

# THE EFFECT OF ORGANIC-Cr DIETARY SUPPLEMENTATION ON STRESS RESPONSE IN TRANSPORT-STRESSED BEEF CATTLE

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## ABSTRACT

Transportation over long distances resulted in stress at animal. Under these circumstances, animal usually manifest depression and the impact on physiological condition changes and loss of body weight. The objectives of the research were to examine effect supplementation of organic-Cr type into diets in transport-stress beef cattle on physiological condition, haematochemical (included were haematological condition and blood chemical) and body weight changes. The experiment was conducted using 16 beef cattle those were transported by truck for a distance of 400 km from Malangbong to Tangerang. The experiment was arranged by Completely Randomized Design with four treatments and four replications. The dietary treatments consisted of R0 (basal diet without Cr supplemented), R1 (R0+3ppm organic-Cr resulted of alkali hydrolysis), R2 (R0+3 ppm organic-Cr resulted from bioprocess), R3 (R0+3 ppm organic-Cr resulted from bioremediation). The result indicated that type of organic-Cr supplementation at 3 ppm in diet did not influence physiological condition, haematochemical and body weight at beef cattle transported for seven hours. There was indication that beef cattle fed on control diet (without Cr) showed a stress symptom, their loss of body weight were higher (5.41%) compared to beef given diet contains organic-Cr (3.72%, 5.04% and 4.83%, respectively for R1, R2 and R3).

*Keywords: beef cattle, organic-Cr, stress, transportation*

## INTRODUCTION

Transportation over long distances is a significant cause of stress in animal. This effect is exacerbated by denying the animals feed and water during transportation. These circumstances results in reduced microbial fermentation in the rumen and decreased alimentary canal motility (Al-Mufarrej *et al.*, 2008) and metabolic dysfunction (Parker *et al.*, 2003).

Transportation stress in animal in physiologically occurred when animal can not maintain homeostasis process that needs some metabolites like glucose, electrolyte (for example  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ) and water to overcome the stress. As consequence, during transportation, animal must be fed on dietary supplement of glucose source to avoid glycogen catabolism. Transport-induced immunosuppression and resulting in productivity losses (Colditz and Hennessy, 2001). In transportation studies using *Bos taurus* calf model, leukocytosis associated with neutrophilia has been reported (Kannan *et al.*, 2000 and Stanger *et al.*, 2005). Such responses may be

indication of a physiological or pathological change, but also may indicate an epinephrine-induced response (Tyler and Cummins, 2003; Nairab and Bonneaub, 2006).

Chromium (Cr) is essential microelement related to insulin activity, mixed up with glucose metabolism, protein, and fat at animal tissue. As the active component from Glucose Tolerance Factor (GTF), Cr is required as cofactor insulin for transported glucose from circulation into peripheral tissue (Anderson and Kozlovsky, 1985; Mertz, 1992). The other research shows addition Cr (chelate-amino acid) increases the milk production (Yang *et al.*, 1996) and improves diet efficiency in beef experiencing of stress (Chang and Mowat, 1992), and improves insulin cordage with specific receptor at target organ (Anderson and Kozlovsky, 1985; Parker *et al.*, 2007). In Cr deficient livestock, Cr supplementary will improve the usage of glucose by insulin for muscle forming and adipose tissue (McNamara and Valdez, 2005). According to Knowles and Warriss (2000), long transportation (18-24 hours) can cause beef cattle loss of body weight by 3%-

11%.

Supplementation of Cr in the diet should be in form of organic-Cr, because nontoxic and 25-30% can be absorbed by the body (Chang and Mowat, 1992). Organic-Cr is complex Cr that bound in protein. Structure GTF is compiled from complex between  $\text{Cr}^{3+}$  with 2 acid molecules of nicotinate and 3 amino acids that is glutamate, glycine and cystein (Linder, 1992). Addition Cr into the diet will be useful, specifically for improving immunity system at livestock experienced stress of transportation (Al-Mufarrej *et al.*, 2008). The objectives of the present study were to explore the effect of organic-Cr dietary supplementation on stress responses of transport-stressed beef cattle.

## MATERIAL AND METHODS

### Animals, Diet and Experimental Design

This experiment was conducted to 16 Brahman Cross bulls ( $469.1 \pm 32.35$  kg), transported by truck for a distance of 400 km from Malangbong to Tangerang, or equal to seven hours transportation time. Beef cattle were given a diet containing 3 ppm Cr for three days prior to be transported. Type of organic-Cr that given was organic-Cr result of skin waste hydrolysis by alkali NaOH, organic-Cr result of *Saccharomeces cerevisiae* bioprocess, and organic-Cr result of Duckweed bioremediation.

The research was arranged based on completely randomized design with four treatments and four replications, respectively. The dietary treatment were R0 = control diet without Cr supplemented (containing 30% rice straw, 70% concentrate), R1 = R0 + 3 ppm organic-Cr by alkali hydrolysis, R2 = R0 + 3 ppm organic-Cr by *Saccharomeces cerevisiae* bioprocess, R3 = R0 + 3 ppm organic-Cr by Duckweed bioremediation. All of diets contained 82.5% dry matter, 11.3% ash, 9.8% crude protein, 31.1% crude fibre and 69.8% TDN.

### Sample Collection and Analysis

Variables perceived were physiological and haematochemical (included were haematological condition and blood chemical), loss of body weight and percentage of carcass. Measurement was conducted after arrive at target location. The body weight change was measured by weighing the body weight at start and after transportation.

Parameter of physiological condition were body temperature, respiration frequency, and pulses frequency. Haematochemical was

measured by taking blood from vena jugularis using Li-Heparin LH (9ml) tube (Sarstedt Inc., Newton, NC). Plasma from each sample was collected and stored at  $-20^{\circ}\text{C}$  until needed for analysis of blood electrolyte (Na, K). Haematological (included value haematocrit, haemoglobin, erythrocyte, and leukocyte) measured as follows : (a) haematocrit was measured by centrifugation of whole blood in capillary haematocrit containing heparin at 3000 rpm for 15 minutes. The value of haematocrit was determined by using microcapillary reader, (b) Haemoglobin (Hb) measurement was conducted by the method of Haemometer Sahli. (c) erythrocyte cell was determined by using haemocytometer. (d) Measurement of leucocytes cells was similar to the method of erythrocyte measurement.

Blood chemical included were glucose, Chromium (Cr), Potassium (K) and Sodium (Na). Glucose concentration of the blood plasma was determined using glucose oxidase/peroxidase enzyme and dianisidine dihydrochloride (Sigma) as described previously (Regmi *et al.*, 2008), and the absorbance was determined by a microplate spectrophotometer (UV-1601). Net glucose disappearance was calculated by subtracting the amount of glucose in incubation media of treatment flasks from that of 0-min control.

The Cr, K and Na in the blood plasma were determined by commercial kits, absorbance of each sample was read *versus* blank tube by UV-1601 Spectrophotometer at 600 nm wavelength. The body weight loss was measured from difference of body weight before and after transportation.

### Statistical Analysis

Data were analyzed by analysis of variance (Steel and Torrie, 1981) using the SPSS ver 12 to know the effect of organic-Cr on stress response (SPSS, 2004).

## RESULTS AND DISCUSSION

The beef cattle that suffer stress can be seen from physiological condition like body temperature, rate of respiration and rate of pulses. At this research, researchers investigating effect organic-Cr on transport stress in cattle have primarily employed beef cattle model. Beef cattle are commonly transported by commercial truck carriers. This study showed that there were no

significant effect ( $P>0.05$ ) of organic-Cr dietary supplementation on transport-stressed beef cattle (Table 1). Some studies also showed that Cr had no significant effects on growth performances (Van de Ligt *et al.*, 2002; Mostafa-Tehrani *et al.*, 2006).

The contrary results suggested that both transportation-related handling and travel have been identified as potential stressful events for cattle (Feuz and Umberger, 2003), and recent literature reviews addressed the potential impact of transportation on cattle health and performance (Eicher, 2001; Swanson and Morrow-Tesch, 2001; Fike and Spire, 2006), and increasing reactive oxygen species (ROS) include superoxide anion, hydrogen peroxide and derivative hydroxyl radical and hydroxide (Zheng *et al.*, 2010).

Transportation has been associated with increasing of morbidity, stress responses and immune response (Stanger *et al.*, 2005), reducing

body weight, and reduces physiological condition (White *et al.*, 2009). PUFA dietary supplementation not significantly to performance of transported-stressed beef cattle (Araujo *et al.*, 2010).

Effect of Cr on transport-stressed beef cattle has been reported. Kegley *et al.* (2000) reported that supplemental Cr, as chromium-L-methionine, increased glucose clearance rate after an insulin infusion and increased the insulin response to an intravenous glucose challenge in growing calves with functioning rumens. Vincent (2004) reported that various trivalent chromate compounds have been used as nutritional supplements, weight-loss agents, and muscle-development agents in humans and as feed additives in domestic animals. Wang *et al.* (2009) showed that trivalent chromium (Cr (III)) is a component of GTF and is vital in carbohydrate, fat, and protein metabolism presumably by potentiating the action of insulin.

The effects of supplemental organic-Cr on

Table 1. Stress Response of Transport-stressed Beef Cattle

Item	Treatment			
	R0	R1	R2	R3
<b>Performance</b>				
Lose weight (%)	5.41	3.72	5.04	4.83
Carcass (%)	53.70	54.20	54.30	54.20
<b>Some Physiological Condition</b>				
Body temperature (°C)	39.0	38.5	39.0	39.3
Rate of respiration per minute	48.8	55.0	55.1	47.1
Rate of pulses per minute	103.9	111.7	100.0	96.4
<b>Haematochemical</b>				
Erythrocyte ( $\times 10^6$ cell/mL)	842	718	821	660
Leucocyte ( $\times 10^3$ cell/mL)	81	69	82	80
Hematocrit (%)	34.40	29.00	33.60	34.20
Haemoglobin (%)	82.50	74.20	79.20	71.90
Blood Glucose (mmol/L)	31.25	56.25	48.25	40.88
Sodium (Na, ppm)	0.34	0.33	0.33	0.34
Potassium (K, ppm)	0.03	0.03	0.03	0.03
Chromium (Cr, ppm)	2.21	2.21	2.22	2.22

R0 = control diet without Cr supplemented, R1 = R0 + 3 ppm organic-Cr by alkali hydrolysis, R2 = R0 + 3 ppm organic-Cr by *Saccharomyces cerevisiae* bioprocess, R3 = R0 + 3 ppm organic-Cr by Duckweed bioremediation

the performance (carcass percentage and body weight) are presented in Table 1. There were no significant ( $P>0.05$ ) effect of organic-Cr on carcass percentage and body weight. The role of Cr in regulation of body composition is still controversial. Cr-Pic is the most widely used organic chromium complex to increase lean body mass or carcass leanness.

Wang *et al.* (2007) reported the carcass effects of trial comparisons and found a mean increase in longissimus muscle area of 2.7 cm<sup>2</sup> (about 7%; with 13 numerically positive responses out of 15 comparisons) and a mean reduction in 10<sup>th</sup> rib backfat depth of 3.3 mm (about 13%; with 13 positive responses out of 15 comparisons). The lack of a consistent response may be relate to Cr levels of the diets, Cr status of subjects, and amino acid levels of diet (Zha *et al.*, 2007).

In the present study, supplementation of 3 ppm Cr/kg diet decreased the lose body weight and increased carcass percentage, although not significant with control groups. These results were not in line to the striking effect on carcass composition of finishing pigs (Wang and Xu, 2004), which implicated that Cr nanoparticle may be a more bioavailable source of Cr and potentially be used as an effective carcass-modifier for animals.

Data in Table 1 showed that effect of various organic-Cr in diet on physiological condition of beef cattle i.e. body temperature, rate of respiration and rate of pulses at beef were not significantly different. Body temperature of beef were ranged at 38.5-39.3°C, rate of respiration were 47.1-55.1 times per minutes, and rate of pulses were 96.4-111.7 times per minutes that were not significantly different. In another words, source type Cr was not influence the physiological condition of beef cattle transported for seven hours.

The haematological value are often used as guideline of livestock health condition. Red blood count relates to ability in transporting nutrient to all body cells, while leucocyte amount relates to the immunity, haematocrit relates to plasma ratio with blood cell, and haemoglobin relates to ability oxygen transport to whole body. The value of beef cattle haematochemical that given diet contains various of organic-Cr after transportation is presented at Table 1.

Table 1 showed that addition organic-Cr into

beef diet did not influence ( $P>0.05$ ) haematochemical value. Nevertheless there was indication that red blood count and haemoglobin at R0 was higher than that of given organic-Cr in their diets. This condition indicated that beef cattle receiving control diet (R0) experienced stress as indication of haemoglobin level. Haemoglobin plays a part in oxygen supply that required by animal if they experiences of stress.

Direct effect of stress transportation consequence is decreasing of body weight. Beef cattle given diet containing Cr generally loss their body weight smaller than beef under control diet (R0). Among type of organic-Cr, in the organic-Cr that was processed by alkali hydrolysis method gives impact anti stress better than other organic-Cr. The best effects occured in the hydrolysis of organic-Cr low solubility of the company enable the consequences occurred either initial rumen (7.18%) and also up to the abomasum (22.27%) compared to the treatment of organic-Cr produce from bioprocess-Cr and bioremediation. Meaningful this condition that organic-Cr was more absorbable and exploited by the body for further metabolism to gluconeogenesis process.

Transportation effects in this research was not clearly emerge, in which this condition were related to long time of transportation conducted only seven hour. Knowles *et al.* (1999) recommend long time of transportation was ideally 24 hour with diet and drinking water available to speed up physical recovery after transportation. Addition Cr was not influences body weight or score of body condition at beef cattle due to heat grasp (Al-Saiady *et al.*, 2004)

This study showed that organic chromium supplementation increased the blood glucose (Table 1). Therefore, organic-Cr was a bioavailable source of chromium, altering glucose and insulin metabolism, especially in the cell membrane transport. Odore *et al.* (2004) and Gupta *et al.* (2007) stated that Cr as chromium propionate had a greater effect on increasing insulin secretion than on insulin sensitivity. An observation using rat pancreas showed that supplemental dietary Cr as chromium chloride increased insulin secretion. Further work must be conducted to elucidate the impact of supplemental bioavailable Cr on ruminants.

The K concentration in blood tended to be constant, however, no differences between treatments for K of plasma or Na (Table 1). This result showed that reabsorption of Na and K may

be decreased due to a concentration gradient favoring net diffusion of Na from interstitial fluid to lumen of renal tubular cells. Nevertheless, the level of haematochemical were normal showing that Na and K play a role to glucose transport. Kumar *et al.* (2010) stated that the glucose is co-transported with Na across the apical plasma membrane into the epithelial cell. It moves through the cell to the basal surface, where it passes into the blood via Glutathion-2, a passive glucose uniporter. The Na- K-ATPase continues to pump Na outward to maintain the Na gradient that drives glucose uptake.

Although the organic-Cr dietary supplementation did not effect directly on the blood electrolyte, but this study showed that organic-Cr play role the general metabolism, especially water turn over to maintain the Na and K level in the blood plasma.

### CONCLUSIONS

The 3 ppm of organic-Cr supplementation in diet did not influence on stress response of transport-stressed beef cattle, included the some physiological condition, haematochemical and body weight loss due to transportation for seven hours. beef cattle fed on control diet (without Cr) showed a stress symptom, their loss of body weight were higher (5.41%) compared to beef given diet contains organic-Cr (3.72%; 5.04% and 4.83%, respectively for R1, R2 and R3).

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