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Dietary citral increase growth and health performance of shrimp *Penaeus vannamei*

Penambahan citral pada pakan untuk peningkatan kinerja pertumbuhan dan kesehatan udang vanname

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ABSTRACT

Phytoadditives have been acknowledged to have some beneficial effects on metabolic processes and health of some aquaculture species. This study aimed to evaluate the effect of dietary citral on carbohydrate utilization, growth and health performance of vannamei shrimp *P. vannamei*. This study used a completely randomized design (CRD) with four dietary treatments with different citral doses (0, 50, 75, and 100 mg citral/kg feed) in triplicates. Shrimp were reared for 60 days using fiber containers (50×50×80cm) of 30 shrimp/container. The frequency of feeding is given four times a day. Dietary citral supplementation of 100 mg/kg resulted in higher shrimp growth and feed utilization efficiency than those of the control. The level of amylase enzyme, post-prandial blood glucose, muscle, and liver glycogen were higher in the treatments with dietary citral than those of the controls. The shrimp immune response was also higher in the shrimp fed with dietary citral diets as shown by the higher total hemocyte count, respiratory burst, and phenoloxidase activity compared to those of the control. At the same time, antioxidant capacity in the hepatopancreas as indicated by superoxide dismutase and malondialdehyde level were also improved in shrimp with citral treatment. It can be concluded that dietary citral could increase the utilization carbohydrate, growth performance and health of shrimp at a concentration 75 mg/kg.

Keywords: citral, carbohydrate, growth, health, *P. vannamei*

ABSTRAK

Secara umum telah diketahui bahwa fitoaditif memiliki pengaruh yang menguntungkan pada proses metabolisme dan kesehatan beberapa spesies akuakultur. Penelitian ini bertujuan untuk mengevaluasi pengaruh penambahan citral pada pakan terhadap pemanfaatan karbohidrat, kinerja pertumbuhan dan kesehatan udang vanname. Penelitian ini menggunakan rancangan acak lengkap (RAL) dengan empat perlakuan dosis citral yang berbeda (0, 50, 75, dan 100 mg citral/kg pakan) dengan tiga ulangan. Udang vanname dipelihara selama 60 hari menggunakan wadah bak fiber (50×50×80cm) sebanyak 30ekor/wadah. Frekuensi pemberian pakan diberikan sebanyak empat kali sehari. Penambahan citral 100 mg/kg menghasilkan pertumbuhan udang yang lebih tinggi dan efisiensi pemanfaatan pakan dibandingkan kontrol. Tingkat enzim amilase, glukosa hemolim post-prandial, glikogen otot, dan glikogen hati lebih tinggi pada perlakuan penambahan citral dibandingkan kontrol. Respon imun udang juga lebih tinggi pada udang yang diberi penambahan citral seperti yang ditunjukkan oleh jumlah total hemosit, respiratory burst, dan aktivitas fenoloksidase yang lebih tinggi dibandingkan dengan kontrol. Pada saat yang sama, aktivitas antioksidan dalam hepatopankreas udang yang ditunjukkan oleh Superoksida dismutase dan Malondialdehida juga meningkat pada udang dengan perlakuan citral. Dapat disimpulkan bahwa penambahan citral dapat meningkatkan pemanfaatan karbohidrat, pertumbuhan dan kesehatan udang pada konsentrasi 75.

Kata kunci: citral, karbohidrat, kesehatan, pertumbuhan, udang vaname

INTRODUCTION

Heretofore, shrimp culture are still experiencing some obstacles, especially disease treatment and provision of high quality feed related. Feed is one of main factor in the success of aquaculture production. The efficiency of feed utilization is one of the indicator of aquaculture success, both from production aspect and economic aspect. The efficiency of feed utilization can be evaluated from protein in feed utilization which is the most expensive component in feed formulation. Protein in aquaculture feed has a very important role for optimal fish growth and health.

Protein utilization should be attempted optimally and efficiently due to the price is expensive and only focused on growth, while the use of other nutrient sources from carbohydrates and fats could be an energy sorce through protein sparing effect mechanism (Kamalam *et al*., 2017). Carbihydrate is a potential nutrient used for energy source in shrimp feed therefore the protein can be utilized for shrimp growth. Otherwise, carbohydrate plays important role in physiology, shrimp nutrition, and immunology (Kamalam *et al*., 2017). The metabolism of carbohydrate in shrimp is played by hepatopancreas in shrimp's digestive tract as location of first carbohydrate absorption that will be carried out to all over shrimp's body through hemolymph (Kamalam *et al*., 2017).

Carbohydrate requirements in shrimp are reported relatively high due to the shrimp growth is strongly influenced by shrimp molting rate that associated with chitin synthesis requires more glucose as precursor for shell formation (Wang *et al*., 2016). However, shrimp has limited ability to utilize carbohydrate, therefore shrimp can not digest high carbohydrate feed (Wang *et al*., 2016). One of the strategy to increase utilization of carbohydrate in shrimp is through natural ingredient supplementation such as essential oil (Sutili *et al*., 2019). Essential oil is a feed additive with antioxidant contains phytochemical with a role against stress response in animal (Kasote *et al*., 2015). Moreover, one of the ingredient used as supplement in feed is citral.

Citral is main component (80%) from essential oil extracted from lemongrass (*Cymbopogon citratus*), *Litsea cubeba*, *Melissa officinalis*, *Verbena officinalis*, *Lemon myrtle* (Mori *et al*., 2019). Citral consist of two isomers of aldehyde monoterpene, geranial and neral in antibacterial and anti fungal activity. Katsukawa *et al*. (2010) mentioned that citral in lemongrass oil has a role to activate receptor gene expression of peroxisome proliferator (PPARs) for fat and carbohydrate metabolism, cell differentiation and proliferation, and inflammation. Citral can induce mRNA gene expression from PPARα carnitine palmitoyltransferase 1 and PPARγ protein binding fatty acid indicates that citral activates PPARα and γ and regulates COX-2 gene expression (Ganjewala *et al*., 2012). Apart from functioning in carbohydrate metabolism, citral is also reported to be used as anti microbe compound (Adukwu *et al*., 2016; Chatrath *et al*., 2022; Gao *et al*., 2020; Kpoviessi *et al*., 2014), anti inflamasi (Ortiz *et al*., 2011) dan anti korosif (Korenblum *et al*., 2013).

The utilization of citral as feed supplement in aquaculture has not been widely used, and the effect against organism thought to be influenced by the dose (Zheng & Bossier, 2023). A previous study by Mori *et al.* (2019) reported that citral has been used as raw material addition in common snook (*Centropomus undecimalis*) fish feed by dose of 440 mg–1760 mg/kg feed. Meanwhile, a study about the utilization of citral in shrimp has never been carried out before. Therefore, this study aims to evaluate the effect of citral addition in shrimp feed against growth performance and health of vaname shrimp.

MATERIALS AND METHODS

Experimental design

This study used completely randomized design with four treatmets of different citral supplementation level in vanname shrimp feed, that were 0 mg/kg, 50 mg/kg, 75 mg/kg, and 100 mg/kg with three replications. Dosage determination was obtained based on previous study of Mori *et al*. (2019) and Mishra *et al.* (2019). Tested feed that used in this study was commercial feed containing 33% of protein (Table 1). Citral addition in feed was carried out by coating, adding citral into 2 g of egg white as binder, then spraying evenly onto the feed using a sprayer. Feed with citral then was dried in the oven at 40°C. Feed proximat analysis includes moisture content measurement, protein, fat, ash content, crue fiber content, and nitrogen-free extract was carried out based on AOAC (2005).

Fish maintenance

Shrimp used in this study was vanname shrimp (*L. vannamei*) with specific pathogen free certified. Eight shrimp at post larvae stage was reared in fiber tub container with volume approximately of 6350 m^3. The shrimp was reared until the size reached ± 2 g. Shrimp (2.05 ± 0.01) g then were stocked in rearing container (50×50×80cm) as much as 30 shrimps/container and reared for 60 days. Shrimp were fed four times a day at 06.00, 10.00, 14.00, and 18.00 WIB with initial feeding of only 8% from biomass weight.

Shrimp growth sampling activity was carried out every 14 days of rearing with total of 30% from each rearing unit. Water quality was maintained by changing the water with total of 30% every week. Water quality measurements that carried out every morning were temperature, pH, and dissolved oxygen (DO), meanwhile ammonia, nitrite, and nitrate were carried out every week by using test kit. The range of water parameter during shrimp rearing were 28.0-30.5 °C for water temperature, 4.2-7.4 for dissolved oxygen, 6.5-7.8 for pH, 0.00-1.42 mg/L for ammonia, 0.01-0.75 mg/L for nitrite (NO_2), and 0.0-20 mg/L for nitrate (NO_3).

Parameter

Evaluated parameter in this study were growth performance, carbohydrate utilization performance, and shrimp health performance. Growth performance parameter that was observed in this study is specific growth rate, feed efficiency, and survival rate. Carbohydrate utilization performance includes amylase enzyme measurement, level of hemolymph glucose after feeding (post-prandial), muscle glycogen and hepatopancreas was measured at the end of rearing according to Wedemeyer & Yasutake's method (1977), protein retention, and fat retention was carried out by measuring protein and fat content in shrimp at the initial and end of shrimp rearing. Protein and fat retention produced from the increase of protein and fat percentage from protein and fat provided.

Those method is according to AOAC (2015). Shrimp health performance includes total haemocyte count (THC), respiratory burst (RB), and phenoloxidase (PO). Antioxidants performance that observed in this study were superoxide dismutase (SOD) analysis and malondialdehyde (MDA), both of them were observed in hepatopancreas. Proximate analysis was carried out by AOAC method (2005) in feed sample, in shrimp body at the initial and end of the study, and shrimp flesh at the end of study.

Data analysis

All the data from this study is tabulated by using Microsoft Excel 2010 software. The assumption of data normality and homogeneity is tested by using Kolmogorov-smirnov test and Levene test. Data analysis of growth performance and shrimp health is carried out using analysis of variance (ANOVA) by SPSS ver 22.0. If the result is significantly different, Duncan test is performed with 95% of confidence interval.

RESULT AND DISCUSSION

Feed palatability describes shrimp response toward feed with citral addition. Feed palatability with citral addition for vanname shrimp was not significantly different (Figure 1). Feed palatability in this study provide information that shrimp still have good response toward feed compared with those without citral addition. There are some of previous studies about citral addition in fish feed,

Table 1. Nutrient composition of vanname shrimp with dietary citral in different concentration.

Ingredients	Citral treatments (%)			
	0 mg/kg	50 mg/kg	75 mg/kg	100 mg/kg
Moisture (%)	8.85	8.61	8.35	8.83
Protein (%)	33.82	33.30	33.68	33.48
Fat (%)	7.14	7.47	7.72	7.62
Crude fiber (%)	2.25	2.26	2.58	2.84
NFE[1] (%)	36.41	36.73	36.20	34,.6
Ash (%)	11.52	11.64	11.48	12.36
Citral (%)	0.00	0.005	0.075	0.01
GE[2] (kkal GE/kg)	4058	4072	4095	4020

Notes: [1]NFE = nitrogen-free extract; [2]GE = *Gross energy*; GE of protein = 5.41 kkal GE/g; GE of fat = 9.40 kkal GE/g; GE of carbohydrate = 4.11 kkal GE/g (Watanabe, 1998).

such as silver catfish (*Rhamdia quelen*) (Sutili *et al*., 2019), common snook (*Centropomus undecimalis*) (Mori *et al*., 2019), and Nile tilapia (*Oreochromis niloticus*) (Brum *et al*., 2017).

Carbohydrate utilization performance is measured by amylase enzyme and blood glucose level in vanname shrimp. Amylase enzyme level was measured in vanname shrimp's intestine after 60 days rearing, the shrimp was fasted before, meanwhile hemolymph glucose level was measured after two hours of feeding (post-prandial). According to statistical test analysis, amylase enzyme level in this study showed that there were differences among treatment (Figure 2). The addition 100 mg/kg of citral in shrimp feed produced high amylase enzyme level. Hemolymph glucose level in shrimp experienced significant increase in citral addition treatment compared to the control (P<0.05), yet among citral treatments weren't significantly different (P>0.05) (Gambar 2b). Highest hemolymph glucose found in 50 mg/kg of citral addition.

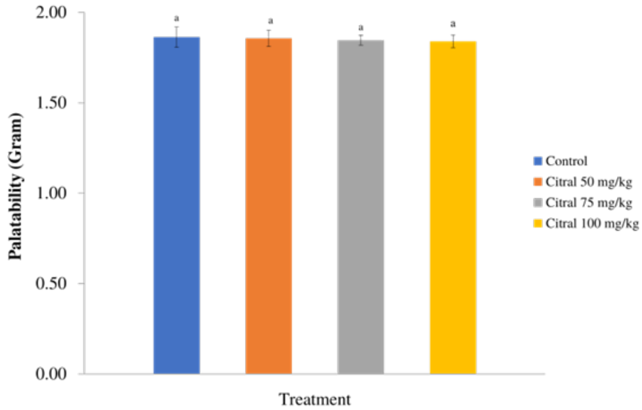

Figure 1. Palatability of the vanname shrimp experimental diet with dietary citral in different concentration.

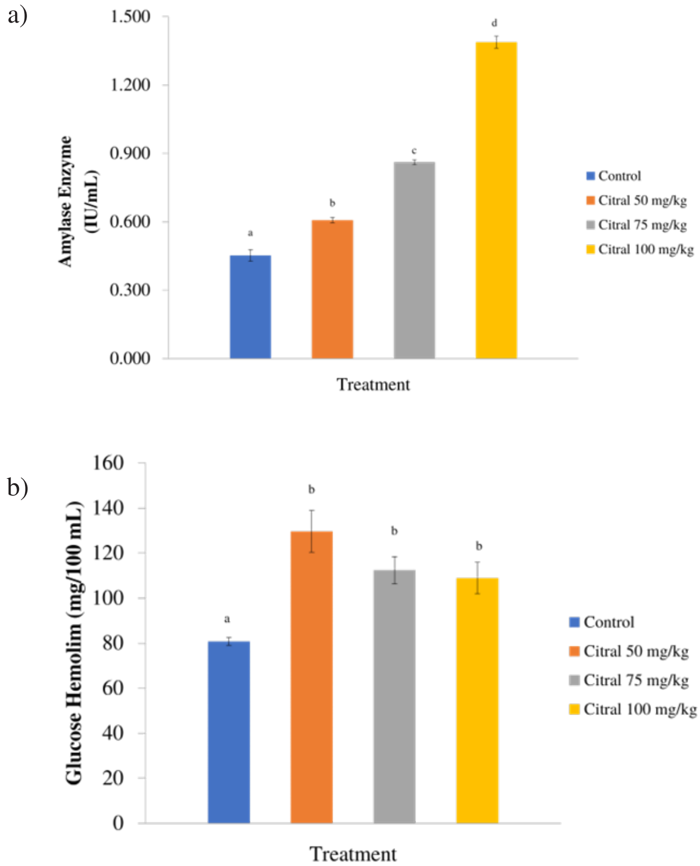

Figure 2. Performance of carbohydrate utilization (a) Amylase enzyme and (b) Glukosa Hemolim *post-prandial* with dietary citral in different concentration on vanname shrimp.

The value of protein retention increased as much as 12% by the addition of 100 mg/kg citral, yet the value of fat retention was not significantly different among treatment. Meanwhile the value of muscle and liver glycogen increased too with the addition of citral in feed compared to control, yet there was not significantly different between treatments with different concentration of citral addition.

Vanname shrimp fed with citral addition in their feed showed that the growth performances were not significantly different after 60 days rearing (Table 4). Survival rate (SR) of shrimp fed with citral addition treatment in their feed was higher than control yet significant differences were only seen in 50 and 75 mg/kg of citral addition, meanwhile 100 mg/kg of citral addition did not show significant difference both with other citral addition treatments and control eventhough the value was higher than control. Weight and final biomass and also specific growth rate of shrimp fed with citral addition in their feed shows higher value than the control, and also showed significant different result especially in 75 and 100 mg/kg of citral addition treatment. Highest feed efficiency value showed in 100/mg of citral addition treatment and was significant different with control (P<0.05).

Table 2. Protein retention, fat retention, muscle glycogen and hepatopancreas in vannamei shrimp dietary citral with different concentrations.

Treatments	Protein retention (%)	Fat retention (%)	Muscle glycogen (mM glucose unit/ gr of sample)	Liver glycogen (mM glucose unit/ gr of sample)
Control	27.87 ± 0.86[a]	13.10 ± 0.72[a]	2.95 ± 0,06[a]	5.51 ± 0.10[a]
50 mg/kg	28.86 ± 0.62[ab]	13.08 ± 0.70[a]	3.91 ± 0,03[b]	6.26 ± 0.27[b]
75 mg/kg	30.37 ± 1.09[b]	13.96 ± 0.82[a]	3.87 ± 0,06[b]	6.23 ± 0.38[b]
100 mg/kg	33.19 ± 1.28[c]	13.87 ± 0.87[a]	3.90 ± 0,08[b]	6.30 ± 0.20[b]

Data represents the average value ± standard deviation (n=3); the same superscript in same column shows that the results were not significantly different at 5% of test level (Duncan's confidence interval test).

Table 3. Growth performance of vanname shrimp fed with different citral addition in feed.

Treatments	SR (%)	Initial weight (g/ekor)	Final weight (g/ ekor)	Initial biomass (gram)	Final biomass (gram)
Control	77.78 ± 5.09[a]	2.05 ± 0.01[a]	12.64 ± 0.36[b]	61.57 ± 0.07[a]	294.96 ± 14.65[ab]
50 mg/kg	88.89 ± 5.09[bc]	2.06 ± 0.01[a]	12.82 ± 0.59[b]	61.70 ± 0.26[a]	323.86 ± 28.56[bc]
75 mg/kg	92.22 ± 5.09[c]	2.04 ± 0.01[a]	13.63 ± 0.55[b]	61.24 ± 0.26[a]	364.10 ± 43.43[c]
100 mg/kg	83.33 ± 3.33[ab]	2.05 ± 0.03[a]	14.86 ± 0.76[c]	61.61 ± 1.04[a]	371.91 ± 30.25[c]

Data represents the average value ± standard deviation (n=3); the same superscript in same column shows that the results were not significantly different at 5% of test level (Duncan's confidence interval test).

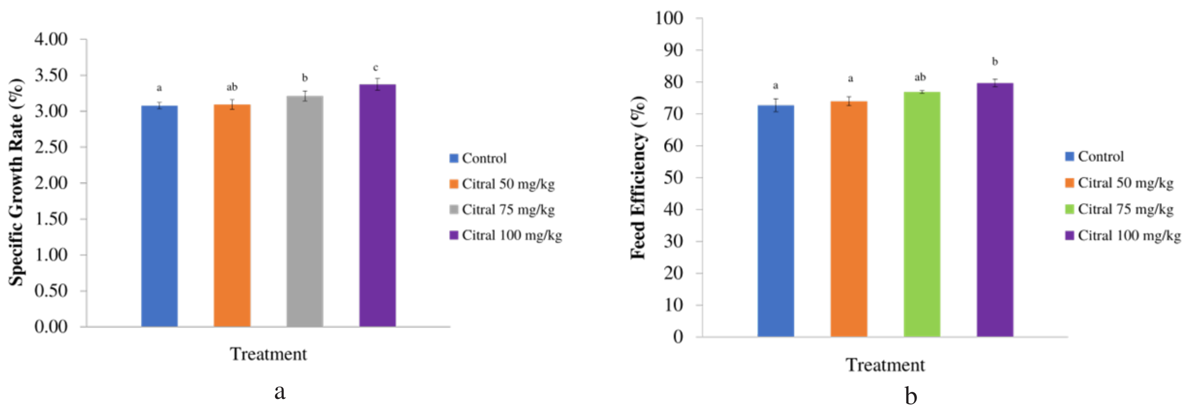

Figure 3. Growth performance (a) Specific growth rate (b) Feed efficiency with different concentrations of dietary citral in vanname shrimp.

Vanname shrimp health status include total hemocyte count (THC), respiratory burst, phenoloxidase, meanwhile antioxidant performance consist of superoxide dismutases (SOD) and malondialdehyde (MDA). The result were significant different among treatments (Table 4). The best vanname shrimp health status showed in treatment of 75 mg/kg of citral addition in feed.

Discussion

The use of phytoadditives such as eesential oil in feed are reported able to increase growth, immune response, and resistance to disease (Brum *et al*., 2017). One of the component of essential oil with high potential for fish feed is citral. Citral is bioactive compound found many in lemongrass and monoterpenes family. Mononterpene is main component from essential oil which includes isoprenoid, lipophilic, and volatile.

The chemical structure of citral consists of two isomeric acyclic monoterpene aldehydes, namely geranial (trans-citral, citral A), and neural (cis-cistral, citral B) (Sharma *et al*., 2021). Essential oil has distinctive characteristic, such as odors that can affect attractibility and feed palatability. The result of feed palatability in this study showed that the addition of citral in feed did not affect shrimp feeding activity therefore tested feed could still be consumed. One of the cheapest energy-nutrient source in feed is carbohydrate.

Carbohydrate is macronutrient in feed contain organic compound such as carbon, hydrogen, oxygen, and usually comes from plant sources. The utilization of carbohydrate as main energy source in aquatic microorganism is not better than birds and mammals (Kamalam *et al*., 2017). Those utilization very depends on the structure of carbohydrate source and amylase enzyme activity in digestive tract (Wang *et al*., 2016). Amylase enzyme work as catalyst in breaking down complex carbohydrate into simple sugars that will be used by the body as energy source. Optimal amylase enzyme activity can increase digestive performance, nutrient absorption, and increase growth performance (Akter *et al*., 2016).

In this study, the citral addition can increase amylase enzyme activity in shrimp digestive tract by the increase of citral concentration in feed. This is in line with the previous study in common snock that showed the citral addition in feed could increase digestive tract enzyme such as amylase, lipase, and pepsin enzyme (Michelotti *et al*., 2020), yet it contrary to the study by Jelenkovic *et al*. (2014) that reported the citral could inhibit alpha-amylase enzyme activity. The effect of essential oil in digestive enzyme performance are reported different depends on concentration provided (Jelenkovic *et al*., 2014).

Dinardo *et al*. (2020) stated that the use of essential oil from oregano leaves (*Origanum vulgare L*) with 80% main component is carvacrol that can actually reduce amylase enzyme activity in digestive tract of European seabass (*Dicentrarchus labrax*). High amylase enzyme activity affected post prandial hemolymph glucose level after two hours feeding. Level of digestibility of carbohydrate into glucose for fulfillment of energy source will increase blood plasma glucose level (Schrama *et al*., 2018). However the increasing trend of amylase enzyme activity was not followed by the increasing trend of hemolymph glucose level after feeding, still the glucose level was still higher than control.

This is thought to be caused by two things, the first is an inhibition in glucose absorption process from intestine into the blood or the second is an increase in the rate of blood glucose absorption into the liver tissue or other organ. The previous study in rat showed that citral could decrease

Table 4. *Total hemocyte count* (THC), *respiratory burst* (RB), *phenoloxidase* (PO) activity, *superoxide dismutase* (SOD) activity, and *malondialdehyde* (MDA) in vanname shrimp with dietary citral at different concentrations for 60 days of rearing.

Treatments	THC (10^3 sel/mm)	RB (O.D 630 nM)	PO (0.D 490 nM)	SOD (% inhibition)	MDA (μM)
Kontrol	10.05 ± 0.79^{a}	0.146 ± 0.023^{a}	0.072 ± 0.002^{a}	58.68 ± 3.94^{a}	0.42 ± 0.033^{c}
50 mg/kg	23.30 ± 2.71^{bc}	0.204 ± 0.015^{b}	0.078 ± 0.004^{a}	62.85 ± 2.62^{a}	0.37 ± 0.011^{b}
75 mg/kg	28.10 ± 3.00^{d}	0.262 ± 0.009^{c}	0.086 ± 0.007^{b}	76.74 ± 3.18^{b}	0.31 ± 0.004^{a}
100 mg/kg	25.18 ± 2.89^{cd}	0.243 ± 0.006^{c}	0.074 ± 0.006^{a}	60.77 ± 1.59^{a}	0.38 ± 0.042^{bc}

Data represents the average value ± standard deviation (n=3); the same superscript in same column shows that the results were not significantly different at 5% of test level (Duncan's confidence interval test).

blood glucose level and increase insulin plasma concentration in diabetic rat (Mishra *et al*., 2019; Xu *et al*., 2018). Xu *et al*., 2018 showed that therapeutic effect of citral in glucose metabolism associated with the increase of insulin sensitivity and the decrease of endogenous glucose production in diabetic rat, furthermorem citral concentration influenced these effects. Some of secondary metabolite in citral such as tannins, steroids, and also triterpenoids are able to accelerate glucose absorption process in the blood.

Tannins are able to shrink intestinal epithelium membrane to reduce food absorption in which can ultimately cause the increase of the rate of blood glucose level are not too high (Iskandar, 2019), steroids can stimulate the release of insulin from pancreatic beta cells, meanwhile triterpenoids help in glucose absorption by stimulating GLUT4 in cells, therefore glucose level in blood will be decreased (Djahi *et al*., 2021; Sundhani *et al*., 2016). Glucose utilization for energy source is influenced by pancreatic hormones activity , namely somastostatin and insulin hormones secretion (Kamalam *et al*., 2017). The impact of high glucose level in blood can cause a deficiency in beta cells produced insulin thereby it can inhibit shrimp growth. Flavonoid compound in citral are able to regenerate beta cells to release more insulin therefore glucose will experience faster absorption process to be used as energy source for fish. The positive role of citral on carbohydrate metabolism is also seen in liver and muscle glycogen concentrations.

Excess carbohydrate will be stored in glycogen and this study showed that the addition of citral in feed could increase glycogen level in hepatopancreas and in shrimp muscle (Tabel 3). Tannins concentration in citral can increase glycogenesis process, this indicates a decrease in total glucose in blood (Djahi *et al*., 2021). Glycogen in hepatopancreas of almost crustacea are used for chitin precursor synthesis in molting cycle (Cuzon *et al*., 2000). High glycogen in hepatopancreas and muscle can show the increase of carbohydrate utilization in feed and glucose absorption level that used for energy is already efficient therefore the excess is stored in the form of glycogen through glycogenesis process (Wang *et al*., 2016).

The value of protein retention and fat retention describes the utilization of nutrient in feed in somatic growth (Lovell, 1989). This study result showed that protein retention in shrimp fed with citral addition is higher than control. This indicates that the role of citral in protein utilization is more efficient for growth and there is a contribution of non-protein nutrient in replacing the role of protein as energy source (*protein sparing effect*). Mishra *et al*. (2019) stated that citral is able to increase a carbohydrate utilization through regulation of the activity of the enzymes glucokinase, hexokinase, and pyruvate kinase.

Apart from citral is thought to be able to increase plasma-insulin that regulate carbohydrate metabolism through enzyme activity control in liver by modifying glucose intake and consumption in target organs such as kidney, muscle, and adipose tissue. The increase of protein retention is also shown at shrimp growth performance. Shrimp growth experiences an increase as the addition of citral in feed. The addition of citral with the concentration of 100 mg/kg in feed resulted in high specific growth rate (3.37 ± 0,08%) and high feed efficiency.

The efficiency of feed utilization occurs because the protein can be utilized optimally, therefore glucose absorption produces an energy to prevent protein catabolism into energy for growth. Citral are reported having effect towards health performance, including shows its antioxidant characteristic in vanname shrimp and common snook (Mori *et al*., 2019), and also able to increase immunomodulators in Nile tilapia (*Oreochromis niloticus*) (Brum *et al*., 2017). In this study, citral has positive effect in shrimp health performance as shown by the increasing of measured parameters. Hemocyte is a cell that plays an important role in immune system of crustacea including cellular and humoral immune response.

Immune system includes detect pathogen, phagocytic activity, cytotoxicity, encapsulation, wound healing, and proPO activation (Xu *et al*., 2018). The result of this study showed that THC in shrimp reared with the addition of citral treatment in feed was higher than control. Total number of hemocyte in shrimp is closely related to stimulating the proPO system activation to produce phenoloxidase activity that will be activated by immunostimulant. ProPO system activation produces melanin to inactivate pathogen and protect the host body from the process of spreading those particles (Kulkarni *et al*., 2021).

The phenoloxidase activity in the end of study showed the highest at the treatment of 75 mg/kg of citral addition in feed. The higher PO activity cause shrimp ability to recognize pathogen inside the body improve better to increase shrimp protection due to pathogen infection (Garcia *et al*., 2008). Respiratory burst activity is a mechanism of ROS releasing consisted of hydrogen peroxide, superoxide anion, and hydroxyl radicals that play role in killing and minimizing pathogen include microbes (Liu *et al*., 2020). The respiratory burst in the end of the study experienced an increasing in citral treatment.

The increase of RB was also in line with THC value that could cause the increase of the number of reactive oxygen species (ROS) that is a main product of respiratory burst. ROS is produced by phagocytic cells that interacted with pathogen during phagocytosis process (Kulkarni *et al*., 2021). Stress in aquatic organism can occur due to environmental interference, unbalanced metabolism, as well as the presence of free radicals during metabolism process (oxidative stress) (Lubkowska *et al*., 2023). MDA test result with citral treatment addition in feed provided significant effect compare to treatment without citral addition. The addition of citral with concentration of 75 mg/kg provided lower 35% of MDA value from control.

It showed that oxidative stress was lower and SOD level was higher as the addition of citral with concentration of 75 mg/kg. SOD level described that shrimp was able to overcome free radicals such as hydrogen peroxide due to ROS produced by metabolism process (Lubkowska *et al*., 2023). The previous study from Mishra *et al*. (2019) explained that the addition of citral can improve SOD activity, catalase enzyme, GPx, and GR because phytochemical compound namely terpenoids removes free radicals inside of the body and decrease ROS level. The trend of survival rate of shrimp within citral treatments are higher than control.

In this parameter, the increase of citral concentration up to 75 mg/kg was followed by the increase of SR, yet it decreased at 100 mg/kg of citral concentration. This decreased was not followed by specific growth rate at 100 mg/kg of citral concentration which was higher than other treatments, respectively. A similar trend was also seen at health performance parameter observed in this study, which was THC level, RB, PO, and SOD in vanname shrimp fed with citral addition were higher than control, yet the highest number found at the treatment of citral concentration of 75 mg/kg. This result is also shown at previous study of Hasanpour *et al*. (2019), the application of green tea extract in feed provided best growth performance with only 25 mg/kg of citral concentration (low concentration), but the SOD enzyme activity was lower than in Siberian sturgeon (*Acipenser baeri*). These condition indicated that at high concentration, citral can repress health response yet for growth, higher citral concetration is more needed.

CONCLUSION

The addition of citral can increase carnohydrate utilization, growth performance, and enhance shrimp health at the concentration of 75 mg/kg citral.

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