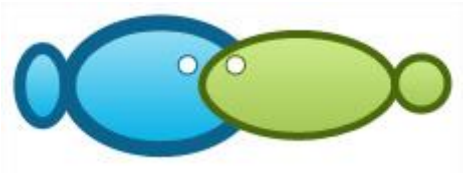

# Fish waste by-product in formulated diet for climbing perch, *Anabas testudineus*

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**Abstract**. An eight weeks experiment was conducted to investigate the effect of fish waste by-product (FWP) in formulated diet on growth, survival and feed utilization of climbing perch, *Anabas testudineus.* FWP composed mainly of visceral organ of fish, was hydrolyzed before testing in formulated diet. Three isoproteic (40%) and isolipidic (10%) diets, F0, F25 and F50, were tested: a control diet with 100% of fish meal (FM) and 0% FWP and two treatments formulated with 25% and 50% FWP inclusion, as a FM substitute. The experiment was conducted in a cemented tank where net cages were installed, each one stocked at 30 fish (initial body weight 0.86±0.10, mean±SD, n=270). Experimental diets were fed to fish in triplicate, twice daily, to apparent satiation. Fish fed with control F0 demonstrated significantly higher weight gain compared to other treatments ($P<0.05$). However, there were no significant differences on the total feed intake among the treatments. As for the feed conversion ratio, fish fed F0 performed better compared to other diets ($P<0.05$). In this trial, 100% of survival was observed in all treatments. The body proximate composition showed that fish fed with FWP diets contained higher level of protein, compared to F0. However, the lipid composition in F0 was the highest, although no significant difference was found with the treatment F25 ($P>0.05$). Fatty acids content in fish fed with FWP showed no significant difference in saturated fatty acid (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) contents, compared with F0 ($P>0.05$). In conclusion, fish fed F0 demonstrated a higher growth performance. However, a FM replacement level up to 25% of FWP did not deteriorate the growth of *A. testudineus* and showed a specific growth rate similar to F0, demonstrating its potential as feed ingredient.

**Key Words**: alternative ingredient, fish meal replacement, feed conversion ratio, protein source.

**Introduction**. Annually, the world is facing enormous challenges from the increasing number of human populations that urges to supply protein sources. In mitigating the food security risks, the fisheries industry, including aquaculture, plays an important role. Aquaculture is one of the globally predominant sectors that provide seafood as a staple diet for human consumption (Chotipontu & Avakul 2010). It is estimated that fisheries products contributed about 50% of the world protein source in 2018 (FAO 2020).

Fish meal (FM) is globally known as a conventional ingredient primarily used to provide proteins in diets (Sogbesan & Ugwumba 2008). FM is obtained from various methods of processing; cooking, pressing, drying and milling fresh meat, trash fish and fish trimmings (Einarsson et al 2019). Basically, FM can be produced using any types of seafood, but in general the processed food comes from less valuable, wild-caught, small marine fishes, considered not appropriate for human consumption as fresh meat (Schipp 2008). However, the saturation of fisheries production and the high cost of FM determined the search for alternative protein sources to replace FM.

Fish waste is one of the cost-efficient and easily obtained ingredients from the fisheries industries, not yet fully investigated and exploited (Harnedy & Fitsgerald 2012; Rana et al 2009). Fish waste generally includes internal organs, gills, head, skin and bones (Khoddami et al 2009). Studies showed that fish waste can be converted into useful ingredients that can be used in aquafeed production (Fum et al 2017).

In order to transform the fishery products into more beneficial and commercial products, additional processing is needed. Improved product can be yielded after processing operations such as hydrolysis or fermentation (Nolsøe & Undeland 2009). Hydrolysis methods like enzymatic hydrolysis are generally used as chemical approaches for protein recovery and widely utilized especially in food industry (Gao et al 2006). However, acid hydrolysis has also become one of the interesting industrial approaches, due to its benefits such as low-cost, short hydrolysis period and straightforward operation (Gao et al 2006). However, there are limited studies on the use of fish waste product (FWP) in aquaculture, after hydrolysis.

In South-East Asia and especially in Thailand, climbing perch (*Anabas testudineus*) is commonly marketed fresh and sometimes it is sold still alive. This species is very important and highly demanded due to its notable nutritive and medicinal benefits (Chotipotu & Avakul 2010; Ahmed et al 2007). It was reported that *A. testudineus* possessed a high level of iron and copper that are crucial in haemoglobin synthesis (Kumar et al 2013). Besides, this fish is also a good protein source for human consumption, as it contains a large spectrum of essential amino acids (Cappell et al 2007). This species is vulnerable in some areas lacking of established cultural practices, as it is exposed to over-fishing and pollution (Mijkherjee et al 2002). There is no available information regarding the nutritional requirement in the culture of this species (Bhaskar et al 2015).

The present study was conducted in order to determine the possibility of using FWP as an alternative protein source in the diet of *A. testudineus*, by examining the effect on growth performance, feed utilization, body indices and body composition after being fed with FWP under culture conditions.

## Material and Method

***Raw material***. In this study, fish waste by-product (FWP) was produced mainly from the discarded of visceral organs including the gut, intestines and liver. The raw material FWP was obtained from a local fish market in Kota Kinabalu, Sabah, Malaysia. The FWP were cut into small chunks, minced using meat mincer and homogenized. The homogenized FWPs were kept in frozen (-20$^{o}$C) until further treatment. In this study, acetic acid was used to hydrolyse FWP (Wisuthipaet & Kongkruang 2015).

***Treatment for FWP***. FWP was weighed to approximately 500 g in 3 L of Erlenmeyer flask (Pyrex). A volume of distilled water was added into the flask with a ratio of 1 part of distilled water to 2 parts of FWP. The mixture was stirred until homogenous. Then, 50 mL of acetic acid solution 2M were added into the mixture and the pH was recorded before autoclaving process. The mixture was autoclaved at 121$^{o}$C for 90 minutes (Wisuthipaet & Kongkruang 2015). After that, the mixture was cooled down, and the pH was recorded in order to adjust the pH to 6.00-6.50 using NaOH solution 6M. The mixture was dried in oven at 100$^{o}$C for 8 hours and subjected to proximate analysis. The analysis showed that the FWP contained 59.0±1.7%, 13.5±2.4% and 7.2±0.1% of crude protein, lipid and ash, respectively.

***Experimental diets***. The FWP was introduced into formulated diets to replace fish meal protein. Three experimental diets were formulated: a control diet (0% replacement, 100% fish meal), 25% and 50% replacement of fish meal with FWP, labeled as F0, F25 and F50 respectively. All diets were formulated to obtain 40% of crude protein (Hossain et al 2012) and 10% crude lipid (Table 1).

Table 1

Experimental diets formulation for *Anabas testudineus* 100 $g^{-1}$ (dry weight basis) and feed composition in experimental diets

| *Ingredients (g 100 $g^{-1}$)* | *Experimental diets* | | |
|---|---|---|---|
| | *F0* | *F25* | *F50* |
| Fish meal[a] | 50.2 | 37.6 | 25.1 |
| FWP[b] | - | 17.2 | 34.5 |
| Fish oil | 6.4 | 4.9 | 3.4 |
| Tapioca starch | 25.0 | 25.0 | 25.0 |
| Vitamin mixture[c] | 3.0 | 3.0 | 3.0 |
| Mineral mixture[d] | 2.0 | 2.0 | 2.0 |
| Carboxyl methylcellulose | 3.0 | 3.0 | 3.0 |
| $\alpha$-cellulose | 10.3 | 7.1 | 3.9 |
| Total | 100.0 | 100.0 | 100.0 |
| | Feed composition (%) | | |
| Protein | 38.37±0.18[a] | 36.06±0.16[b] | 37.61±0.24[c] |
| Lipid | 10.27±0.39[c] | 9.54±0.39[b] | 10.51±0.47[a] |
| Moisture | 19.60±0.37[b] | 10.08±0.12[a] | 15.05±0.03[c] |
| Ash | 17.57±0.24[a] | 21.95±0.42[b] | 24.20±0.65[c] |

[a]Fish meal: crude protein 79.65, crude lipid 7.09; [b]Fish waste product: crude protein 57.96; crude lipid 13.85; [c]Vitamin mixture (Dexchem Industries Sdn. Bhd.), containing (g $kg^{-1}$ dry weight): ascorbic acid 45 g inositol 5; choline chloride 75; niacin 4.5; riboflavin 1; pyridoxine HCl 1; thiamine HCl 0.92; dicalcium pantothenate 3; retinyl acetate 0.6; vitamin D3 0.08; menadione 1.67; dialpha tocopherol acetate 8; d-Biotin 0.02; folic acid 0.09; vitamin B12 0.001; cellulose; [d]Mineral mixture (Dexchem Industries Sdn. Bhd.), containings (g $kg^{-1}$ dry weight): calcium phosphate monobasic 270.98; calcium lactate 327; ferrous sulphate 25; magnesium sulphate 132; potassium chloride 50; potassium iodide 0.15; copper sulphate 0.785; manganese oxide 0.8; cobalt carbonate 1; zinc oxide 3; sodium selenite 0.011; calcium carbonate 129.27.

***Feeding trial***. *A. testudineus* were obtained from a fish hatchery in Khlong Hoy Khong, and the experiment was conducted in Songkhla Inland Fisheries Development and Research Centre, Khlong Hoy Khong, Hatyai, Thailand. All the fish were acclimatized in tank prior to experiment and fed with a local commercial feed (First One brand, 30% dietary protein)**.** In order to maintain a good health and prevent fungi and ectoparasite infections, the fish were given a prophylactic treatment with 3% NaCl solution for 10 seconds and sufficient amount of oxygen was supplied through artificial aeration before placing the acclimatization tank (Dey et al 2014). After 2 weeks of acclimatization, the fish were distributed into experimental cages.

The experiment was conducted in a large cemented tank (6 x 10 x 2 m) in Songkhla Inland Fisheries Development and Research Centre, Khlong Hoy Khong. Nylon net cages inside each of 1.5 x 2 x 1.5 m (length x width x depth, n=9) were installed inside the cemented tank. The water depth was maintained at a maximum of 1.2 m using an overflow PVC pipe and the outlet was covered with a fine meshed net. The fish, initially averaging a body weight of 0.86±0.10 g, were weighed and distributed randomly into the 9 cages with 30 specimens in each cage. Triplicate cages were randomly assigned to each of the dietary treatment. During the eight weeks of feeding trial, fish was hand-fed two times daily, morning and evening (08:00 and 16:00) until apparent satiation. The water quality was observed daily and maintained at acceptable range; pH 6.8-8.2, temperature 27.7-32.4$^{o}$C, dissolved oxygen 5-7.4 mg $L^{-1}$. As for the maintenance of good water quality, 10-20% of water exchange was performed daily and supplied with flow-through water (hill streamed, filtered water at an exchange rate of 5 L $mL^{-1}$) and mild aeration. Besides, a black garden net was used to cover the cages in order to prevent intrusion of predators such as birds.

***Sample and data analysis***. During the feeding trial, body weight measurement was performed biweekly, on a sample of ten specimens randomly collected from each cage, and recorded. At the end of the feeding trial, all fish were counted to calculate the survival, body weight and length were measured. For the proximate and fatty acid

biochemical analysis, ten fish specimens were sacrificed from each cage. Five more fish specimens were sampled from each cage to determine the body condition. All samples for biochemical analysis were kept in polyethylene zipped bag and preserved in -20°C for further analysis. The growth performance, feed utilization and body indices of the fish were determined using the following formulas (Ayisi et al 2017):

$$\text{Body weight gain (BWG) (\%)} = \frac{final\ body\ weigh\ (g) - Initial\ body\ weight\ (g)}{initial\ body\ weigh\ (g)} \times 100$$

$$\text{Specific growth rate (SGR) (\%)} = \frac{\ln(final\ body\ weigh\ (g)) - \ln(initial\ body\ weigh\ (g))}{Time\ (days)} \times 100$$

$$\text{Survival (\%)} = \frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish} \times 100$$

$$\text{Feed Intake (FI) (g fish}^{-1}\text{)} = \frac{Total\ feed\ intake\ for\ 8\ weeks\ (g)}{Total\ number\ of\ fish}$$

$$\text{Feed conversion ratio (FCR)} = \frac{Feed\ intake\ (g)}{Wet\ weight\ gain\ (g)}$$

$$\text{Protein efficiency ratio (PER)} = \frac{Wet\ weight\ gain\ (g)}{Total\ protein\ intake\ (g)}$$

$$\text{Net protein utilization (NPU)} = \frac{Final\ fish\ body\ protein - Initial\ fish\ body\ protein}{Total\ protein\ intake} \times 100$$

$$\text{Condition factor (CF)} = \frac{Final\ body\ weight\ (g)}{Total\ length\ (cm)^3} \times 100$$

$$\text{Hepatosomatic index (HSI)} = \frac{Liver\ weight\ (g)}{Body\ weight\ (g)} \times 100$$

$$\text{Viscerosomatic index (VSI)} = \frac{Visceral\ weight\ (g)}{Body\ weight\ (g)} \times 100$$

***Proximate analysis***. Proximate analysis, such as: ash, crude lipid and protein, were done according to AOAC (1990). The moisture content was determined by oven drying the sample at 105°C for 24 hours, and then the sample was incinerated in a muffle furnace at 550°C for 5 hours, in order to determine the crude ash content. Lipid was determined using Soxtec Lipid Analyzer (Soxtec™ 2043, Foss, Sweden). Crude protein was determined using Kjeltec Protein Analyzer (Kjeltec™ 2300, Foss, Sweden).

***Fatty acid analysis***. All samples were extracted following the modified method of Folch et al (1957) using chloroform and methanol (2:1, v/v) extraction. Sample analyses were conducted in Borneo Marine Research Institute, UMS, Sabah, Malaysia. The crude lipid was then transmethylized, following method Yoshiraka & Satoh (1989), into fatty acid methyl esters (FAME), using sodium hydroxide in methanol (NaOH-methanol) and hydrogen chloride in methanol (HCl-methanol) before analysing in a gas chromatograph (Shimadzu GC-2010, Shimadzu Corporation, Japan). The chromatograph peaks obtained from the samples were then identified by comparing the retention times of each peak with known standards of FAME (Supelco™ 37 Component FAME mix, Supelco Inc., USA).

***Statistical analysis***. All data were subjected to statistical verification by Duncan's new multiple range test, with Microsoft Excel and SPSS/PC+ software (Walpole & Myers 1978).

**Results**. The growth performance and feed efficiency of *A. testudineus* after the feeding trial is presented in Table 2. The highest percentage of weight gain (WG) was 1753.33±147.55% in treatment fed F0 diet (control), significantly higher than in other treatments ($P<0.05$). This was followed by *A. testudineus* fed F25 and F50. However, the WG percentage showed a decreasing pattern with the increasing level of FWP. Specific

growth rate (SGR) showed a similar pattern with the WG. SGR ranged from 5.26±0.35 to 4.04±0.11. The highest SGR was observed in F0 however, it was not significantly higher than in F25 (P>0.05).

As for feed utilization, no significant difference was observed between treatments and the feed intake ranged from 877.87±65.26 g to 887.93±22.72 (P>0.05). The feed conversion ratio (FCR) in this study ranged from 1.46±0.12 to 3.39±0.28. Fish fed with F0 performed significantly better than other treatments, followed by fish fed with F25, which showed a FCR significantly better compared with fish fed with F50 (P<0.05). A similar pattern was observed on protein efficiency ratio (PER). Fish fed with F0 showed a significantly higher value at 1.54±0.10 and PER in fish fed with F50 was the lowest, at 0.72±0.04 (P<0.05). In this study no mortality was recorded: the survival rates from all treatments were 100%.

Table 2

Growth performance of *Anabas testudineus* in eight weeks of feeding trial

| *Growth performance* | *F0* | *F25* | *F50* |
|---|---|---|---|
| Initial BW (g) | 0.98±0.14 | 0.88±0.08 | 0.82±0.05 |
| Final BW (g) | 18.53±1.48$^{a}$ | 12.07±0.61$^{b}$ | 7.87±0.22$^{c}$ |
| Weight gain (%) | 1753.33±147.55$^{a}$ | 1107.00±60.85$^{b}$ | 687.33±21.94$^{c}$ |
| Specific growth rate (% day$^{-1}$) | 5.26±0.35$^{a}$ | 4.686±0.08$^{a}$ | 4.041±0.11$^{b}$ |
| Total feed intake (g) | 887.93±22.72$^{a}$ | 881.43±67.11$^{a}$ | 877.87±65.26$^{a}$ |
| Feed conversion ratio | 1.46±0.12$^{b}$ | 2.30±0.07$^{b}$ | 3.39±0.28$^{a}$ |
| Protein efficiency ratio | 1.54±0.10$^{a}$ | 1.14±0.03$^{b}$ | 0.72±0.04$^{c}$ |
| Survival (%) | 100 | 100 | 100 |

*Values are expressed as mean ± SD (n=3);*Superscripts a and b denote significant differences among the treatments at p<0.05, with the Duncan test (within rows).

The results of body indices and proximate composition analysis of *A. testudineus* are presented in Table 3. The condition factor ranged from 1.93±0.07 to 2.05±0.03 without a significant difference among all the treatments. Regarding the hepatosomatic index (HSI) of this study, all treatments were not significantly different, ranging from 1.66±0.51 in the F25 diet to 2.20±0.20 in the F50 diet (P>0.05). Unlike the pattern in HSI, the viscerosomatic index (VSI) was significantly higher in the F50 diet, at 9.64±1.34 compared to the treatments F0 and F25 (P<0.05).

Table 3

Body indices and proximate composition of *Anabas testudineus* after eight weeks of feeding trial

| *Parameter* | *F0* | *F25* | *F50* |
|---|---|---|---|
| Condition factor | 2.05±0.03$^{a}$ | 1.93±0.07$^{a}$ | 1.94±0.19$^{a}$ |
| Hepatosomatic index (HSI) | 1.83±0.48$^{a}$ | 1.66±0.51$^{a}$ | 2.20±0.20$^{a}$ |
| Viscerosomatic index (VSI) | 7.71±0.38$^{b}$ | 7.20±0.63$^{b}$ | 9.64±1.34$^{a}$ |
| Protein | 50.41±0.61$^{c}$ | 52.22±0.42$^{b}$ | 56.30±0.29$^{a}$ |
| Lipid | 26.54±0.76$^{a}$ | 23.51±0.47$^{ab}$ | 14.15±3.68$^{b}$ |
| Moisture | 66.78±0.12$^{c}$ | 67.50±0.03$^{b}$ | 68.97±0.14$^{a}$ |
| Ash | 11.95±0.42$^{c}$ | 14.20±0.65$^{b}$ | 16.21±0.23$^{a}$ |

*Values are expressed as mean ± SD (n=3); *Superscripts a and b denote significant differences among the treatments at p<0.05, with the Duncan test (within rows).

The results for whole body composition of *A. testudineus* showed that protein content was significantly higher in the treatment F50 and the lowest in the treatment F0. However, opposite patterns were observed between the body protein content and the body lipid content: a significantly higher body lipid content was shown in the treatment F0, while the lowest lipid content was measured in the treatment F50 (P<0.05). Body moisture and ash contents showed a similar trend with the body protein content. The

treatment F50 showed the significant highest moisture and ash contents, and the lowest in the treatment F0 (P<0.05). Significant differences of body moisture and ash contents were observed between all the treatments.

Fatty acids compositions of FWP and whole body of *A. testudineus*, after treatment with formulated feed, are illustrated in Table 4. Generally, the major fatty acids fraction in FWP was constituted of saturated fatty acids (SFA, 46.77%), followed by mono unsaturated fatty acids (MUFA, 35.76%) and poly unsaturated fatty acids (PUFA, 21.88%). The fatty acid profile of the *A. testudineus* followed the same pattern regardless of the treatments. The different dietary treatments did not lead to significant differences between the SFA and MUFA of the body fatty acids (P>0.05). However, the treatment with F50 caused a significantly higher PUFA (P<0.05) than in other treatments. Fish under the F25 and F50 treatments contained a higher level of C18:2n6 (linoleic acid), C20:5n3 (eicosapentaenoic acid) and C22:6n3 (docosahexaenoic acid) compared to F0, while there were no significant differences between the treatments with FWP (P>0.05).

Table 4

Fatty acid composition (% of total fatty acids) in FWP and body of *Anabas testudineus*

| *Fatty acids* | *FWP* | *F0* | *F25* | *F50* |
|---|---|---|---|---|
| C12:0 | ND | 0.46±0.02 | 0.48±0.02 | 0.47±0.00 |
| C14:0 | 3.40±0.15 | 5.84±0.03 | 5.85±0.27 | 5.94±0.00 |
| C15:0 | 0.85±0.03 | 0.63±0.02 | 0.64±0.03 | 0.66±0.02 |
| C16:0 | 30.22±0.63 | 30.00±0.38 | 30.41±1.06 | 30.22±0.63 |
| C17:0 | 1.03±0.11 | 0.72±0.30 | 0.90±0.06 | 0.67±0.29 |
| C18:0 | 9.62±0.57 | 5.40±0.02 | 5.43±0.22 | 5.47±0.09 |
| C20:0 | 0.30±0.14 | 0.31±0.02 | 0.31±0.00 | 0.30±0.00 |
| C21:0 | 0.63±0.06 | ND | ND | ND |
| C22:0 | 0.22±0.05 | ND | ND | ND |
| C24:0 | 0.50±0.09 | ND | ND | ND |
| ∑ SFA | 46.77±9.85 | 43.36±8.57$^{a}$ | 44.02±8.68$^{a}$ | 43.73±8.64$^{a}$ |
| C14:1 | 0.01±0.00 | 0.25±0.01 | 0.25±0.00 | 0.91±0.08 |
| C16:1 | 3.27±0.21 | 5.72±0.29 | 6.12±0.02 | 5.90±0.50 |
| C17:1 | 0.28±0.01 | 0.26±0.01$^{a}$ | 0.09±0.00$^{b}$ | 0.16±0.15$^{ab}$ |
| C18:1n9 | 13.57±0.27 | 23.22±0.83 | 23.06±0.42 | 23.63±0.33 |
| C20:1 | 0.65±0.10 | 3.59±0.09 | 2.66±1.86 | 3.77±0.00 |
| C22:1n9 | 0.50±0.04 | 2.78±0.00 | 2.85±0.03 | 1.85±0.30 |
| C24:1 | 0.31±0.05 | 0.55±0.01 | 0.54±0.01 | 0.54±0.00 |
| ∑MUFA | 35.76±7.15 | 36.37±7.36$^{a}$ | 35.57±7.41$^{a}$ | 35.76±7.15$^{a}$ |
| C18:2n9t | 0.16±0.02 | 0.25±0.10 | 0.23±0.13 | 0.38±0.00 |
| C18:2n6c | 5.93±0.38 | 3.17±0.00$^{b}$ | 3.28±0.03$^{a}$ | 3.25±0.04$^{a}$ |
| C18:3n3 | 0.87±0.18 | 0.54±0.04 | 0.56±0.01 | 0.59±0.00 |
| C20:3n6 | ND | 0.12±0.00 | 0.14±0.00 | 0.13±0.00 |
| C20:4n6 | ND | 0.59±0.01 | 0.30±0.00 | 0.47±0.08 |
| C20:5n3 | 4.00±0.18 | 3.47±0.05$^{b}$ | 3.67±0.00$^{a}$ | 3.71±0.07$^{a}$ |
| C22:6n3 | 21.10±0.92 | 12.06±0.31$^{b}$ | 12.97±0.00$^{a}$ | 13.23±0.18$^{a}$ |
| ∑PUFA | 21.88±4.11 | 20.26±3.76$^{c}$ | 21.36±4.05$^{b}$ | 21.88±4.11$^{a}$ |

*Values are expressed as mean ± SD (n=3) except ∑, where values are expressed as sum ± S.D (n=3); *Superscripts a, b and c denote significant differences among the treatments at p<0.05, with the Duncan test (within rows); *Fatty acid values less than 0.10 were not mentioned in the table.

**Discussion**. As the human population is increasing, the demand of protein sources from seafood is also rising, indirectly contributing to the increase of waste from the fisheries industry. Recent year, FWP is gaining research interest as an alternative protein source in aquaculture (Fum et al 2017; Chitmanat et al 2009; Sotolu 2009; Obasa et al 2011). The proximate composition of this product provides primary information on its quality, through its ingredients. A protein source is characterised as protein rich when it contains

more than 35% of crude protein (NRC 2011). The present study demonstrated that FWP contained 59.0±1.7% of crude protein and 13.5±2.4% of crude lipid after being hydrolysed with acetic acid and the value was higher compared to the FWP before treatment (data not shown). FM is reported to contain 66% to 75% of crude protein and 7% to 8% of crude lipid. Rendered animal meals contain 53% to 94% of crude protein and 1% to 18% of crude lipid, while some key plant protein meals such as soybean, lupin, pea and canola meals provide 25% to 49% of crude protein and 1% to 20% of crude lipid (Glencross 2016). Although FWP contained lower crude protein levels compared with FM, it is still comparable to other protein source meals or higher than most plant protein meals. The crude protein and lipid content of FWP in the present study was almost comparable to a study that used tilapia and catfish as the main sources of fish waste meal, reporting crude protein of 61.6% and crude lipid of 9.5% (Sotolu 2009). While Obasa et al (2011) reported that fish waste meal, produced from discarded smoked bonga fish (*Ethmalosa fimbriate*) and *Sardinella* sp., contained 45.9% and 16.4% crude protein and lipid, respectively. This indicates that quality of the FWP or fish waste meal depends on the type of fish or component used for the production of the meal. The high crude protein content in FWP in this study suggested that it can be a promising ingredient to replace FM as protein source in aquaculture.

In the present study FWP was tested and partially replaced FM at 25% and 50% in diets of *A. testudineus*. Growth performance of the *A. testudineus* fed with the FWP diets did not perform as good as the fish under the control F0 diet. However, the SGR of fish fed with F25, at the end of the trial, showed no significant differences with those fed with F0, the control diet. This suggests that 25% inclusion level of FWP or below can be added into the diet for *A. testudineus*. The present study was also consistent with a study on tilapia which also suggested FWP inclusion at less than 25% to replace fishmeal (Chitmanat et al 2009), while a study on African catfish *Clarias gariepinus* (initial body weight 18.2 g), that tested FWP inclusion at 10% and 15%, showed that the fish fed 15% of FWP (meal from *Oreochromis* sp. and *Clarias* sp. as main components) performed significantly better than fish fed FWP 10% inclusion (Sotolu 2009). Another study tested on smaller size of *C. gariepinus* (initial body weight 5.2 g) showed that the inclusion of FWP (meal from discarded of *E. fimbriata* and *Sardinella* sp.) as partial replacement of fish meal at 50% and below did not affect the feed efficiency although a significantly lower growth was observed compared to a full fishmeal diet, as control (Obasa et al 2011). In a study using chicken waste meal to replace fishmeal in diet for seabass, *Lates calcarifer,* Nandakumar et al (2013) showed that the replacement level can reach 10% without significantly affecting the growth of the fish. However, a replacement level higher than 10% significantly reduced the growth of seabass juveniles. However, a study that utilized squid by-product to replace fishmeal showed that replacement of fishmeal can be effective up to 30% to 60% in diets of juvenile red sea bream (*Pagrus major*) and Japanese flounder (*Paralichthys olivaceus*) (Kader et al 2011, 2012a). It is also reported that, a combination of fish soluble, squid by-product blended with fermented soybean meal and scallop by-product blended with fermented soybean meal at a ratio of 2:1:1 respectively, enhanced the growth of sea bream at 100% fishmeal replacement (Kader & Koshio 2012b).This indicates that the utilization of FWP or other waste meals in aquafeed depends on the type of waste material used, quality of the waste meal, species of fish tested, the size of the fish and other factors.

Feed ingredients play an important role in determining in palatability of the formulated diets. Diminishing appetite of the fish can reduce the feed intake and lead to poor growth performance (Kader et al 2010, 2012a). In the present study, however, the feed intake was almost similar among the treatments without significant difference. This indicates that the fish accepted all the feed well and FWP did not cause palatability problem to the juvenile of *A. testudineus*. Despite the similar feed intake, inclusion of FWP in F25 and F50 caused lower growth and feed efficiency than in fish fed with control, as observed in the poor FCR and PER, which indicated that the amount of protein or feed consumed did not efficiently contributed to growth. Although the apparent digestibility coefficient of the nutrient is not performed in the present study, the lower growth of *A. testudineus* fed with the test diets could be due to the presence of indigestible

components in the fish waste, as dietary ingredients, directly influencing the digestible energy of the feed (Morales et al 1994). Besides, the present study showed that the VSI of fish fed F50 was significantly higher than other treatments. This could be due to high level of FWP in the diet that causing a visceral response to less digestible feed. Study on pacific tuna showed that visceral weight of fish increased in relatively to the body weight, enhancing the digestibility when feeding on extruded formulated feed which is less digestible compared to fresh feed (Kondo et al 2016). Besides, the size of the fish may be smaller in the present study (El-Sayed 1999). The digestive system of a smaller fish in general is not as developed as in bigger fish (Zambonino-Infante et al 2008). Thus, administering less digestible feed to the smaller fish increases the indigestibility of nutrients, compared to the bigger fish. In the present study, the VSI of fish fed with F25 was not significantly different from the VSI in the fish fed with the control diet. This suggests that the 25% FWP feed did not lead to significant changes on the visceral weight. Future studies can be conducted to test the effects of feeding with lower levels than 25% of FWP on bigger *A. testudineus* specimens.

The present study showed that fish fed with FWP diets contained higher body protein, compared to fish fed with the control diet. Similarly, the ash content showed higher trends in fish fed with FWP diets. This is consistent with Jamil et al (2007) that reported similar trends of body protein and ash contents under diets added with increasing levels of animal by-products in red snapper, *Lutjanus campechanus*. However, the present study showed that the increasing levels of FWP decreased the body lipid content, possibly due to a slower growth and to indigestibility issues when body lipids are used for energy and bodily functions, while retaining the body protein, thus causing a relative increase of the body protein content. The fatty acids profile was not significantly affected by the treatments, except for PUFA levels which showed higher trends in the body of fish fed with FWP diets. This could be due to the selective utilization of SFA and MUFA for energy and thus causing a relative increased of the PUFA level in fish fed with FWP diets.

**Conclusions**. In general, a FM replacement level at 25% did not deteriorate the growth of *A. testudineus*, while at a replacement level of 50% significant lower growth and feed utilization are observed. Hence, FWP at 25% or lower can be considered as a potential source of protein, able to replace fishmeal. This can reduce the dependency of fishmeal in aquafeed, simultaneously lessening the waste generated from fishery industries.

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