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Stress responses of transportation on red tilapia which given feed containing chromium

Respons stres transportasi pada ikan nila merah yang diberikan pakan berkromium

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ABSTRACT

This study was conducted to evaluate stress responses of transportation on red tilapia *Oreochromis* sp. which given feed containing chromium. Three isonitrogenous and isocaloric experimental feeds were prepared, these diets were control (without chromium), CrPic 1 mg/kg, and CrYst 2 mg/kg supplementation in feed, all group were arranged triplicate. Satiation feeding was done three times a day. After a 60-day feeding experiment, the experimental fishes were fasted and distributed in polyethylene bags (N=60 fish/bag) containing 3 L of water, subjected to condition of transport simulation for 13 hours. Survival rate, levels of plasma cortisol, blood glucose, superoxide dismutase (SOD), and malondialdehyde (MDA) enzyme were observed at before transportation, after transportation, one day, and two days after transportation. The result showed that chromium supplementation reduced the levels of plasma cortisol before and after transportation, one day, and two days after transportation. Also, it decreased blood glucose compared with control significantly before transportation and one day after transportation. The SOD enzyme concentration increased significantly after fish was fed with feed containing chromium for 30 days, while the MDA enzyme concentration increased significantly after two days of transportation. However, there was no significant difference in the survival of red tilapia between treatments. The best result was obtained in the treatment of fish which fed with feed containing chromium. A 1 mg/kg of CrPic supplementation and 2 mg/kg CrYst increased the body resistance in red tilapia by decreasing the negative effect of stress while transportation.

Keywords: stress, transportation, red tilapia, chromium

ABSTRAK

Penelitian dilakukan untuk mengevaluasi respons stres transportasi ikan nila merah *Oreochromis* sp. yang diberikan pakan yang mengandung kromium. Pada penelitian ini digunakan tiga jenis pakan, terdiri atas pakan tanpa suplementasi kromium (kontrol), pakan bersuplementasi kromium pikolinat (CrPic 1 mg/kg), dan kromium yeast (CrYst 2 mg/kg), semua perlakuan diulang sebanyak tiga ulangan. Pemberian pakan sebanyak tiga kali sehari dan dilakukan secara *at satiation*. Setelah 30 hari pemeliharaan, ikan uji dipuasakan dan didistribusikan dalam plastik polietilen (N=60 ekor ikan/kantong plastik) yang berisi 3 L air, dilakukan dengan simulasi transportasi selama 13 jam. Parameter yang diamati pada penelitian ini adalah kelangsungan hidup, kortisol, glukosa darah, enzim superoksida dismustase (SOD), dan malondialdehida (MDA) saat sebelum transportasi, sesaat setelah transportasi, sehari, dan dua hari setelah transportasi. Hasil yang didapatkan adalah suplementasi kromium menurunkan konsentrasi kortisol secara signifikan sebelum transportasi, sesaat, sehari, dan dua hari setelah transportasi. Suplementasi kromium menurunkan glukosa darah secara signifikan pada saat sebelum transportasi dan sehari setelah transportasi. Konsentrasi enzim SOD meningkat secara signifikan setelah pemberian pakan bersuplementasi kromium selama 30 hari, sedangkan konsentrasi enzim MDA meningkat secara signifikan setelah dua hari transportasi pada ikan yang diberi pakan bersuplementasi kromium. Namun, tidak ada perbedaan yang signifikan pada kelangsungan hidup ikan nila merah antarperlakuan. Hasil terbaik diperoleh pada perlakuan ikan dengan suplementasi kromium. Suplementasi 1 mg/kg CrPic dan 2 mg/kg CrYst dapat meningkatkan daya tahan tubuh pada budidaya ikan nila merah dengan menurunkan pengaruh negatif stres akibat transportasi.

Kata kunci: stres, transportasi, nila merah, kromium

INTRODUCTION

Nile tilapia *Oreochromis* sp. is such a potential species to be developed and cultured in many regions because of high economic value (Suprayudi *et al*., 2013). Moreover, Nile tilapia has high tolerance towards many environmental conditions, such as pH, temperature, nitrogen waste, low dissolved oxygen, and easy handling. Those advantages cause Nile tilapia is widely emerged and developed (Noor *et al*., 2010). However, the intensive growing of Nile tilapia requires excellent innovation and advanced technology in order to respond condition changes in aquaculture activity, including fish transportation.

Fish transportation consists of fresh fish transport and live fish transport. Nile tilapia transport is an important aspect because transportation is a method to transfer a number of fishes in a certain period of time, simultaneously by maintaining fish quality and high survival rate to the destination. This transportation was done on the Nile tilapia juvenile to be reared and for breeding purpose (Orina *et al*., 2014), and also for market size (Wyne & Wurts, 2011). Live fish transport is quiet complicated process in aquaculture activity because it tends to change water quality in transportation media, such as carbon dioxide accumulation, ammonia, and suspended solids (Emmanuel *et al*., 2013). Apart from water quality, fish transportation also depends on other factors, such as a period of time, temperature, density, size of the fish, the physical condition of the fish, stress level, and packing (Iverson *et al*., 2005; Ashley, 2007; Tang *et al*., 2009). Water quality changes during transportation affects stress level and psychological changes of the fish.

Stress level is described as common psychological respond along with threatening condition. Repeating and prolonged stress condition might become negative influence towards growth and developing level, immunity, and reproduction mechanism (Schreck & Tort, 2016). Decreasing on stress level during transportation is a crucial aspect to support survival rate and growth performance (Navarro *et al*., 2016). This aspect can be done through feed supplementation, one of them is chromium (Cr) supplementary in the feed.

The main role of chromium in fish metabolism is to help insulin through its existence on the organometallic molecule, glucose tolerance factor (GTF) (Yan *et al*., 2010). Insulin metabolism affects lipid peroxidation (Refaie *et al*., 2009); Cr as insulin potentiator. Therefore, Cr is assumed functioning as an antioxidant (Lai, 2008). Some of the experiments stated that chromium supplementary is scientifically proved in reducing the negative effect of environmental stress in some livestocks. (Bahrami *et al*., 2012; Huang *et al.,* 2015). Supplementary of chromium picolinate (CrPic) 1 mg/kg and Cr *yeast* (CrYst) 2 mg/kg were being able to increase growth performance and blood biochemical activity on red tilapia (Rakhmawati *et al.,* 2018). This study aimed to evaluate stress level caused by transportation on red tilapia fed with chromium-containing feed.

MATERIALS AND METHODS

Tested feed

The composition of ingredient and proximate tested feed is shown in Table 1. This study used three kinds of feed with an equal amount of protein and energy, they are feed without chromium supplementary (control), feed containing 1 mg/kg chromium picolinate (CrPic), and feed containing 2 mg/kg Cr *yeast* (CrYst). CrPic, which is used in this study, is originally come from Sigma Aldrich and CrYst is from Alltech Chemistry Corporation. The ingredients and composition of treatment feed in this study were referred to Rakhmawati *et al.* (2018).

Experimental fish and rearing activity

As many of 180 monosex male red tilapia juveniles with average body weight 13.79 ± 0.13 g and average body length 9.18 ± 0.06 cm were acquired from Department of Aquaculture, IPB. Before this study was done, all of the experimental fish were reared for one week and fed using commercial feed with a protein content of 32% for acclimatization purpose to experiment condition. Furthermore, experimental fishes were randomly distributed into 12 aquaria (35×45×90 cm^3) with density of 20 fishes each aquaria. Feeding activity was done three times a day with at satiation method (08.00, 12.00, and 16.00). The experimental fish was reared for 30 days. Continuous aeration was applied with water renewal about 25% every 24 hours. The fecal matter was siphoned at 07.00 a.m. every day. During the experiment, water quality parameters were maintained on a normal level (temperature 27−28 °C; dissolved oxygen 6.8−7.6 mg/L; pH 6.5−6.8).

Table 1. The composition of ingredient and proximate Cr picolinate (CrPic) 1 mg/kg supplementary tested feed and Cr yeast (CrYst) 2 mg/kg supplementary tested feed.

Ingredient (g/100 g)	Chromium supplementary		
	0 (Control)	1 mg/kg CrPic	2 mg/kg CrYst
Fish meal	3	3	3
Soybean meal	30	30	30
Meat bone meal	15.5	15.5	15.5
Pollard	42.5	42.5	42.5
Wheat flour	2.68	2.68	2.68
Polimetilolcarbamide (PMC)	0.2	0.2	0.2
Fish oil	1.5	1.5	1.5
Corn oil	2	2	2
Vitamin and mineral	2.62	2.62	2.62
CrPic (mg/kg)	0	1	0
CrYst (mg/kg)	0	0	2
The result of proximate analysis (g/100 g) (dry weight) and feed energy			
Protein (%)	28.53	28.95	28.97
Lipid (%)	7.33	7.58	7.83
Crude fiber (%)	12.47	13.29	12.36
NFE (%)	38.74	36.95	37.96
Ash (%)	14.14	15.59	15.61
Energy (kcal/100 g)	438.63	439.36	442.15
Cr in feed			
Cr (mg/kg)	0.958	1.478	1.778

Fish transportation

After rearing activity for 30 days, all of the fishes were fasted for 24 hours. Thereafter, transportation simulation was done for 13 hours. A number of 60 fishes was packed into polyethylene bags loaded with freshwater in density 20 fishes/L (SNI 7584: 2010). Oxygen was also infused into the polyethylene bags with comparison 2:1 for oxygen and water, respectively. When packing was finished, polyethylene bags were put in styrofoam and then it was cautiously sealed. After that, styrofoam was put into a container which loaded with water flow, so that styrofoam was moved (Budiyanti, 2010). After transportation was finished, the fishes were put back into the rearing container to be observed the survival rate and blood biochemical condition after one and two days post transportation. During sample collection, the fishes were fasted.

Biochemical analysis

Biochemical analysis consists of glucose level, plasma cortisol, superoxide dismutase (SOD) activity, and malondialdehyde (MDA). Blood sample collecting was done to quantify glucose level and plasma cortisol. The fish liver was collected to quantify enzymatic activity of SOD and MDA. Measurement of glucose level, plasma cortisol, and enzymatic activity of SOD and MDA were done before transportation, shortly after transportation, a day after, and two days after transportation. Three sample fishes each treatment were randomly taken and was anesthetized using 0.2 g/L tricaine methanesulfonate (MS−222) (Ahmed *et al.*, 2012). Fish blood was taken from vena caudalis using sterile syringe after the inner part was rinsed with 1 mL sodium citrate 3.8%. Right after the blood was taken, it was put into centrifuge at 3000 rpm for 10 minutes. The supernatant solution was collected to observed plasma biochemistry. Entirely serum was collected in a microtube and labeled, and then stored in temperature 20 °C until the analysis was conducted. Plasma cortisol quantity was determined using ELISA method from a commercial kit (DRG Cortisol ELISA EIA−1887, Germany) and plasma glucose was also determined using a calorimetric enzymatic test

from commercial kit glucose liquicolor (Human mbH Germany) according to the kit procedure. Absorbance readings used spectrophotometer with wavelength 500 nm (HITACHI−U−2001).

MDA measurement was done using procedure according to Singh *et al.* (2002). As many of 0.5 g fresh fish liver was chopped in cold condition along with 1 mL phosphate buffer saline (PBS) containing 11.5 g/L KCl and pH 7.4, all those solutions were homogeneously stirred. The homogenate was centrifuged for 20 minutes at 10000 rpm. As many as 0.5 mL supernatant solution was added with a mixture of 2.23 mL concentrated HCl, 10 g TCA, 0.38% TBA, and 100 mL of distilled water. This mixture was incubated at 80 °C for one hour. After the mixture got rather cold, the mixture was centrifuged at 3000 rpm for 20 minutes. The supernatant solution was poured into another tube for absorbance readings with spectrophotometer 532 nm wavelength. TEP was used as standard solution.

SOD activity measurement was determined according to the method by Misra and Fridovich (1972). As much as 1 g of the chopped fish liver was added with 2 mL buffer phosphate pH 7.4 and made into homogenate using tissue grinder. Thereafter, it was centrifuged at 10000 rpm for 20 minutes. The supernatant (I) was poured into Eppendorf and SOD ready to analyze. From supernatant (I), as much as 0.25 mL supernatant (I) was collected to another tube and added with 0.4 mL chloroform and ethanol mixture (3:5), and then centrifuged at 3000 rpm for 10 minutes. The supernatant (II) was collected as much as 100 µL and added 3 mL buffer carbonate pH 10.2 in 30 °C and 100 µL ephinephrine (0.05 mg/10 mL HCl 0.01 N) and read by spectrophotometer with wavelength 480 nm, absorbance was read at 1st, 2nd, and 3rd minute after epinephrine was added. Absorbance changes were used for calculation. HCl 0.01 N was used as blank solution. Control solution consists of 100 µL of distilled water added with 3 mL buffer carbonate and 100 µL epinephrine.

Statistical analysis

Entirely data which presented in this study was average value ± standard error of three replications each treatment. All data were analyzed using one−way ANOVA and posthoc Duncan test using SPSS statistic ver.22. A significant difference was assumed on $P<0.05$.

RESULTS AND DISCUSSION

Result

Survival rate

The survival rate of experimental fish was shown Table 2. There was no significant difference in red tilapia survival rate after 30 days of rearing, after 13 hours of transportation, one day, and two days post transportation. The survival rate of red tilapia ranged from 98.33−100%.

Glucose level and plasma cortisol

Glucose level and plasma cortisol on red tilapia were shown in Table 3. CrPic and CrYst supplementary were significantly proved to reduce glucose level and plasma cortisol before transport phase, briefly after transport, and one day after transport ($P<0.05$). Each treatment had an increasing result on blood glucose level briefly after transport. One day after transport, blood glucose was noted decreasing. The most significant blood glucose decreasing on red tilapia was CrYst supplementary, while CrPic supplementary was less significant ($P<0.05$). On control treatment, blood glucose was decreased on two days after transport.

Plasma cortisol on both supplementary showed similar a result, however, control treatment was different. All treatments had increasing value on plasma cortisol briefly after transport, but both Cr supplementary feed treatment significantly

Table 2. Survival rate on red tilapia fed with 1 mg/kg chromium picolinate (CrPic) supplementary feed and 2 mg/kg chromium yeast (CrYst) during transport stress level test.

Monitoring time	Survival rate (%)		
	Control	CrPic	CrYst
After 30-day of rearing, before transport	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]
After 13-hour transport	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]
One day after transport	100 ± 0.00[a]	100 ± 0.00[a]	100 ± 0.00[a]
Two days after transport	98.33 ± 2.89[a]	100 ± 0.00[a]	100 ± 0.00[a]

*) The same superscript letter behind each standard error indicate no significant difference ($P>0.05$).

Table 3. Glucose level and plasma cortisol changing on red tilapia fed with 1 mg/kg Cr picolinate (CrPic) and 2 mg/kg Cr yeast (CrYst) supplementary feed during transport stress level test.

Monitoring time	Glucose level and plasma cortisol change					
	Glucose			Cortisol		
	Control	CrPic	CrYst	Control	CrPic	CrYst
Before transport	87.70 ± 3.28[b]	78.16 ± 1.99[a]	82.97 ± 1.59[b]	75.05 ± 7.66[b]	52.73 ± 12.92[a]	39.19 ± 4.90[a]
After transport	112.18 ± 4.62[a]	106.43 ± 2.27[a]	107.81 ± 1.85[a]	188.30 ± 32.2[b]	79.44 ± 9.19[a]	77.72 ± 17.86[a]
One day after transport	101.46 ± 1.01[c]	86.57 ± 3.66[b]	74.20 ± 5.73[a]	172.39 ± 42.2[b]	61.53 ± 2,99[a]	54.94 ± 15.61[a]
Two days after transport	82.07 ± 5.25[a]	87.86 ± 2.25[a]	85.00 ± 4.87[a]	65.05 ± 2.00[a]	66.57 ± 4.15 [a]	64.12 ± 3.75[a]

*) Different superscript letter behind each standard error on the same row indicate significant difference (P<0.05)

resulted in lower level of plasma cortisol (P<0.05) compared with control. One day after transport, plasma cortisol was decline. Decreasing plasma cortisol on red tilapia fed with Cr supplementary feed (CrPic and CrYst) was significantly more rapidly (P<0.05) compared with control. Red tilapia on control treatment was able to reduce its plasma cortisol in two days after transport.

Malondialdehyde (MDA) and superoxide dismutase (SOD) enzymatic activities

Malondialdehyde (MDA) and superoxide dismutase (SOD) enzymatic activity were shown in Table 4. MDA activity was not significantly different (P>0.05) before transport, briefly after transport, and one day after transport. MDA activity on two days after transport was significantly increased in both Cr supplementary treatment (P<0.05). MDA enzyme activity before transport or after a 30−day of feeding treatment was not any significant difference (P>0.05). MDA activity briefly after transport didn't result in any significant difference in each feed supplementary and control treatment. Simply, one day after transport, all treatments had decreased level of MDA enzyme activity. Both feed supplementary feed had plenty more decreasing level 42.40% (CrPic) and 20.07% (CrYst), compared with the control which had less significant decreased level 4.66%. Meanwhile, MDA enzyme activity in two days after transport on Cr supplementary treatment was increased, while control treatment was still decreased.

Supplementation of Cr picolinate and Cr yeast was able to increase SOD enzyme activity before transport (P<0.05), but statistically there

Table 4. Malondialdehyde (MDA) and superoxide dismutase (SOD) enzymatic activity on red tilapia fed with 1 mg/kg Cr picolinate (CrPic) and 2 mg/kg Cr *yeast* (CrYst) during transport stress level test

Monitoring time	Enzyme activity					
	Malondialdehyde			Superoxide dismutase		
	Control	CrPic	CrYst	Control	CrPic	CrYst
Before transport	19.95 ± 7.82[a]	39.35 ± 18.27[a]	35.69 ± 11.38[a]	4.22 ± 0.38[a]	6.58 ± 1.34[ab]	7.73 ± 2.0[1]b
After a 13-hour transport	18.44 ± 7.79[a]	40.97 ± 15.87[a]	33.44 ± 12.16[a]	6.13 ± 2.01[a]	7.11 ± 1.54[a]	5.42 ± 0.15[a]
One day after transport	17.58 ± 3.7[a]	23.60 ± 13.94[a]	26.73 ± 16.93[a]	5.24 ± 1.00[a]	4.99 ± 1.25[a]	5.30 ± 0.71[a]
Two days after transport	11.69 ± 2.98[a]	31.45 ± 7.87[b]	47.16 ± 12.86[b]	5.91 ± 0.54[a]	6.13 ± 0.46[a]	6.49 ± 3.64[a]

*) Different superscript letter behind each standard error on the same row indicate significant difference (P<0.05)

was no significant difference on SOD enzyme activity before transport, one day, and two days after transport. The highest level of SOD enzyme activity on red tilapia fed with CrYst supplementary and then CrPic respectively ($P<0.05$), compared with control treatment before transport. Briefly after transport, SOD activity was increased on red tilapia fed with CrPic supplementary feed and control treatment. One day after transport, SOD enzyme activity on red tilapia fed with CrYst supplementary feed was increased, whereas red tilapia fed with CrPic supplementary feed and control treatment was decreased. SOD activity was increased in two days after transport on each treatment.

Discussion

In this study, plasma cortisol changes moved proportionally with blood glucose level. Both parameters mentioned before, were essential stress indicators, plasma cortisol as primary stress indicator, while blood glucose level is secondary stress indicator (Porchas *et al.,* 2009; Zahl *et al.,* 2009). The hormonal stress response is an essential physiological adaptation for hemostasis mechanism. From the physiological view, stressor caused different plasma cortisol and epinephrine response for certain time. Epinephrine increases in seconds and nevermore involved in circulation, while cortisol increases in minutes to hours in responding stressor (Vijayan *et al.,* 2010). The neuroendocrine response towards stress is characterized by an excessive level of gluconeogenesis, glycogenolysis, and insulin resistance. Hyperglycemia is caused by an increasing in liver activity, especially in stress condition. Some of cortisol metabolic impact is increase in blood glucose level through enzyme activity on gluconeogenesis and inhibition of glucose intake in peripheral tissue, such as skeletal muscle. Both epinephrine and norepinephrine induce gluconeogenesis and glycogenolysis, where norepinephrine increases glycerol supply to the liver through lipolysis (Dungan *et al*., 2009; Bartness *et al*., 2010).

Cortisol and blood glucose level increased in each treatment briefly after transport indicated stress on experimental fish. This kind of result is similar to cortisol and blood glucose level increase at 60th and 90th minute on tilapia after electric shock (Barreto & Volpato, 2006), similarly when tilapia is injected with different anesthesia during stress level test (Navarro *et al.,* 2016). This result was also shown on common carp which experienced cortisol increase, but insignificantly after a 12−hour transport (Dobsikova *et al.,* 2009). One day after transport, cortisol was decreased. Nevertheless, cortisol on control treatment was significantly still higher than fish fed with CrPic and CrYst supplementary, briefly after transport, one day, and two days after transport.

When fish is exposed to a stressor, physiological responses are started with threat recognizing by the central nervous system. Cortisol secretion is started in hypothalamic−pituitary−interrenal axis along with corticotropin−releasing hormone (CRH) or corticotropin−releasing factor (CRF), especially from hypothalamus gland which induce corticotropic cell from anterior hypophysis to secrete an adrecorticotropic hormone (ACTH) (Barton, 2002). A related experiment on rainbow trout, showed that blood glucose level modulates cortisol secretion caused by ACTH on rainbow trout (Conde−Sieira *et al*., 2013). In vitro cortisol secretion is reinforced by the high level of glucose and decreasing on cytocrine−B (glucose transport inhibitor). It proves glicemia condition and nonpancreatic−releasing hormone. It shows that hyperglycemia is related to cortisol synthesis and its secretion under stress condition. In this mechanism, adrenaline did not only release glucose, but also as hyperglycemia signal, a prerequisite for ACTH in cortisol secretion.

Blood glucose level increased briefly after transport. In stress condition, fish needs more energy to adapt towards transport condition. Stress induces adrenaline to release energy, followed with corticosteroids (cortisol) from adaptation response; cortisol regulates longer energy distribution after adaptation process and recovers normal condition, include re−regulated set point to control hemostasis (Koolhaas *et al*., 2011). Overall, stress response obtained temporarily hyperglycemia in providing energy source against stress. Nerve system is direct functions towards chromaffin cell in glycolytic adrenaline secretion on 2nd minute basis. After that, cortisol takes over to maintain hyperglycemia for some minutes to hours and enable redistribution and reallocate adaptive energy (Koolhaas *et al.,* 2010; 2011).

One day after transport, blood glucose level was decreasing. The most significant decreasing of blood glucose level occurred on CrYst supplementary feed treatment and CrPic supplementary feed treatment respectively, compared with control treatment. Decreasing of blood glucose level in control treatment occurred two days after transport. Therefore, CrPic and

CrYst supplementary feed enable to reduce blood glucose level more rapid than without Cr supplementation. Chromium is potential to induce insulin sensitivity so that more effective in utilizing glucose, also found that CrYst had more insulin sensitivity compared with CrPic (Rakhmawati *et al*., 2018). Yeast contained in CrYst held an essential role in glucose and insulin mechanism (Liu *et al.*, 2015).

Two days after transport, blood glucose level was increasing in Cr supplementary treatment after stress response was eliminated. A possible cause was gluconeogenesis mechanism in order to recover energy loss while fasting. In the beginning of fasting phase, cortisol induced gluconeogenesis (glucose production from non−carbohydrate source) and activated anti−stress and anti−inflammatory path. Cortisol also contributed on liver and muscle glyconeolysis (glycogen to glucose−1−phosphate and glucose) (Martin & Crump, 2003). However, at the end of fasting phase, cortisol increased glycogenesis, liver took over unused glucose molecule by peripheral tissue and turned it into glycogen deposit that will be used during fasting phase (Baynes & Dominiczak, 2009). Furthermore, cortisol against insulin and contributed to insulin resistance by reducing glucose transporter translocation, especially glucose transporter which sensitive to insulin (GLUT)−4 to the cell membrane (Brown & Brown, 2012).

Stress was caused free radical production which potentially destructs cell membrane produced by binary unsaturated fatty acid peroxidation in a membrane cell. The peroxidation lipid expressed by MDA enzyme activity (Talas & Duran, 2012). MDA value on experimental fish fed by CrPic and CrYst supplementary feed reared for 30 days was not significantly different compared control treatment, however, it had a lot more decreasing in MDA enzyme activity one day after transport. It was assumed caused by chromium stimulatory on insulin activity. Chromium held an important role in increasing insulin sensitivity and affect major cells related to insulin activity (El−Fattah, 2016). Supplementary of 2 mg/kg CrYst and 1 mg/kg CrPic significantly increased insulin sensitivity on red tilapia. Both supplementary treatment increased glucose intake and its utilization in the cell (Rakhmawati *et al.,* 2018). Insulin also increases lipid transport from triglyceride and push it to be catabolized, so that lipoprotein lipase activity in plasma will be increased (Yan *et al.*, 2008). Therefore, chromium supplementary will decrease oxidative damage caused by stress.

In this study, SOD activity after 30 days of feeding CrPic and CrYst supplementary feed is significantly increased compared to control treatment. It showed that chromium is potentially increased supply and SOD antioxidants activity. SOD enzyme is one of the most important antioxidants to prevent oxidative stress. SOD enzyme functioned as a free radical reducer, so that cell damages could be avoided (Ognjanovic *et al.*, 2008; Li *et al.*, 2011) and it worked by cleaning free radical or reactive oxygen species (ROS) enzymatically and turn it into more a stable product. SOD enzyme catalyzed superoxide dismutase reaction (O^{2-}) into hydrogen peroxide and an oxygen molecule, so that it is no longer harmful to the cell (Halliwell, 2006).

The antioxidant and anti−inflammatory activity of CrPic has been studied in some experiments and explainable in many mechanisms, one of them is induced the decreasing of nitrite serum production, which inhibits nitrite reaction with superoxide and reduces peroxynitrite production (Cefalu *et al*., 2010; Seif, 2015). Similarly to CrYst, yeast contained in the feed was assumed to affect antioxidant activity. Mannan oligosaccharide (MOS) is gluco−mannoproteins complex which originally comes from *Saccharomyces cervesiae* cell wall (Sohn *et al*., 2000). Mannose receptor (MR) is endocytic receptor expressed by macrophage and endothelium cell which recognized glycoprotein and glycan microbe ligand (Ringo *et al*., 2010; 2014). Grisdale-Helland *et al*. (2008) evaluated feed effect which contains 10 g/kg MOS on Atlantic salmon for 4 months. The result showed that indigestion was significantly increased. While Sang *et al*. (2009; 2011) found that MOS feeding on Marron lobster *Cherax tenuimanus* resulted positive effect towards immune response, survival rate, and stress response and 1 and 2 g/kg MOS feeding significantly increase phagocytosis, anion superoxide production, and SOD activity on sea cucumber compared with non−MOS feeding (Gu *et al.,* 2011). However, after transport, there was no significant difference in SOD concentration between treatment. It means SOD activity during stress was affected by chromium supplementation.

CONCLUSION

The present study showed that 1 mg/kg CrPic and 2 mg/kg CrYst supplementary feed were the best results among treatment. Therefore, it can be

concluded that chromium supplementary feed is profoundly able to increase immune system on red tilapia and reduce the negative impact of fish transport.

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