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Blood Profile of Lactating Cows Supplemented with Organic Chromium and *Ganoderma lucidum*

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Abstract: The aim of this experiment was to determine blood profile (hemoglobin, hematocrit, red blood cells, white blood cells), differential leucocytes count (neutrophils, lymphocytes, monocytes, eosinophils) and chromium in blood of lactating cows supplemented with organic chromium (Cr) and *Ganoderma lucidum* in ration. Fifteen lactating cows grouped and allocated in the five treatments in randomize block design, were fed a basal diet composed of 60% grass and 40% concentrate. Supplementation on basal diet as treatment were: A = basal diet (control), B = 3 ppm inorganic Cr in $CrCl_3.6H_2O$, C= 3 ppm organic Cr (fermentation product with *Ganoderma lucidum*), D = *Ganoderma lucidum* (5 g 50 kg^{-1} live weight) and E = Organic Cr+*Ganoderma lucidum*. Blood profile, differentiation of leucocytes and chromium in blood were investigated. The result showed that there were no significant differences on blood profile ($p>0.05$). Total leucocytes count was 7120±1.44 cell/µl in treatment E to 9270±2.32 cell/µl in control. Also, organic Cr and *Ganoderma lucidum* supplementation had no significant effect on differential leucocytes counts and chromium in blood ($p>0.05$). It can be concluded that blood profile, differential leucocytes count and chromium in blood of lactating cows have not been affected by supplementing organic chromium and *Ganoderma lucidum* in ration.

Key words: Blood profile, differential leucocytes, cow, chromium, *Ganoderma lucidum*

INTRODUCTION

Chromium has been established as an essential micromineral for animals (NRC, 1997). Elemental Cr is not absorbed and has no nutritional value (Ducros, 1992). Trivalent chromium (Cr^{3+}) is the most stable oxidation state in which Cr is found in living organisms. It does not have the capacity to cross cell membranes easily (Mertz, 1993). Trivalent chromium is essential to normal carbohydrate, protein and lipid metabolism, hormonal regulation and immune function (Pechova and Pavlata, 2007). The physiological function of chromium is as an integral component of biologically active chromium or Glucose Tolerance Factor (GTF) (NRC, 1997). Chromium may be present in the form of inorganic compounds or organic complexes (Pechova and Pavlata, 2007). Inorganic chromium is very poorly absorbed (0.4-2.0%). It must be converted to an organic complex to enable the physiological functioning of chromium. Conversion of inorganic chromium (e.g., chromium chloride) in the liver or kidney to the bioactive form may be slow (Pechova and Pavlata, 2007). Organic chromium can be synthesized by using a fungi *Ganoderma lucidum* that can incorporate Cr into their cell (Yang *et al*., 2006). Supplemental organic chromium had improved milk yield and immune response of stressed cows (Yu *et al*., 2006; Bryant *et al*., 2004; Al-Saiady *et al*., 2004). Lymphocytes from cows supplemented with 0.5 mg Cr/kg diet, from Cr amino acid had increased blastogenic responses to concavalin A stimulation (Burton *et al*., 1993). Supplementation with 5 mg Cr/day increased antibody responses following vaccination with tetanus toxin in dairy cows (Faldyna *et al*., 2003). Chromium provided in basal diets ranged from 0.79 to 1.60 mg/kg of dry matter and the amount of supplemented chromium ranged from 5.5 mg/day up to 10 mg/day (NRC, 1997). Chromium from blood is relatively quickly absorbed by bones, accumulating also in the spleen, liver and kidney, lungs and the large intestine (Pechova and Pavlata, 2007).

Ganoderma lucidum (Fr) Karst, is the species of the class Basidiomycetes which commonly known as Ling Zhi on China and Reishi, Manentake or Shachitake in Japan. It is one of the most traditional China medicinal herbs, used as healthy food. It is non toxic and it can restore the body to its natural state, enabling all organs to function normally. It is primarily composed of complex

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carbohydrates called polysaccharides, triterpenoids, protein and amino acids (Chang and Miles, 2004). *Ganoderma lucidum* has been shown to possess potent antioxidant in multiple research studies with little or no side effect (You and Lin, 2003; Chang and Miles, 2004). The major compounds with pharmacology activities appear to be triterpens, polysaccharides, bioactive proteins and nucleic acids which function as immune-modulator (Gao *et al*., 2004a; Gao *et al*., 2004b; Lin and Zhang, 2005; Gao *et al*., 2005; Boh *et al*., 2007; Zengtao *et al*., 2011); antioxidant (Zhao *et al*., 2004; Chen *et al*., 2005; Sun *et al*., 2004; Yuen and Gohel, 2009); anticancer; Yuen *et al*., 2008; Yuen and Gohel, 2009). *Ganoderma lucidum* can enhance the body immune system and improve blood circulation, thus improving better health conditions. *Ganoderma lucidum* can also be used as carrier to synthesize organic chromium (Yang *et al*., 2006).

During lactation, dairy cows can be stressed (Akers, 2002; Barnes, 2007), so that it may induce a Cr deficiency state. Stressed cause the increase in glucose metabolism and cause mobilization of Cr from the body's reserve (Pechova and Pavlata, 2007). Changes typical of stress response are seen in blood profile, in total and differential leucocytes counts of cows at lactation (Jain, 1986). Circulating leucocytes numbers vary considerably among cows. This variation may be ascribed to several factors, in particular to emotional state of animal (stress) at the time of blood sampling. Changes in lymphocyte numbers may depend on the degree of stress (Jain, 1986). The number of leukocytes in the blood is often an indicator of disease. Stress condition will cause the high loose of chromium. Cr deficiency increase hemoglobin, hematocrit, erythrocytes, leucocytes. Chromium supplemented can be function in stress condition, Supplementation of chromium is thought to prevent Cr losses during lactation so it can maintain cows in good health. *Ganoderma lucidum* supplementation is thought to improve immune response of lactating cows. Studies examining the effects of dietary Cr on health in dairy cows are limited and little information is available about the effect of organic chromium and *Ganoderma lucidum* on blood profile, differential leucocytes counts and chromium in blood. Therefore, the objectives of this study were to investigate blood profile, differential leucocytes count and chromium in blood of lactating cows supplemented with organic chromium and *Ganoderma lucidum* in ration.

MATERIALS AND METHODS

Animals and diets: Fifteen lactating cows in late lactation were randomly allocated from experimental block based on milk production. Animals was fed a basal diet consisted of 60% grass and 40% concentrate and was supplemented with chromium (organic Cr and inorganic Cr), fruiting bodies of *Ganoderma lucidum* and combination of organic chromium and *G. lucidum*. Supplementation on basal diet as treatment were: A = basal diet as control (un supplemented), B = 3 mg/kg inorganic Cr $CrCl_3$, C = 3 mg/kg organic Cr (fermentation product with *Ganoderma lucidum*), D = *Ganoderma lucidum* (5 g/50 kg live weight) and E = organic Cr+*Ganoderma lucidum*. Basal diet for lactating cows consisted of grass and concentrate (Table 1). Chromium was provided as an inorganic Cr ($CrCl_3.6H_2O$) and organic Cr. This experiment was conducted in two periods: preliminary period (2 weeks) and collecting period (8 weeks).

Table 1: Composition of basal diets (%) and nutrient value (%) and chromium content (mg/kg dry matter) of the experimental diets

Ingredient	%
Grass	60
Concentrate	40
Total	100
Nutrient:	
Crude Protein	12.1
Crude Fiber	25.68
Total Digestible Nutrient (TDN)	67
Chromium (mg/kg dry matter)	8.39

The animals were fed twice a day according to body weight and milk production, based feeding scale (NRC, 2001), whereas water was available ad libitum. The sample of grass and concentrate were collected in collecting period. Blood profile such as hemoglobin, hematocrit , red blood cell or erythrocyte, white blood cell or leucocytes were evaluated. The differential leucocytes count such as neutrophils, lymphocytes, monocytes and eosinophils were also counted during lactating period. Number of chromium in blood were measured.

Blood samples: Blood samples were collected from each cow before preliminary period and at the end of collecting period for blood profile, differential leucocytes count, chromium count in blood and blood glucose analysis. Blood samples were kept on ice box immediately until analysis.

Chemical analysis: Crude protein, crude fiber, dry matter and organic matter analysis of feed were carried out with proximate analysis (AOAC, 2000). Chromium content analysis of feed and blood were started with wet ashing and were determined by Atomic Absorption Spectrometry (AAS).

Statistical analysis: Statistical analysis were performed with General Linear Model (GLM) procedure of statistical analysis system (SAS, 1997). Analysis of variance was performed by ANOVA procedure.

RESULTS

Blood profile: Supplementation of chromium and fruit body of *Ganoderma lucidum* and its combination in lactating cows diet did not affect blood profile (p>0.05) (Table 2). Hemoglobin concentration was 9.33±0.58 g/dl in organic chromium treatment while control was 10.33±1.15 g/dl. Hematocrit value statistically was not difference (p>0.05) by adding chromium and *Ganoderma lucidum*. It values was 27.67±2.52% in inorganic chromium treatment while control was 24.33±0.58%. Erythrocytes count was also not difference (p>0.05) with value 6.28±0.73 million/µl in control up to 7.14±1.08 million cell/µl in combination treatment (Cr+*G. lucidum*).

Total leucocytes count was 7120±1.44 cell/µl in combination treatment (organic chromium + *Ganoderma lucidum*), while control was 9270±2.32 cell/µl and this value was not significantly different (p>0.05). Average of minimum and maximum ambient temperature during the experimental period was 22.85±0.41°C in the morning and 32.62±0.85°C in the afternoon, respectively.

Differential leucocytes counts: The amount of neutrophils were no difference statically (p>0.05) on cows supplemented with chromium and *Ganoderma lucidum* or cows not supplemented (Table 3). The lowest neutrophil value is 2530 cell/µl or 34.67% of total leucocytes which is found on cows receiving *Ganoderma lucidum* and then is followed by combination treatment of *Ganoderma lucidum* and organic chromium with value is 2620 cell/µl or 37% of total leucocytes, while amount of neutrophli in control (cows not receiving *Ganoderma lucidum* and chromium) was 3310 cell/µl.

The same result also was found in lymphocyte count. Its count showed no difference (p>0.05). The lowest lymphocyte count is founded in supplementation of *Ganoderma lucidum* and organic chromium with number 3860 cell/µl or 53.67% of total leucocytes, while control was 4960 cell/µl. Monocytes count statistically was not difference (p>0.05). Monocytes number of treatment supplemented with *Ganoderma lucidum* and combination of organic Cr with *Ganoderma lucidum* in lactating cows give the lowest monocytes number (63 cell/µl or 1% of total leucocytes), when it is compared to another treatment (200-290 cell/µl or 2-3% of total leucocytes).

The amount of eosinophils showed no difference statistically (p>0.05) with a number of 553 cell/µl or 7.33% of total leucocytes in *Ganoderma lucidum* treatment, while control was 908 cell/µl or 10% of total leucocytes.

Chromium in blood: Chromium in blood in lactating cows to organic chromium and supplementation were

Table 2: Blood profile (hemoglobin, hematocrit, erythrocytes, leucocytes count) of lactating cows supplemented with chromium and *Ganoderma lucidum* in ration

	Treatment				
Parameters	Control	inorganic Cr	Organic Cr	Ganoderma	Organic Cr+Ganoderma
Hemoglobin (g/dl)	10.33±1.15	10±1	9.33±0.58	10±1	10±1
Hematocrit (%)	24.33±0.58	27.67±2.52	26.33±2.52	27.33±1.53	26.67±2.08
Erythrocytes (10^6/µl)	6.28±0.73	7.09±1.43	6.96±0.28	6.46±0.97	7.14±1.08
Leucocytes (10^6/µl)	9.27±2.32	9.18±3.21	9.07±1.31	7.23±1.89	7.12±1.44

Means in the same row were not significantly different (p>0.05)

Table 3: Differential leucocytes count (neutrophil, lymphocyte, monocytes, eosinophil) and of leucocytes of lactating cows supplemented with chromium and *Ganoderma lucidum* in ration

	Treatment				
Parameters	Control	inorganic Cr	Organic Cr	Ganoderma	Organic Cr+Ganoderma
Leucocytes (10^3/µl)	9.27±2.32	9.18±3.21	9.07±1.31	7.23±1.89	7.12±1.44
Differentiation of Leucocytes:					
Neutrophils (%)	37±8.72	36±2.00	37.33±0.58	34.67±2.31	37±1.73
Lymphocytes (%)	52.67±5.51	51.67±2.08	48.33±4.73	54.33±5.51	53.67±4.16
Monocytes (%)	2±1	3.33±2.31	3.33±2.08	3±1.73	1±1
Eosinophil (%)	10±2	9±1	11±5.57	7.33±2.52	8.67±2.52
Basofiphl (%)	0	0	0	0	0
Differensiation of Leucocyte:					
Neutrophils (10^3/µl)	3.31±0.36	3.34±1.33	3.38±0.44	2.53±0.79	2.62±0.49
Limphocytes (10^3/µl)	4.96±1.74	4.76±1.70	4.35±0.40	3.87±0.67	3.86±1.03
Monocytes (10^3l/µl)	0.201±0.14	0.260±0.08	0.293±0.17	0.220±0.15	0.063±0.06
Eosinophils (10^3/µl)	0.908±0.18	0.813±0.26	1.045±0.62	0.553±0.32	0.597±0.12
Basofil (10^3cell/mm^3)	0	0	0	0	0

Means in the same row were not significantly different (p>0.05)

Table 4: Chromium in blood of lactating cows supplemented with chromium and *Ganoderma lucidum* in ration

	Treatment				
Parameters	Control	inorganic Cr	Organic Cr	Ganoderma	Organic Cr+Ganoderma
Chromium in blood (ppm)	0.58±0.15	0.52±0.10	1.25±0.92	0.73±0.51	0.92±0.36

Means in the same row were not significantly different (p>0.05)

not significantly different (p>0.05) (Table 4). Chromium in blood in treatment organic chromium was 1.25±0.92ppm, while control was 0.58±0.15ppm.

DISCUSSION

Hemoglobin concentration of lactating cows supplemented with chromium and *Gamoderma lucidum* is relatives stable. Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells. It releases the oxygen to burn nutrients to provide energy to power the functions of the organism and collects the resultant carbon dioxide to bring it back to the respiratory organs to be dispensed from the organism. Decrease of hemoglobin, with or without an absolute decrease of red blood cells, leads to symptoms of anemia. Hemoglobin deficiency can be caused either by decreased amount of hemoglobin molecules, as in anemia, or by decreased ability of each molecule to bind oxygen at the same partial pressure of oxygen. Normal values of hemoglobin for cattle have been reported by many investigators that is 8-15 g/dl (Jain, 1986). This blood composition value relate mainly to physiological factors such as ambient temperature, water balance and quality of nutrition. Maximum temperature in this study was higher than maximum critical temperature for dairy cattle (Agustin *et al*., 2011), in which critical temperature for dairy cattle is 26° Celcius (Jones and Stallings, 1999). Average of minimum and maximum ambient temperature during the experimental period was 22.85±0.41°C in the morning and 32.62±0.85°C in the afternoon, respectively (Agustin *et al*., 2011) and it can be predicted that it have caused heat stress for cows in the afternoon. Stress condition will cause the high loose of chromium. Cr deficiency has also led to an increase in hematological parameters (hemoglobin, hematocrit, erythrocytes, leucocytes and mean erythrocyte volume). Chromium supplemented can be function in stress condition but statistically was not difference. Hemoglobin synthesis is influenced by nutrient such as protein and chromium used in protein metabolism.

In this study, chromium (in organic Cr and organic Cr) and *Ganoderma lucidum* supplementation showed no difference in blood profile after treatment for 8 weeks experiment. This result is consistent with Kalili *et al*. (2011) who also founded that blood hematology were not affected by chromium methionine supplementation (5 g/day) from the fifth week prior to parturition until 12 weeks thereafter when fed to dairy cows in transition period, where forage was the major source of energy.

The hematocrit (Ht or HCT) or Packed Cell Volume (PCV) or Erythrocyte Volume Fraction (EVF) is the volume percentage (%) of red blood cell (erythrocyte) of total blood volume (Jain, 1986). Hematocri value in this research is still in normal values for cattle that is 24.0-46.0% (Jain, 1986). However, there is indication that supplementation of chromium (inorganic Cr or organic Cr) and *Ganoderma lucid*um or combination of organic Cr with *Ganoderma lucidum* give hematocrit value higher (26.33 to 27.67%) than control. This PCV profile is the same with erythrocyte profile. Erythrocytes also play a part in the body's immune response, in which their hemoglobin releases free radicals which break down the pathogen's cell wall and membrane, killing it. Lactating cows fed chromium and *Ganoderma lucidum* were in good health (Agustin *et al*., 2011). In this research, leucocytes concentration of cows was in normal value for healthy cows with normal value is 7000-10 000 cell/mm^3 (Jain, 1986). Leucocytes are cells of the immune system involved in defending the body against both infectious disease and foreign materials. Supplementation of chromium and *Ganoderma lucidum* were not significantly different (p>0.05) but there was indication that supplementation of *Ganoderma lucidum* and combination of organic Cr with *Ganoderma lucidum* in lactating cows give the lowest leucocytes number (7120 cell/mm^3) when it is compared to another treatment (9270 cell/mm^3). The low leucocytes count is related with the low somatic cell count in milk. This is indicated that cows are in healthy condition and they have not been infected by bacteria (Jain, 1986). The number of leukocytes in the blood is often an indicator of disease. Supplementation of chromium is not followed by the increase of leucocytes numbers and can be predicted that the role of *Ganoderma lucidum* did not to stimulate leucopoiesis but its role was to increase the activity of leucocytes. The decrease of leucocytes number of lactating cows supplemented with *Ganoderma lucidum* indicate that there is a migration of leucocytes cells to mammary gland to defend mammary because of *Ganoderma lucidum* function as immunomodulator (Zengtao *et al*., 2011). Some leukocytes migrate into the tissues of the body to take up a permanent residence at that location rather than remaining in the blood. These cells still serve a role in the immune system. In our research (Agustin *et al*., 2011), the numbers of Somatic Cell Count (SCC) was the lowest in lactating cows supplemented with combination of *Ganoderma lucidum* with chromium that is 310×10^{3}cell/ml, while control was 800×10^3 cell/ml. Supplementation of *Ganoderma lucidum* and its

combination with organic Cr beside can reduce Somatic Cell Count (SCC) up to 310×10^3 cell/ml, it can also reduce number of bacteria up to <100 estimate CFU/ ml, so dairy cattle is not in mastitis condition,

Differential leucocytes count: Neutrophils defend against bacterial or fungal infection and other very small inflammatory processes that are usually first responders to microbial infection. The amount of neutrophils or its profile follows the moving of leucocytes. Neutrophils response of lactating cows to *Ganoderma lucidum* supplementation indicated the lowest neutrophil count and then is followed by combination treatment of *Ganoderma lucidum* and organic chromium. These is because of the role of *Ganoderma lucidum* as immunomodulator (Boh *et al*., 2007; Zengtao *et al*., 2011) and the biological function of chromium in immune function (Pechova and Pavlata, 2007). Neutrophils are very active in phagocytosing bacteria. Neutrophils are the most common cell type seen in the early stages of acute inflammation.

The same result also was found in lymphocyte count. The lowest lymphocyte count is founded in supplementation of *Ganoderma lucidum* and organic chromium (3860 cell/mm^3). This indicated that both *Ganoderma lucidum* and organic chromium can work together to improve immune system of animal. This is because active compounds of *Ganoderma lucidum* can improve immune system of dairy cows under subclinical condition. Beta-D glucan content in *Ganoderma lucidum* can stimulate the growth of T cells (You and Lin, 2003; Lin and Zhang, 2005; Ikawati *et al*., 2011) that can attack infected cells (Baratawijaya, 2006). Glycoprotein fraction of *Ganoderma lucidum* (15 amino acids and 2 kinds of saccharides) showed the response on stimulation index and there was no immunosuppressive response identified from T and B lymphocyte cells (Darusman, 2011). The blood has three types of lymphocytes that are B cells, T cells and Natural Killer cells. Lymphocytes are much more common in lymphatic system. B cells make antibodies that bind to pathogen to enable their destruction. T cells are important in the defense against intra cellular bacteria and are able to kill damage cells. Natural killer cells are able to kill cells of the body that are displaying a signal to kill them (Baratawijaya, 2006). Absorbed Cr circulates in blood bound to the β-globulin plasma fraction and is transported to tissues bound to transferrin or other complexes at the physiological concentration. Transferrin receptors are insulin-sensitive; an increase of this hormone in blood stimulates the transport of transferrin receptors from the vesicles inside cells to the plasmatic membrane (Kandror, 1999). Chromium has an improving effect on insulin binding and increases the number of insulin receptors on the cell surface and sensitivity of pancreatic β-cells together with an overall increase of insulin-sensitivity (Anderson, 1997). Chromium acts as a cofactor for insulin and therefore, Cr activity in the organism is parallel to insulin functions. The immune function may be affected in association with insulin and/or cortisol activity but it can just as well be mediated by production regulation of certain cytokines (Borgs and Mallard, 1998).

Monocytes play multiple roles in immune function, mainly in response to inflammation signals. Monocytes number of treatment supplemented with the combination of organic Cr and *Ganoderma lucidum* in lactating cows give the lowest monocytes number. Monocytes are a type of leucocytes that can move quickly to sites of infection in the tissues. The number of leukocytes in the blood is often an indicator of disease. Supplementation of combination organic chromium and *Ganoderma lucidum* is not followed by the increase of monocytes numbers and can be predicted that the role of *Ganoderma lucidum* in immune function increased with organic chromium. Once monocytes move from the bloodstream out into the body tissues, they undergo changes (differentiate) allowing phagocytosis and then known as macrophages.

Eosinophils are white blood cells that are one of the immune system components. Eosinophils primarily deal with parasitic infections. In this research, eosinophils count of cows supplemented with *Ganoderma lucidum* was the lowest. It means that *Ganoderma lucidum* can reduce parasite count although statistically is not difference. Eosinophils are also the predominant inflammatory cells in allergic reactions. Eosinophils developed and mature in bone marrow. After maturation, eosinophils circulate in blood and migrate to inflammatory sites in tissue. In addition, eosinophils play a role in fighting viral infections.

Chromium in blood: In this research, chromium is present in diets in the form of inorganic compounds and organic complexes. Concentration of Cr in blood of lactating cows supplemented with organic Cr was higher than inorganic form and this is not related with the number of absorbed chromium. Organic chromium absorbed from small intestine of lactating cows supplemented with organic chromium is the same with inorganic chromium absorbed. Concentrations of Cr in the blood of cattle with respect to the Cr content in pasture plants in a region characterized by an increased Cr level have been studied by Sahin *et al*. (1996). They founded that Cr in blood detected ranged from 9 to 92 μg/l, depending on the Cr content in the plants. Pechova *et al*. (2002) found the Cr concentrations in blood of dairy cows during the peripartal period to be 3-5 μg/l while supplementation with 10 mg Cr per animal/day had no effect on the Cr concentrations in blood.

Supplementation of chromium or its combination with *Ganoderma. lucidum* could not change the blood glucose concentration (Agustin *et al*., 2011). Each animal could reach a normal level of blood glucose for ruminant (Agustin *et al*., 2011) which according to Larson (1985) that normal level of glucose is 52 mg/dl. Chromium is needed to improves glucose transporter or to increases the activities of insulin like growth factor-I (IGF-I) receptor. However, An-Qiang *et al*. (2009) had reported that concentration of blood glucose and ratio of blood glucose to insulin of Holstein cows under heat stress were increased by adding chromium.

Conclusion: We concluded that blood profile, differential leucocytes count, chromium in blood of lactating cows have not been affected by supplementing organic chromium and *Ganoderma lucidum* in ration.

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