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Original Article

Effects of crude Anthocyanins from three plants on lipid contents in white leg shrimp (*Penaeus vannamei*)♣

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Abstract

This study aimed to evaluate the crude extracts from three anthocyanin rich plants for fat reduction in shrimp meat. The types of anthocyanin plentiful in local plants included delphinidin-3,5-diglucoside derivative from butterfly pea petals, delphinidine-3-sambubioside from roselle calyx, and cyanidin-3-glucoside from malabar fruit. These were mixed in formulated feed at 5% (weight/weight dry basis) substitution levels. Farmed white leg shrimp, *Penaeus vannamei* (25.78±0.71g) were subjected to a feed experiment for 45 days of rearing. The results show no effects on growth performance. The cyanidin-3-glucoside derivatives from malabar fruit were accumulated the most in the experimental shrimp, with a highly significant difference (p<0.01) from other anthocyanins. Proximate analysis indicated that total fat and energy in shrimp meat differed highly significantly (p<0.01) from those for the control group, with lower levels induced by all feeds with crude anthocyanins. Butterfly pea and malabar fruit additives gave lower levels of total cholesterol in shrimp meat than in the control group, with highly significant (p<0.01) differences. In summary, crude anthocyanins provided in the feed as anti-oxidants could be accumulated in live shrimp and reduced the fat content of shrimp meat, which has implications for the health concerns associated with shrimp consumption.

Keywords: crude anthocyanin, plant sources, feed, *Penaeus vannamei*, lipid contents

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1. Introduction

Marine shrimp products, whether they are products from wild catch or farmed, have high cholesterol content. Almost 90 % of shrimp production is farmed and fed with a commercial pellet feed. Recently, alternative sources of protein and lipid have been substituted in feed for cost reduction. The agricultural by-products used in feed formulations can elevate fat content of shrimp meat (Hicks & Verbeek, 2016; Mirabella, Castellani, & Sala, 2014).

The marine shrimp as a seafood product is a favorite of consumers, because it provides good nutrition with high protein and fat contents. However, the fats in the pellet feed will accumulate in shrimp meat and are subsequently transferred to humans via the trophic pathway. These can be harmful if accumulated in fat compounds and in some parts of the human body, for example contributing to heart disease and stroke, high blood pressure, and many other conditions (Piche, Poirier, Lemieux, & Despres, 2018).

In the last decade, medical research has attempted to address the harmful effects caused by cholesterol. Anthocyanins have been tested on mice in laboratories and have been widely applied to health treatments of high cholesterol patients. Accordingly, many anthocyanin rich plant products have been tested, such as purple corn, blueberries, purple sweet potato, black soybean, blackberries, strawberries, etc. (Han *et al.*, 2006; Jayaprakasam, Olson, Schutzki, Tai, & Nair, 2006; Kaume, Gilbert, Brownmiller, Howard, & Devareddy, 2012; Kwon *et al.*, 2007; Zafra-Stone *et al.*, 2007;). Anti-obesity effects in mouse from anthocyanin treatments were demonstrated with decreased body weight in 8 weeks (Wu, Jiang, Yin, Long, & Zheng, 2016).

Moreover, rainbow trout (*Oncorhynchus mykiss*) has been treated with experimental anthocyanin from purple corn (*Zea mays*) mixed in the pellet feed. High levels of polyunsaturated fatty acids (PUFAs) were found in the treated fish, with significant reduction in adiposity, and moreover also the HDL levels in plasma had increased significantly (Villasante *et al.*, 2015). Apparently, the antioxidant properties of anthocyanins may affect lipids and thereby the health of various animals. Anthocyanins are a class of flavonoids that impart blue, red, and purple colors to fruits and vegetables (Tonon, Brabet, & Hubinger, 2010). The most common types of anthocyanins are cyanidin, delphinidin, pelargonidin, peonidin, petunidin, and malvidin.

This study evaluated the crude extracts from three anthocyanin-rich plants for fat reduction in white leg shrimp (*Penaeus vannamei*) meat. The experiment included 4 experimental feeds, namely control feed and feeds with 3 derivatives of anthocyanin rich local tropical plants: delphinidin-3,5-diglucoside derivative form butterfly pea petal (*Critoria ternatea*), delphinidin-3-sambubioside from roselle calyx (*Hisbicus sabdariffa*), and cyanidin-3-glucoside from malabar fruit (*Melastoma malabathricum*) (Jamil, Zairi, Nasim, & Pa'ee, 2018; Singh, Karlo, & Pandey, 2014; Wong, Yusof, Ghazali, & Che Man, 2002). These were mixed in feed pellets used in a feed experiment for 45 days. The observed parameters include growth performance, nutritional values, total cholesterol, and fatty acid profile of experimental shrimp. The main outcomes of this study focused on the quality of shrimp product, as regards the lipid content, with implications for the health of consumers of shrimp products.

2. Materials and Methods

2.1 Feed formulations

2.1.1 Crude anthocyanin powder preparation and total anthocyanin analysis

The anthocyanin-rich butterfly pea petals and roselle calyx were purchased from a local market, and ripe malabar berries were collected from natural growth in Surat Thani province. A part of butterfly pea petals, roselle calyx and ripe malabar fruit meat were separated, the wet weights were recorded, and the samples were put in polyethylene bags for freezing at -40°C over 1 day. The frozen plant tissues were freeze-dried (by freeze dryer model Beta 2–8 LSCplus, Martin Christ®, Osterode am Harz, Germany) for 24-36 hours, then the weights of samples were recorded for wet/dry ratio calculations, and total anthocyanins were also quantified (Table 1). Anthocyanin-rich plants were ground with a grinder to powder of about 30 µm particle diameter and were kept in a refrigerator prior to use.

Total anthocyanin analysis: Crude powders of the anthocyanin-rich plants, experimental feeds, and shrimp samples (approximately 0.1 g each in weight) were put in 60 mL dark bottles for anthocyanin analysis. Then 7.5 mL of 95% ethanol was added and mixed on a horizontal shaker in a dark room for 12 hours at room temperature, to extract anthocyanins. After that the solutions were placed in 50 mL centrifuge tubes, and sediment debris was separated by high-speed centrifuging at 15,000 rpm (Z 36 HK, Hermle Labortechnik GmbH, Germany) and 4°C for 10 minutes. The clear supernatant solutions were measured by the pH differential method (Lee, Durst, & Wrolstad, 2005).

The reagents prepared included 0.025 M potassium chloride buffer (pH 1.0) and 0.4 M sodium acetate buffer (pH 4.5). The appropriate dilution factor for each sample was found by diluting with the potassium chloride buffer until the absorbance at $\lambda_{vis\text{-}max}$ (543 nm for delphinidine-3-glucoside, 520 nm for delphinidine-3-sambubioside, and 530 nm for cyanidin-3-glucoside monomeric (Stintzing & Carle, 2004)) was within the linear range. The final volume of the sample was divided by its initial volume to obtain the dilution factor. There were two dilutions, one with potassium chloride buffer,

Table 1. The characteristics of three plant tissues used in this study as feed additives (mean ± SD, n=5).

Parameter	Butterfly pea petal (delphinidin-3,5-diglucoside)	Roselle calyx (delphinidin-3-sambubioside)	Malabar fruit (cyanidin-3-glucoside)
Wet/dry mass ratio	10.18 ± 0.29^{c}	7.73 ± 0.13^{b}	3.21 ± 0.19^{a}
Total anthocyanins (mg/g. dw)	2.67 ± 0.03^{a}	6.18 ± 0.10^{b}	34.95 ± 0.15^{c}

Different superscripts indicate significant differences within a row (Tukey's post hoc test, p<0.05).

and the other with sodium acetate buffer. The previously determined dilution factor was used, and these dilutions were allowed to equilibrate for 15 min before measuring at $\lambda_{vis\text{-}max}$ nm and at 700 nm, against deionized water as the blank. It is noted that the samples to be measured were clear, not cloudy or with sediment. The absorbance A of the diluted sample was calculated from:

$$A = (A\ \lambda_{vis\text{-}max} - A700)\ pH\ 1.0 - (A\ \lambda_{vis\text{-}max} - A700)\ pH\ 4.5 \quad (1)$$

The monomeric anthocyanin pigment concentration in the sample was calculated as follows.

$$\text{Monomeric anthocyanin pigment (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon \times 1) \quad (2)$$

where

MW (molecular weight) = 518.5 g/mol for delphinidin-3-glucoside, 597 g/mol for delphinidin-3-sambubioside and 449.2 g/mol for cyanidin-3-glucoside

DF = dilution factor

1= pathlength in cm

ε (molar absorptivity) = 29,000 for delphinidin-3-glucoside, 23,700 for delphinidin-3-sambubioside and 26,900 for cyanidin-3-glucoside (Chu *et al.*, 2017; Giusti & Wrolstad, 2001; Juliani *et al.*, 2009).

10^3 = factor for conversion from g to mg.

2.1.2 Feed formulations

The shrimp feed formulations are shown in Table 2. Five percent (w/w) dried powder of a plant (as feed additive portion) was added to one of the 3 experimental feeds, one for each plant. On the other hand, five percent (w/w) of cellulose was mixed in the control feed. The feeds were manually homogenized with 60% (v/w) water added to the formulations in Table 2. Feed was processed to pellets of 5 mm diameter by using a meat mincer at room temperature, and were then dried in a hot air oven at 60°C for 36 hours (until dry), and were kept in plastic bags in the dark in a refrigerator prior to use.

2.2 Experimental shrimp

Live farmed shrimps (white leg shrimp, *Penaeus vannamei*) with 25.78±0.71g initial body weight were purchased from an outdoor shrimp farm in Kanchanadit district, Surat Thani province. They were allowed to acclimatize for 7 days to the rearing conditions in a 3x3x1.5 m concrete pond with a water depth of 50 cm, at 18 pcs/m^3 density (81shrimp per pond) and 3 replicate ponds in each treatment. Then, the experimental feeds were provided at 5% of body weight as 4 meals per day at 06.00 a.m., 12:00 p.m., 06:00 p.m., and midnight. The water quality was optimal with 30 ppt salinity and 180 mg-$CaCO_3$/L alkalinity. All the experimental ponds had nitrification reactors to remove nitrogenous wastes (ammonia and nitrite) and had a blower for continuous aeration that was provided during the 45 days of experiment. The monitored water quality measures included salinity measured using a reflecto-salinometer, pH measured using a pH meter, and alkalinity measured with titration (Boyd & Tucker, 1992), while ammonia and nitrite were measured following Strickland & Parsons (1972) method. These levels were recorded every 4 days during the

Table 2. Feed formulations and proximate analyses of shrimp experimental feed

Ingredient (%)	Experimental diet			
	Control	Butterfly pea petal	Roselle calyx	Malabar fruit
Soybean meal	52.7	52.7	52.7	52.7
Fish meal	6.3	6.3	6.3	6.3
Squid meal	8.7	8.7	8.7	8.7
Wheat starch	11	11	11	11
Corn starch	6.1	6.1	6.1	6.1
Vitamin-mineral premix[1]	2.5	2.5	2.5	2.5
Vitamin C	0.1	0.1	0.1	0.1
Dicalcium phosphate	2.5	2.5	2.5	2.5
Lecithin	1.2	1.2	1.2	1.2
Fish oil	3.9	3.9	3.9	3.9
Cellulose	5	-	-	-
Butterfly pea powder	-	5	-	-
Roselle powder	-	-	5	-
Malabar fruit powder	-	-	-	5
Proximate analysis (mean ± SD)				
Total Anthocyanin (mg/g. dw)	0.17±0.03	0.30±0.00	0.77±0.01	1.20±0.00
Protein (%)	31.72±0.09	31.58±0.02	30.54±0.23	30.81±0.08
Total fat (%)	3.86±0.12	3.28±0.33	3.26±0.30	3.62±0.47
Total Cholesterol (mg/100g)	313.00±6.39	305.40±4.63	314.27±4.00	274.45±6.88
Energy (cal/g)	4,027.43±12.79	4,024.50±7.14	3,999.07±19.29	4027.17±8.78
Ash (%)	0.10±0.00	0.11±0.00	0.10±0.00	0.10±0.00
Moisture (%)	0.92±0.00	0.92±0.00	0.92±0.00	0.92±0.01
Fiber (%)	3.6±0.10	3.97±0.12	4.07±.012	4.30±0.10

[1] (per kg premix): Vitamin A 2,105,000 IU, Vitamin D_3 421,000 IU, Vitamin E 20,000 mg, Vitamin B_1 6,000 mg, Pantothenic acid 1,000 mg, Vitamin B_6 6,000 mg, Vitamin E 500 mg, Nicotinic acid 3,000 mg, Folic Acid 500 mg, Choline 500,000 mg, Inositol 12,500 mg, Biotin 100 mg, Sodium 7.85 g, Zinc 2,500 mg, Iron, 5000 mg, Manganese 2,000 mg, Copper 430 mg

rearing, showing no abnormal levels at any time and no consistent or large differences between the treatments (Table 3).

2.3 Sample preparation

Twenty randomly sampled white leg shrimps were treated with cold seawater to slow down the metabolism and movement, and weighed. Individual weights were recorded for growth performance calculation. Shrimp samples were symmetrically dissected and then put in polyethylene plastic bags to -40°C for 6 hours, after which they were freeze-dried for 24-36 hours. Individual dry sample weights were recorded. Both experimental feed and dried shrimp samples were ground to powders for analysis, representing each experimental group.

2.4 Proximate analysis

The proximate analysis parameters including protein, total crude fat, ash, fiber and energy were analyzed in Aquatic Nutritional Laboratory at Chonburi Aquatic Animal Feed Research and Technology Development Center. Moisture was determined by drying the samples at 105 °C for 2 h in an oven to a stable weight, and ash by incineration at 600 °C in a muffle furnace for 2 hours according to the method of AOAC (2016). For total protein analysis, approximately 0.1 g of sample was placed in a tin foil cup for the carbon/nitrogen analyzer (Truspec CN, LECO®) and an EDTA capsule (LECO®) was used for the calibration. Total crude fat in an 0.1 g sample was added with LECO-Dry and then 3 mL of 70% ethanol was added to a thimble, which was placed in the Fat Extractor TFE2000 (LECO®) analyzer. Determination of energy was done using a bomb calorimeter, the AC500 (LECO®) analyzer. Fiber analysis was performed by ALS Laboratory Group (Thailand) with an AOAC (2016) standard method.

2.5 Cholesterols and fatty acids analysis

For cholesterol levels, samples of both feed and shrimp meat were quantified by ALS Laboratory Group (Thailand) with a standard method for cholesterol analyses (Al-Hasani, Hlavac, & Carpenter, 1993). For the fatty acids analysis, the crude fats extract from the Fat Extractor TFE 2000 (LECO®) was converted to fatty acid methyl esters (FAME), followed by acidic transmethylation with 12% (v/v) BF_3 in methanol (at 100 °C for 30 min). Phase separation was achieved with a saturated solution of sodium chloride (Carvalho & Malcata, 2005). FAME were analyzed by gas chromatography on an Agilent GC system (Agilent, Technologies, Palo Alto, CA) using Mix GLC-30, GLC-40, GLC-50, GLC-70 and GLC-80 (Supelco, Inc) of standard fatty acids, at the Office of Scientific Instruments and Testing, Prince of Songkla University. To determine the index of atherogenicity (IA), the formula used was: IA = [C12:0+(4 × C14:0)+C16:0]/ΣUFA (Chen & Liu, 2020)

2.6 Statistical analysis

The analyzed observations were growth performance measures (initial weight, weight gain, average daily growth, and survival rate), proximate analysis results (protein, total fats, ash, fiber, and energy), fat contents (total cholesterols and fatty acids), and total anthocyanins in the raw materials (butterfly pea petal powder, roselle calyx powder, and malabar fruit powder), in the feeds, and in the shrimp edible muscle tissues. Significant differences of means from an analysis of variance by treatments were subjected to Tukey's *post hoc* test in SPSS® software. Differences with $p<0.05$ were considered statistically significant.

3. Results and Discussion

3.1 Water quality and shrimp growth performance

After the shrimps had been reared with experimental feeds for 45 days, they were randomly sampled from their ponds. The water quality parameters pH, salinity, alkalinity, ammonia, and nitrite were monitored during rearing, and these all remained in their optimal ranges for shrimp rearing (Table 3). Toxic inorganic nitrogen in the forms of ammonia and nitrite was treated with a nitrification reactor, designed for inorganic nitrogen control to maintain the levels below critical limits (Venkateswarlu, Seshaiah, & Behra, 2019) during the experiment.

Growth performance and survival rate were quantified. The results show that weight gain was in the range 9.1-9.3 g and average daily growth was 0.20-0.21 g/day, with 60-65% survival rate (Table 4) during the rearing. These growth performance measures and survival rate did not show statistically significant differences. The alternative feed formulations had one of three anthocyanin rich plants added at 5% level, while in the control group without anthocyanin supplementation 5% of cellulose was substituted in the feed. The selected plant supplements did not affect main nutritional values, such as protein or lipids, but contributed slightly to fiber content (Table 2) as a common phytoproduct. The

Table 3. The ranges of water quality parameters in shrimp ponds over 45 days of rearing

Parameter	Optimum range	Experimental diet			
		Control	Butterfly pea petal	Roselle calyx	Malabar fruit
pH	7.5-8.5	7.58-8.10	7.51-8.10	7.50-8.09	7.50-8.10
Salinity (ppt)	12-25	28.00-30.00	28.00-30.00	28.00-30.00	28.00-30.00
Alkalinity (mg-$CaCO_3$/L)	>120	150.00-170.00	150.60-169.8	150.00-170.00	150.00-166.40
Ammonia (mg-N/L)	< 1.0	0.10-0.35	0.10-0.35	0.14-0.29	0.10-0.43
Nitrite (mg-N/L)	< 0.5	0.16-0.37	0.16-0.40	0.22-0.34	0.21-0.32

Optimum ranges cited from Venkateswarlu, Seshaiah, & Behra (2019)

anthocyanin supplements in the experimental feeds of this study did not affect shrimp growth performance. Also, the survival rate results indicate that the experimental shrimps consistently responded to pond rearing in captivity, with no difference by dietary treatment.

3.2 Total anthocyanin accumulation

3.2.1 Total anthocyanin supplementation

The total anthocyanin levels in crude materials from the 3 plants were 34.95±0.15 mg/g dw in malabar fruit powder, 6.18±0.10 mg/g dw in roselle calyx powder, and 2.67±0.03 mg/g dw in butterfly pea petals (Table 1). The feed formulations with anthocyanin phyto-pigment rich additives at 5% (w/w) had total anthocyanin concentrations 1.20±0.00 mg/g dw in malabar fruit feed, 0.77±0.01 mg/g dw in roselle calyx feed, and 0.30±0.00 mg/g dw in butterfly pea petals feed (Table 2). The 5 percent (w/w) supplementation of experimental shrimp feeds was chosen because for aquatic animals the anthocyanins need to remain water soluble. In a previous study, Valle and Coutteau (2015) suggested that 3% of phytobiotics could be used in shrimp feed as additive level. In this study, the anthocyanins from plants are easily degraded by elevated temperature or ultraviolet radiation, and there can be losses from dissolution in water. During 60˚C drying of the pellet feeds, the total anthocyanin levels in the feeds decreased 29.13, 8.03, and 8.9-fold, respectively. Time profiles of temperature during feed processing and storage may affect total anthocyanins, due to thermal instability of delphinidin-3-5-glucoside and delphinidin-3-sambubioside derivatives (Aurelio, Edgardo, & Navarro-Galindo, 2008; Cisse, Vaillant, Acosta, Dhuique-Mayer, & Dornier, 2009; Lee, Abdullah, & Hung, 2011; Marpaung, Handoko, & Kartawiria, 2018). Further, the derivatives of anthocyanins from plants interact with sugar moieties so that a moderate 60˚C temperature should be used in drying to reduce moisture and avoid fungal contamination, but even this can degrade the anthocyanin content. A low 60˚C temperature during heating, and storage in dark cool conditions have been recommended (Brownmiller, Howard, & Prior, 2008; Khanal, Howard, & Prior, 2010).

3.2.2 Metabolized anthocyanins in shrimp meat

Experimental shrimps that ingested anthocyanins as feed supplements showed total anthocyanins accumulation in muscle (flesh) of 0.80±0.02 mg/g dw in malabar fruit fed group, 0.55±0.03 mg/g dw in roselle fed group, 0.30±0.01 mg/g dw in butterfly pea fed group, and 0.09±0.01 mg/g dw in control group (Table 5). The differences between feed experiment groups were significant (p<0.01). The metabolized anthocyanins did not only accumulate in shrimp muscles but can also be enriched in the compound eyes, hepatopancreas, chromatophores in epidermis layer, and in white muscle (Putthasri, 2015). The edible part of a marine shrimp is its abdominal muscles, hence the metabolized anthocyanins in muscles can contribute as functional foods for a consumer. The metabolized anthocyanins in muscles showed accumulation levels matching the anthocyanin supplementation levels in feed. Of course, the metabolized anthocyanins in any reservoirs in the body may contribute to

Table 4. Growth performance and survival rate of experimental shrimp fed with anthocyanin supplementation for 45 days. (mean ± SD, n=3)

Growth parameter	Experimental diet			
	Control	Butterfly pea petal	Roselle calyx	Malabar fruit
Initial weight (g)	26.03±0.82	25.38±1.27	25.07±1.37	26.65±1.19
Weight gain (g)[1]	9.3±0.48	9.15±0.43	9.38±0.50	9.1±0.15
ADG (g/day)[2]	0.21±0.01	0.20±0.01	0.21±0.01	0.20±0.00
Survival rate (%)[3]	65.0±5.0	60.0±0.0	65.0±5.0	65.0±5.0

[1]Weight gain (g) = final weight–initial weight
[2]Average Daily Growth (ADG) = Weight Gained/Length of Feeding Trial (days) [3]survival rate (%) = (Final number of shrimp /Initial number of shrimp) x 100

Table 5. The average metabolized anthocyanin concentrations and nutritional composition analysis of muscles (dry basis) of experimental shrimp fed with anthocyanin supplemented feed for 45 days. (mean ± SD, n=20)

Proximate analysis	Experimental diet			
	Control	Butterfly pea petal	Roselle calyx	Malabar fruit
Metabolized anthocyanin (mg/g. dw) in muscle	0.09±0.01^{a}	0.30±0.01^{b}	0.55±0.03^{c}	0.80±0.02^{d}
Protein (%)	81.56±0.22^{a}	83.66±0.16^{c}	87.86±0.78^{d}	82.60±0.05^{b}
Total crude fat (%)	3.04±0.13^{c}	2.38±0.08^{a}	2.74±0.14^{b}	2.33±0.11^{a}
Total cholesterols (mg/100g)	1,837.46±27.41^{c}	1,692.00±19.81^{b}	2,000.24±23.61^{d}	1,594.00±21.64^{a}
Metabolic energy (cal/g)	4,720.93±27.28^{d}	4,438.23±10.86^{b}	4,554.97±6.17^{c}	4,349.10±14.17^{a}
Ash (%)	0.08±0.00^{a}	0.09±0.00^{b}	0.09±0.00^{b}	0.09±0.00^{b}
Wet/dry ratio	5.02±0.18^{c}	4.93±0.40^{c}	4.60±0.33^{b}	4.28±0.59^{a}

Different superscripts in a row indicate significant differences by treatment (Tukey's post hoc test, p<0.05)

antioxidant activity, induce the immune system, and facilitate the weight loss in animals (Karupiah & Ismail, 2015; Onsanit *et al.*, 2020; Prior, 2010; Yilmaz, 2019).

3.3 Nutritional value of experimental shrimp

3.3.1 Crude Protein and growth performance

The proximate analysis showed crude protein and ash in edible shrimp meat, and water content of shrimp muscle (evaluated as wet/dry ratio) in roselle and malabar fed groups was significantly different from the control group (p<0.01). The water content can be associated with apparent muscle firmness. Moreover, ash content was significantly (p<0.01) higher in anthocyanins fed group than in control group (Table 5). The crude protein levels in shrimp muscles were 87.86±0.78% in roselle calyx fed group, 83.66±0.16% in butterfly pea petals fed group, 82.60±0.05% in malabar fruit fed group and 81.56±0.22% in control fed group, with significant differences (p<0.01). In a prior study on phytobiotic supplementation in shrimp feeds, the survival rate and feed conversion ratio were improved over control but without effect on growth performance (Valle & Coutteau, 2015). Similarly, our results show no growth performance differences of the shrimp (p>0.05), in Table 4. This is consistent with an earlier report on Nile tilapia (*Oreochromis niloticus*) indicating that anthocyanin did not promote growth performance (Yilmaz, 2019).

3.3.2 Fat content and energy

Total crude fats were extracted from shrimp muscles. Interestingly, the crude fats with anthocyanin supplementation were significantly decreased in quantity relative to the control group (p<0.01), especially in the butterfly pea petals and the malabar fruit fed groups. The relative reductions were 23.36% in malabar fruit fed group, 21.71% in butterfly petals fed group, and 9.87% in roselle calyx fed group, from that of the control group (Table 5). Regarding energy in shrimp muscle dry matter, less energy was found in anthocyanin supplemented groups than in control group. The main sources of energy in shrimp muscles are carbohydrates, lipids and proteins. In previous reports on vertebrate animals such as rats, broilers, or rabbits fed anthocyanin supplements, the supplementation inhibited lipogenesis and adipocytes (Lee, Lee, Lefevre, & Kim, 2014; Lee *et al.*, 2017), contributing to decreased fat or having anti-obesity activity in the animals (Dani, Rusman, & Zuprizal, 2019). In addition, when anthocyanins were fed to broilers the fat content in muscles decreased. It can be concluded that anthocyanins have anti-obesity properties and protect against weight gain (Karupiah & Ismail, 2015; Kaume, Gilbert, Brownmiller, Howard, & Devareddy, 2012).

3.3.3 Cholesterol and fatty acid profiles

Total cholesterols in shrimp muscles were quantified. The results show that total cholesterol concentrations were significantly decreased (p<0.01) although the roselle calyx group had significantly higher level than the control group (Table 5), accompanied by crude fat decreases. The decreases from control group were 13.25% in malabar fruit fed group, and 7.92% in butterfly pea petals fed group, while the roselle calyx fed group showed an 8.89% increase in total cholesterols in shrimp muscles. In a prior study of nano-encapsulated *Melastoma malabathricum* fruit extract, it reduced cholesterols in broiler meat (Dani *et al.*, 2019). This is consistent with treatments of the fish rainbow trout (*Oncorhynchus mykiss*) with experimental anthocyanins (mainly cyanidin-3-glucoside derivatives form purple corn (*Zea mays*)) mixed in pellet feed significantly reducing adiposity, and moreover also the HDL cholesterol levels in plasma were significantly increased (Villasante *et al.*, 2015). Metabolized anthocyanins may reduce lipogenesis or inhibit adipocytes, causing reduced cholesterol levels in shrimp meat. As regards fatty acids, our results show that the levels of ΣMUFA and ΣPUFA in anthocyanin fed groups of shrimps were below those of the control group (Table 6). This is in contrast with farmed cattle and goats fed with anthocyanin supplementation, from *Clitoria ternatea*, which improved ΣUFA and decreased mono-saturated fatty acids in meat (da Silva Pereira *et al.*, 2020; Tahuk, Dethan, & Sio, 2018). The effects on fatty acids may be due to decreased total lipids, caused by anthocyanins in shrimp feed. Anthocyanin influences on both the level and the quality of fatty acids in aquatic animals need further research, as they are not fully understood. The index of atherogenicity (IA) in shrimp fed with anthocyanin rich plant powder supplement was reduced from that of the control shrimp. The shrimp fed with malabar fruit powder had the lowest IA. It has been demonstrated that the consumption of foods or products with a lower IA can reduce the levels of total cholesterol and LDL-C in human blood plasma (Chen & Liu, 2020).

4. Conclusions

Anthocyanins from 3 plant species, when supplemented in shrimp feed, accumulated in muscles of the marine shrimp *Penaeus vannamei*, and the cyanidin-3-glucoside as main derivative from malabar fruit powder had strong effects on reducing total crude fats in shrimp meat, as well as its total cholesterol levels. Overall, the anthocyanin metabolites reduced fat content in shrimp meat, indicating potential health implications to consumers for shrimp as seafood.

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Table 6. Fatty acid profiles of muscles of shrimp fed with anthocyanin supplementation for 45 days. (*n*=20)

Fatty acid profile (%)	Experimental diet			
	Control	Butterfly pea petal	Roselle calyx	Malabar fruit
Saturated fatty acids (SFA)				
Caprylic acid (C8:0)	0.00	0.01	0.00	0.00
Nonanoic acid (C9:0)	0.01	0.01	0.02	0.00
Lauric acid (C12:0)	0.03	0.03	0.03	0.02
Myristic acid (C14:0)	0.35	0.31	0.28	0.26
Pentadecanoic acid (C15:0)	0.31	0.32	0.32	0.31
Palmitic acid (C16:0)	17.06	15.61	15.63	15.22
Heptadecanoic acid (C17:0)	1.00	1.22	1.18	1.16
Stearic acid (C18:0)	9.61	10.53	9.83	9.86
Arachidic acid (C20:0)	0.24	0.3270	0.2879	0.3152
Behenic acid (C22:0)	10.92	11.62	12.29	11.79
Lignoceric acid (C24:0)	0.65	0.54	0.52	0.53
Monounsaturated fatty acids (MUFA)				
Palmitoleic acid (C16:1)	1.21	1.10	1.05	1.00
Oleic acid (C18:1)	13.03	11.89	11.81	11.24
Eicosanoic acid (C20:1)	0.61	0.51	0.52	0.53
Erucic acid (C22:1)	0.40	0.36	0.42	0.51
Polyunsaturated fatty acids (PUFA)				
Linoleic acid (C18:2)	14.40	13.81	13.59	14.32
Linolenic acid (C18:3)	0.66	0.63	0.60	0.58
∑ SFA	40.17	40.53	40.38	39.48
∑ MUFA	15.24	13.86	13.80	13.28
∑ PUFA	15.06	14.44	14.19	14.90
Index of Atherogenicity (IA)[1]	0.61	0.60	0.60	0.58

[1]IA = [C12:0 + (4 × C14:0) + C16:0]/ΣUFA

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