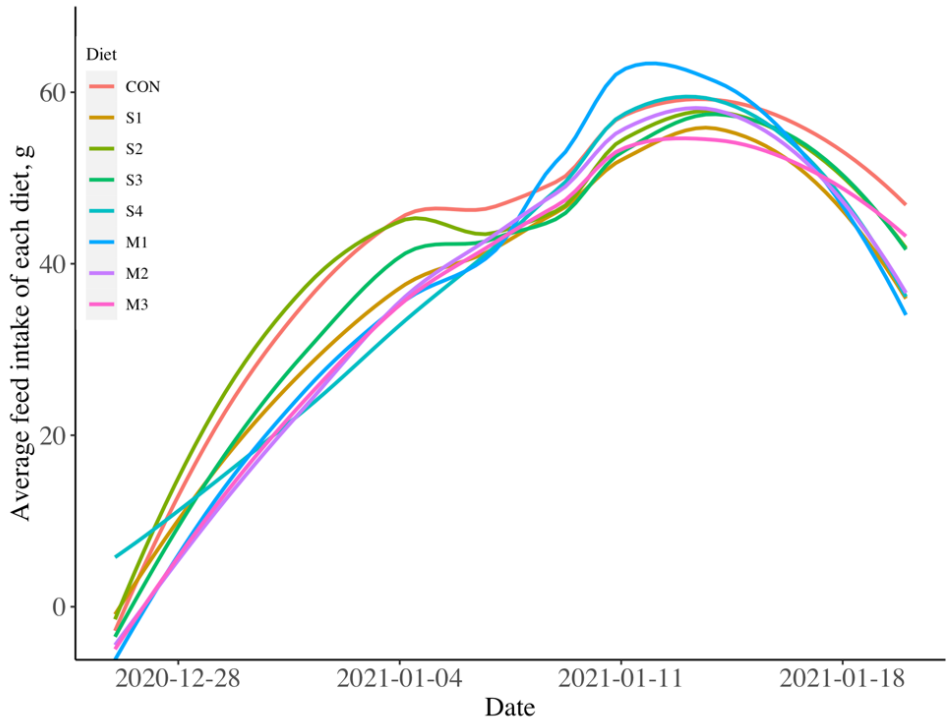

Figure 4-2 Feed intake of plant protein diets by largemouth bass.

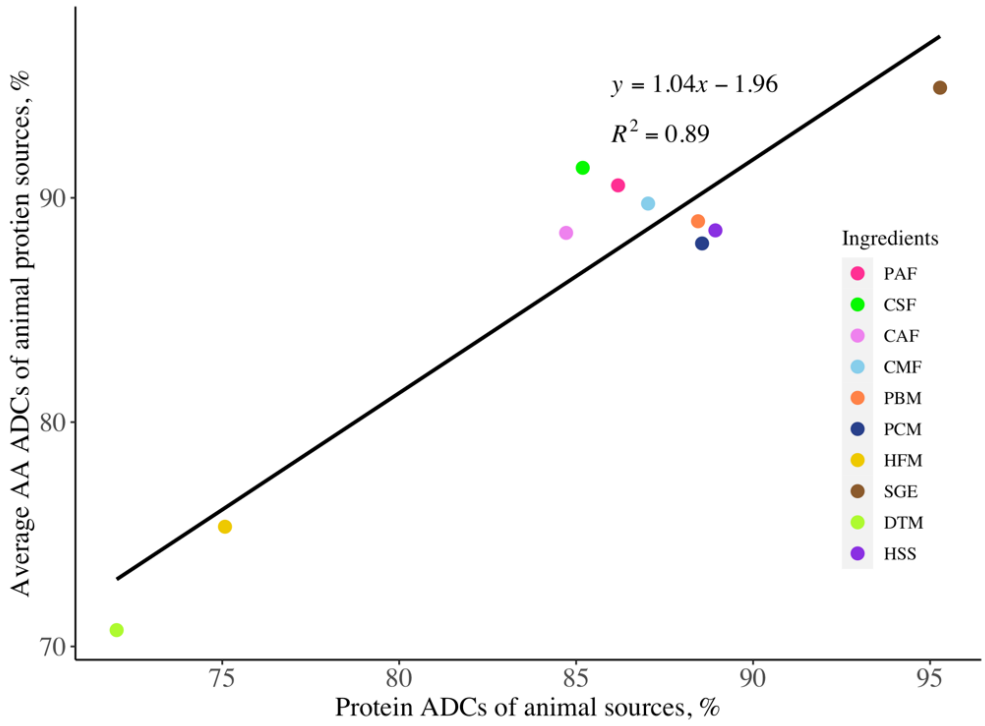

Figure 4-3 Relationships between average amino acids ADCs and protein ADCs of animal ingredients.

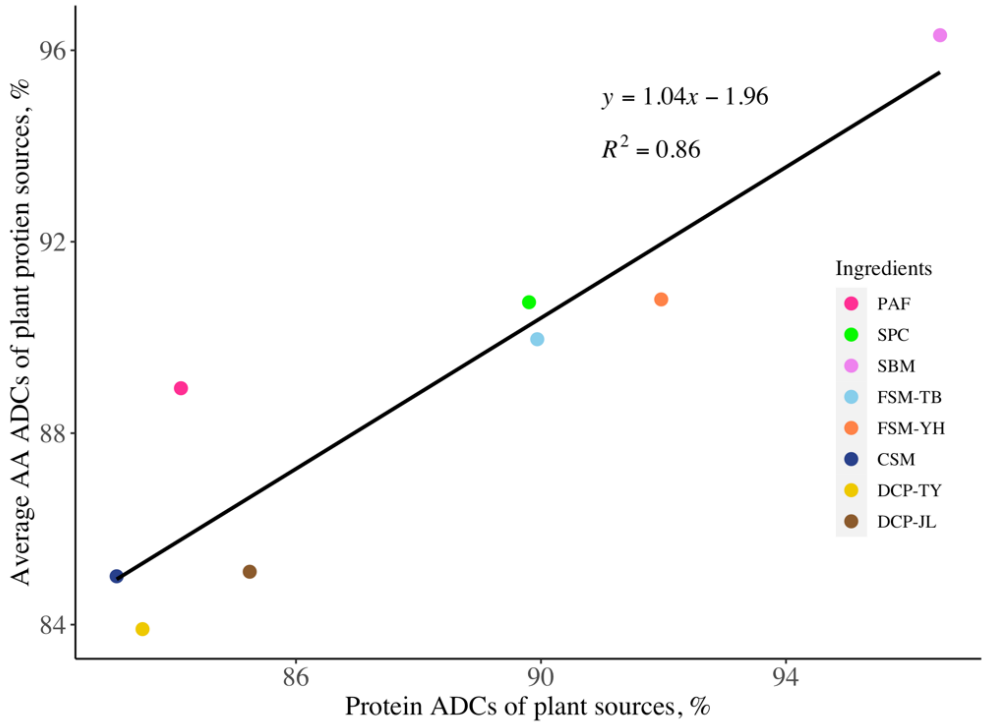

Figure 4-4 Relationships between average amino acids ADCs and protein ADCs of plant ingredients.

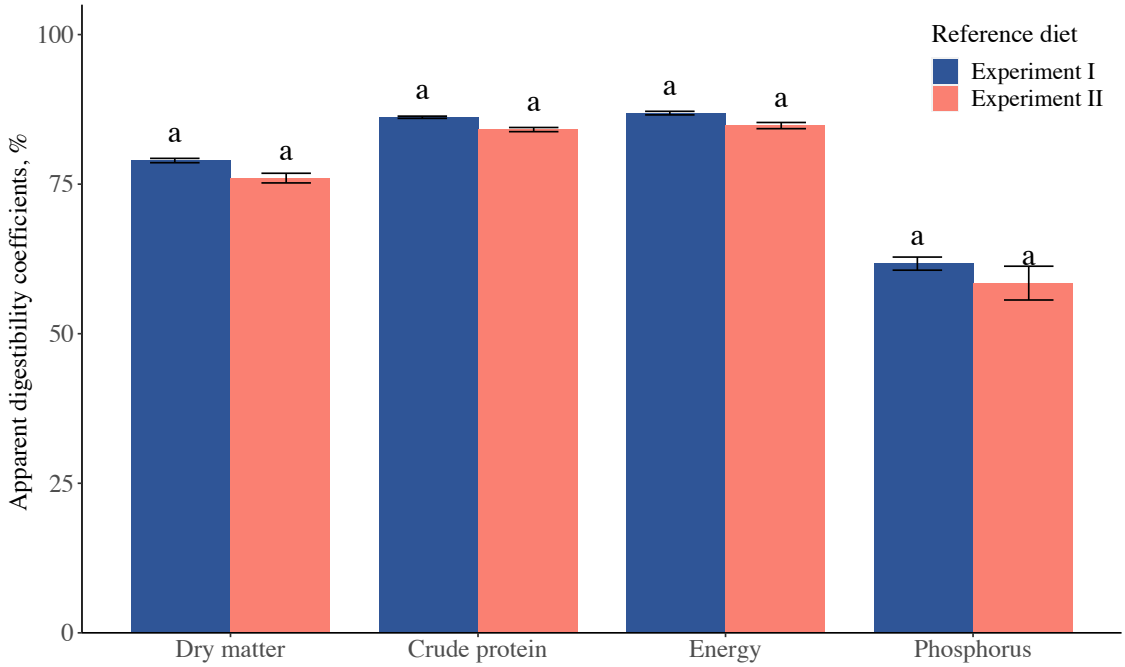

Figure 4-5 ADCs of Peruvian anchovy fishmeal used in two experiments.

Table 4-15 Amino acids ADCs of animal protein ingredients in largemouth bass, %.

	PAF	CSF	CAF	CMF	PBM	PCM	HFM	SGE	DTM	HSS
Essential amino acids										
Arg	94.1±0.14^{ab}	93.7±0.29^{bc}	92.3±0.16^{c}	93.4±0.15^{bc}	92.9±0.24^{bc}	92.2±0.52^{c}	85.6±0.50^{d}	95.5±0.55^{a}	80.2±0.37^{e}	92.2±1.15^{c}
His	90.7±0.45^{ab}	92.2±0.64^{a}	88.3±0.88^{ab}	90.2±0.96^{ab}	90.4±1.26^{ab}	87.2±0.91^{b}	60.3±3.13^{c}	90.9±1.52^{ab}	62.8±0.38^{c}	89.4±1.15^{ab}
Ile	89.1±0.24^{bc}	90.7±0.89^{b}	88.6±0.47^{bcd}	89.0±0.36^{bc}	87.2±1.22^{cd}	85.5±1.27^{d}	79.2±1.14^{e}	95.9±0.61^{a}	69.3±0.02^{f}	87.7±1.88^{bcd}
Leu	92.5±0.14^{bc}	94.1±0.59^{b}	91.0±0.25^{cd}	92.6±0.27^{bc}	91.6±0.76^{cd}	90.3±0.99^{d}	80.5±0.89^{e}	98.0±0.44^{a}	77.4±0.23^{f}	91.6±0.98^{cd}
Lys	93.1±0.20^{c}	95.9±0.38^{ab}	92.6±0.13^{c}	93.7±0.38^{bc}	92.6±0.63^{c}	92.0±0.81^{c}	64.3±1.98^{f}	96.6±0.71^{a}	80.3±0.22^{e}	87.4±1.00^{d}
Met	92.4±0.04^{c}	93.9±0.21^{bc}	92.8±0.42^{c}	93.0±0.18^{c}	95.6±0.65^{b}	92.6±0.45^{c}	74.8±1.00^{f}	98.8±0.93^{a}	84.5±1.32^{e}	89.9±1.40^{d}
Phe	91.3±0.15^{c}	93.5±0.45^{b}	89.3±0.19^{c}	90.2±0.38^{c}	90.3±0.82^{c}	89.3±0.89^{c}	81.6±0.91^{d}	97.3±0.48^{a}	76.9±0.23^{e}	91.2±1.29^{c}
Thr	89.9±0.19^{bc}	91.6±0.30^{b}	88.2±0.25^{cd}	89.8±0.54^{bc}	87.8±0.73^{cd}	88.0±0.57^{cd}	74.2±0.99^{e}	93.7±1.13^{a}	66.2±0.29^{f}	86.2±0.79^{d}
Val	90.8±0.23^{bc}	92.3±0.92^{b}	89.8±0.16^{bcd}	90.4±0.46^{bc}	88.8±0.78^{cd}	87.3±0.78^{d}	79.9±0.88^{e}	96.9±0.56^{a}	70.6±0.14^{f}	89.0±1.73^{cd}
Non-essential amino acids										
Ala	92.3±0.23^{b}	93.4±0.71^{b}	90.2±0.04^{cd}	92.0±0.15^{bc}	91.5±0.58^{bcd}	90.1±0.97^{cd}	78.5±1.20^{e}	98.1±0.49^{a}	75.8±0.05^{f}	89.9±0.66^{d}
Asp	84.5±0.47^{b}	81.9±1.00^{bc}	78.0±0.55^{c}	79.8±1.40^{c}	78.9±1.16^{c}	78.8±0.35^{c}	47.3±3.25^{d}	91.1±2.10^{a}	48.3±0.55^{d}	80.7±0.80^{bc}
Cys	75.3±0.57^{bc}	75.4±0.53^{bc}	80.1±2.16^{b}	71.5±1.28^{c}	75.3±0.53^{bc}	73.7±0.57^{c}	64.0±1.49^{d}	92.4±2.09^{a}	21.8±3.87^{e}	79.3±0.13^{b}
Glu	93.4±0.15^{bc}	94.4±0.27^{b}	91.4±0.35^{d}	93.0±0.39^{bcd}	92.2±0.34^{cd}	91.5±0.57^{d}	74.5±1.17^{e}	97.2±0.79^{a}	73.7±0.30^{e}	91.9±0.53^{cd}
Gly	87.6±0.39^{b}	86.4±1.45^{bc}	81.5±0.46^{d}	86.1±1.09^{bc}	86.5±0.37^{bc}	83.6±0.36^{cd}	76.5±0.82^{e}	91.3±2.96^{a}	72.8±0.20^{f}	85.6±0.28^{bc}
Pro	90.9±0.39^{bc}	91.8±0.84^{b}	89.0±0.25^{cd}	90.7±0.54^{bc}	89.7±0.20^{bcd}	89.8±0.12^{bc}	87.6±0.28^{d}	95.3±1.65^{a}	77.6±0.75^{e}	91.2±0.25^{bc}
Ser	89.2±0.22^{bc}	91.1±0.33^{a}	87.7±0.53^{cd}	89.3±0.56^{abc}	87.5±0.73^{cd}	87.9±0.44^{bcd}	80.2±0.73^{e}	87.1±1.05^{d}	71.8±0.28^{f}	89.7±0.36^{ab}
Tyr	89.6±0.26^{b}	89.1±0.59^{b}	88.6±0.18^{bc}	88.8±0.60^{bc}	86.1±1.33^{cd}	83.6±1.67^{d}	69.1±1.22^{e}	93.8±0.85^{a}	66.6±0.31^{e}	85.6±0.93^{d}
Total amino acids	90.6±0.21^{bc}	91.3±0.57^{b}	88.4±0.17^{d}	89.8±0.50^{bcd}	89.0±0.60^{cd}	88.0±0.61^{d}	75.3±1.09^{e}	94.9±0.98^{a}	70.7±0.32^{f}	88.6±0.65^{cd}

Data in the table are represented by means ± SE. In each row, data with different letter superscripts indicate significant differences (P<0.05).

Table 4-16 Amino acids ADCs of plant protein ingredients in largemouth bass, %.

	PAF	SPC	SBM	FSM-TB	FSM-YH	CSM	DCP-TY	DCP-JL
Essential amino acids								
Arg	92.9±0.35^{c}	95.8±0.86^{b}	98.4±0.14^{a}	95.1±1.12^{bc}	95.3±1.17^{b}	93.8±0.16^{bc}	93.8±0.43^{bc}	94.5±0.38^{bc}
His	89.2±0.21^{b}	91.8±0.84^{b}	99.0±0.50^{a}	90.4±2.30^{b}	92.2±2.46^{b}	83.3±0.40^{c}	81.8±1.50^{c}	83.4±1.26^{c}
Ile	87.0±0.53^{c}	90.7±1.08^{bc}	96.7±1.43^{a}	92.3±2.07^{abc}	94.7±1.69^{ab}	77.2±2.22^{d}	76.1±2.39^{d}	76.67±2.06^{d}
Leu	91.0±0.43^{b}	91.2±1.14^{b}	95.6±0.71^{a}	91.9±1.38^{ab}	94.8±1.35^{ab}	80.4±1.13^{c}	80.3±1.71^{c}	82.1±1.16^{c}
Lys	91.9±0.33^{a}	93.0±1.14^{a}	94.9±0.77^{a}	86.4±1.96^{b}	86.9±1.68^{b}	72.9±2.47^{c}	67.3±2.09^{d}	71.9±1.32^{cd}
Met	91.2±0.13^{a}	90.4±0.74^{a}	92.2±2.98^{a}	85.8±2.58^{a}	89.2±1.89^{a}	69.3±3.13^{b}	72.3±1.45^{b}	75.1±0.89^{b}
Phe	89.5±0.47^{cd}	92.1±0.43^{bc}	97.3±0.85^{a}	92.4±1.56^{bc}	94.3±1.64^{ab}	90.2±1.18^{cd}	88.4±1.07^{d}	88.5±0.47^{d}
Thr	88.0±0.46^{b}	88.8±1.44^{b}	95.7±0.95^{a}	88.1±2.34^{b}	89.4±2.71^{b}	78.8±1.57^{c}	78.1±1.78^{c}	77.7±1.89^{c}
Val	89.4±0.32^{a}	90.4±0.85^{a}	95.4±1.46^{a}	89.5±2.38^{a}	92.3±2.71^{a}	82.0±1.54^{b}	81.6±1.61^{b}	79.2±2.57^{b}
Non-essential amino acids								
Ala	90.9±0.48^{b}	88.0±1.97^{b}	96.4±0.68^{a}	89.5±1.34^{b}	91.0±2.08^{b}	77.4±1.11^{c}	75.3±1.95^{c}	79.3±1.29^{c}
Asp	82.1±0.18^{bc}	87.0±1.40^{b}	95.8±0.83^{a}	86.6±2.36^{b}	87.2±2.62^{b}	79.1±2.23^{c}	77.5±1.29^{c}	78.9±1.69^{c}
Cys	72.5±0.57^{bcd}	66.3±2.17^{d}	81.5±1.79^{a}	71.7±2.23^{cd}	70.0±3.86^{cd}	78.7±2.38^{ab}	74.8±0.32^{abc}	78.7±0.99^{ab}
Glu	92.1±0.34^{cd}	94.8±0.76^{b}	98.0±0.28^{a}	93.7±0.95^{bc}	93.3±1.14^{bcd}	91.4±0.50^{d}	91.3±0.49^{d}	92.3±0.58^{cd}
Gly	85.8±0.29^{b}	83.8±2.62^{bc}	96.3±0.62^{a}	82.9±1.97^{bc}	81.8±3.92^{bc}	78.2±1.71^{c}	77.1±0.99^{c}	79.3±1.84^{bc}
Pro	90.0±0.45^{bc}	92.4±0.50^{ab}	96.5±0.53^{a}	88.6±1.53^{bc}	89.0±2.05^{bc}	86.1±0.92^{cd}	83.4±0.70^{de}	81.0±2.68^{e}
Ser	87.1±0.48^{c}	91.6±1.26^{b}	97.6±0.38^{a}	91.7±1.66^{b}	92.5±2.19^{b}	84.9±0.74^{c}	85.2±1.02^{c}	85.5±1.20^{c}
Tyr	87.0±0.55^{c}	89.7±2.07^{bc}	96.7±0.45^{a}	91.4±1.85^{b}	93.7±0.61^{ab}	80.5±1.07^{d}	80.7±1.64^{d}	82.8±0.86^{d}
Total amino acids	88.9±0.34^{b}	90.7±1.10^{b}	96.3±0.64^{a}	90.0±1.62^{b}	90.8±1.79^{b}	85.0±1.09^{c}	83.9±0.97^{c}	85.1±1.04^{c}

Data in the table are represented by means ± SE. In each row, data with different letter superscripts indicate significant differences (P<0.05).

5 Discussion

5.1 Formulas of evaluating ADCs

Formulas for evaluating ingredients digestibility are divided into diet replacement method and ingredient replacement method (Aksnes et al., 1996). As mentioned above, the diet replacement means that test ingredients substitute a part of the reference diet, and the ingredient replacement method refers to the test ingredients substitute a single component in the reference diet. Therefore, they are different in calculation of digestibility.

In diet replacement method, the reference diet is replaced at 30% inclusion of test ingredients (Cho et al., 1982), and digestibility formula was:

$$ADC_I, \% = \frac{(ADC_D - 0.7 * ADC_c)}{0.3}$$

where ADC_I, ADC_D and ADC_C represent the apparent digestibility of ingredient, test diet and reference diet, respectively. However, this formula does not take actual nutrients contribution of test ingredients and reference diet into account, but simply uses ratios to calculate. In fact, the nutrients in test ingredients and reference diet varies a lot, and digestibility of ingredients is determined by protein, lipid and amino acids composition which has no direct connection with the substitution ratio. In addition, Aksnes et al. (1996) also proposed that using ingredient replacement method rather than diet replacement was more repeatable in different experiments. Therefore, the ingredient replacement method was used in this experiment.

In this experiment, reference diet was mainly composed of PAF as the sole protein source and tapioca as the sole source of starch, in order to avoid interaction between different raw materials. Each test ingredients substituted 30% PAF and the other components were completely consistent with the reference diet. The ADCs of protein, amino acids and phosphorous of test ingredients was calculated using the following formula:

$$ADC_I, \% = \frac{(ADC_D - P_c * ADC_c)}{P_I}$$

where ADC_I, ADC_D and ADC_C represent the apparent digestibility coefficients of test ingredients, test diets and PAF respectively, and P_C and P_I represent the nutrient ratios of PAF and test ingredients in test diets. ADCs of protein, amino acids and phosphorous of PAF were equal with the reference diet since PAF was the sole protein and phosphorous source in reference diet. Based on this, ADCs of test ingredients can be calculated. This formula not only measures the digestibility of ingredients in reference diet, but also considered the actual nutritional contribution of each component in the diet which ensured the reliability of the digestibility values.

However, there are also some weak points in this formula. PAF was not the sole source of dry matter and energy in reference diet. Therefore, the reference diet should be separated into two parts when calculating. Part a was the replaced part of formulation which included PAF and test ingredients. Part b was the unchanged part of formulation in all diets. The ADCs of energy and dry matter were calculated using the following formula:

$$ADC_I, \% = \frac{(ADC_D - P_b * ADC_b)}{P_a}$$

where ADC_I, ADC_D and ADC_b represent ADCs of test ingredients, test diets and part b, respectively. P_a and P_b are the energy or dry matter ratios in the diets. Ideally, the digestibility of part b in all diets should be same. Then the ranges of ADC_b can be calculated by assuming the ADC_I values from 0 to 1. For example, ADC_b of energy in all animal protein diets can be calculated by this way which ranged from 86.2% to 100%. Meanwhile, the digestibility of part b should be lower than that of part a in reference diet, and the ADCs of energy in the reference diet was 86.4 %. Therefore, the ADC_b was was found to range from 86.2% to 86.4%. Since the gap of this range was really small, then we used the ADCs of the reference diet as the ADC_b then the digestibility of each ingredient can be obtained.

5.2 Usage of inert markers

Cr_2O_3 and Y_2O_3 are widely used in most studies, but some studies have reported that Cr_2O_3 cannot be completely recovered (Austreng et al., 2000). Therefore, a high dosage of Cr_2O_3 (5-

10 g kg^{-1}) is needed in the diets in order to ensure the accuracy of results. However, high levels of Cr_2O_3 reduced the lipid content of feces and affected the intestinal flora (Ringø, 1993). Moreover, chromium is toxic even at low concentrations, and may cause allergy in some cases (Austreng et al., 2000). Y_2O_3 has the same intestinal transit rate as the other nutrients. It can be incorporated into the diets evenly even at low concentrations. Yttrium oxide does not interfere with the digestion and metabolism of animals or intestinal flora. Therefore, Y_2O_3 was used as an inert marker in this experiment.

5.3 Collection of feces

Stripping and collection from water are methods mostly used in collection feces in current studies. Stripping is a way that forced fish to excrete. But feces may be contaminated with mucus and urine in the feces. Thus, the measured digestibility may be lower than the true value. Collection from water is a way that collect the feces naturally excreted by the fish. The longer the feces in the water, the more nutrients will leach so that the measured digestibility is higher than real value. In this experiment, feces were easily broken into particles with water flow, so the feces are collected by stripping.

5.4 ADCs of dry matter

5.4.1 ADCs of dry matter of animal protein ingredients in experiment I

Dry matter digestibility provides a quantitative estimate of digested and absorbed materials. The lower the apparent digestibility, the more indigested raw materials were excreted (Li et al., 2013; Luo et al., 2008). ADCs of dry matter in SGE was 96.4% in this experiment, which was significantly higher than that of other ingredients, indicating that nutrients in SGE is readily digested by largemouth bass. ADCs of dry matter of four sources of fish meal, PBM and PCM were also high (77.9%-84.2%) but significantly lower than that of SGE. One main reason for this may be related to ash content. Ash is an insoluble content in ingredients which decreases the digestibility and the ash content of four sources of fish meal. Ash contents of PBM and PCM was four times that of SGE in this experiment. Likewise, the correlation between ash content in these ingredients and dry matter digestibility was -0.71. Similar results also found

by Rahman et al. (2016) and he pointed out that high ash content in raw materials would increase feces and cause mineral imbalance.

ADCs of dry matter for HFM (69.5%) and DTM (67.0%) were lowest in this experiment though the ash content were low. The reason for low digestibility of HFM may be related to keratin. Keratin contains strong, poorly digestible disulfide bonds, which is a main component of feather protein. Although, keratin can be hydrolyzed into available amino acids, Cheng et al. (2021) believed that hydrolysis is incomplete even with high temperature and pressure. This result was consistent with Yu et al. (2013), Chi et al. (2017), Booth et al. (2013) and Davies et al. (2009), who reported that ADC of dry matter of HFM was significantly lower than other animal ingredients. For DTM, chitin, the main composition of its carbohydrates, is may be characterized as an antinutrient which was difficult to digest and utilize for fish (Hua et al., 2019). Besides, chitin also affects the digestibility of other nutrients. Shiau & Yu (1999) and Fontes et al. (2019) all confirmed that digestibility of dry matter decreased as the content of chitin increased. Chemello et al. (2020) also indicated that the increase of chitin will significantly lower the ADC of protein for ingredients. In addition, DTM accounted for 25.1% in diet which was very high, thereby ADC of dry matter was lowest. These results indicated that HFM and DTM should not be used as the major protein sources in diets.

5.4.2 ADCs of dry matter of plant protein ingredients in experiment II

ADC of dry matter represents the overall digestion of raw materials. Low digestibility of dry matter reflects high content of indigestible substances in the raw materials (Zhang et al., 2015). Compared with ADC of dry matter in PAF, the corresponding digestibility in plant proteins was significantly lower, except for SPC and SBM. This was consistent with Lee (2002) and Lee et al. (2020) which reported that the digestibility of dry matter for animal proteins was higher than that of plant proteins by carnivorous fish, and the reason may be related to the higher content of fibre or antinutrients in plant ingredients. Zhou & Yue (2012) and Zhang et al. (2015) also reported that ADC of dry matter for plant proteins was negatively correlated with fibre content and antinutrients. Although SPC and SBM had some antinutrients, the ash content of both was significantly lower than PAF, thereby no significant difference was found in ADC of dry matter for SPC, SBM and PAF. This result is consistent with Masagounder et al. (2009), Portz et al. (2004) and Cheng et al. (2021). In general, fermented soybean has less antinutrients and the quality of ingredients is improved by hydrolysis. Li et al. (2020) and He

(2020) also approved that fermented soybean had higher ADC of dry matter than SBM. While ADC of dry matter of FSM-TB (61.5%) and FSM-YH (63.4%) were significantly lower than SBM in this experiment. The differences between here may be caused by the quality differences of raw materials since ADC of dry matter of two FSM in this experiment were significantly lower than the results reported by He (2020) and Wang et al. (2008).

There was no significant difference in ADC of dry matter among the 3 cottonseed products, but the digestibility of dry matter was low and ranged from 55.0%-61.9%. Theoretically, DCP was expected to have higher dry matter digestibility compared with CSM because DCP does not contain gossypol and the protein as well as amino acids are more efficient with low temperature processing. This lack of effect of gossypol may due to the short digestive tract in carnivorous fish where free gossypol is quickly excreted (He, 2016). Meanwhile, the fiber contents in the three cottonseed products were higher than what was found with SBM, and the amino acid compositions were unbalanced. Therefore, the digestibility of three cottonseed products was significantly lower than that of SBM. This result was consistent with Zhou & Yue et al. (2012) and Zhou et al. (2008) which proved the digestibility of CSM and DCP by tilapia and bluntnose black bream (*Megalobrama amblycephala*) had no significant difference but both lower than soybean, respectively. These results showed that SPC and SBM digested efficiently by largemouth bass and can be used as main protein sources in diets while other plant ingredients were not recommended.

5.5 ADCs of protein and amino acids

5.5.1 ADCs of protein and amino acids in animal protein ingredients

Protein quality is the main factor affecting fish growth and protein digestibility. It is also the major indicator to measure the availability of nutrients in raw materials (Yu et al., 2013). The present study showed that ADC of crude protein for SGE was significantly higher than that of other raw materials. Although the digestibility of SGE has not been previously reported in fish, the high digestibility of it had been demonstrated in other animals (Norberg et al., 2004; Zhang, S. et al., 2015). Carnivorous fish utilises fish meal efficiently, as demonstrated by crude protein digestibility ranging from 84.7% to 87.0% in the four sources of fish meal in this experiment. However, the ADC values of crude protein were lower than the values obtained in most studies

(Chi et al., 2017; Lee et al., 2020; Rahman et al., 2016; Yu et al., 2013; Zhou & Yue, 2012). The difference may be attributed to the fish species, fish meal quality, water temperature and feces collection methods. There was no significant difference in protein digestibility between PAF and the 3 Chinese fish meals. This indicates that although the chemical composition of different sources of fish meal varied, protein ADC of different sources of were quite close, and also the quality of Chinese fishmeal has improved over time. ADCs of protein for PBM, PCM and HSS in this experiment were significantly higher than that of four sources of fish meal but lower than SGE. This suggests that those ingredients were digested efficiently by largemouth bass. In addition, protein ADC values of HFM (75.1%) and DTM (72.0%) were lowest. This result is consistent with the results obtained by Yu et al., (2013) and Cheng et al., (2021) who found that ADC of HFM and DTM was about 70% and significantly lower than that of poultry by-products. Several studies indicated that the low digestibility of HFM and DTM wase related to the high content of keratin (Campos et al., 2017; Zhang, C.-x. et al., 2015) and chitin (Chemello et al., 2020; Fontes et al., 2019; Longvah et al., 2011), respectively. Unbalance amino acids in HFM may also an important factor causing low protein digestibility.

Generally, the total amino acids digestibility follows the digestibility of proteins (Rahman et al., 2016; Zhang, C.-x. et al., 2015). The relationship between them in this experiment was shown in Figure 4-3. However, digestibility of different essential amino acids in raw material varied. ADCs of nine essential amino acids in SGE were significantly higher than that of other 9 animal protein ingredients, and the digestibility of each amino acid in SGE was quite close. SGE has balanced amino acid composition and the concentration of essential amino acids is higher than fish meal. Meanwhile, SGE was produced by spray drying, which is known to protect the integrity and availability of sensitive amino acids (Sosnik & Seremeta, 2015). Therefore, amino acids in SGE utilized efficiently by largemouth bass. While the digestibility of essential amino acids for HFM and DTM was the lowest and ADC of each amino acid varied from 60.3% to 85.6% and 62.8% to 84.5%, respectively. The reason of this for HFM was caused by the high content of cysteine combined into cystine through disulfide bond, which increases the difficulty of digestion further. For DTM, high content of chitin may the key factor causing low digestibility. Chitin is a non-starch polysaccharide, which is usually considered as an antinutrient that affecting the digestibility of fish. Studies found that chitin could bind with protein strongly and affected the hydrolysis and digestion of protein and amino acids of DTM (Piccolo et al., 2017). The digestibility of each essential amino acid in all test ingredients also varied. ADC of histidine (60.3%–92.2%), lysine (64.3%–96.6%) and isoleucine (69.3%–

95.9%) varied the most. The digestibility of histidine and lysine was lowest in HFM, and that of isoleucine was lowest in DTM, while these amino acids in four sources of fish meal, PBM, PCM, SGE and HSS did not show significant difference. These results indicate the importance of balancing diets with crystalline amino acids and paying strong attention to combination value of different protein-rich feed ingredients when using HFM and DTM as protein sources.

The results showed that the four sources of fish meal, PBM, PCM, SGE and HSS could be used as main protein sources for largemouth bass in the diet, while HFM and DTM should be combined with other ingredients and balanced with crystalline amino acids when used.

5.5.2 ADCs of protein and amino acids in plant protein ingredients

Protein digestibility is the primary indicator to measure the quality of protein ingredient (Lee, 2002). In this experiment, ADCs of protein in eight ingredients were high which were 83.1% to 96.5% indicating that the protein quality of these ingredients was high. Digestibility of protein in PAF was lower than that in previous experiments (Chi et al., 2017; Glencross et al., 2005; Mo et al., 2019; Rahman et al., 2016). Also, research showed that the digestibility of fish meal was significantly higher than cottonseed products (Lee et al., 2020; Lee, 2002; Zhou & Yue, 2012). This difference may be attributed to the processing of fishmeal. In plant proteins, protein ADC of SBM has reached 96.5%, which was significantly higher than that of other ingredients (89.8%–92.0%). Although the digestibility of soybean meal was the same as that of Portz & Cyrino (2004) and Zhou et al. (2008), it was inconsistent with most studies which indicated that ADC of SBM was significantly lower than that of SPC and FSM (Dong et al., 2010; Glencross et al., 2005; Mo et al., 2019; Tibbetts et al., 2006; Zhou & Yue, 2012). In general, SPC and FSM have less antinutrients, and protein digestibility is higher due to low temperature processing and microbial fermentation. The difference between here may be related to the quality of SBM used in this experiment. The appearance of SBM in this experiment was bright yellow which indicated that heat intensity, and thereby Maillard reaction was weak during processing, thus ensuring high availability of protein. At the same time, Dersjant-Li (2002) also proposed that adult fish had stronger tolerance to antinutrients than juveniles. The digestibility of cottonseed products had no significant difference but was lower than that of soy products. This result was consistent with Zhou et al. (2008), Zhou & Yue (2012), Cheng et al. (2021and Lee et al. (2020). Although gossypol has affected digestibilities in some studies, there was no difference between CSM and DCP. The possible reason for this

may be related to the non-starch polysaccharides both in CSM and DCP which increased the viscosity of feed and reduced the digestion time, thereby reducing the effect of gossypol. Overall, the results in this experiment showed that the digestibility of protein in all plant ingredients was similar or even higher than PAF, and these plant proteins could be used as protein sources of largemouth bass diet when the substitution was 30%.

The digestibility of protein depended on the composition of amino acids. Imbalance or lack of essential amino acid will lead to the decreased of protein utilization (Lee et al., 2020). In this study, a highly positive correlation between protein ADC and amino acids ADC, as shown on Figure 4-4. But the utilization of each amino acid in plant protein ingredients were different. ADCs of nine essential amino acids in SBM was highest and quite close (92.2%-99.0%). This result indicated that amino acids in SBM were utilized efficiently by largemouth bass. ADC of essential amino acids for CSM, DCP-TY and DCP-JL were low and variable. In nine essential amino acids, ADC of arginine in CSM, DCP-TY and DCP-JL were highest, followed by phenylalanine, while ADC of lysine and methionine were the lowest. Kumar et al. (2014) suggested that gossypol in cotton product would bind with amino acids, thereby reducing its availability, especially for lysine. Overall, the ADC of amino acids for plant ingredients by largemouth bass was high, especially for soy products. For each amino acid in all ingredients, ADC of arginine (92.9% to 98.4%) and phenylalanine (88.4% to 97.3%) were the least variable. ADC of lysine, methionine and isoleucine varied the most, around 20%. The result indicated that lysine, methionine and isoleucine should be supplemented to ensure amino acid balance and improve the digestibility of feed when using soy products and cottonseed products.

5.6 ADCs of energy

5.6.1 ADCs of energy in animal protein ingredients

High positive correlation (r=0.93) was observed between the digestibility of energy and dry matter in animal protein ingredients except HSS in this experiment. Although energy value and dry matter digestibility of HSS was close to PAF, ADC of energy of HSS was significantly lower. This may be due to the chemical composition of HSS which mainly consists of carbohydrates and protein. Lipid provided more energy than carbohydrates and protein while

lipid content in PAF was much higher than that in HSS. Therefore, energy digestibility of HSS was lower than that of PAF.

The energy digestibility of HFM (77.6%) and DTM (75.6%) were lowest in this experiment, which indicates that these raw materials cannot digested efficiently by largemouth bass. The reasons for this may be related to the keratin and antinutrients, respectively. ADC of energy of PAF, CSF, CAF and CMF were high, and ranged from 86.9% to 94.0%. Similar results were also found in olive flounder, indicating that energy digestibility of fish meal ranged from 83.5% to 93.9% (Rahman et al., 2016). Digestibility of PBM and PCM were higher than fish meal in this experiment which was different from other researchers (Booth et al., 2013; Chi et al., 2017; Lee et al., 2020; Yu et al., 2013). The reason may be related to experiment fish, rearing environment and feed processing.

5.6.2 ADCs of energy in plant protein ingredients

ADCs of energy for PAF, SPC and SBM were highest in this experiment and did not show significant differences. This result was different from Glencross et al. (2005) which reported that energy digestibility of fish meal and SPC were significantly higher than that of SBM in rainbow trout. Similar result was also found in largemouth bass (Masagounder et al., 2009). The reason of this may be related to the ingredient composition and processing. ADCs of protein and amino acids of SBM was as high as PAF which indicated that the content of fibre and antinutrients was low or they did not affect the digestibility of SBM significantly. Ash content of SBM was the lowest of all ingredients, resulting this feed ingredient being highly digestible. Apart from this, the processing of SBM was mild since the appearance of it was bright yellow rather than brown. This indicates that heating was sufficient to inactivate protease inhibitors, but not excessive so that Maillard reaction or strong cysteine-binding were made. ADC of energy for FSM-YH, FSM-TB and three cottonseed products were significantly lower than SBM and SPC. This may be caused by the chemical composition and antinutrients in ingredients. Zhou et al (2008) and Zhou &Yue (2012) also illustrated energy ADC of cottonseed was lower than soybean in bluntnose black bream and hybrid tilapia.

Overall, SBM and SPC can replace fish meal perfectly when the substitution was 30%, but the digestibility of other plant ingredients cannot be used as main protein sources with 30% inclusion.

5.7 ADCs of phosphorous of six animal protein ingredients

Phosphorus is an essential mineral for fish which builds up fish bone and scales and has numerous metabolic functions. It is first limiting factor for plant production in freshwater and demands in predatory fish must be met by the diet. Therefore, phosphorus in diets to satisfy the demands of the fish is extremely important (NRC, 2011; Sugiura et al., 2004). However, high content of phosphorus in water will leads to eutrophication, so it is important to pay strong attention to ADCs of phosphorus in raw materials.

The ADCs of phosphorus of four sources of fish meal by largemouth bass ranged widely (37.2%-61.7%) and were generally low except for PAF (61.7%). Research on phosphorus digestibility of fish meal in other carnivorous fish species has been done. Luo et al. (2009) indicated that the digestibility of phosphorous of white fish meal in *Synechogobius hasta* was 63.97%. Similar result was also found by Yu et al. (2013) which illustrated ADCs of phosphorous of Peruvian fish meal in snakehead (*Ophiocephalus argus*) was 65.2%. These results were consistent with the ADC of phosphorous for PAF in this experiment but higher than that of 3 Chinese fish meal in this experiment. Li et al. (2007) measured the ADCs of phosphorus for red fishmeal, white fishmeal and meat and bone meal by large yellow croaker (*Pseudosciaena crocea*), and it was found that the digestibility of phosphorous in meat and bone meal was only 27.6%. The main reason of this was that most of the phosphorus in meat and bone meal was insoluble hydroxyphosphate lime and calcium phosphate, which was not suitable for digestion and absorption by fish. Similarly, three Chinese fish meal used in this experiment contains high amount of fish bones or shrimp and crab shells, which were water-insoluble calcium phosphate, thereby reducing the digestibility of phosphorous. The low ADC of phosphorus of PBM and PCM may also linked with this.

5.8 ADC of PAF in two experiments

No significant difference was found in ADCs of dry matter, protein, energy, phosphorous of PAF used in two experiments. This indicated that the experiment was repeatable, and that the results of ADCs were reliable.

6 Conclusion

In animal protein ingredients, ADCs of dry matter, crude protein, amino acids and energy of SGE were highest, followed by PBM and PCM, ADCs of four sources of fish meal and HSS were slightly lower. The digestibility of HFM and DTM were lowest. The results showed that all animal protein ingredients except HFM and DTM can be used as main protein sources of largemouth bass, while HFM and DTM is not recommended. Meanwhile, the digestibility of phosphorus is affected by the composition of raw materials. Insoluble hydroxyphosphate lime and calcium phosphate was not suitable for digestion and absorption by fish.

In plant protein ingredients, the apparent digestibilities were highly variable. ADCs of PAF, SPC and SBM showed no significant difference. Those of FSM-TB, FSM-YH, CSM, DCP-TY and DCP-JL were significantly lower. Therefore, SPC and SBM can replace fish meal as the main protein source for largemouth bass, while the usage of others should be limited in the diets.

There was no significant difference of PAF in reference diet tested in two experiments. This indicates that different RAS did not affect the results significantly and that the data obtained in these experiments were repeatable and reliable.

Recalculating the ADCs of animal and protein ingredients using the mean value of PAF in two experiments showed that the apparent digestibility of animal protein ingredients was significantly higher than that of most plant protein ingredients. Dry matter, protein, amino acids and energy ADC of SGE were significantly higher than other ingredients, followed by PBM, PCM, four sources of fish meal and HSS. ADC of nutrients in SPC and SBM were lower than the ingredients above. While ADC of protein and amino acids of HFM and DTM were significantly lower than that of SPC and SBM, no significant difference was found on ADCs of dry matter and energy. The apparent digestibility of FSM-TB, FSM-YH, CSM, DCP-TY and DCP-JL were the lowest.

Overall, SGE was the best performing feed ingredient among these key ingredients for largemouth bass. PBM, PCM, PAF, CSF, CAF, CMF and HSS can also be used as high-quality protein ingredients for this species. At the same time, SBM and SPC can be used as the main

protein source when the amino acids are balanced. The other ingredients tested in this project should have limited use, due to low apparent digestibilities of varies nutrients and/or energy.

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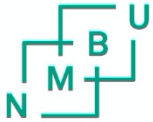

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