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Amino acid compounds released by the giant freshwater prawn *Macrobrachium rosenbergii* during ecdysis: a factor attracting cannibalistic behaviour?*

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Abstract: Ecdysis is a common phenomenon that happens throughout the life phase of the giant freshwater prawn *Macrobrachium rosenbergii*. It is vital to better understand the correlation between cannibalism and biochemical compound that exists during the moulting process. The objective of the present study was to determine the amino acid profile released by *M. rosenbergii* during the ecdysis process that promotes cannibalism. To accomplish this, changes in amino acid levels (total amino acid (TAA) and free amino acid (FAA)) of tissue muscle, exoskeleton, and sample water of culture medium from the moulting (E-stage) and non-moulting (C-stage) prawns were analysed using high-performance liquid chromatography (HPLC). Comparison study revealed that among the TAA compounds, proline and sarcosine of tissues from moulting prawn were found at the highest levels. The level of FAA from water that contains moulting prawns (E-stage) was dominated by tryptophan and proline. Significant values obtained in the present study suggested that these amino acid compounds act as a chemical cue to promote cannibalism in *M. rosenbergii* during ecdysis. The knowledge of compositions and compounds that were released during the moulting process should be helpful for better understanding of the mechanism and chemical cues that play roles on triggering cannibalism, and also for future dietary manipulation to improve feeding efficiencies and feeding management, which indirectly impacts productivity and profitability.

Key words: Amino acid compound; Cannibalism; Chemical cue; Giant freshwater prawn; Moulting
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1 Introduction

The giant freshwater prawn *Macrobrachium rosenbergii* is an important aquatic species in Malaysia, with high domestic and global demand. Prawn aquaculture plays a pivotal role in stimulating economic growth towards attaining high income and sustainability for a few countries.

However, the output of farmed *M. rosenbergii* in Malaysia fell, while at the same time rapidly expanding in other Asian countries, particularly China, India, and Thailand. The fall in its production was due to many factors, one of which is low productivity caused by cannibalism (Azra et al., 2019). Cannibalism

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is an unwanted behaviour that is a major contributor to high mortality rate as it seriously impacts productivity and profitability especially in *M. rosenbergii*, an economically importance species in Asia (Justo et al., 1991).

Various factors influencing cannibalism include stocking densities, size heterogeneity (aggressive environment), availability of refuge, limitation of food, and attack made on prawns undergoing moulting (ecdysis) (Peebles, 1978; Marshall et al., 2005; McCallum et al., 2018). Ecdysis is a moulting process and commonly cycles happen throughout the life phases of *M. rosenbergii*. In order to increase size, prawns need to moult (shed) their confining exoskeleton. During this process, they experience fluctuations in hormone level, biochemical compounds, physiological and prawn behaviours. Romano and Zeng (2017) stated that biochemical compounds glucose (Cuzon et al., 1980), protein (Waterman, 1960), and lipid (Luvizotto-santos et al., 2003) play important roles in the moulting process, especially amino acid (protein) that is released during the moulting process, and can be a triggering factor for cannibalism (Barki et al., 2011).

According to Barki et al. (2011), during and after moulting, a variety of chemical cues are released into the water. These will stimulate chemoreceptor activity and enhance feeding behaviour, which indirectly promotes cannibalism. This is supported by Romano and Zeng (2017) indicating that chemical cues which trigger cannibalism are generated during the moulting process. Prawns which have newly moulted are particularly susceptible to cannibalism. Some crustaceans have the ability to identify the moulting status of conspecifics via a hormone, for example ecdysone. The effect of hormones during the moulting process has been well studied, with a number of studies finding the relationship between moulting and hormones. Studies have shown that moulting crustaceans are controlled by moult-inhibiting hormone (MIH) (Skinner, 1985; Chang et al., 1993; Lachaise et al., 1993; Gäde and Marco, 2006) and crustacean hyperglycemic hormone (CHH) (Webster and Keller, 1986; Yasuda et al., 1994; Sefiani et al., 1996; Zarubin et al., 2009). However, most studies only focused on the hormone rather than biochemical compounds, especially free amino acids (FAAs) (odours), which may also influence agonistic/aggressive behaviour (Hay, 2011).

Studies have revealed the proximate composition of *M. rosenbergii* in the wild, particularly that from muscle samples in natural culture environments (Bhavan et al., 2010). The utilization of FAAs is relatively different among each species. Nonetheless, several amino acids including glycine, glutamine, proline, and alanine are frequently found in crustacean muscle (Abe et al., 1999; Fujimori and Abe, 2002; McNamara et al., 2004; Augusto et al., 2007a, 2007b, 2009; de Faria et al., 2011). Consequently, biochemical compounds directly promote cannibalism. This could clearly have a destructive effect on aquaculture production, resulting in unprofitability (Romano and Zeng, 2017). There has been limited research on the chemical cue/communication in the area of crustacean aquaculture, and limited information is available on the impact of amino acid content on cannibalism during the moulting process of *M. rosenbergii*.

Despite the growing evidence for amino acid profiling of *M. rosenbergii*, there remains a significant research gap in terms of differences with regard to the amount of amino acids released during the moulting stages. Therefore, the present study was conducted to determine the amino acid profile released by *M. rosenbergii* during the ecdysis process. Knowledge of biochemical compounds during the moulting process is important for an understanding of cannibalism. In addition, current knowledge gaps on the roles of chemical cues that play on triggering cannibalism from an aquaculture perspective is very limited and no report or published work has been found so far on the relationship between cannibalism and biochemical compounds during this moulting process.

2 Materials and methods

2.1 Animals, rearing and sampling

Fully grown *M. rosenbergii* with body weight range from 24.83 to 46.52 g were sampled from a commercial farm situated at Negeri Sembilan, Malaysia. The prawns were then transported to the marine hatchery at the Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu, Terengganu, Malaysia. All prawns were subjected to two weeks of maintenance process in stocking tanks to enable acclimation to current surroundings at 27–29 °C. After that, prawns were maintained individually in a

static aquarium (volume: 25 L, 28 cm (height)×45 cm (length)×20 cm (breadth)) and fed twice a day (08:00 and 16:00) with commercial finisher pellets (35% (mass fraction) protein) at a rate of 10% total body weight, for moulting stage determination.

2.2 Determination of moulting stage

The determination of moulting stage was made as observable characteristics according to the method described by Kamaruding et al. (2017). The 3rd pleopod was cut and mounted in distilled water on a glass slide. Transparent appendage and developing setae (setogenesis) were chosen as detectable features. Microscopic examination (10× magnification) of a single exopodite from the 3rd pleopod of the prawns was performed to analyze the moulting stages.

2.3 Determination of amino acid profile

To identify and characterize the potential chemical compounds from the moulting prawn which gave cues that lead to cannibalism, amino acids of muscle tissue, exoskeleton, and culture water samples from moulting (E-stage, ecdysis) and non-moulting (C-stage, inter-moulting) prawns were examined. In brief, prawns at the C-stage and D_1-stage (pre-moulting) were cultured separately in a static aquarium with 5 L of water. It should be noted that the prawn at the C-stage was used as a control in this study. Each day for one week, 50% of the water in the aquarium was changed and there was no food supply throughout the experiment. The aquarium with the D_1-stage prawn was watched carefully up to moult. The exoskeleton and muscles from prawn which has newly moulted (E-stage) were immediately extracted from aquariums and frozen in ice instantly. Subsequently, the harvested tissue was stored at −80 °C. Next, 500 mL of culture water samples in the moulting and non-moulting aquariums were filtered and kept at −80 °C for total amino acid (TAA) and at 4 °C for FAA for subsequent analysis. The concentrations of TAA and FAA in moulting and non-moulting prawns (five each group) were quantified. Collected tissues and water samples were freeze-dried in a vacuum freeze-dryer until used.

2.3.1 Determination of total amino acids

The compositions of TAAs in tissue muscle, exoskeleton, and culture water of the freeze-dried samples were extracted based on the method described by Liu and Boykins (1989). Oxidations of cysteine and methionine were performed using performic acid prior to the protein hydrolysis. Performic acid (CH_2O_3) is an oxidation solution that was prepared raw solution through mixing formic acid (CH_2O_2) with 30% hydrogen peroxide (H_2O_2) (9:1, volume:volume ratio), which was then incubated at room temperature for 1 h. A total of 55 μg of powdered sample was dissolved in 20 μL of formic acid, heated at 50 °C for 10 min, and then 100 μL of the oxidation solution was added to the mixture. The oxidation was carried out for 30 min. The excess reagent was removed from the sample in a vacuum centrifuge. The oxidation proteins were acid-hydrolyzed with 20 mL of 5.7 mol/L hydrochloric acid (HCl, as hydrolysis solution) and 200 μL of hydrolysis solution was added to 500 μg of sample in a hydrolysis tube. The sample tube was sealed after substituting oxygen with nitrogen gas. The sample was kept in the oven for 90 min at 150 °C. Then the collected solution sample was filtered through a 25-μm membrane filter and injected into a high-performance liquid chromatography (HPLC) for the quantification of TAA.

TAA tryptophan is unstable for acid digestion in the presence of trace amounts of oxygen. Thus, a separate analysis for TAA tryptophan was estimated using gas-phase hydrolysis (Yano et al., 1990). Tryptophan oxidation at the time of hydrolysis was precluded using thioglycolic acid (TGA) as the reducing acid. This hydrolysis solution consists of 7 mol/L hydrochloric acid, 10% trifluoroacetic acid, 20% TGA acid, and 1% phenol. A total of 50 μg of the freeze-dried sample was placed in a big tube with 200 μL of hydrolysis solution. The tube was sealed in vacuum (approximately 50 μm of mercury) to vaporize the TGA. Later, the tube was heated to 166 °C for 30 min. After hydrolysis, the tube was dried in vacuum for 5 min to remove the residual acid. Then the collected solution sample was filtered through a 25-μm membrane filter and injected into the HPLC for the quantification of TAA.

2.3.2 Determination of free amino acids

The technique described by Okuma and Abe (1994) and adapted from Shinji et al. (2012) was used to extract FAAs in the muscles and exoskeleton. A total of 2 g tissue sample was homogenized with 8 mL of 15% perchloric acid. The sample was then

centrifuged at 18000*g* for 10 min at 4 °C. The supernatants were collected and filtered through a 0.45-μm nylon filter membrane prior to HPLC analysis. Culture water samples from moulting (E-stage) and non-moulting (C-stage) aquariums were filtered using a 0.45-μm nylon filter membrane. The solid-phase extraction (SPE) Sep-Pak-C18 cartridge was conditioned through rinsing with 30 mL of methanol and 10 mL of distilled water. The culture water sample (200 mL) was loaded onto the conditioned cartridge, and the retained compounds were eluted with 5.0 mL of 10% ethanol. The obtained elute was collected and evaporated to dry in a rotary vacuum evaporator. Subsequently, the residue was dissolved in 1.0 mL of distilled water and filtered through a 0.45-μm nylon filter membrane prior to the HPLC analysis (Wang et al., 2010).

2.3.3 Analysis of amino acids

The identity and quantity of amino acids were assessed by referring to the retention time and peak areas of the standard amino acids (Sigma-Aldrich, St. Louis, MO, USA) and were carried out based on the methods of Henderson et al. (2000) using an Agilent 1200 SL series system equipped with a binary pump delivery system (G1312B) and well plate automatic liquid sampler (WPALS) with injection program (G1376C). Agilent ZORBAX Eclipse plus C18 stationary phase column (1.8 μm, 2.1 mm×100 mm) was used as an analytical column. The Agilent method was used to set the chromatography conditions. Chromatography conditions such as temperature of the column oven and flow rate were 40 °C and 0.42 mL/min, respectively. The injection volume was 1 mL. The diode array detector (DAD) was set at 338 nm (orthophthalaldelyde (OPA)) and 262 nm (9-fluorenylmethyl-chloroformate (FMOC)) to monitor the derived amino acids.

2.4 Statistical analysis

All data for amino acid (TAA and FAA) levels are presented as mean±standard error (SE) and analyses were carried out using two-way analysis of variance (ANOVA). Data between the groups (moulting and non-moulting) were compared using two-way ANOVA with a posterior analysis (Tukey's post-hoc test) to identify which compound means differed. The significance level was set at P=0.05.

3 Results

Amino acid standards were detected, where peak identification was done using representative elution profiles. Tables 1 and 2 demonstrate amino acid profiles (TAA and FAA) in tissue muscle, exoskeleton, and culture water from moulting and non-moulting prawns. Based on the peak areas of the standard amino acids, a total of 23 amino acids including 10 essential amino acids (EAAs) and 13 non-essential amino acids (NEAAs) were detected. The EAAs were histidine, threonine, arginine, valine, methionine, tryptophan, phenylalanine, isoleucine, leucine, and lysine. The NEAAs studied were aspartic acid, glutamatic acid, asparagine, serine, glutamine, glycine, alanine, tyrosine, cysteine, norvaline, hydroxyproline, sarcosine, and proline.

3.1 Total amino acids

The different TAA compound levels from moulting prawn, E-stage (in tissue muscle, exoskeleton, and culture water sample), and non-moulting prawn, C-stage (in tissue muscle and culture water sample), are shown in Table 1. The two-way ANOVA for TAA analysis results indicates that the F-value (25.39) was higher than the F-critical value (2.40). There was a significant difference in the TAA levels of the tissue muscle, exoskeleton, and culture water sample between the moulting and non-moulting prawns with a P-value of 1.87×10^{-18}. Moreover, among the 23 amino acids profiled, there was a significant difference between the individual amino acid concentrations based on a factor test (P=5.66×10^{-22}). Based on the Tukey's post-hoc test, proline ($(17.39\pm0.67)\times10^{-2}$ mg/g) and sarcosine ($(8.64\pm0.68)\times10^{-2}$ mg/g) were the compounds detected at the highest levels in the tissue muscle of moulting prawns. Moreover, it was found that histidine ($(10.78\pm0.62)\times10^{-2}$ mg/g) was the compound detected at the highest level in the tissue muscle of non-moulting prawns. The results indicated that the TAA isoleucine was the only compound that can be detected in culture water sample from moulting ((1.98±0.30) mg/dL) and non-moulting ((1.90±0.10) mg/dL) prawns. Nonetheless, there were no significant differences between these two groups.

There was a significant difference in summation of TAA levels detected in the tissue muscle and exoskeleton between the moulting and non-moulting prawns (P=0.006; Fig. 1). Tukey's post-hoc analysis

Table 1 Total amino acid (TAA) concentrations in the muscles, exoskeleton, and culture water samples from moulting and non-moulting prawns

Amino acid	TAA in culture water (mg/dL)		TAA in muscle ($\times10^{-2}$ mg/g)		TAA in exoskeleton from moulting prawn ($\times10^{-2}$ mg/g)
	Non-moulting	Moulting	Non-moulting	Moulting	
Essential					
Histidine			10.78±0.62[a]	3.95±0.14[b,c,d]	0.91±0.11[b]
Threonine			1.81±0.10[c,d]	3.19±0.11[c,d]	
Arginine			0.85±0.15[c,d]		1.33±0.96[b]
Valine			1.53±0.15[c,d]	3.08±0.18[c,d]	1.22±0.15[b]
Methionine				2.31±0.77[c,d]	0.61±0.18[b]
Tryptophan			9.91±0.34[a]	5.71±0.33[b,c]	
Phenylalanine				0.10±0.04[d]	
Isoleucine	1.90±0.10[a]	1.98±0.30[a]	0.85±0.15[c,d]	1.66±0.96[c,d]	
Leucine			0.87±0.24[c,d]	3.54±0.15[c,d]	
Lysine				2.00±0.67[c,d]	
Non-essential					
Aspartic acid					0.61±0.76[b]
Glutamic acid			0.59±0.19[c,d]		0.91±0.66[b]
Asparagine			0.90±0.30[c,d]	1.17±0.15[d]	0.46±0.33[b]
Serine			4.87±0.79[b,c]	2.87±0.97[c,d]	0.88±0.11[b]
Glutamine			1.74±0.14[c,d]	0.87±0.02[c,d]	
Glycine				0.56±0.08[d]	0.44±0.05[b]
Alanine			0.85±0.62[c,d]	3.39±0.37[c,d]	1.23±0.88[b]
Tyrosine			0.84±0.16[c,d]	3.43±0.36[c,d]	1.35±0.97[b]
Cysteine				1.53±0.88[c,d]	0.63±0.18[b]
Norvaline			1.12±0.38[c,d]	0.71±0.41[d]	0.28±0.05[b]
Hydroxyproline				1.83±0.11[c,d]	
Sarcosine			7.95±0.41[a,b]	8.64±0.68[b]	6.34±0.17[a]
Proline			3.06±0.31[c,d]	17.39±0.67[a]	8.24±0.15[a]

Each value represents mean±standard error (SE) of four individuals. Significant differences at $P<0.05$ are indicated by different superscripts within a column

revealed that there was a significant difference ($P=1.01\times10^{-24}$) between individual amino acid concentrations of proline (0.2563 mg/g) and sarcosine (0.1498 mg/g). Thus, the compounds with the highest levels detected in the moulting prawn were proline and sarcosine, while the compound with the lowest level detected was phenylalanine (0.0010 mg/g). In comparison with the control prawns (non-moulting), histidine (0.1078 mg/g) and tryptophan (0.0991 mg/g) were the highest compounds, whereas glutamic acid (0.0059 mg/g) was the lowest compound detected in the control muscles. It should be noted that a total of seven TAAs such as methionine, phenylalanine, lysine, aspartic acid, glycine, cysteine, and hydroxyproline were not detected in non-moulting prawns.

3.2 Free amino acids

The FAA contents of moulting prawns, E-stage (in tissue muscle, exoskeleton and culture water sample), and non-moulting prawns, C-stage (in tissue muscle and culture water sample), are shown in Table 2. The two-way ANOVA results indicate that the F-value (11.36) was higher than the F-critical value (2.40). There was a significant difference in the FAA levels of tissue muscle, exoskeleton, and culture water sample between moulting and non-moulting prawns with a P-value of $1.14\times10^{-0.8}$. There was a significant difference between the individual amino acid concentrations ($P=7.05\times10^{-29}$). A Tukey's post-hoc test revealed that tryptophan ((9.57±0.53) mg/dL) and

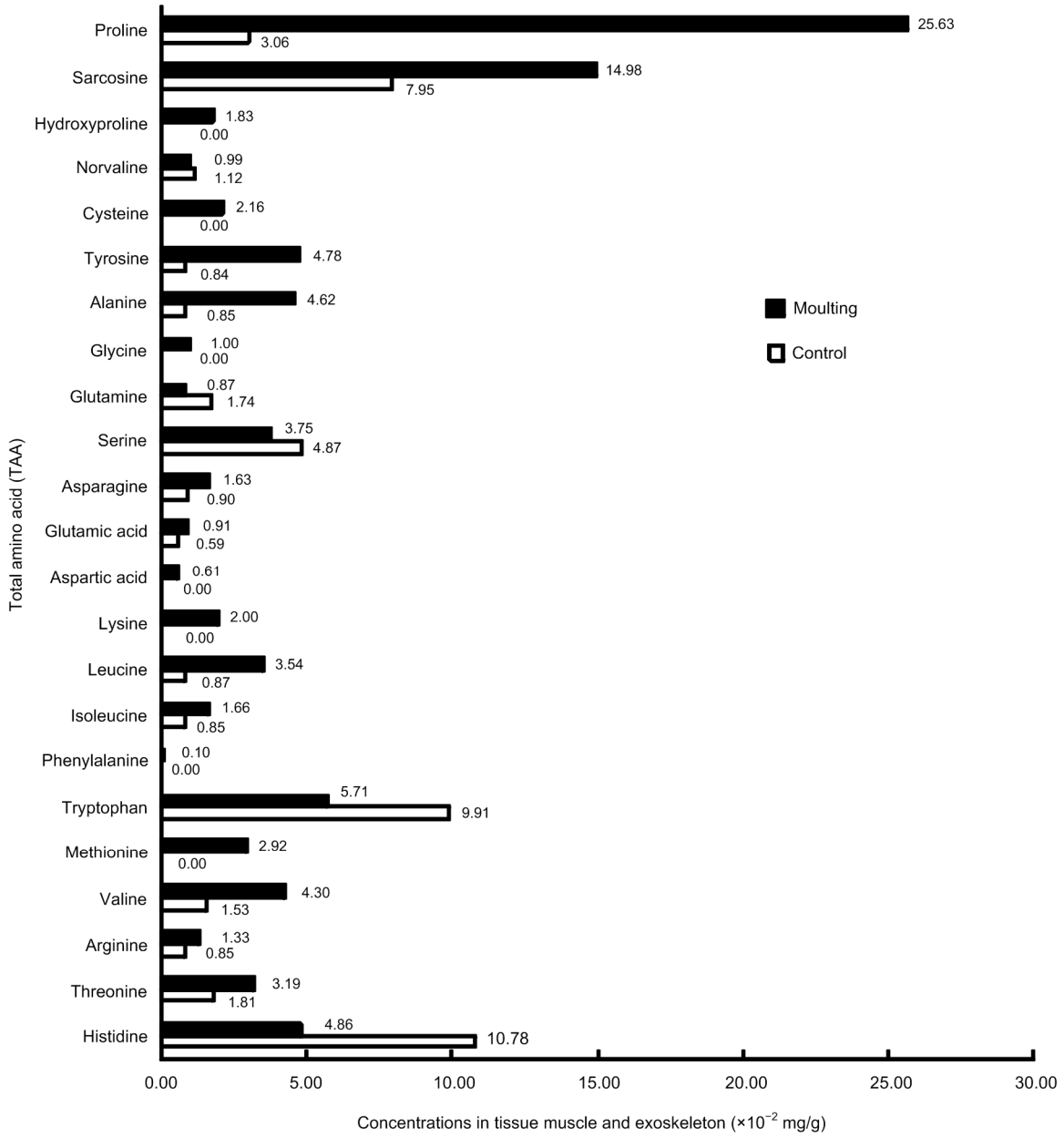

Fig. 1 Summation of total amino acids (TAAs) in tissue muscle and exoskeleton samples released from moulting and non-moulting (control) prawns

proline ((9.54±0.25) mg/dL) were the highest FAAs detected in moulting water, followed by sarcosine ((9.02±0.70) mg/dL) and serine ((4.22±0.82) mg/dL). Meanwhile, histidine ((7.03±0.92)×10^{-2} mg/g) was detected in the control prawn tissue. In the control muscle, the lowest FAAs detected were cystine ((0.37±0.06)×10^{-2} mg/g) and phenylalanine ((0.38±0.17)×10^{-2} mg/g). In the exoskeleton, the lowest FAA detected was norvaline ((0.40±0.05)×10^{-2} mg/g).

There was a significant difference in summation of FAA levels detected in the tissue muscle and exoskeleton during the moulting and non-moulting prawns (P=6.22×10^{-11}; Fig. 2). Tukey's post-hoc test revealed that there was a significant difference between individual amino acid concentrations (P=1.87×10^{-18}) of tryptophan (0.1252 mg/g), sarcosine (0.1023 mg/g), histidine (0.0814 mg/g), proline (0.0536 mg/g), and alanine (0.0525 mg/g), which were

Table 2 Free amino acid (FAA) concentrations in the muscles, exoskeleton, and culture water samples from moulting and non-moulting prawns

Amino acid	FAA in culture water (mg/dL)		FAA in muscle ($\times10^{-2}$ mg/g)		FAA in exoskeleton from moulting prawn ($\times10^{-2}$ mg/g)
	Non-moulting	Moulting	Non-moulting	Moulting	
Essential					
Histidine		4.04±0.26^{b,c}	7.03±0.92^{a}	5.44±0.72^{a,b,c}	2.70±0.16^{b}
Threonine		0.89±0.15^{b,c,d}		2.89±0.97^{c,d,e}	
Arginine					1.28±0.25^{b}
Valine	1.69±0.62^{a}	3.12±0.18^{b,c,d}			1.54±0.54^{b}
Methionine				1.52±0.88^{d,e,f}	0.82±0.19^{b}
Tryptophan		9.57±0.53^{a}	6.08±0.35^{a}	5.98±0.35^{a,b}	6.54±0.38^{a}
Phenylalanine		0.97±0.52^{b,c,d}	0.38±0.17^{c}		
Isoleucine			2.21±0.13^{b,c}	0.87±0.87^{e,f}	
Leucine			2.12±0.51^{b,c}	1.79±0.13^{d,e,f}	
Lysine	0.65±0.18^{a,b}	0.66±0.15^{d}	0.73±0.23^{c}	1.31±0.51^{e,f}	0.79±0.12^{b}
Non-essential					
Aspartic acid					
Glutamic acid					1.18±0.25^{b}
Asparagine		0.86±0.17^{b,c,d}	0.88±0.02^{b,c}	1.19±0.52^{e,f}	
Serine		4.22±0.82^{b}	4.13±1.38^{a,b}	2.99±0.35^{c,d,e}	0.92±0.15^{b}
Glutamine				0.86±0.03^{e,f}	1.83±0.16^{b}
Glycine					
Alanine				3.35±0.16^{b,c,d,e}	1.90±0.11^{b}
Tyrosine		1.68±0.60^{b,c,d}		3.40±0.02^{b,c,d,e}	
Cysteine	0.78±0.20^{a,b}	0.77±0.13^{c,d}	0.37±0.06^{c}		1.24±0.17^{b}
Norvaline			1.48±0.17^{c}	0.71±0.08^{e,f}	0.40±0.05^{b}
Hydroxyproline	0.91±0.52^{a,b}				
Sarcosine		9.02±0.70^{a}	4.09±0.18^{a,b}	7.88±0.24^{a}	2.35±0.13^{b}
Proline		9.54±0.25^{a}	0.89±0.23^{b,c}	4.17±0.75^{b,c,d}	1.19±0.11^{b}

Each value represents mean±standard error (SE) of four individuals. Significant differences at $P<0.05$ are indicated by different superscripts within a column

the highest compounds detected in the moulting prawn. Meanwhile, the lowest compound detected in the moulting prawn was isoleucine (0.0087 mg/g). Histidine (0.0703 mg/g) and tryptophan (0.0608 mg/g) were the highest FAA compounds detected in the control muscles. On the other hand, cysteine (0.0037 mg/g) and phenylalanine (0.0038 mg/g) were the lowest FAA compounds detected in the control muscles.

In the present study, a total of eight FAAs were not detected in control prawns including methionine, valine, tyrosine, alanine, arginine, threonine, glutamine, and glutamic acid. Meanwhile, phenylalanine was only detected in non-moulting prawn (control), whereas hydroxyproline, glycine or aspartic acid could not be detected in either test group.

4 Discussion

M. rosenbergii is aggressive and cannibalistic when reared at high densities. A low stocking rate is one suggestion as a possible action to reduce cannibalism. However, in an intensive culture system, a culture maximum density is needed and it is not the practice to provide substrates for hiding places. The materials do not appear practical for intensive rearing systems. Chemical communications have received very little attention in the field of crustacean aquaculture research. This study identified and characterized the potential chemical compounds from the moulting prawn that could give a cue to cannibalism. Cannibalism associated with moulting is also observed in

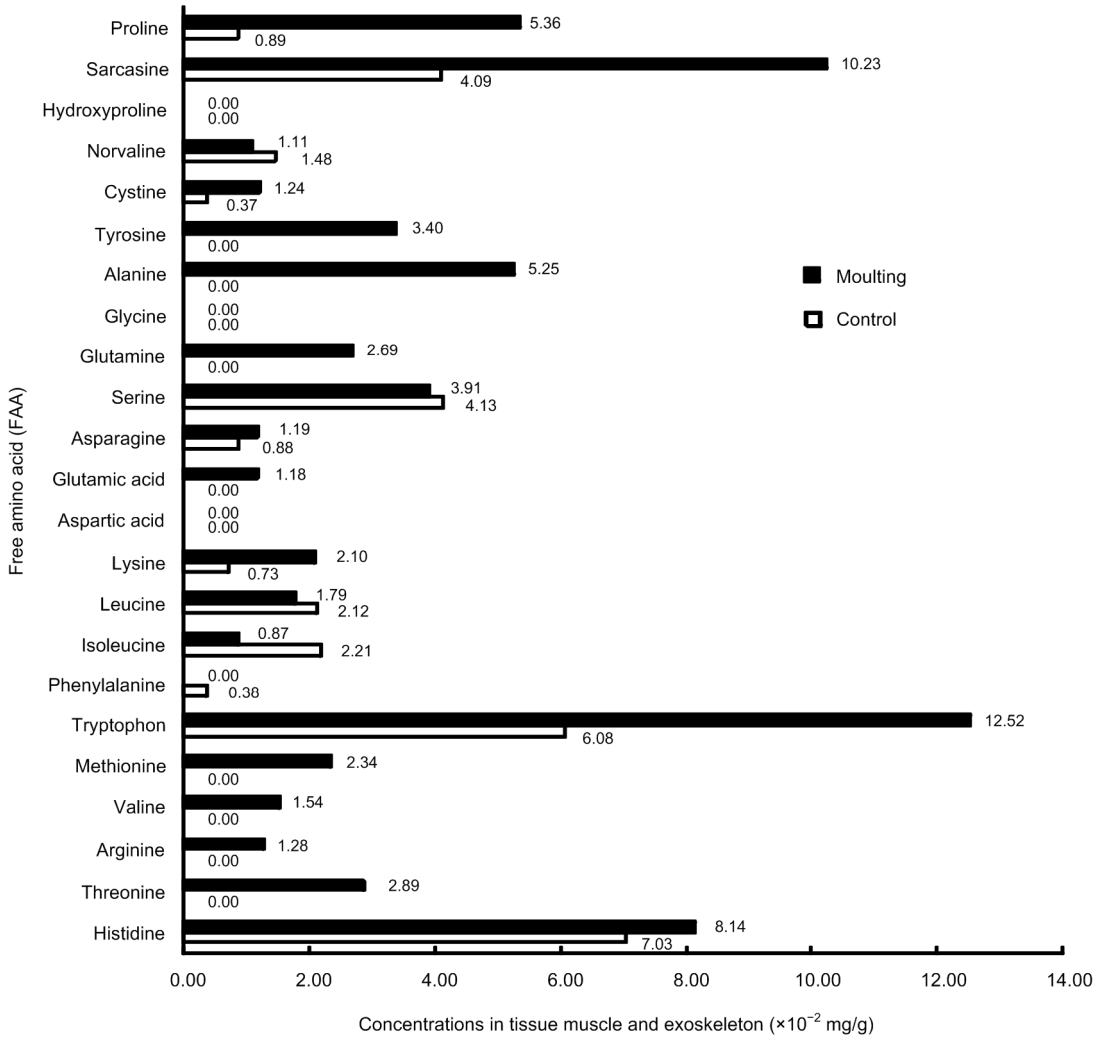

Fig. 2 Summation of free amino acids (FAAs) in tissue muscle and exoskeleton samples released from moulting and non-moulting (control) prawns

species of other crustaceans such as mud crab, *Scylla serrata* (Keenan and Blackshaw, 1999), and juvenile *M. rosenbergii* (Peebles, 1978; Nair et al., 1999).

Findings in amino acid profiles revealed that there was no obvious difference in TAA content between non-moulting and moulting prawns but there was a significant difference in the distribution pattern of FAAs relative to the moulting process. As such, only four FAA compounds (valine, lysine, cysteine, and hydroxyproline) were detected in the culture water of non-moulting prawns (C-stage). In contrast, 12 FAA compounds (tryptophan, proline, sarcosine, serine, histidine, valine, tyrosine, phenylalanine, asparagine, threonine, lysine, and cysteine) were detected in water samples from moulting prawns (E-stage) (Fig. 3). These gave further understanding of the involvement of protein turnover that was responsible for a large fraction of the energy budget. During ecdysis, protein in the prawn body was broken down (degradation) and was recycled back into the protein FAA pool via synthesis (Carter and Mente, 2014). Fractional rates of the protein synthesis act as a major factor in the removal of FAAs through protein turnover when there is a high demand of the feed consumption during pre-moulting and inter-moulting stages (Brodsky et al., 2017). There was a positive correlation between FAA content and its flavour, which acts as an important factor that facilitates cannibalism (Kato et al., 1989). Supported by Barki et al. (2011), the blends of these flavours yielded amino acids that act as chemical

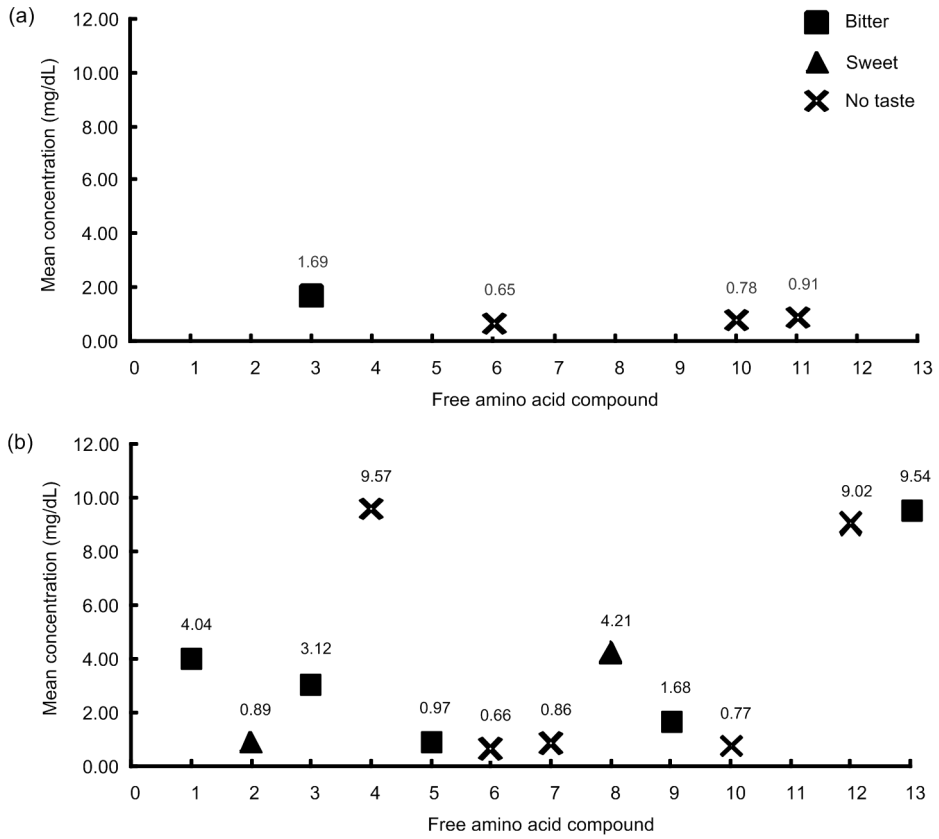

Fig. 3 Scatter plot showing the comparisons between the mean concentrations of free amino acids (FAAs) existing in culture water released by non-moulting (a) and moulting (b) prawns with the different tastes

1. Histidine; 2. Threonine; 3. Valine; 4. Tryptophan; 5. Phenylalanine; 6. Lysine; 7. Asparagine; 8. Serine; 9. Tyrosine; 10. Cysteine; 11. Hydroxyproline; 12. Sarcosine; 13. Proline

attractant and stimulate chemoreceptor activity of crustaceans. This was because of higher levels of several FAAs associated with sweet tastes, such as sarcosine, proline, serine, and threonine, detected in the culture water of moulting prawns. On the other hand, FAAs such as and histidine, valine, tyrosine, and phenylalanine contribute to a bitter taste.

Generally, crustaceans grow in a chemosensory environment suffused with chemical signals and underwater indicators (Derby and Sorensen, 2008; Caprio and Derby, 2010; Schmidt and Mellon, 2010). It should be noted that *M. rosenbergii* possesses two varieties of chemosensory organs similar to other crustaceans, including sensilla dense arrays on the mouthparts and walking legs. In the course of the moulting process, smell (as a chemical signal) could convey an indication regarding the organism. This condition has been proposed as an important mechanism that influences or attracts the animals. In addition, among the 12 FAAs detected in the culture water samples from moulting prawns (E-stage), tryptophan and proline were at the highest levels. Because of their hydrophobic nature, both of these amino acids have long been used as feed additives to improve feed intake in aquaculture (Wu et al., 2013, 2014). For an instance, dietary supplementation of tryptophan showed effectiveness in reducing cannibalism up to 70% on juvenile *M. rosenbergii* (Nair et al., 1999). Application of dietary tryptophan has also been shown to prevent aggression in the juvenile grouper *Epinephelus coioides* (Hseu et al., 2003), juvenile Atlantic cod *Gadus morhua* (Höglund et al., 2005), and juvenile mud crab *S. serrata* (Laranja et al., 2010). Therefore, a higher level of tryptophan detected in culture water from moulting prawns has become an attraction factor that triggers the occurrence of cannibalism, which is used to be as an additional food source. Further understanding on the underlying physiological mechanism

could be used as a cannibalism management behaviour in aquaculture. As such, FAA that has been detected in the current study could assist in better understanding the role of signals generated as a chemical cue during the moulting process.

Significant results in the present study were found when comparing the amino acid compounds of the moulting prawn with those of the non-moulting prawn. These help improve understanding of the physiological relationship and amino acid metabolism. It reveals how energy resources were utilised by prawns during ecdysis with the involvement of protein degradation (amino acid) (Mente et al., 2002). High levels of FAA compounds were found released during the ecdysis by moulting prawns that spread a chemical cue/signal and then enhance chemoreceptor activity, which indirectly promotes cannibalism. It was proven that high level of essential FAA, especially tryptophan, has become a factor for non-moulting prawn to attack the prawn which newly moulted and a necessary source of feed to fulfil the dietary requirement and high appetite during pre-moulting and inter-moulting stages (Carter and Mente, 2014).

5 Conclusions

In summary, the current study demonstrated that amino acid contents in muscle, exoskeleton, and the culture water samples were different between moulting and non-moulting prawns of *M. rosenbergii* and may influence cannibalism. Actual cannibalism was not studied in this experiment, so this is only hypothesis, not proven by observation or experiment. The higher levels of tryptophan and proline detected in the moulting prawn bodies and culture water signify the involvement of amino acids in chemical cues and this information is useful for studies that seek to develop inhibitors of cannibalism. Extrapolation of data from this study could be a guideline for more robust scientific development in *M. rosenbergii* dietary improvement and manipulations to improve feeding efficiencies. Through continuous research like this, the ultimate goal of the sustainable and profitable *M. rosenbergii* aquaculture industry can be achieved.

Contributors

Abu Seman JUNETA-NOR and Mhd IKHWANUDDIN conceived and designed the study, drafted the paper, and took responsibility for the integrity of the data and the accuracy of the data analysis. Noordiyana Mat NOORDIN, Hong-yu MA, and Norainy Mohd HUSIN developed the statistical methods and interpreted the data. Mohamad Nor AZRA revised the manuscript. All authors agree to be accountable for all aspects of the work and have read and approved the manuscript.

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Compliance with ethics guidelines

Abu Seman JUNETA-NOR, Noordiyana Mat NOORDIN, Mohamad Nor AZRA, Hong-yu MA, Norainy Mohd HUSIN, and Mhd IKHWANUDDIN declare that they have no conflict of interest.

All institutional and national guidelines for the care and use of laboratory animals were followed.

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中文概要

题　目：罗氏沼虾蜕皮过程中释放的氨基酸组分是引发同类残杀的因素？

目　的：确定罗氏沼虾在蜕皮过程（该过程引起同类残杀）中释放的氨基酸组分，并明确其中差异的氨基酸成分。

创新点：了解罗氏沼虾蜕皮过程中生化组分的变化对于理解同类残杀的重要意义。目前，还没有关于同类残杀和蜕皮过程与生化组分之间关系的研究报道。本研究以此为切入点，采用统计学方法探究二者之间的关联，并鉴定其中差异的氨基酸成分。

方　法：在人工饲养条件下，测定了蜕皮阶段和非蜕皮阶段的罗氏沼虾肌肉、外骨骼和养殖水体中的总氨基酸及自由氨基酸组分，采用双因素方差分析法比较分析了两个阶段氨基酸组分的差异，同时鉴定了差异的氨基酸成分。

结　论：在蜕皮阶段，组织中的总氨基酸以脯氨酸和肌氨酸的含量最高，而水体中的自由氨基酸含量以色氨酸和脯氨酸为主。研究结果表明，这些氨基酸组分在罗氏沼虾蜕皮过程中释放了促进同类残杀的化学信号。

关键词：氨基酸组分；同类残杀；化学信号；罗氏沼虾；蜕皮