Original article DOI: 10.19027/jai.23.1.79-91

Dietary evaluation of cinnamaldehyde supplementation with different protein energy levels and ratios in Pacific whitleg Shrimp *Litopenaeus vannamei*

Evaluasi pemberian sinamaldehid pada pakan dengan kadar dan rasio energi protein berbeda pada udang vaname *Litopenaeus vannamei*

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(Received August 21, 2023; Accepted September 28, 2023)

ABSTRACT

This study aimed to evaluate the growth performance and carbohydrate metabolism of Pacific whiteleg shrimp after feeding with different cinnamaldehyde concentrations and protein-energy ratios. The study used a completely randomized design with six treatments in triplicates. The treatments were S003213; treatment S053213; treatment S052814; treatment S102814; treatment S052815; and treatment S102815. The study was conducted for 56 days in a 76 L volume aquarium using shrimps with 1.38 ± 0.01 g at 200 individuals/m^3. The results showed that the S053213 treatment was significantly different (P<0.05) compared to other treatments for the specific growth rate (SGR). Hexokinase (hk) and phosphoenolpyruvate carboxykinase (pepck) produced by the S053213 treatment were significantly different (P<0.05) from the S003213 treatment. The S052815 and S102815 treatments produced higher protein retention (PR) and protein efficiency ratio (PER) compared to other treatments (P<0.05) and also produced the same final average weight (FW) as the S003213 treatment. This research shows that vannamei fed by 0.10% supplementation dose of cinnamaldehyde with a decreased feed protein up to 28% and C/P ratio 14 and 15 are able to utilize feed as well as protein 32% without cinnamaldehyde. The addition of cinnamaldehyde with a higher C/P ratio requires a higher dose of cinnamaldehyde than the optimal dose.

Keywords: carbohydrate metabolism, cinnamaldehyde, growth, feed energy ratio, Pacific whiteleg shrimp

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi kinerja pertumbuhan dan metabolisme karbohidrat udang vaname *Litopenaeus vannamei* yang diberikan kadar sinamaldehid pada protein dan rasio energi pakan berbeda. Penelitian menggunakan rancangan acak lengkap (RAL) dengan enam perlakuan dan tiga ulangan. Adapun perlakuan terdiri dari perlakuan kontrol S003213; perlakuan S053213; perlakuan S052814; perlakuan S102814; perlakuan S052815; dan perlakuan S102815. Penelitian dilakukan selama 56 hari pemeliharaan pada akuarium volume 76 L menggunakan udang vaname berukuran 1,38 ± 0,01 g dengan kepadatan 200 individuals/m^3. Hasil penelitian menunjukkan bahwa perlakuan S053213 berbeda nyata (P<0,05) dibandingkan perlakuan lainnya untuk parameter laju pertumbuhan spesifik (SGR). Parameter heksokinase (*hk*) dan *phosphoenolpyruvate carboxykinase* (*pepck*) yang tertinggi dihasilkan oleh perlakuan S053213 dan berbeda nyata (P<0,05) dengan perlakuan S003213. Pada perlakuan S052815 dan S102815 menghasilkan retensi protein (PR) dan rasio efisiensi protein (PER) lebih tinggi dibandingkan dengan perlakuan lainnya (P<0,05) serta menghasilkan bobot rata-rata akhir (FW) sama dengan perlakuan S003213. Penelitian ini menunjukkan bahwa udang vaname yang diberikan suplementasi sinamaldehid sebesar 0,10% dengan protein pakan 28% dan rasio C/P menjadi 14 dan 15 mampu pemanfaatan protein pakan yang sama dengan protein pakan 32% tanpa suplementasi sinamaldehid. Penambahan sinamaldehid dengan rasio C/P yang lebih tinggi membutuhkan dosis sinamaldehid yang lebih tinggi dari dosis optimal.

Kata kunci: metabolisme karbohidrat, pertumbuhan, rasio energi pakan, sinamaldehid, udang vaname

INTRODUCTION

Pacific whiteleg shrimp *Litopenaeus vannamei* is one of the aquaculture commodities with high economical value, intriguing the shrimp culturists to improve their production and utilize the land maximally. Nevertheless, diseases and high feed cost are drawbacks in shrimp culture (Fan *et al*., 2016; Anderson *et al*., 2019). Feed cost in shrimp culture reaches up to 60%, which needs an appropriate feed selection in quantity and quality as an important factor in culture activities. Feed selection should notice on the appropriate nutrients in shrimp at ponds for efficient utilization and proportional requirement. Appropriate nutrient compositions, either macro- or micronutrients, become an important condition to support the shrimp growth.

One of the issues related to nutrient efficiency is the capability of shrimp to utilize non-protein energy, like carbohydrates or lipids. Protein is an important nutrient for shrimp for meat formation, tissue repairment, body functional maintenance, and a main ingredient in formulated feed for growth acceleration (Wang *et al*., 2016). Lipids and carbohydrates are non-protein energy sources that affect growth, while protein is utilized for growth. However, the protein ingredient source availability is declining and causes the feed cost tends to be expensive, while shrimps require protein as the main source for growth optimization (Kriton *et al*., 2018; Li *et al*., 2023). Therefore, an alternative energy source that can be converted for shrimp growth is necessary.

A component of energy source in artificial feed for shrimp is carbohydrates. Carbohydrates are energy components that can act as a protein-sparing effect and help reduce the feed cost due to high protein ingredient source in aquaculture activities (Wang *et al*., 2015; Wang *et al*., 2016). Carbohydrates are quite available everywhere as the cheapest energy source for animal nutrients and as a primary energy source (Wang *et al*., 2016; Takahashi *et al*., 2018; Li *et al*., 2019). Nevertheless, shrimp has a trouble utilizing carbohydrates, mainly glucose, as rising up the carbohydrates in feed can impact on the growth reduction (Wang *et al*., 2016).

A strategy to improve the carbohydrate utilization in shrimp is through bioactive compounds dietary supplementation such as cinnamaldehyde, isolated mainly from cinnamon (*Cinnamomum verum*). Cinnamaldehyde is a bioactive compound from cinnamons, that can improve animal growth and feed efficiency (Zhu *et al*., 2017; Chapman *et al*., 2019; Zhou *et al*., 2020), including the carbohydrate metabolism regulation (Kumar *et al*., 2012). Cinnamaldehyde absorption can induce the expression of insulin-like growth factor (IGF-1) mRNA and activate insulin through insulin-receptor (IR) (Nikzamir *et al*., 2014). Moreover, insulin induces the expression of glucose-transporter mRNA that transports glucose into the cells (Kipmen-Korgun *et al*., 2009). This condition can also increase the use of glucoses as an energy source (Zhu *et al*., 2017) and decrease the lipogenesis process. In addition, the IGF-1 mRNA has roles in protein accumulation, mainly for muscle formation and fish biomass elevation through protein biosynthesis and collagen in the tissues (Takasao *et al*., 2012).

Several studies have reported the dietary cinnamaldehyde or cinnamon supplementations for aquatic animals, namely striped catfish *Pangasius hypophthalmus*, common carp *Cyprinus carpio*, Nile tilapia *Oreochromis niloticus,* grass carp *Ctenopharyngodon idella*, dan pacific whiteleg shrimp *Litopenaeus vannamei* (Setiawati *et al*., 2016a; Laheng *et al*., 2016; Jusadi *et al*., 2016; Amer *et al*., 2018; Ghafoor, 2020; Zhou *et al*., 2020; Abd El-Hamid *et al*., 2021; Mousa *et al*., 2021; Shan *et al*., 2021; Junior *et al*., 2022; Zhou *et al*., 2023). Dietary supplementation of cinnamaldehyde improves the disease resistance and reduces the lipid accumulation in whiteleg shrimp (Safratilofa *et al*., 2015; Friedman, 2017; Doyle & Stephens, 2019; Chen *et al*., 2022). The striped catfish fed with 1% of cinnamon-supplemented diet showed improved growth, feed efficiency, protein retention, and meat quality (Setiawati *et al*., 2014; Setiawati *et al*., 2015; Rolin *et al*., 2017; Wahyudi *et al*., 2023). In shrimp, the application of cinnamaldehyde as a diet supplement is still limited. Therefore, this study aimed to evaluate the dietary supplementation of cinnamaldehyde on the growth performance and carbohydrate metabolism in Pacific whiteleg shrimp fed with different protein-energy levels and ratios.

MATERIALS AND METHOD

Materials

The Pacific whiteleg shrimps that were used in this study had an average weight of 1.38 ± 0.01 g and were obtained from the *PT. Syaqua Indonesia*, Banten, West Java. These shrimps were previously

reared for 40 days from the post-larvae (PL) period. Shrimps were reared in the Laboratory of Fishery Production, College of Vocational Studies, IPB University, Bogor, Indonesia. Cinnamaldehyde as the supplement in this study was a refined product of *cinnamaldehyde* (C_9H_8O) *GRM3277-Himedia.*

Methods

This study was conducted in the IPB University. Shrimp rearing and sampling were performed in the Laboratory of Fishery Production, College of Vocational Studies, IPB University, Bogor, Indonesia. Proximate and enzymatic analyses were performed in the Laboratory of Fish Nutrition, while gene expression was analyzed in the Laboratory of Aquatic Organism Reproduction and Genetics, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, Indonesia. A further gene expression analysis was continued in the Advanced Research Laboratory, IPB University, Bogor, Indonesia.

Experimental diets

The diet fed to the shrimp was a formulated diet with cinnamaldehyde supplementation in different protein-energy levels and ratio (C/P). The treatments were composed of S003213 (0% cinnamaldehyde supplementation in 32% feed protein and C/P ratio 13); treatment S053213 (0,05% cinnamaldehyde supplementation in 32% feed protein and C/P ratio 13); treatment S052814 (0.05% cinnamaldehyde supplementation in 28% feed protein and C/P ratio 14); treatment S102814 (0.10% cinnamaldehyde supplementation in 28% feed protein and C/P ratio 14); treatment S052815 (0.15% cinnamaldehyde supplementation in 28% feed protein and C/P ratio 15); and treatment S102815 (0.20% cinnamaldehyde supplementation in 28% feed protein and C/P ratio 15). As all diets were proportional with the treatments and C/P applied, the compositions were different among the treatments (Table 1). Proximate analysis was also conducted to determine the nutrient contents of the diets,

Table 1. Composition of the experimental diets for each treatment for pacific whiteleg shrimp.

Ingredients (%)	Treatment					
	S003213	S053213	S052814	S102814	S052815	S102815
Fishmeal	20.00	20.00	15.00	15.00	15.00	15.00
Corn gluten meal	8.00	8.00	10.00	10.00	10.00	10.00
Meat bone meal	10.00	10.00	10.00	10.00	11.00	11.00
Wheat pollard	15.00	15.00	18.00	18.00	20.00	20.00
Corn meal	14.00	14.00	17.20	17.50	14.45	14.90
Soybean meal	17.00	17.00	11.80	12.85	11.50	11.00
Cassava starch	4.00	3.95	4.00	3.50	4.00	4.00
Squid oil	2.00	2.00	1.88	1.85	3.00	3.00
Fish oil	2.00	2.00	1.87	2.00	3.00	3.00
Cinnamaldehyde	**0**	**0.05**	**0.05**	**0.10**	**0.05**	**0.10**
Lysine	0.30	0.30	0.30	0.30	0.30	0.30
Lecithin	0.80	0.80	0.80	0.80	0.80	0.80
DL-Methionine	0.30	0.30	0.30	0.30	0.30	0.30
Choline chloride	0.30	0.30	0.30	0.30	0.30	0.30
Mono-calcium phosphate	1.00	1.00	1.00	1.00	1.00	1.00
Cholesterol	0.30	0.30	0.30	0.30	0.30	0.30
Vitamin Mix	1.00	1.00	2.00	2.00	1.00	1.00
Mineral Mix	1.00	1.00	2.00	2.00	1.00	1.00
Carboxymethylcellulose (CMC)	3.00	3.00	3,00	3.00	3.00	3.00

Note: The cinnamaldehyde is a clear colorless or yellow liquid with a strong cinnamon odor at a density of 1.045-1.055 g/mL and a purity of 98%.

following the procedures of the Association of Official Analytical Chemists (AOAC, 2012).

Maintenance and sampling of shrimp

Shrimps were reared for 56 days in glass aquarium volume 76 L and stocking density of 200 individuals/m^3. Shrimps were fed with the treatment diets four times a day at 07.00, 11.00, 15.00, and 19.00 WIB. The rearing procedure was in accordance with Wiyoto *et al*. (2017) and Ramadhani *et al*. (2022). The shrimp sampling was performed at the initial rearing period, weekly, and the final rearing period by measuring the shrimp weight and counting the number of shrimps.

The water quality during rearing was maintained at optimal level by removing the diet waste once a week and replacing the water gradually at 20-30% of the water volume. Water quality was maintained gradually and remained at a temperature of 28–30°C, a dissolved oxygen of 4.7–6.4 mg/L, a pH of 6.8–8.0, an ammonia concentration of 0.02–0.05 mg/L, a salinity of 25-26 g/L. The parameters observed in this study were composed of gene expression related to carbohydrate metabolism, glycogen content measurement in hepatopancreas and body muscle, growth performance, and diet utilization.

Gene Expression Analysis

Gene expression analysis was observed at the final rearing period by taking the shrimp hepatopancreas at 12 hours after rearing. Carbohydrate metabolism was evaluated by the qRT-PCR through gene expression parameter associated with carbohydrate metabolism: 1) glucose transport: *glut-1*; 2) glycolysis: hexokinase (*hk*), and phosphoenolpyruvate carboxykinase (*pepck*). The total RNA was extracted by crushing the hepatopancreas in 200 µL of *GENEzol™ Reagent* solution (*Geneaid*, *Taiwan*), following the recommended protocols. The concentrated RNA was measured with a spectrophotometer at 260 nm absorbance wavelength, multiplied by the RNA constant and dilution factor. The RNA integrity was evaluated by the 2% agarose gel diluted in 0.1% diethylpyrocarbonate (DEPC) solution. The RNA purification and cDNA synthesis were performed using 1 µg of the total RNA with *RevetraAce qPCR RT mastermix* and *gDNA remover kit* (*Toyobo*), following the recommended protocols.

The cDNA synthesis success was assessed from the β-actin amplification. The gene expression analysis was performed using the real-time PCR (qPCR). The qPCR analysis was performed in a *Rotor-gene 6000* machine (*Corbett, USA*). The reaction in the process was occurred in the total volume of 20 µL, containing 10 µL of 2× *SensiFAST SYBR NO-ROX* (*Bioline, UK*), 0.8 µL (10 µM) of primer F and R, 10 ng of cDNA from the hepatopancreas, and 4.4 µL of water.

Primers were amplified through the PCR cycle, containing pre-denaturation at 95°C for 2 min, and 45 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 20 s, and extension at 72°C for 20 s. The melting curve analysis was evaluated with primer specification in amplification program. The gene expression level was determined by normalizing the β-actin and following the protocols (Livak & Schmittgen, 2001). The mRNA gene primers used in carbohydrate metabolism expression are presented in Table 3.

Glycogen Analysis

The shrimp hepatopancreas and body were preserved and homogenized by chopping. Each

Table 2. Proximate analysis of feed for each treatment.

Composition	S003213	S053213	S052814	S102814	S052815	S102815
Moisture (%)	7.93	7.84	8.71	8.55	8.75	8.63
Ash (%)	8.96	9.16	7.89	7.97	6.37	6.31
Protein (%)	32.03	32.04	27.99	27.88	27.96	27.98
Lipid (%)	8.26	8.30	7.74	7.45	9.31	9.13
Crude fiber (%)	4.18	3.54	5.88	5.92	5.07	5.06
NFE	38.64	39.12	41.79	42.24	42.54	42.89
GE (kcal/kg)[g]	4154.10	4178.48	4008.11	3992.88	4184.72	4183.93
C/P	13.0	13.0	14.3	14.3	15.0	15.0

Note: *NFE = nitrogen-free extract = 100 - (protein + lipid + moisture + ash + crude fiber); **GE = gross energy = (% protein × 5.6 kcal) + (% lipid × 9.4 kcal) + (%NFE × 4.1 kcal) (Watanabe, 1988).

of these samples were weighed at 0.5-2.0 g and dried in an oven at 100°C for 24 h to measure the moisture content of the samples. Furthermore, glycogen analysis was performed, following Takeuchi (1988).

Growth performance and feed utilization

Growth performance determined in this study was composed of final weight, specific growth rate (SGR), feed conversion ratio (FCR), and survival rate (SR). The SGR (Yang *et al*., 2022), SR, FCR (Cai *et al*., 2022), RP, and PER (Zhang *et al*., 2023) were calculated at the final rearing period with the following formula.

$$\text{SGR (\%/ day)} = \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{days}}$$

$$\text{SR} = \frac{\text{final shrimp number}}{\text{initial shrimp number}} \times 100$$

$$\text{FCR} = \frac{\text{feed consumption}}{(\text{final biomass} - \text{initial biomass})}$$

$$\text{PR} = \frac{\text{Protein deposition in the shrimp body}}{\text{Protein weight consumed by shrimp}} \times 100$$

$$\text{PER} = \frac{\text{final biomass} - \text{initial biomass}}{\text{feed intake} \times \text{dietary protein content}} \times 100$$

Data analysis

The data results were tabulated in *MS. Excel* and analyzed with an ANOVA method using *SPSS 22.0* stastistics software. The homogeneity level was previously determined using a one-sample Kolmogorov-Smirnov test. As a significant difference among the treatment data was occurred, data were continuously analyzed the Duncan's test at 95% of confidence level.

RESULTS AND DISCUSSION

Results

Growth performance

The growth performance of Pacific whiteleg shrimp fed with cinnamaldehyde-supplemented diets and different protein-energy ratios (C/P) for 56 days is presented in Table 4. The dietary

Table 3. Primer sequences of genes related to carbohydrate metabolism in shrimp analyzed in this study.

Gen	Symbol	Sequence	Function	References
Glucose transporter 1	*GLUT1*	F: TGG CAT TGA GCA ACT TCT TG R: TAG GGC TCT TCG TGC TTC AT AAC GTG TCA GCC TTC TCT TC	Glucose Transporter	(Lage *et al*., 2017)
Heksokinase	*HK*	F: AGT CGC AGC AAC AGG AAG TT R: CGC TCT TCT GGC ACA TGA TA	Glycolysis	(Lage *et al*., 2017)
Fosfoenolpiruvat karboksikinase	*PEPCK*	F: GATGTCACCATCACCTCGTG R: CTCATGGCTCCTCCTACCAG	Gluconeogenesis	(Lage *et al*., 2017)
β-actin	*β ACT*	R: GAGCAACACGGAGTTCGTTGT F: CATCACCAACTGGGACGACATGGA	Housekeeping	Gen Bank accession no. AF300705

Table 4. Growth performance of Pacific whiteleg shrimp *Litopenaeus vannamei* after feeding with cinnamaldehyde-supplemented diet and different protein-energy ratios.

Treatment	IW (g)	FW (g)	SR (%)	SGR (%/day)	FCR
S003213	1.38 ± 0.01^{a}	6.68 ± 0.08^{c}	93.33 ± 0^{a}	2.86 ± 0.02^{c}	1.43 ± 0.03^{c}
S053213	1.38 ± 0.02^{a}	7.86 ± 0.08^{d}	95.56 ± 3.85^{a}	3.16 ± 0.03^{d}	1.30 ± 0.04^{a}
S052814	1.39 ± 0.01^{a}	5.90 ± 0.05^{a}	93.33 ± 0^{a}	2.61 ± 0.02^{a}	1.47 ± 0.04^{c}
S102814	1.36 ± 0.01^{a}	6.20 ± 0.05^{b}	93.33 ± 0^{a}	2.73 ± 0.03^{b}	1.48 ± 0.04^{c}
S052815	1.38 ± 0.01^{a}	6.25 ± 0.05^{b}	95.56 ± 3.85^{a}	2.74 ± 0.01^{b}	1.37 ± 0.04^{b}
S102815	1.39 ± 0.01^{a}	6.63 ± 0.03^{c}	95.56 ± 3.85^{a}	2.83 ± 0.02^{c}	1.36 ± 0.03^{ab}

Note: IW = average weight of shrimp at the initial rearing period, FW = average weight of shrimp at the final rearing period (H56), SGR = specific growth rate, FCR = feed conversion ratio, SR = survival rate. Uppercase letters behind the mean (±standard deviation) in the same row indicate a significant difference (P<0.05).

supplementation of 0.05% cinnamaldehyde with 32% protein (S053213 treatment) provides a significant different value on the FW and SGR parameters (P<0.05). Meanwhile, the dietary supplementation of cinnamaldehyde had no significant effect on SR parameter. In general, the shrimp weight gain in the S053213 treatment in the diet produced a higher FW than other treatments (S003213; S052814; S102814; S052815 and S102815). The FCR value S102815; S052815 and S053213 was lower than other treatments.

Nutrient utilization parameters

The nutrient utilization parameters in shrimp, namely protein retention (PR) and protein-efficiency ratio (PER), are presented in Table 5. The results indicate the highest PR is shown in the S102815 treatment and significantly different (P<0.05) from the S003213, S053213, S052814 and S102814 treatments, but showing an insignificant difference with the S052815 treatment. The highest PER value was obtained from the S102815 treatment at 2.63 ± 0.06, but was insignificantly different from the S052815 treatment and significantly different from the S003213, S053213, S052814, S102814 treatments (P<0.05).

Glucose parameters

The glucose, muscle glycogen, and liver glycogen contents are presented in Table 6. The glucose value at S003213 treatment was higher than other treatments (P<0.05). The S053213 treatment produced a higher muscle glycogen than other treatments (P<0.05). Meanwhile, the liver glycogen content had no significant difference among the treatments.

Shrimp body proximate parameters

The nutrient composition of shrimps after feeding with cinnamaldehyde-supplemented diets is presented in Table 7. The results showed that the S053213 treatments produced a higher protein content than other treatments, but was insignificantly different from other treatments. Treatments S003213 and S053213 produced less fat compared to other treatments (P<0.05).

Expression of carbohydrate metabolism genes

The carbohydrate metabolism-related genes were measured from the hepatopancreas samples of the shrimp at the final rearing. The gene expression parameters were composed of *glut1, hk*, and *pepck* (Figure 1). In *glut1* gene expression, the S003213 treatment obtained the lowest value and a significantly different value (P<0.05) among other treatments (S053213, S052814, S102814, S052815 and S102815). The *hk* gene expression in the S053213 treatment obtained the highest value, compared to other treatments, but showing an insignificant difference. The highest *pepck* gene expression was obtained from the S053213 treatment and significantly different from the S003213 treatment (P<0.05), but showing

Table 5. Protein retention (%), protein efficiency ratio at the final rearing in Pacific whiteleg shrimp.

Treatment	PR (%)	PER
S003213	29.30 ± 0.64 [a]	2.03 ± 0.03 [a]
S053213	35.76 ± 0.71 [c]	2.39 ± 0.07 [b]
S052814	33.63 ± 0.90 [b]	2.41 ± 0.06 [b]
S102814	35.53 ± 0.75 [c]	2.47 ± 0.08 [b]
S052815	37.98 ± 0.38 [d]	2.60 ± 0.08 [c]
S102815	37.88 ± 0.77 [d]	2.63 ± 0.06 [c]

Note: PR = protein retention; PER: protein eficiency ratio.

Table 6. Blood glucose, muscle and liver glycogen at the final rearing period of Pacific whiteleg shrimp

Treatment	Glucose (mg/100mL)	Muscle glycogen (mg/g)	Liver glycogen (mg/g)
S003213	44.032 ± 0.485 [b]	6.882 ± 0.043 [a]	1.252 ± 0.012 [a]
S053213	42.635 ± 0.441 [a]	7.669 ± 0.216 [b]	1.278 ± 0.014 [a]
S052814	42.797 ± 0.231 [a]	6.762 ± 0.076 [a]	1.259 ± 0.008 [a]
S102814	42.359 ± 0.646 [a]	6.823 ± 0.111 [a]	1.259 ± 0.008 [a]
S052815	42.359 ± 0.646 [a]	6.871 ± 0.085 [a]	1.246 ± 0.014 [a]
S102815	42.289 ± 0.449 [a]	6.893 ± 0.208 [a]	1.267 ± 0.009 [a]

Note: Different superscript letters at the same row indicate a significant difference value (P<0.05).

Table 7. Proximate analysis of Pacific whiteleg shrimp at the final rearing period (% in wet base).

Treatment	Moisture	Ash	Protein	Lipid	Crude fiber	NFE
S003213	77.90 ± 0.09 ^{d}	3.77 ± 0.03 ^{a}	14.20 ± 0.38 ^{a}	1.32 ± 0.09 ^{a}	1.34 ± 0.07 ^{ab}	1.43 ± 0.34 ^{b}
S053213	77.43 ± 0.30 ^{cd}	4.59 ± 0.20 ^{bc}	14.79 ± 0.15 ^{a}	1.27 ± 0.04 ^{a}	1.47 ± 0.15 ^{b}	0.47 ± 0.23 ^{a}
S052814	77.24 ± 0.47 ^{cd}	4.23 ± 0.05 ^{bc}	14.26 ± 0.49 ^{a}	2.63 ± 0.27 ^{b}	1.36 ± 0.10 ^{ab}	0.34 ± 0.12 ^{a}
S102814	76.65 ± 1.07 ^{bc}	4.16 ± 0.47 ^{bc}	14.68 ± 0.07 ^{a}	2.54 ± 0.01 ^{b}	0.98 ± 0.02 ^{a}	1.02 ± 0.66 ^{ab}
S052815	75.26 ± 0.18 ^{a}	4.92 ± 0.55 ^{c}	14.74 ± 0.55 ^{a}	2.38 ± 0.40 ^{b}	1.38 ± 0.10 ^{ab}	0.99 ± 0.64 ^{b}
S102815	76.01 ± 0.34 ^{ab}	4.29 ± 0.13 ^{ab}	14.77 ± 0.45 ^{a}	2.31 ± 0.51 ^{b}	1.27 ± 0.48 ^{ab}	1.34 ± 0.09 ^{b}

Note: Different superscript letters at the same row indicate a significant difference value (P<0.05).

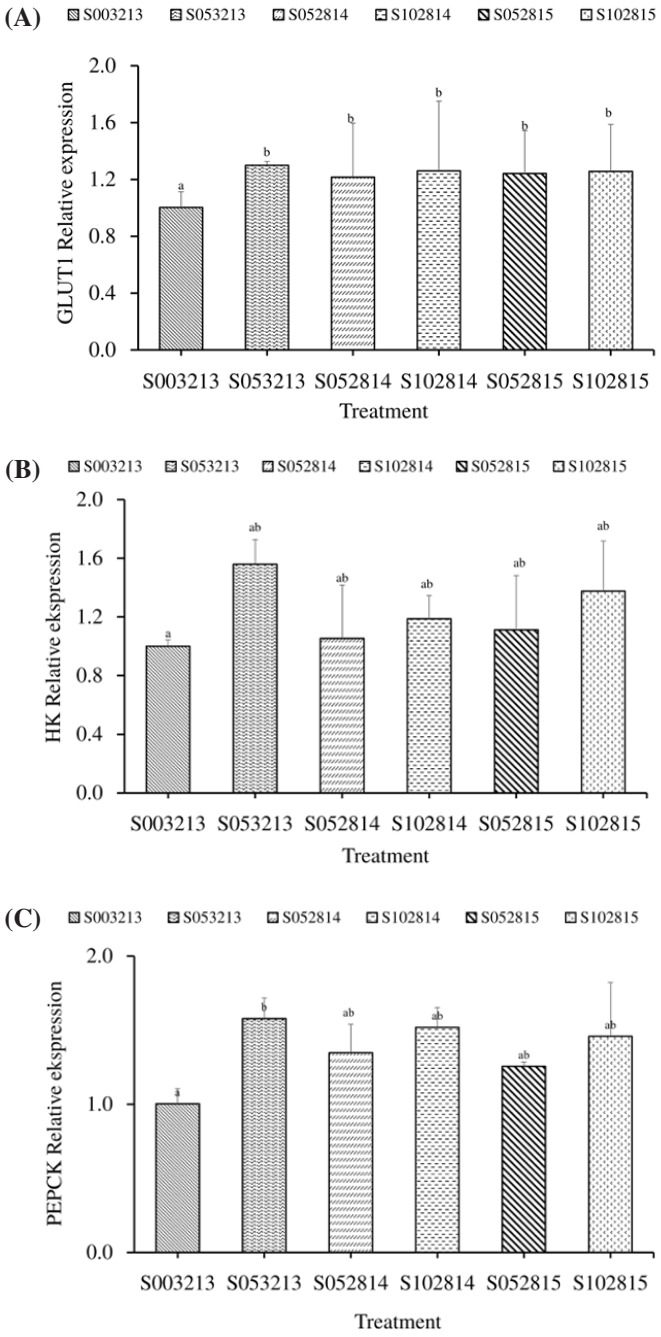

Figure 1. Carbohydrate metabolism gene expressions in Pacific whiteleg shrimp after feeding with cinnamaldehyde-supplemented diets at different protein-energy levels and ratios: (A) Glucose transporter 1 (*glut1*); (B) hexokinase (*hk*); (C) phosphoenolpyruvate carboxykinase (*pepck*).

no significant difference on other treatments (S052814, S102814, S102814, S052815, and S102815).

Discussion

This study indicates that the S053213 treatment with 32% protein content and 0.05% cinnamaldehyde supplementation provides a better utilization in carbohydrates, impacting on the weight gain and specific growth rate in whiteleg shrimp. According to Hendriana *et al.,* (2023), the supplementation of 0.05% cinnamaldehyde has roles in non-protein energy utilization improvement. A similar condition was also reported by Samocha *et al*. (2004), through the application of 32% protein that obtained a better growth rate. Shrimps can utilize protein at 30-60% for their growth (Yun *et al*., 2015).

The results showed that the 0.10% cinnamaldehyde supplementation, higher than the optimal dose, with low protein content (28%) and protein-energy ratio of 14 and 15 could improve the protein retention at the S052815 and S102815 and reduce the FCR value significantly, compared to the S003213 without cinnamaldehyde supplementation. Several factors that affect FCR are feed quality, fish or shrimp species in utilizing the feed, fish or shrimp size, and water quality during rearing. The amount of FCR determined the feed efficiency level. Moreover, total carbohydrates are considered to help regulate the body metabolism (Handajani, 2006).

The results showed that shrimps can utilize the 32% protein more effectively by utilizing the non-protein energy from the diet due to cinnamaldehyde supplementation. According to Suprayudi *et al*. (2014), the utilization of non-protein energy in the diet (protein-sparring effect) as energy source can be applied for growth. Carbohydrate assimilation efficiency depends on the enzymatic activity regulation, besides feed quality and quantity *(*Rosas *et al*., 2001). Positive roles of cinnamaldehyde in carbohydrate metabolism may be related to the positive effect of cinnamaldehyde in insulin activity, as reported in mice (Guo *et al*., 2017). Insulin has an important role in carbohydrate metabolism, starting from blood glucose balance, blood glucose transportation to body cells, and glucose utilization as an energy source.

The crustacea hyperglycemic hormone (CHH), insulin-like peptides, and insulin-like growth factor (IGF-I and IGF-II) are hormone in crustacean hemolymph for glucose regulation. Cinnamaldehyde can also activate the insulin-like growth factor (IGF-1) for regulating the protein and collagen biosynthesis in body tissue (Takasao *et al*., 2012). Increased carbohydrate utilization by shrimps is expected to reduce the protein content in the formulated diet. Growth performance and feed utilization efficiency significantly are significantly influenced by the carbohydrate level in feed. Previous studies reported that cinnamaldehyde had positive roles in elevating the SGR, PR, and PER values, and reducing the FCR value in striped catfish (Setiawati *et al*., 2016a; Tartila *et al*., 2021), Nile tilapia (Amer *et al*., 2018), grass carp (Zhou *et al*., 2021), and rainbow trout (Ravardshiri *et al*., 2021). According to Enes *et al*. (2010), insulin has important roles in glucose homeostasis and postprandial glucose uptake induction through peripheral tissue.

The plasma insulin secretion increase is correlated with the blood glucose level decrease (Anand *et al*., 2010). Cinnamaldehyde can support the insulin content and reduces the glucose level by regulating the glucose utilization in muscle and reducing the gluconeogenesis process in the liver. The glycogen content increased along with the cinnamaldehyde supplementation, which indicates that available glucose is stored as glycogen (Zhang *et al*., 2020). Glycogen in crustacean hepatopancreas is an important precursor in chitin synthesis, glucose supply, and molting cycle (Cuzon *et al*., 2000).

Glycogen can be utilized, when there no feed supply through glycogenolysis (Brosnan & Watford, 2015). Glycogen becomes a short-term reserved-energy that can be used rapidly, when the available energy is depleted (Setiawati *et al*., 2015). When the glycogen is depleted, animals will mobilize and transform lipid and protein as glucose through gluconeogenesis to sustain the blood glucose level. Gluconeogenesis occurs in the shrimp hepatopancreas (Reyes-Ramos *et al*., 2018; Berry *et al*., 2019). When gluconeogenesis no longer produces adequate glucose levels, the organism will experience a number of detrimental side effects, even death (Brosnan & Watford, 2015).

Feeding high carbohydrates can trigger hyperglycemia, glycogen deposition, increase lipid biosynthesis in liver tissue (Zhang *et al*., 2019; Su *et al*., 2021), and low growth to high mortality (Chen *et al*., 2022). Growth performance will have an impact on the quality of nutrients in the shrimp's body. Cinnamaldehyde

plays a role in increasing the insulin receptor (*IR*), so the use of carbohydrates as an energy source can increase. In addition, cinnamaldehyde can also increase the fatty acid oxidation through Carnitine palmitoyl transferase-1a enzyme (*Cpt-1a*) (Nikzamir *et al*., 2014; Zhu *et al*., 2017*)*, characterized by a reduction in the lipogenesis process and a decreased fat levels in the body (Lopes et al., 2015).

The carbohydrate metabolism genes in Pacific whiteleg shrimp were measured through gene expression parameters, including *glut1*, *hk* and *pepck* genes, which were examined under the fasting conditions for 24 hours. Several studies reported that increasing the carbohydrate content in feed could transform the gene expression, especially those related to carbohydrate metabolism (Rocha *et al*., 2015; Chen *et al*., 2017). High *hk* and *pepck* values indicate an increased glycolysis activity in muscle and liver, which was thought to occur because the *hk* gene has reached its maximum point to work in the conversion of glucose into glucose-6-phosphate. The expression of the *hk* gene is known to have a high ability to bind to glucose but its activity can be inhibited by high levels of glucose-6-phosphate.

The *hk* enzyme acts as a gene encoded key enzyme as a limiting factor in the glycolysis process (Enes *et al*., 2009). Meanwhile, increased *pepck* gene expression value indicates the gluconeogenesis process. The results of this study indicate that administration of cinnamaldehyde at 0.05% with 32% protein content in diet can increase the expression of genes related to carbohydrate metabolism as an energy source through the glycolysis process, which describes that carbohydrate metabolism occurs more actively.

CONCLUSION

The Pacific whiteleg shrimp fed with 0.1% cinnamaldehyde and 28% protein with the C/P ratios of 14 and 15 provides a better retention protein and feed conversion ratio, than 32% protein without cinnamaldehyde dietary supplementation. A higher cinnamaldehyde inclusion beyond the optimal dose is recommended, when a higher C/P value in the formulated diet is applied.

ACKNOWLEDGMENTS

Authors would like to thank The College of Vocational Studies, IPB University Bogor, who have facilitated this research.

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