Research Article

Potential Enhancement of Proximate, Fatty Acid, and Amino Acid Compositions through the Hybridisation of *Pangasianodon hypophthalmus* (Sauvage, 1983) (♀) and *Pangasius nasutus* (Bleeker, 1976) (♂)

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Abstract | This study investigated the biochemical composition of a hybrid, crossbred between *Pangasianodon hypophthalmus* (PH) (♀) and *Pangasius nasutus* (PN) (♂), denoted as hybrid PH×PN, in comparison to its parents' species in terms of proximate compositions, fatty acid, and amino acid profiles. The results revealed that the biochemical composition of hybrid PH×PN was comparable with either one of its parents' species. The crude lipid and total saturated fatty acids (SFAs) were significantly higher in hybrid PH×PN and *P. hypophthalmus*. Also, crude protein and docosahexaenoic acid (22:6*n*-3, DHA) were higher in the hybrid PH×PN and *P. nasutus*. However, there were no significant differences recorded between the hybrid and its parents for omega-3 (*n*-3PUFA), total polyunsaturated fatty acids (PUFAs), and eicosapentaenoic acid (C20:5*n*-3, EPA) and (C22:5*n*-3, EPA). Likewise, the total essential amino acids (EAAs) was significantly higher in hybrid PH×PN and *P. nasutus*. In summary, certain components did not deviate much from either of their parents' species or even enhanced in the hybrid compared to *P. nasutus*. Therefore, the production of this hybrid could be an alternative to meet the market demand for *P. nasutus*.

Keywords | Amino acids, Fatty acids, Hybrid, *Pangasianodon hypophthalmus*, *Pangasius nasutus*

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INTRODUCTION

Fisheries and aquaculture represent an important source of food, nutrition and employment for millions of people in the industrialised nations as well as many of those who struggle to maintain reasonable livelihoods in resource-limited countries (Al-Rasheed et al., 2018, 2020; Klinger et al., 2017). The industry has become the fastest-growing agricultural industry due to recent advances in the genetic, nutritional as well as cultural and management systems (Small et al., 2016). The expansion of the aquaculture sector occasioned by the genetic improvements, especially in the

production of hybrid fishes with improved desirable traits like flesh quality has made investments in the sector more profitable, hence the increase in demands for fish products (Guillen et al., 2019; Supartini and Yagi, 2018).

Fish is a vital source of animal protein (Jabeen and Chaudhry, 2011). It has been recognised as one of the healthy and high-quality sources of animal protein as it contains imperative components such as low cholesterol, high omega-3 polyunsaturated fatty acid (*n*-3 PUFA), eicosapentaenoic acid (EPA: C20:5*n*-3) and docosahexaenoic acid (DHA: C22:6*n*-3) which are highly recommended for human dietary component (Wang et al., 2014; Shahidin and Ambigaipalan, 2018; Gutiérrez et al., 2019). In addition, supplementary intake of essential amino acids (EAAs) has been shown to enhance and extend health-span via multiple integrated mechanisms, which include regulation of glucose and lipid metabolism, increase mitochondrial biogenesis and energy balance and enhanced immune capabilities (Bifari et al., 2017). Some of the amino acids (i.e., glutamic acid, aspartic acid) play important role in tissue regeneration due to their roles in the inflammatory processes and synthesis of collagen (Barchitta et al., 2019; Corsetti et al., 2017).

The quality of fish flesh is influenced by internal and external factors which include genetic background, size, feeding sources, environmental conditions, temperatures, etc. (Fuentes et al., 2010). More recent attention has been focusing on the use of external methods to improve flesh quality through the supplementation of amino acids, fatty acids, or mineral elements in the diet (Wang et al., 2015; Jiang et al., 2016; Sobczak et al., 2020) or by modifying the processing or slaughters methods (Essid et al., 2020; Varga et al., 2013). Hybridisation is commonly used in different aquaculture genetic improvement programs, due to its robustness (Hulata, 2001). It is primarily being applied to address a shortage in animal protein (Monalisa et al., 2013) as well as to improve economically important traits including growth rate, disease resistance, maturation and nutritional qualities by taking advantage of different strains (Xio et al., 2019; Lhorente et al., 2019). The genetic program has been widely applied in terrestrial animals (Zhang et al., 2018; Blasco et al., 2019), hybrid fishes (Suresh, 1991; El-Hawarry, 2012; Neira et al., 2016) and crustaceans (Nolasco-Alzaga et al., 2018). Hence, it has become the most widely used genetic improvement program (So and Fortes, 2016).

This study was initiated due to the high market demand for the superior flesh of *P. nasutus*. In Malaysia, catfish from the family of Pangasidae, *Pangasius nasutus*, locally known as Patin buah has outstanding organoleptic qualities, thus commanding high price whenever it is available. The economic interest in this species has increased; nevertheless, mass production is still limited due to difficulty in production. The production of seedling from native *P. nasutus* is almost non-existent due to the lack of available broodfish. Farmers are relying heavily on the wild catch to fulfil the market demands. In the hope to produce fish of similar and/or better flesh qualities, a hybrid was introduced through artificial fertilisation from the crossing between *P. hypopthalmus* (♀) and *P. nasutus* (♂). *P. hypopthalmus* is renowned for its sought-out freshwater fishes in Southeast Asian Countries (Sriphairoj et al., 2018) owing to its outstanding breeding performances (Islam et al., 2019) and excellent flesh qualities (Orban et al., 2008). Despite the rising demands for this hybrid in local markets, the nutritional quality, particularly fatty acid and amino acid profiles, has not been documented. Therefore, this study was conducted to determine the proximate composition, fatty acids and amino acids of hybrid PH×PN in comparison with its parents' species.

MATERIALS AND METHODS

Sampling

Samples (n=5) of *P. hypophthalmus* (total length, TL: 50.67±3.76 cm; body weight, BW: 1.50±0.09 kg) and hybrid PH×PN (TL: 49.67±2.88 cm; BW: 1.41±0.02 kg) were obtained from the local breeding fish farm, while samples of cultured *P. nasutus* (TL: 51.33±4.50 cm; BW: 1.52±0.15 kg) were bought from local fishermen. Six months prior to the commencement of the experiment, both the *P. hypophthalmus*, *P. nasutus* and their hybrids were cultured in different cages in the same rearing area at the Temerloh River, Pahang, Malaysia (coordinates were 3° 27' 11" North, 102° 25' 28" East). The fishes were fed with the same commercial diet, Tilapia starter (protein: 32%; lipid: 4%) twice daily *ad libitum*.

For the experimental purpose, live fishes were packed with oxygen in plastic bags and transported in insulated iceboxes to the Aquatic Animal Health Laboratory, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. The fish were euthanized within 24 h upon arrival at the laboratory via ice-cold water (hypothermia) immersion. The fish were then gutted, washed, and filleted from head to the caudal peduncle before mincing with a grinder. On the other hand, the ground fillet was dried (at a temperature of 105°C for 48 h) for proximate analysis. Meanwhile, a 0.2 mg of ground fish was preserved at −80°C for amino and fatty acids analysis.

Determination of proximate composition

Proximate analyses for crude protein, crude lipid, moisture, and ash were carried out according to the Association of Official Analytical Chemists (AOAC) method (Latimer, 2016). Crude protein was determined by the Kjeldahl

method where the conversion factor of 6.25 was used to convert the total nitrogen (N) to crude protein (N × 6.25) and the distillation process was carried out using a Kjeltec 2400 machine with the aid of a semi-auto Kjeltec 2400 machine (Gerhard, Malaysia). The crude lipid was determined with the help of the Soxhlet apparatus using non-polar organic solvent petroleum ether. The moisture content was determined by drying the samples at 105°C until constant weight and ash content was attained, followed by further drying in a muffle furnace (Jiangsu, China) at 550°C overnight. Total carbohydrate was derived by subtracting 100% to the sum of % crude lipid (CL), % crude protein (CP), % moisture (M) and % ash (A) contents (total carbohydrate = CL-CP-M-A) (Jabeen and Chaudhry, 2011).

Fatty acid analysis

Lipids were extracted according to the method described by Folch et al. (1957). Briefly, 5.0 g of frozen samples were thawed at room temperature and homogenised in chloroform/methanol (2:1, v/v) using an Ultra-Turrax T5 FU homogenizer (IKA Analysentechnik GmBH, Germany). The mixture was evaporated under a stream of nitrogen and the lipid was determined gravimetrically. The extracted lipids were converted to fatty acids methyl esters (FAMEs) through transmethylation according to methods of Metcalfe and Schmitz (1961). In order to determine the concentration of the fatty acids, heneicosanoic acid (21:0) (Sigma®, USA) was added prior to the transmethylation process. The crude lipid extract was prepared via transmethylation with the aid of boron trifluoride after saponification with saponified with NaOH (R and M Chemicals, Essex, UK) in methanol.

Hewlett-Packard 5890 Gas Chromatography (Agilent Technologies, USA) was used to analyse the FAMEs. Ultra-pure nitrogen (99.99 %) was applied as the carrier gas (2 ml min^{-1} and split ratio was 30:1), while the column temperature was set between 100–190°C with the increment of 7.2°C min^{-1} to obtain optimal separations. Moreover, individual fatty acids were identified based on the retention times and peaks according to the chain length of a standard mix of FAs (Bellefonte, PA, USA).

Amino acids analysis

This was done according to Azilawati et al. (2015). Briefly, about 01-0.2 g of sample was thawed at room temperature. For the hydrolysis process, 5 mL of 6 N hydrochloric acid was added to the sample and incubated in an oven at 110 °C for 24 h according to Nemati et al. (2004). A high purity amino acids standard solution (Sigma Aldrich, USA) was used for the protein hydrolysis.

The mixture was then added with L-Aminobutyric acid (AABA) and further diluted with 100 ml of distilled water. A 0.45 µm cellulose acetate membrane was used to filter the solution and 10 µL of the aliquot was submitted for derivatization. Both AABA and Hyp were prepared at 2.5 Mm in 0.1 N hydrochloric acid and AABA was used as the calibration standard at each calibration point. The calibration standard solutions were prepared from the mixtures of Hyp, AABA, amino acids standard solution and deionized water to form 37.5, 100, 250, 500 and 1000 pmol/µl. A set of derivatizing reagents kits from the Waters AccQ_Fluor™ were purchased from Waters (Massachusetts, USA) and heated at 55°C for 10 min before injecting into the HPLC system (Azilawati et al., 2014).

The chromatographic separation was done in a Waters® Alliance System (2695 separations module) which is equipped with Waters 2475 Multi-k Fluorescence Detector. The column on the other hand has a holding temperature of 36 °C, with a 10 µL of injection volume. Also, a tertiary aqueous solvent (AccQ_Tag™) was applied. A mobile phase buffer (Eluent A) was purchased and used according to standards.

Statistical analysis

The collected triplicate data were expressed as mean value ± standard deviation (SD). Analysis of variance (ANOVA) was used to determine the significant difference in proximate composition, fatty acids, and amino acids analyses among the species and their hybrids. The data were tested for normality prior to statistical analysis by using Shapiro Wilks W test and homogeneity of variances was checked by Levene's test. The post-hoc under the Duncan's multiple range test was performed to ascertain any significant differences between the different species at a significant level of $P < 0.05$. Statistical analyses of the data were carried out using the SPSS software version 17.0.

RESULTS AND DISCUSSION

Proximate analysis

Table 1 shows the proximate analysis of the three species (*P. hypophthalmus*, *P. nasutus* and their hybrid. The protein content of hybrid PH×PN (25.09±0.22%) was found insignificant ($p > 0.05$) with female parent, though *P. hypophthalmus* (24.86±0.34%) was significantly higher ($p < 0.05$) than *P. nasutus* (23.55±0.24%). In a study on a similar hybrid, the protein content was found to be significantly higher ($p < 0.05$) in the hybrid (17.52±0.52%) than both of its parents' species (PH: 14.87±0.07%; PN: 15.07±0.61%) (Suryaningrum et al., 2010). Similarly, Monalisa et al. (2013) reported that higher protein content was recorded in hybrid koi (20.22%) than its native koi (18.05%). The overall, protein contents from the three species studied were found to be lower than the protein contents reported

for other freshwater fishes (Funmilayo, 2016; Zuraini et al., 2006). Traditionally, high protein content in fish flesh is determined by the nature of the feed, its availability as well as habitat (Sudirman et al., 2018).

Table 1: Proximate compositions of hybrid PH×PN and its parents species.

Proximate composition (%)	*P. hypophthalmus* (n=5)	*P. nasutus* (n=5)	Hybrid PH×PN (n=5)
Crude protein	24.86±0.34 [a]	23.55±0.24[b]	25.09±0.22 [a]
Crude lipid	3.48±0.05 [b]	4.51±0.15 [a]	4.34±0.07 [a]
Ash	1.15±0.01[a]	1.08±0.06 [a]	1.23±0.09 [a]
Moisture	67.61±0.01 [a]	67.46±0.44[a]	67.12±0.31 [a]
Carbohydrate	2.90±0.25 [b]	3.39±0.10 [a]	2.22±0.14 [b]

Results are expressed on a wet basis (%) as mean values ± standard deviation with different superscripts within the same row indicate a significant difference ($p < 0.05$).

The percentage of crude lipid of the hybrid was found to be 4.34±0.07%, an intermediate value between its parent species, which was significantly higher ($p < 0.05$) in *P. hypophthalmus* (3.48±0.05%). Nevertheless, in comparison with *P. nasutus* (4.51±0.15%), the difference was not significant ($p > 0.05$). This result is in congruence with the previous study on a similar hybrid as reported where the values were relatively lower than in the current study (Suryaningrum et al., 2010). However, a study on hybrid tilapia demonstrated no significant difference ($p > 0.05$) for lipid, protein and moisture contents to its parents' species (El-Hawarry, 2012). Comparison of crude lipid of *P. hypophthalmus* in the present study was found to be lower than that the proportion reported in Snakehead, Channa spp. studied by Ho and Paul (2009) (2.55%) and Zuraini et al. (2006) (5.7±1.9% – 11.9±4.2%). The lipid content is claimed to vary among species and can be classified into different categories according to their fat contents which are lean fish (< 2%), low (2–4%), medium (4–8%) and high fat (> 8%) (Ackman, 1990). Hence, *P. hypophthalmus* can be considered to be low fat while both the hybrid and *P. nasutus* as medium-fat content.

Water content is one of the most analyzed parameters among seafood due to its influence on the physical properties, sensorial quality, microbiological stability and shelf life of fish (Da-Silva et al., 2008). In the present study, no statistically significant difference ($p > 0.05$) was observed between these three species (67.12±0.31 - 67.61±0.01%). Larsen et al. (2010) specified that most of the moisture contents in fish flesh are above 70%, and the variability across different species can be used to indicate the composition of lipid, protein and energy in it. It is important to note that high moisture content in fish flesh is not desirable as it tends to increase the rate of spoilage by the microbial pathogens, which results in reduced

Table 2: Fatty acid profiles of hybrid PH×PN and its parents species.

Fatty acids	*P. hypophthalmus* (n = 5)	*P. nasutus* (n = 5)	Hybrid PH×PN (n = 5)
Lauric acid (C12:0)	1.72±0.26 [b]	2.92±0.27 [a]	3.46±0.26 [a]
Myristic acid (C14:0)	4.24±0.26 [a]	2.66±0.30 [b]	3.18±0.69 [b]
Palmitic acid (C16:0)	29.37±3.19 [a]	28.83±1.46 [b]	29.00±2.38 [a]
Stearic acid (C18:0)	13.42±1.30 [a]	10.91±1.67 [b]	11.14±1.17 [b]
Σ Saturated fatty acids (SFAs)	48.74±1.92 [a]	44.25±1.79 [b]	48.53±1.46 [a]
Palmitoleic acid (C16:1)	1.38±0.20 [b]	2.89±0.17 [a]	1.78±0.51 [b]
Cis-9-Oleic acid (C18:1*n*-9)	24.81±0.79 [ab]	27.16±3.00 [a]	23.36±1.42 [b]
Σ Monounsaturated fatty acids (MUFAs)	26.18±0.96 [b]	29.44±0.82 [a]	25.66±0.84 [b]
Cis-9,12-Linoleic acid (C18:2*n*-6)	10.37±0.16 [ab]	8.89±0.89 [b]	11.03±0.90 [a]
α-Linoleinic acid (C18:3*n*-3)	4.26±0.67 [b]	6.55±1.31[ab]	4.88±0.20 [a]
Arachidonic acid (C20:4*n*-6)	1.47±0.27 [b]	1.99±0.54 [ab]	2.59±0.23 [a]
cis-5,8,11,14,17-Eicosapentaenoic acid (C20:5*n*-3) (EPA)	5.51±0.85 [a]	5.65±1.63 [a]	5.72±0.24 [a]
cis-5,8,11,14,17-Eicosapentaenoic acid (C22:5*n*-3) (EPA)	0.64±0.10 [b]	0.95±0.11 [a]	0.80±0.15 [ab]
4,7,10,13,16,19-Docosahexaenoic acid (C22:6*n*-3) DHA	2.83±0.43 [a]	1.66±0.28 [b]	1.31±0.31[b]
n-6PUFA (omega-6)	11.84±0.30 [ab]	10.89±1.20 [b]	13.83±1.38 [a]
n-3PUFA (omega-3)	13.24±1.42 [a]	14.80±2.58 [a]	12.71±0.69 [a]
n-6/*n*-3	0.90±0.10 [ab]	0.75±0.15 [b]	1.27±0.26 [a]
Σ Polyunsaturated fatty acids (PUFAs)	25.08±1.52 [a]	25.69±2.98 [a]	26.32±1.66 [a]

Results are expressed in wet basis (%) as mean value ± standard deviation with different superscripts within the same row indicate significant differences ($p < 0.05$).

flesh quality (Wu et al., 2018). Meanwhile, no significant difference ($p > 0.05$) was recorded in the ash content between the hybrid and its parents' species with values ranging between 1.08±0.06–1.23±0.09%. The findings indicate a statistically significant level of carbohydrate ($p < 0.05$) in the *P. nasutus* species, which conforms with earlier reports (Funmilayo, 2016; Thammapat et al., 2010). Nevertheless, carbohydrate is less reported in fish as it usually presents very low amount to almost non-existence.

Fatty acids

A total of 12 fatty acids were found in this study across the three different species studied where saturated fatty acids (SFAs) predominated the muscle (Table 2). *P. hypophthalmus* (48.74±1.92%) and the hybrid (48.53±1.46%) showed higher SFAs compared to the *P. nasutus* (44.25±1.79%). The total SFAs of *P. hypophthalmus* in the current study is considerably higher than reported in Wangcharoen et al. (2015) on the same species (45.06%), and the value was almost comparable to Mekong giant catfish, *Pangasianodon gigas* (47.43%). In a similar study, total SFAs of *P. nasutus* was comparable to the hybrid catfish, *Pangasius larnaudii* × *P. hypophthalmus* (44.0%). The percentage of lauric acid in hybrid PH×PN (3.46±0.26%) was higher than both of its parents; however, this difference was not significant ($p > 0.05$) with *P. nasutus* (2.92±0.27%). Myristic acid was high for *P. hypophthalmus* (4.24±0.26%) as well as stearic acid (13.42±1.30%), while intermediate level was recorded for the hybrid (3.18±0.69%, 11.14±1.17%, respectively). Palmitic acid was found to be significantly lower ($p < 0.05$) in *P. nasutus* (28.83±1.46%) than hybrid PH×PN (29.00±2.38%) and *P. hypophthalmus* (29.37±3.19%).

The percentage of monounsaturated fatty acids (MUFAs) for these three species accounted for about 23–27% of the total SFAs with oleic acid, representing 90% of the total MUFAs. However, the hybrid (25.66±0.84%) and *P. hypophthalmus* (26.18±0.96%) had comparatively similar concentrations. In relation to the parent species, intermediate level of palmitoleic acid was recorded for the hybrid, while *P. nasutus* (2.89±0.17%) was significantly high ($p < 0.05$). Oleic acid is the major fraction among the MUFAs in lipids of many species in freshwater fishes and marine species (Özogul and Özogul, 2007). High levels of trans-fatty acids in the diet are unfavourable and do not have beneficial effects on low-density and high-density lipoprotein cholesterol (LDL and HDL) (Jabeen and Chaudhry, 2011).

The total PUFAs was slightly higher in the hybrid PH×PN, but the difference is not statistically significant ($p > 0.05$) compared with its parents' species with values ranging from 25.08±1.52–26.32±1.66%. However, the values were slightly lower than reported on another freshwater and marine species (Özogul and Özogul, 2007; Prato and Biandolino, 2012; Roncarati et al., 2012). Among the two important polyunsaturated fatty acids, the total *n*-6 was observed to be considerably higher ($p < 0.05$) in the *P. hypophthalmus* (11.84±0.30%) and hybrid PH×PN (13.83±0.31%). The major contributor to *n*-3 PUFAs was DHA (C22:6*n*-3), followed by EPA (C20:5*n*-3) and EPA (C22:5*n*-3). There was no statistically significant difference ($p > 0.05$) for the total *n*-3 PUFAs and EPA (C20:5*n*-3), meanwhile EPA (C22:5*n*-3) was higher in *P. nasutus* (0.95±0.11%) with no significant difference with hybrid PH×PN (0.80±0.15%) as well. The values were found to be lower than the hybrid between black-ear catfish and striped catfish (*Pangasius larnaudii* × *P. hypophthalmus*), which was to have 4.36% *n*-3 fatty acids (Wangcharoen et al., 2015). Numerous studies have documented the importance of *n*–3 PUFAs in the prevention and treatment of cardiovascular disease, inflammation, aggression, depression, hypertension, autoimmune disorders and cancer as well as playing a significant role during neural development in infants (Osendarp, 2011; Shahidi and Ambigaipalan, 2018; Tørris et al., 2018; Zhang et al., 2020).

The major components of *n*-6 PUFAs comprised of linoleic acid (C18:3*n*-3) and arachidonic acid (C20:3*n*-3) and this was found to be high in the hybrid compared to the parent species. Although the total *n*-6 PUFAs was found to be higher ($p < 0.05$) in hybrid PH×PN (13.83±1.38%), the difference was significant ($p > 0.05$) in relation to *P. hypophthalmus* (11.84±0.30%). In general, the concentration of docosahexaenoic acid (22:6*n*–3, DHA) and eicosapentaenoic acid (20:5*n*–3, EPA), which are all PUFAs, was said to be higher in marine species (Zhang et al., 2020; Tanakol et al., 1999). It is known as important dietary supplements due to the inability of human to effectively synthesize in our body (Sushchik et al., 2007). The value of *n*-6/*n*-3 ratio in hybrid PH×PN and its parents ranged between 0.75±0.15 to 1.2±0.26%. This ratio is one of the key indicators for comparing the nutritional values of different species, and the values in the present study were lower than the maximum value of 4.0 which demonstrate that these three species have lower lipid contents in reference to the recommendation of the UK Department of Health value (Pigott and Tucker, 1990).

Amino acids

As shown in Table 3, 16 amino acids comprising of 7 essential and 9 non-essential amino acids were found in this study. There was no statistically significant difference ($p > 0.05$) for all three species except for arginine, alanine, lysine, total EAAs and NEAAs. In the present study, glutamic acid was found with values ranging from 15.99–16.52%, which is comparable to other freshwater fishes (Funmilayo, 2016; Kumaran et al., 2012). Also, the composition of glutamic acid has been reported to be 60% in skeletal muscle, where it plays a significant role in

cellular proliferation following illness (Wang et al., 2014).

Table 3: Amino acid profiles of hybrid PH×PN and its parents species.

Amino acids	*P. hypophthalmus* (n = 5)	*P. nasutus* (n = 5)	Hybrid PH×PN (n = 5)
Hydroxyproline	1.48±0.76 [a]	1.25±0.50 [a]	1.46±0.22 [a]
Aspartic acid	10.68±0.57 [a]	10.94±0.43[a]	11.56±0.32 [a]
Serine	4.47±0.12 [a]	4.56±0.21 [a]	4.29±0.12 [a]
Glutamic acid	16.52±0.31 [a]	16.13±0.40[a]	15.99±0.47 [a]
Glycine	2.51±0.03 [a]	2.70±0.11 [a]	2.64±0.08 [a]
Arginine	7.81±0.24 [ab]	8.04±0.03[a]	7.29±0.07 [b]
Threonine*	4.82±0.07 [a]	4.53±0.56[a]	4.73±0.16 [a]
Alanine	6.37±0.00 [a]	5.57±0.34 [b]	5.63±0.02 [ab]
Proline	4.44±0.66 [a]	3.35±0.19 [a]	3.62±0.10 [a]
Tyrosine*	3.57±0.03 [a]	3.89±0.13 [a]	3.70±0.04 [a]
Valine*	4.88±0.13 [a]	5.17±0.23 [a]	4.99±0.11 [a]
Methionine*	3.10±0.01 [a]	3.27±0.25 [a]	3.14±0.03 [a]
Lysine*	10.11±0.08 [b]	10.94±0.50[ab]	11.56±0.22 [a]
Isoleucine*	5.27±0.11 [a]	5.32±0.23 [a]	5.27±0.16 [a]
Leunine*	8.51±0.21[a]	8.69±0.42 [a]	8.57±0.04 [a]
Phenylalanine*	4.56±0.10 [a]	4.70±0.31 [a]	4.59±0.08 [a]
ΣEAA	44.82±0.79 [b]	46.53±0.63[a]	46.56±0.76 [a]
ΣNon EAA	54.28.18±0.74[a]	52.54±0.57[b]	52.47±0.70[b]

Results are expressed in wet basis (%) as mean values ± standard deviation with different superscripts within the same row indicate significant difference ($p < 0.05$). *Essential amino acids.

The hybrid PH×PN recorded significantly lower ($p < 0.05$) arginine than in both parents. Likewise, lysine was higher in hybrid PH×PN; alanine was significantly ($p < 0.05$) highest in *P. nasutus* with hybrids showing intermediate levels of alanine compared to the parents' species. Moreover, the hybrid PH×PN and *P. nasutus* contained higher levels of total EAAs compared to *P. hypophthalmus*; while *P. nasutus* had the highest levels of glycine followed by the hybrid PH×PN. Worthy of note is that glycine is very important in tissue healing (Corsetti et al., 2017). On the other hand, lysine which is reported to have a low concentration in cereal was found to be significantly high in the hybrid PH×PN (Leinonen et al., 2019). Hence, dietary supplementation using fish sources is recommended to balance the amino acid requirement and the overall protein quality of a mixed diet in humans (Mariotti and Gardner, 2019).

Overall, the variability observed in the hybrid PH×PN can be attributed to environmental conditions like temperature and salinity of the water, the abundance of food or season of the year (Wang et al., 2014). Several studies had shown a close relationship between the diet introduced to fish where their FA profiles reflected the FA profiles of the given diet (Jobling et al., 2008; Francis et al., 2007). The differences in the fatty acid contents in this current study presumably occurred due to the variability in terms of nutrient intake of the fish, especially considering the fact that all three species studied were kept under the same environmental condition and fed the same diet. It is hypothesised that the variations in flesh are due to the rate of absorption or fish physiology which relates to its swimming behaviors, as supported by Zhao et al. (2018).

Nakamura et al. (2007) implied that physical activity is an important factor that contributes to the variation of protein and lipid portions in the fish muscle. It was observed that *P. nasutus* preferred to stay at the bottom of the cage and was less active as compared to *P. hypophthalmus* which actively swam at the upper part and bottom and occupied the space in the cage, while the hybrid stayed at the bottom of the cage. The differences in swimming behaviours may have partly contributed to the variation in the biochemical composition of each species in the present study.

Wangcharoen et al. (2015) have also suggested that the content of *n*-3 fatty acids, especially EPA and DHA in commercialised freshwater could be manipulated through feeding and hybridisation. The traditional method used by farmers for improved breeding efficiency is by selecting species with desirable traits among the same species and crossbreed them, while hybridisation is commonly used when interspecies features are desired (Hulata, 2001; So and Fortes, 2016). Besides, previous studies have demonstrated similar likelihood with the heritability compositon of fatty acids of Whiteleg shrimp, *Litopenaeus vannamei* in progeny from 79 full-sib families (Nolasco-Alzaga et al., 2018). In this study, the higher values of certain key components in hybrid PH×PN indicates that hybridisation of the two different species may contribute to enhancing certain key components in the hybrid.

CONCLUSIONS AND RECOMMENDATIONS

The present study has revealed that some components of hybrid PH×PN were intermediate and comparable to either one of its parents' species. The crude lipid and SFAs were significantly higher in hybrid PH×PN and *P. hypophthalmus*. Meanwhile, crude protein and DHA (22:6*n* -3) demonstrated significantly higher in hybrid and *P. nasutus*. Likewise, the total essential amino acid (EAAs) was significantly higher in hybrid and *P. nasutus*. However, there were no significant differences recorded between hybrid and its parents for omega-3 (*n*-3PUFA), total PUFAs, and EPA (C20:5*n*-3) and (C22:5*n*-3). These findings revealed the high values of certain important biochemical compositions of hybrid, indicated the enhancement of certain components through hybridisation. In addition,

there was no lacking important nutrients observed in the hybrid. Hybridisation may not be the only factor that contribute to the differences; however, it resulted immediate gains due to the inheritance of desirable traits in hybrid. The present study also makes a noteworthy contribution, where it would benefit the farming industry and consumers with regards to the nutritional value and possibly organoleptic quality of this hybrid. Besides that, the production of this hybrid could be an alternative to meet the market demand as well as reducing the pressure on the almost extinct *P. nasutus*. It would be very interesting to further the research by backcrossing the hybrid to either its parental line or combining hybridisation and selective breeding to see the potential of combining selective breeding and hybridisation effect on flesh quality of produced hybrids.

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NOVELTY STATEMENT

Our results make it conceivable that hybridization may have contributed to the increased concentration of crude lipid, total saturated fatty acids (SFAs), crude protein and docosahexaenoic acid which were all found to be significantly higher in hybrid.

AUTHOR'S CONTRIBUTION

Fairus: Conceptualization, writing original draft and investigation; Annie: Conceptualization, reviewing and Editing; Yuzine: Visualization and supervision; Fadhil: Visualization and supervision; Bashiru: Reviewing and editing; Zaimah: Supervision and resources; Hidayahanum: Visualization, supervision. All authors contributed in revising and have approved the article.

Ethical statement

This study was conducted according to the approval protocol of the Institutional Animal Care and Use Committee of the Universiti Putra Malaysia (AUP-R083/2017).

Conflict of interests

The authors have declared no conflict of interest.

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