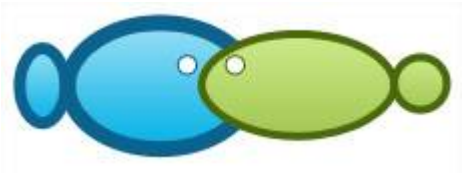

Evaluation of the administration of cinnamaldehyde to feed on the growth performance and carbohydrate metabolism of Pacific whiteleg shrimp (*Litopenaeus vannamei*)

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Abstract. Shrimp have patterns of carbohydrate metabolism, growth, and cellular and humoral immune responses that are different from fish and limited ability to utilize high carbohydrates, although carbohydrates are often included in the feed as a source of protein reserve energy. The use of bioactive compounds such as cinnamaldehyde is one of the strategies to improve utilization of feed carbohydrates. This study aims to evaluate the effect of different levels of dietary cinnamaldehyde on the growth performance and carbohydrate metabolism of Pacific whiteleg shrimp *Litopenaeus vannamei*. This research was conducted at the Fisheries Production Laboratory of the College of Vocational Studies, IPB University. The study used a completely randomized design (CRD) consisting of five dietary treatments with different cinnamaldehyde levels in triplicates, namely control (0%), CN0.05 (dietary cinnamaldehyde of 0.05%), CN0.10 (0.10%), CN0.15 (0.15%) and CN0.20 (0.20%). Pacific whiteleg shrimp with an initial size of 2.17±0.02 g were maintained for 56 days in an aquarium with a volume of 76 L of water at a density of 15 shrimp/aquarium. The results showed that the administration of 0.05% cinnamaldehyde increased the shrimp growth performance as demonstrated by the higher final weight, specific growth rate (SGR), protein retention, and decreasing lipid retention as well as feed conversion ratio (FCR) compared to those of the control and other cinnamaldehyde treatments. Adding cinnamaldehyde to food supplements can increase growth performance through carbohydrate metabolism in Pacific whiteleg shrimp. Thus, cinnamaldehyde plays the role of an alternative energy source which is included in non-protein energy.

Key Words: bioactive compound, feed utilization, feed supplement, non-protein energy, protein sparring effect.

Introduction. Pacific whiteleg shrimp (*Litopenaeus vannamei*) is an economically aquaculture commodity in Indonesia and its production is still continue until nowdays. Increasing shrimp production still faces many obstacles, especially concerning disease and high feed costs (Yousefian & Amiri 2009; Gao et al 2012; Fan et al 2016; Anderson et al 2019). One of the problems related to feed efficiency is the ability of shrimp to utilize non-protein energy sources such as carbohydrates or lipids. Shrimp have patterns of carbohydrate metabolism, growth, and cellular as well as humoral immune responses that are different from fish and limited ability to utilize carbohydrates (Shiau & Jiang 1991). Although carbohydrates are often included in the feed as a replacement of the protein (Cruz-Suarez et al 1994; Cuzon et al 2004; Li et al 2023), the ability of shrimp to utilize carbohydrates is generally relatively low, and an increase in carbohydrates in feed can reduce growth (Wang et al 2016). One of the strategies that can be implemented to increase the utilization of feed carbohydrates in aquaculture organisms is to use bioactive compounds such as cinnamaldehyde, which is abundant in cinnamon (*Cinnamomum verum*).

Cinnamaldehyde is a natural chemical compound from cinnamon that can improve growth performance and feed efficiency for animals (Zhu et al 2017; Chapman et al 2019; Zhou et al 2020) and is still considered a safe, natural ingredient that is well tolerated in humans and animals (Luo et al 2013). One of the functions of cinnamaldehyde reported in previous studies is to activate insulin-like growth factor (IGF-1), which can play a role in increasing protein deposition in the body, especially in building muscle and increasing fish biomass utilizing protein and collagen biosynthesis in body tissues (Plaisier et al 2011; Takasao et al 2012). Research on mice showed that feeding with cinnamaldehyde as much as 20 mg kg^{-1} for four weeks showed normal blood glucose concentrations in diabetes-induced rats (Khare et al 2016). Moreover, trans-cinnamaldehyde could be used as a nutritional supplement to enhance disease resistance and reduce lipid accumulation of Pacific whiteleg shrimp (Chen et al 2022). Several previous studies have reported research on cinnamaldehyde administration to aquaculture organisms. Research on catfish *Pangasianodon hypopthalmus* showed that cinnamaldehyde in feed could cause an increase in fish growth performance (Setiawati et al 2016). Adding 0.1% cinnamaldehyde for 75 days on Nile tilapia *Oreochromis niloticus* fingerlings through feed can increase growth performance, immunity, and antioxidant status (Amer et al 2018). The same results were shown for grass carp *Ctenopharyngodon idella*, where feeding supplemented with cinnamaldehyde for 60 days increased growth performance through increased absorption and digestive capacity (Zhou et al 2021). Rainbow trout *Oncorhynchus mykiss* that were fed with the addition of cinnamaldehyde for eight weeks showed increased parameters related to growth performance, enzyme digestion, blood concentration, and antioxidant activity (Ravardshiri et al 2021). Apart from positively affecting growth, immunity, and antioxidant capacity, feeding with cinnamaldehyde was also reported to improve the liver structure and meat quality on catfish *P. hypophthalmus* (Tartila et al 2021). Research on the use of cinnamaldehyde in shrimp is still limited. Therefore, this study aims to evaluate the effect of adding cinnamaldehyde to feed on carbohydrate metabolism and the growth performance of Pacific whiteleg shrimp.

Material and Method

Materials. The experimental animal was Pacific whiteleg shrimp (*Litopenaeus vannamei*) with an average weight of 2.17±0.02 g from PT Syaqua Indonesia Banten, West Java. This study was conducted on May-June 2022. All shrimp used in this study were cultured in seawater tanks at the Laboratory of Fisheries Production, College of Vocational Studies, IPB University, Bogor, Indonesia. The cinnamaldehyde used is cinnamaldehyde (C_9H_8O) GRM3277-Himedia 500 mL from Intralab Ekatama Company, Bogor, West Java, Indonesia.

Methods. The research was conducted for 56 days at IPB University. The maintenance and sampling shrimp activities were carried out at the Laboratory of Fisheries Production, College of Vocational Studies, IPB University, Bogor, Indonesia. Proximate and enzyme analysis was carried out at the Laboratory of Nutrition and The Gene expression analysis was conducted at the Laboratory of Reproduction and Genetics of Aquatic Organisms, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, IPB University, Bogor, Indonesia. Further gene expression analysis was conducted at Advanced Research Laboratory Unit, IPB University, Bogor, Indonesia.

Ethical approval. All Pacific whiteleg shrimp experimental and rearing procedures were handled complied with the animal welfare under the national accreditation no. SNI 7311:2009 of Republic of Indonesia.

Experimental diets. The research feed was formulated with an iso-protein of 32% with an energy/protein ratio (C/P) of 13 (Table 1) with different levels of cinnamaldehyde addition, namely control CN0 (feed with 0% added cinnamaldehyde), CN0.05 (feed with the addition of 0.05% cinnamaldehyde), CN0.10 (feed with the addition of 0.10%

cinnamaldehyde), CN0.15 (feed with the addition of 0.15% cinnamaldehyde) and CN0.20 (feed with the addition of 0.20% cinnamaldehyde). The proximate feed analysis was carried out following the procedures of the Association of Official Analytical Chemists (AOAC 2012). The results of proximate feed analysis were showed in the Table 2.

Table 1

Experimental diets composition

Ingredients (%)	*Treatments*				
	CN0	*CN0.05*	*CN0.10*	*CN0.15*	*CN0.20*
Fishmeal	20.00	20.00	20.00	20.00	20.00
Corn gluten meal	8.00	8.00	8.00	8.00	8.00
Meat bone meal	10.00	10.00	10.00	10.00	10.00
Wheat pollard	15.00	14.95	14.95	14.95	14.95
Corn meal	14.00	14.00	14.00	14.00	14.00
Soybean meal	17.00	17.00	17.00	17.00	17.00
Cassava starch	4.00	4.00	3.95	3.90	3.85
Squid oil	2.00	2.00	2.00	2.00	2.00
Fish oil	2.00	2.00	2.00	2.00	2.00
Cinnamaldehyde	0.00	0.05	0.10	0.15	0.20
Lysine	0.30	0.30	0.30	0.30	0.30
Lecithin	0.80	0.80	0.80	0.80	0.80
DL-Methionine	0.30	0.30	0.30	0.30	0.30
Choline chloride	0.30	0.30	0.30	0.30	0.30
Mono-calcium phosphate	1.00	1.00	1.00	1.00	1.00
Cholesterol	0.30	0.30	0.30	0.30	0.30
Vitamin mix	1.00	1.00	1.00	1.00	1.00
Mineral mix	1.00	1.00	1.00	1.00	1.00
Carboxymethylcellulose (CMC)	3.00	3.00	3.00	3.00	3.00

Note: The cinnamaldehyde is a clear colorless or yellow liquid with a strong cinnamon smell, with a density of 1.045-1.055 g mL^{-1} and a purity of 98%. Vitamin mix contained 900 IU kg^{-1} retinole (A), 200 mg kg^{-1} ascorbic acid (C), 200 IU kg^{-1} cholecalciferol (D), 10 mg kg^{-1} menadione (K3), 100 mg kg^{-1} α-tocopherol (E), 1000 mg kg^{-1} choline, 100 mg kg^{-1} inositole, 15 mg kg^{-1} thiamine (B1), 20 mg kg^{-1} riboflavin (B2), 15 mg kg^{-1} pyridoxine (B6), 50 mg kg^{-1} d-pantothenic acid (B5), 75 mg kg^{-1} nicotinic acid, 0.5 mg kg^{-1} biotin, 0.05 mg kg^{-1} cyanocobalamin (B12), 5 mg kg^{-1} folic acid. Mineral mix contained 0.5 mg kg^{-1} Co ($CoCl_2.6H_2O$), 5 mg kg^{-1} Cu ($CuSO_4.5H_2O$), 50 mg kg^{-1} Fe ($FeSO_4.7H_2O$), 4 mg kg^{-1} I (KI), Cr ($CrCl_3.6H_2O$) 0.1 mg kg^{-1}, 150 mg kg^{-1} Mg ($MgSO_4.7H_2O$), 25 mg kg^{-1} Mn ($MnSO_4.H_2O$), 0.1 mg kg^{-1} Se ($NaSeO_3$), 100 mg kg^{-1} Zn ($ZnSO_4.7H_2O$).

Table 2

Results of the proximate analysis of experimental diets

Composition	*CN0*	*CN0.05*	*CN0.10*	*CN0.15*	*CN0.20*
Moisture (%)	7.93	7.84	8.04	7.80	6.80
Ash (%)	8.86	9.16	9.20	9.64	9.21
Protein (%)	32.11	32.15	32.13	32.02	32.15
Lipid (%)	7.80	7.60	7.92	7.99	7.55
Crude fiber (%)	4.18	3.54	3.88	3.77	4.30
NFE	39.11	39.72	38.83	38.79	39.99
Energy (kJ kg^{-1})	406.55	406.82	407.1	407.37	407.64

Note: GE gross energy = (% protein * 23.4 kJ g^{-1}) + (% lipid * 38.5 kJ g^{-1}) + (%NFE *17.2 kJ g^{-1}) (Takeuchi et al 2002); NFE = nitrogen-free extract.

Maintenance and sampling of shrimp. The maintenance of shrimp was conducted for 56 days in an aquarium measuring 60 x 40 x 40 cm^3 with a stocking density of 15 individuals per aquarium. During maintenance, the shrimp were fed four times a day, at 07.00, 11.00, 15.00, and 19.00 using experimental diet. Maintenance procedure refers to Wiyoto et al (2017) and Ramadhani et al (2022). Shrimp sampling was carried out at the beginning of rearing every week and at the end by measuring the weight and counting the number of shrimps. During the rearing period, the water quality was always tried to

be optimum by siphoning leftover feed once in week and changing the water regularly 20-30% of total aquarium volume. Water quality was monitored periodically and showed that during the maintenance period water temperature was in the range of 28-30°C, dissolved oxygen concentration 4.8–6.5 mg L^{-1}, pH 6.8-7.8, ammonia concentration 0.03-0.05 mg L^{-1} and salinity 25-26 g L^{-1}.

Measurement of parameters. Parameters observed in this study included analysis of gene expression related to carbohydrate metabolism, measurement of glycogen levels in the hepatopancreas and body muscles of shrimp, growth performance, and feed utilization.

Gene expression analysis. Gene expression analysis was measured at the end of the observation by taking samples of the shrimp hepatopancreas at 0, 12, and 24 hours. Gene expression observed in this study was carbohydrate metabolism including: 1) glucose transport: glut-1; 2) glycolysis: glucokinase (gck), hexokinase (hk), and phosphoenolpyruvate carboxykinase (pepck) using qRT-PCR. Total RNA was extracted by crushing the hepatopancreas in 200 µL of GENEzolTM Reagent solution (Geneaid, Taiwan) according to the recommended protocol. RNA concentrations were measured with a spectrophotometer at an absorbance of 260 nm, then multiplied by the RNA constant and the dilution factor. RNA integrity was evaluated with a water-diluted 2% agarose gel containing 0.1% diethylpyrocarbonate (DEPC). RNA purification and cDNA synthesis were performed using 1 µg of total RNA using RevetraAce qPCR RT master mix with gDNA remover kit (Toyobo) according to the recommended protocol. The success of cDNA synthesis was carried out by amplifying β-actin.

Gene expression was performed using real-time PCR (qPCR). qPCR reactions were performed on a Rotor-gene 6000 machine (Corbett, USA). The response was run with a total of 20 µL consisting of 10 µL 2x SensiFAST SYBR NO-ROX (Bioline, UK), 0.8 µL (10 µM) primers F and R, 10 ng cDNA from the hepatopancreas, and 4.4 µL water. Primary amplification was performed by pre-denaturing PCR cycles at 95°C for 2 min. The 45 cycles included denaturation at 95°C for 15 seconds, annealing at 60°C for 20 seconds, and extension at 72°C for 20 seconds. Melting curve analysis was evaluated with the primary specifications in the amplification program. Gene level expression was performed by normalizing β-actin and following a protocol (Livak & Schmittgen 2001). The mRNA gene primers used in the assay of carbohydrate metabolism are presented in Table 3.

Table 3

Primary sequences of genes related to carbohydrate metabolism in shrimp analysed in this study

Gene	*Symbol*	*Sequence*	*Role*	*References*
Glucose transporter 1	GLUT1	F TGG CAT TGA GCA ACT TCT TG R TAG GGC TCT TCG TGC TTC AT	Glucose transporter	Lage et al (2017)
Glucokinase	GCK	CTC GTG TTG CTG TTG TTC TTG AAC GTG TCA GCC TTC TCT TC	Glycolysis	Lage et al (2017)
Hexokinase	HK	AGT CGC AGC AAC AGG AAG TT CGC TCT TCT GGC ACA TGA TA	Glycolysis	Lage et al (2017)
Phosphoenol-pyruvate carboxykinase	PEPCK	F: GATGTCACCATCACCTCGTG R: CTCATGGCTCCTCCTACCAG R: CCG TGG ATC ATC TCG TAG GT	Gluconeo-genesis	Lage et al (2017)
Pyruvate kinase	PK	R: AGCTGTCTCAGCAGGTCCAT R: AGCGAGGCCTGTACTTTGAA	Glycolysis	Lage et al (2017)
β-actin	β ACT	R: GAGCAACACGGAGTTCGTTGT F:CATCACCAACTGGGACGACATGGA	House keeping	Munaeni et al (2020)

Glycogen analysis. Hepatopancreas and shrimp bodies were taken and homogenized by chopping. Then the liver and meat samples were weighed as much as 0.5-2 g each and dried in an oven at 100°C for 24 hours to measure the moisture content of the samples. Furthermore, glycogen analysis was carried out based on Wedemeyer & Yasutake (1977).

Growth performance and feed utilization. Growth performance includes final shrimp weight, feed consumption, specific growth rate (SGR), feed conversion ratio (FCR), and survival rate. SGR, FCR, survival rate, protein retention (PR) and lipid retention (LR) were calculated at the end of the rearing period based on the following formulae:

Specific growth rate (SGR). The formula used to calculate SGR is provided below:

$$SGR\ (\%\ day^{-1}) = \frac{Ln(Wt) - Ln(W0)}{day}$$

where: SGR = specific growth rate (% day^{-1});
Wt = final weight of shrimp (g);
W0 = initial weight of shrimp (g);
Ln = the natural log.

Survival rate (SR). The formula used to calculate SR is provided below:

$$SR = \frac{Nt}{N0} \times 100$$

where: SR = survival rate (%);
Nt = number of shrimp at the end of rearing period (shrimp);
No = number of shrimp at the beginning of rearing period (shrimp).

Feed conversion ratio (FCR). The formula used to calculate FCR is provided below:

$$FCR = \frac{F}{Wt - W0}$$

where: FCR = feed conversion ratio;
F = amount of feed during rearing (g);
Wo = weight of biomass at the beginning of shrimp rearing period (g);
Wt = weight of biomass at the end of shrimp rearing period (g).

Protein retention and lipid retention. The formulae used to calculate PR and LR are provided below:

$$PR = \frac{Pu}{Pc} \times 100$$

$$LR = \frac{Lu}{Lu} \times 100$$

where: PR = protein retention (%);
LR = lipid retention (%);
Pu = protein deposition in the shrimp body (g);
Pc = protein weight consumed by shrimp (g);
Lu = the amount of lipid stored in the body (g);
Lc = lipid weight consumed by shrimp (g).

Statistical analysis. The data obtained are tabulated in MS. Excel and analyzed using the statistical program SPSS 22.0, including analysis of variance (ANOVA). Duncan further tested significant differences at a 95% confidence interval.

Results. This study resulted the parameter of expression of carbohydrate metabolism genes and growth performances in each treatments.

Genes expression of carbohydrate metabolism. The genes involved in glucose metabolism have been measured, including GLUT1, HK, PEPCK, GCK and PK. The study of gene expression was done after 0, 12, and 24 hours. The result showed that at 0 hours, there were no significant differences ($p > 0.05$) between treatments. While at 12 hours, the CN0.05 treatment showed the highest increase in expression of the GLUT1, HK, and GCK genes compared to other treatments. In contrast, the expression of the PEPCK gene at the 12^{th} hour of the CN0.10 treatment showed the highest value. At the 24^{th} hour, the

expression of carbohydrate metabolism genes, except for the PK gene, did not appear to differ significantly between treatments and tended to decrease compared to the 12th hour. At the 24th hour, the expression of the PK gene in the CN0.05 treatment showed a significant increase and was higher than the other treatments. In contrast, at the 12th hour, there was no significant difference in the expression of the PK gene between all treatments (Figure 1).

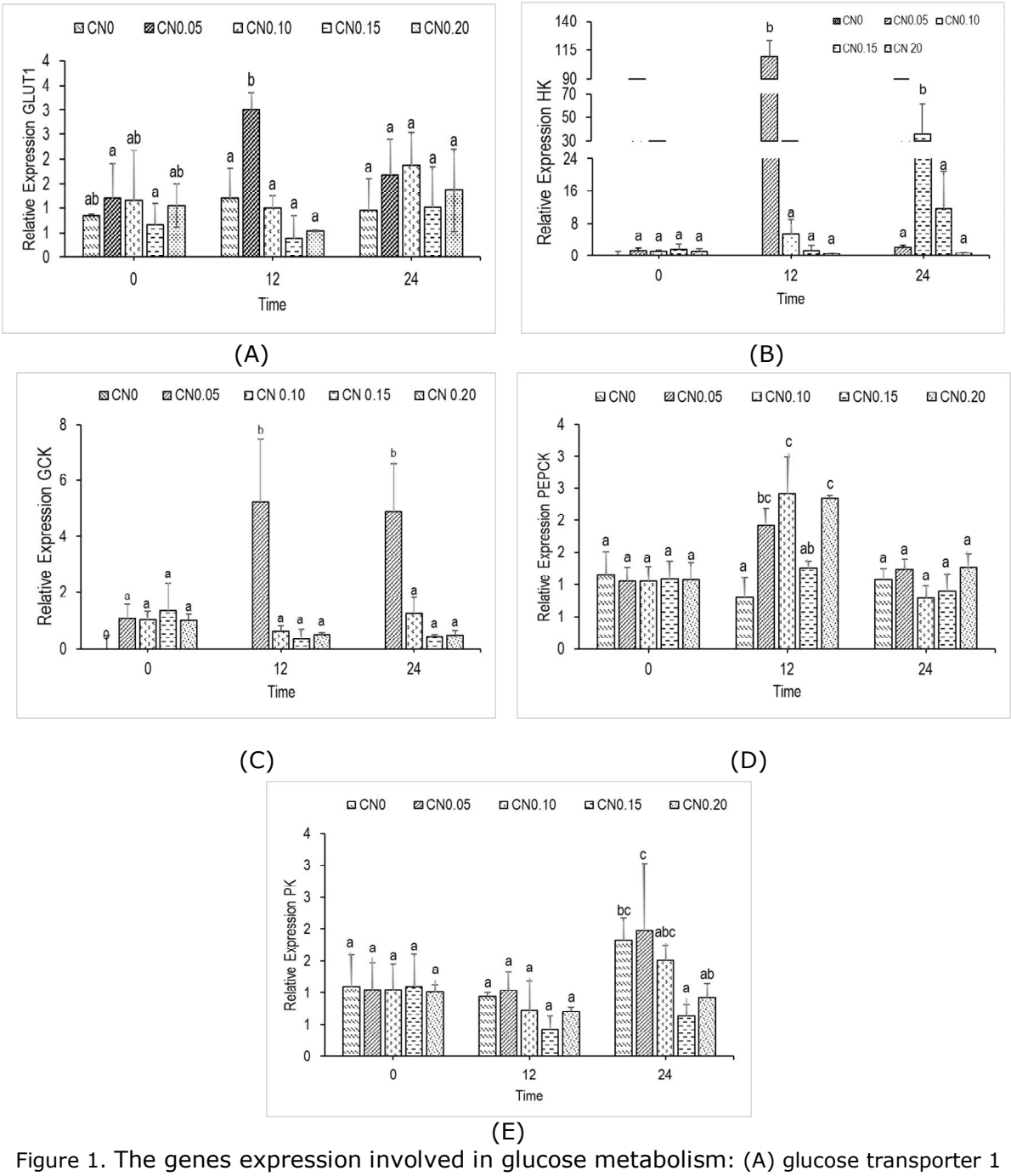

Figure 1. The genes expression involved in glucose metabolism: (A) glucose transporter 1 GLUT1, (B) hexokinase (HK), (C) phosphoenolpyruvate carboxykinase PEPCK, (D) glucokinase GCK, (E) pyruvate kinase (PK), in Pacific whiteleg shrimp treated different cinnamaldehyde doses under fasting conditions. CN0 (feed with doses of 0% added cinnamaldehyde), CN0.05 (feed with doses of 0.05% cinnamaldehyde), CN0.10 (feed with doses of 0.10% cinnamaldehyde), CN0.15 (feed with doses of 0.15% cinnamaldehyde) and CN0.20 (feed with doses of 0.20% cinnamaldehyde).

Growth performances. The growth performances measured include initial weight (Wo), final weight (W56), amount of feed consumed (FC), specific growth rate (SGR), feed

conversion ratio (FCR), survival rate (SR), protein retention (PR), lipid retention (LR). The growth performance of Pacific whiteleg shrimp given cinnamaldehyde supplementation with various proteins and different feed energy ratios (R/P) during 56 days of rearing is presented in Table 4. The treatment CN0.05 showed significant differrent ($p < 0.05$) in parameters of W56, FC, SGR, FCR, PR, LR, and muscle glycogen compared to treatment CN0. Moreover, the treatment CN0.05 showed the highest value of liver glycogen but not significant different ($p > 0.05$) compared to treatments CN0, CN0.10, and CN0.20, meanwhile treatment CN0.05 was significantly different ($p < 0.05$) compared to CN0.15.

Table 4

Growth performance of Pacific whiteleg shrimp (*Litopenaeus vannamei*) for 56 days of the rearing period

Parameter	*Treatments*				
	CN0	*CN0.05*	*CN0.10*	*CN0.15*	*CN0.20*
Wo (g)	2.18 ± 0.02^{a}	2.17 ± 0.02^{a}	2.16 ± 0.02^{a}	2.18 ± 0.02^{a}	2.18 ± 0.02^{a}
W56 (g)	8.30 ± 0.10^{a}	8.89 ± 0.09^{c}	8.50 ± 0.10^{b}	8.52 ± 0.10^{b}	8.40 ± 0.10^{ab}
FC(g)	124.33 ± 5.51^{a}	127.44 ± 3.84^{c}	128.33 ± 3.51^{b}	124.67 ± 5.57^{b}	127.67 ± 18.01^{ab}
SGR (% day^{-1})	2.42 ± 0.03^{a}	2.55 ± 0.02^{c}	2.48 ± 0.02^{b}	2.47 ± 0.15^{ab}	2.44 ± 0.03^{ab}
FCR	1.54 ± 0.02^{b}	1.35 ± 0.03^{a}	1.44 ± 0.08^{ab}	1.45 ± 0.09^{ab}	1.45 ± 0.11^{ab}
SR (%)	91.11 ± 3.85^{a}	93.33 ± 6.67^{a}	91.11 ± 3.85^{a}	93.33 ± 6.67^{a}	93.33 ± 6.67^{a}
PR (%)	30.17 ± 0.54^{a}	37.66 ± 1.14^{b}	33.63 ± 1.85^{a}	32.48 ± 2.68^{a}	32.45 ± 3.47^{a}
LR (%)	13.05 ± 0.29^{b}	10.58 ± 0.44^{a}	10.00 ± 1.91^{a}	10.16 ± 1.33^{a}	12.97 ± 1.35^{b}
Muscle glycogen (mg g^{-1})	14.42 ± 2.18^{a}	18.63 ± 0.59^{b}	18.21 ± 0.37^{b}	13.55 ± 0.18^{a}	12.64 ± 0.66^{a}
Liver glycogen (mg g^{-1})	1.87 ± 0.18^{ab}	2.27 ± 0.30^{b}	2.24 ± 0.20^{b}	1.53 ± 0.16^{a}	1.93 ± 0.44^{ab}

Note: Wo = average weight of shrimp at the start of rearing, W56 = average weight of shrimp at the end of rearing (H56), FC = amount of feed consumed, SGR = specific growth rate, FCR = feed conversion ratio, SR = survival rate, PR = protein retention, LR = lipid retention. Uppercase letters behind the mean (±standard deviation) values in the same row indicate a significant difference ($p < 0.05$).

Discussion. Carbohydrate metabolism was measured in Pacific whiteleg shrimp by measuring expression parameters, including the GLUT1, HK, PEPCK, GCK, and PK genes. Gene expression was examined under fasting conditions for 24 hours; although the half-life of cinnamaldehyde is still unknown, it appears that the process of carbohydrate metabolism is still ongoing at the 12th hour after fasting, PEPCK gene expression increased in all treatments, indicating the occurrence of gluconeogenesis. At the same time, there was an increase in GLUT-1 expression, showing an increase in glucose transport. Another increase was also seen in the expression of the HK and GCK genes, indicating an increase in glycolytic activity in muscle and liver. This increase is thought to occur because the HK gene has reached its maximum point to convert glucose to glucose-6-phosphate. The HK enzyme is known to have a high ability to bind to glucose, but its activity can be inhibited by high levels of glucose-6-phosphate (Enes et al 2009; Devlin 2010), while the GCK enzyme has a low ability to bind to glucose, but its activity is not inhibited by glucose-6-phosphate (Enes et al 2009; Devlin 2010), while PK gene expression showed an increase at 24 hours which may indicate that the last stage of glycolysis involving the pyruvate kinase enzyme is still ongoing at this time. The results of this study indicate that the administration of cinnamaldehyde, especially at a concentration of CN0.05, can increase the expression of genes related to carbohydrate metabolism from glucose transportation to the utilization of glucose as an energy source through the glycolysis process, which may indicate that carbohydrate metabolism in this treatment is more active.

The increased ability to utilize carbohydrates in the CN0.05 treatment was reflected in the growth performance and feed utilization of the shrimp. Previous studies have shown that cinnamaldehyde plays a positive role in increasing SGR, PR, and decreasing FCR in catfish *P. hypopthalmus* (Setiawati et al 2016; Tartila et al 2021), Nile tilapia *O. niloticus*. (Amer et al 2018), grass carp *C. idella* (Zhou et al 2021), and rainbow trout *O. mykiss* (Ravardshiri et al 2021). This was also seen in this study where

treatment CN0.05 showed the highest SGR and PR, as well as lowest FCR value compared to other treatments. Another study in mice reported that the positive role of cinnamaldehyde on carbohydrate metabolism is helps insulin activity (Guo et al 2017). Insulin plays an important role in carbohydrate metabolism, starting from balancing blood glucose, transporting glucose from the blood to the cells body, and utilizing glucose as an energy source. According to Takasao et al (2012), cinnamaldehyde can activate insulin-like growth factor (IGF-1), increasing the biosynthesis of protein and collagen in body tissues.

The liver plays an important role in maintaining normal blood sugar conditions by storing glucose as glycogen (Tella et al 2019). Glycogen becomes a short-term energy store that is quickly depleted when the main energy store is depleted (Setiawati et al 2015). Stored glycogen is utilized when there is no supply from food through the process of glycogenolysis (Brosnan & Watford 2005; Setiawati et al 2015). When glycogen levels are depleted, animals will mobilize and convert stored lipids and proteins into glucose through the process of gluconeogenesis to maintain blood glucose levels. Glycogen in the crustacean hepatopancreas is an essential precursor for chitin synthesis and plays a vital role during the moulting cycle (Cuzon et al 2000, 2004).

Muscle glycogen values were higher in the treatment CN0.05 and CN0.1 compared to the other treatments, meanwhile the value of liver glycogen was not significantly different compared to other treatments. This result is related to the glycogen content, which increases with the cinnamaldehyde supplement given to the feed, indicating an excess of glucose stored in glycogen (Zhang et al 2020). Stored glycogen is utilized when there is no supply of feed through the process of glycogenolysis (Hackett & McCue 2010; Brosnan & Watford 2005). Glycogen becomes a short-term energy store used quickly if the main energy supply runs out (Setiawati et al 2015). When glycogen levels run out, animals mobilize and convert stored lipids and proteins into glucose through gluconeogenesis to maintain blood glucose levels. When gluconeogenesis is no longer able to produce sufficient glucose levels, the organism can experience several adverse side effects, even causing death (Brosnan & Watford 2005). Feeding high carbohydrates can trigger hyperglycemia, glycogen deposition, and increase lipid biosynthesis in liver tissue (Zhang et al 2019; Su et al 2021) as well as causing lower growth, suppressed immunity, and high mortality (Guo et al 2011). Chen et al (2022) also recently reported that shrimp fed with cinnamaldehyde significantly increased lipid metabolism and growth performance which supports the results of this study. This study concluded that adding 0.05% of cinnamaldehyde to feed can increase the utilization of carbohydrates, thereby improving growth performance in Pacific whiteleg shrimp.

Conclusions. The results showed that the administration of 0.05% cinnamaldehyde increased the shrimp growth performance and carbohydrate metabolism in Pacific whiteleg shrimp. Thus, cinnamaldehyde plays the role of yielding alternative energy source which is included in non-protein energy. Cinnamaldehyde with the dosage CN0.05 can be applied to enhance shrimp immune responses.

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Conflict of interest. The authors declare that there is no conflict of interest.

References

Amer S. A., Metwally A. E., Ahmed S. A. A., 2018 The influence of dietary supplementation of cinnamaldehyde and thymol on the growth performance, immunity and antioxidant status of monosex Nile tilapia fingerlings (*Oreochromis niloticus*). The Egyptian Journal of Aquatic Research 44(3):251-256.

Anderson G. L, Valderrama D., Jory D. E., 2019 GOAL 2019: Global shrimp production review. Global Seafood Alliance. Available at: www.globalseafood.org. Accessed: March, 2022.
AOAC, 2012 Official methods of analysis of AOAC International. 19th edition. AOAC International, Maryland, US, pp. 69-84.
Brosnan J. T., Watford M., 2005 Starvation: metabolic changes. In: Encyclopedia of life sciences. John Wiley & Sons, pp. 1-7.
Chapman C. E., Ort S. B., Aragona K. M., Cabral R. G., Erickson P. S., 2019 Effect of cinnamaldehyde on feed intake, rumen fermentation, and nutrient digestibility, in lactating dairy cows. Journal of Animal Science 97(4):1819-1827.
Chen Z., Jing F., Lu M., Su C., Tong R., Pan L., 2022 Effects of dietary trans-cinnamaldehyde on growth performance, lipid metabolism, immune response and intestinal microbiota of *Litopenaeus vannamei*. Fish and Shellfish Immunology 131: 908-917.
Cruz-Suarez E., Ricque-Marie D., Pinal-Mansilla J. D., Wesche-Ebelling P., 1994 Effect of different carbohydrate sources on the growth of *Penaeus vannamei*: economical impact. Aquaculture 123(3-4):349-360.
Cuzon G., Rosas C., Gaxiola G., Taboada G., Van Wormhoudt A., 2000 Utilization of carbohydrates by shrimp. In: Avances en nutrición acuícola V. Memorias del V Simposium Internacional de Nutrición Acuícola. Cruz-Suárez L. E., Ricque-Marie D., Tapia-Salazar M., Olvera-Novoa M. A., Civera-Cerecedo R. (eds). 19-22 Noviembre, 2000, Mérida, Yucatán, pp. 328-339.
Cuzon G., Lawrence A., Gaxiola G., Rosas C., Guillaume J., 2004 Nutrition of *Litopenaeus vannamei* reared in tanks or in ponds. Aquaculture 235(1-4):513-551.
Devlin T. M., 2010 Textbook of biochemistry with clinical correlations. 7th edition. New York: John Wiley & Sons, 1240 pp.
Enes P., Panserat S., Kaushik S., Oliva-Teles A., 2009 Nutritional regulation of hepatic glucose metabolism in fish. Fish Physiology and Biochemistry 35(3):519-539.
Fan L., Wang A., Miao Y., Liao S., Ye C., Lin Q., 2016 Comparative proteomic identification of the hepatopancreas response to cold stress in white shrimp, *Litopenaeus vannamei*. Aquaculture 454:27-34.
Gao W., Tan B., Mai K., Chi S., Liu H., Dong X., Yang Q., 2012 Profiling of differentially expressed genes in hepatopancreas of white shrimp (*Litopenaeus vannamei*) exposed to long-term low salinity stress. Aquaculture 364-365:186-191.
Guo R., Liu Y., Tian L., Xia H., Wang J., 2011 Effects of dietary cornstarch levels on fat metabolism of hepatopancrease in *Litopenaeus vannamei*. Acta Scientarium Naturalium Universitasis Sunyatseni 50(2):105-109.
Guo X., Sun W., Huang L., Wu L., Hou Y., Qin L., Liu T., 2017 Effect of cinnamaldehyde on glucose metabolism and vessel function. Medical Science Monitor 23:3844-3853.
Hackett E. S., McCue P. M., 2010 Evaluation of a veterinary glucometer for use in horses. Journal of Veterinary Internal Medicine 24(3):617-621.
Khare P., Jagtap S., Jain Y., Baboota R. K., Mangal P., Boparai R. K., Bhutani K. K., Sharma S. S., Premkumar L. S., Kondepudi K. K., Chopra K., Bishnoi M., 2016 Cinnamaldehyde supplementation prevents fasting-induced hyperphagia, lipid accumulation, and inflammation in high-fat diet-fed mice. BioFactors 42(2):201-211.
Lage L. P. A., Plagnes-Juan E., Putrino S. M., Baron F., Weissman D., Guyonvarch A., Brugger R., Nunes A. J. P., Panserat S., 2017 Ontogenesis of metabolic gene expression in whiteleg shrimp (*Litopenaeus vannamei*): new molecular tools for programming in the future. Aquaculture 479:142-149.
Li S., Wang J., Xiong Y., Zheng J., Zhou D., Zhao J., Ding Z., 2023 Protein-sparing effect of α-lipoic acid in diets with different protein/carbohydrate ratios for the oriental river prawn, *Macrobrachium nipponense*. Aquaculture Research 2023:5614359.
Livak K. J., Schmittgen T. D., 2001 Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 25(4):402-408.
Luo Q., Wang S. M., Lu Q., Luo J., Cheng Y. X., 2013 Identification of compounds from the water soluble extract of *Cinnamomum cassia* barks and their inhibitory effects against high-glucose-induced mesangial cells. Molecules 18(9):10930-10943.

Munaeni W., Widanarni, Yuhana M., Setiawati M., Wahyudi A. T., 2020 Effect in white shrimp *Litopenaeus vannamei* of *Eleutherine bulbosa* (Mill.) Urb. powder on immune genes expression and resistance against *Vibrio parahaemolyticus* infection. Fish and Shellfish Immunology 102:218-227.
Plaisier C., Cok A., Scott J., Opejin A., Bushhouse K. T., Salie M. J., Louters L. L., 2011 Effects of cinnamaldehyde on the glucose transport activity of GLUT1. Biochimie 93(2):339-344.
Ramadhani D. E., Hendriana A., Wahjuningrum D., Mulya M. A., 2022 Vibrio dynamics and health status of Pacific white shrimp fed with cinnamaldehyde-containing feed. Jurnal Ilmiah Perikanan dan Kelautan 14(2):285-296.
Ravardshiri M., Bahram S., Javadian S. R., Bahrekazemi M., 2021 Cinnamon promotes growth performance, digestive enzyme, blood parameters, and antioxidant activity of rainbow trout (*Oncorhynchus mykiss*) in low-carbohydrate diets. Turkish Journal of Fisheries and Aquatic Sciences 21(7):309-322.
Setiawati M., Sakinah A., Jusadi D., 2015 [Evaluation of growth and meat performance of *Pangasianodon hypopthalmus* which fed with *Cinnamomum burmanni* leaves enriched diet]. Jurnal Akuakultur Indonesia 14(2):171-178. [in Indonesian]
Setiawati M., Jusadi D., Laheng S., Suprayudi M. A., Vinasyiam A., 2016 The enhancement of growth performance and feed efficiency of Asian catfish, *Pangasianodon hypophthalmus* fed on *Cinnamomum burmannii* leaf powder and extract as nutritional supplementation. AACL Bioflux 9(6):1301-1309.
Shiau Y. R., Jiang B. C., 1991 Determine a vision system's 3D coordinate measurement capability using Taguchi methods. International Journal of Production Research 29(6):1101-1122.
Su J., Mei L., Xi L., Gong Y., Yang Y., Jin J., Liu H., Zhu X., Xie S., Han D., 2021 Responses of glycolysis, glycogen accumulation and glucose-induced lipogenesis in grass carp and Chinese longsnout catfish fed high-carbohydrate diet. Aquaculture 533:736146.
Takasao N., Tsuji-Naito K., Ishikura S., Tamura A., Akagawa M., 2012 Cinnamon extract promotes type I collagen biosynthesis via activation of IGF-I signaling in human dermal fibroblasts. Journal of Agricultural and Food Chemistry 60(5):1193-1200.
Takeuchi T., Lu Jun, Yoshizaki G., Satoh S., 2002 Effect on the growth and body composition of juvenile tilapia *Oreochromis niloticus* fed raw spirulina. Fisheries Science 68(1):34–40.
Tartila S. S. Q., Jusadi D., Setiawati M., Fauzi I. A., 2021 Evaluation of dietary supplementation with cinnamon products on growth, blood composition, liver structure, and meat quality of striped catfish (*Pangasianodon hypophthalmus*). Aquaculture International 29:2243-2257.
Tella T., Masola B., Mukaratirwa S., 2019 The effect of *Psidium guajava* aqueous leaf extract on liver glycogen enzymes, hormone sensitive lipase and serum lipid profile in diabetic rats. Biomedicine and Pharmacotherapy 109:2441-2446.
Wang X., Li E., Chen L., 2016 A review of carbohydrate nutrition and metabolism in crustaceans. North American Journal of Aquaculture 78(2):178-187.
Wedemeyer G. A., Yasutake W. T., 1977 Clinical method for the assessment of the effect of environmental stress on fish health. U.S. Fish and Wildlife Service, Technical Paper No. 89, 18 pp.
Wiyoto W., Sukenda S., Harris E., Nirmala K., Djokosetiyanto D., Ekasari J., 2017 The effects of sediment redox potential and stocking density on Pacific white shrimp *Litopenaeus vannamei* production performance and white spot syndrome virus resistance. Aquaculture Research 48(6):2741-2751.
Yousefian M., Amiri M. S., 2009 A review of the use of prebiotic in aquaculture for fish and shrimp. African Journal of Biotechnology 8(25):7313-7318.
Zhang W., Liu K., Tan B., Liu H., Dong X., Yang Q., Chi S., Zhang S., Wang H., 2019 Transcriptome, enzyme activity and histopathology analysis reveal the effects of dietary carbohydrate on glycometabolism in juvenile largemouth bass, *Micropterus salmoides*. Aquaculture 504:39-51.

Zhang Y., Lu R., Qin C., Nie G., 2020 Precision nutritional regulation and aquaculture. Aquaculture Reports 18:100496.
Zhou Y., Jiang W. D., Zhang J. X., Feng L., Wu P., Liu Y., Jiang J., Kuang S. Y., Tang L., Peng Y., Zhou X. Q., 2020 Cinnamaldehyde improves the growth performance and digestion and absorption capacity in grass carp (*Ctenopharyngodon idella*). Fish Physiology and Biochemistry 46(4):1589-1601.
Zhou Y., Feng L., Jiang W. D., Wu P., Liu Y., Jiang J., Kuang S. Y., Tang L., Peng Y., Zhou X. Q., 2021 Cinnamaldehyde improved intestine immune function and alleviated inflammation associated with NF-κB pathways in grass carp (*Ctenopharyngodon idella*) after infection with *Aeromonas hydrophila*. Aquaculture Reports 21:100837.
Zhu R., Liu H., Liu C., Wang L., Ma R., Chen B., Li L., Niu J., Fu M., Zhang D., Gao S., 2017 Cinnamaldehyde in diabetes: a review of pharmacology, pharmacokinetics, and safety. Pharmacological Research 122:78-89.

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