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Mineral Utilization in Rams Fed Ration Supplemented with Different Levels of Chromium, Calcium, and Cation-Anion Balances

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ABSTRAK

Kromium (Cr) adalah mineral penting bagi ternak ruminansia. Metabolisme dan interaksi mineral Cr belum banyak diketahui. Penelitian ini dilakukan untuk mengkaji utilisasi mineral dan pertumbuhan domba garut yang diberi mineral Cr, Ca, dan neraca kation anion ransum (NKAR) berbeda. Perlakuan ransum adalah R0 (ransum dengan NKAR+14); R1 (Ransum dengan NKAR+14 + Cr 3ppm), R2 (Ransum dengan NKAR 0 + Ca); R3 (Ransum dengan NKAR 0 + Cr 3 ppm + Ca). Rancangan penelitian adalah rancangan kelompok menggunakan 24 ekor domba garut jantan berumur 1,5-2 tahun. Suplementasi Cr dalam ransum yang mengandung Ca tidak mempengaruhi konsumsi ransum, pertambahan bobot badan, dan kecernaan bahan kering ransum, tetapi menurunkan penyerapan Cr dan Ca pada ransum rendah Ca. Suplementasi Cr tidak berpengaruh terhadap status Cr, Ca, Zn, dan Mg dalam darah dan semen. Tingkat konsumsi Cr berhubungan negatif dengan tingkat penyerapan Cr dan berhubungan positif dengan kadar Cr darah. Terdapat hubungan positif antara konsumsi Ca dengan tingkat penyerapan Ca dan Mg juga dengan kadar Ca dan Zn darah. Konsumsi Cr dan Ca tidak berhubungan dengan kadar Cr dan Ca semen.

Kata kunci: kromium, kalsium, neraca kation-anion, domba garut

ABSTRACT

Chromium (Cr) is an essential mineral for ruminants. Its metabolism and interactions with other minerals has not been widely known. This experiment was designed to evaluate the utilization of minerals and growth of Garut ram fed ration supplemented with Cr and different Dietary Cation Anion Balance (DCAB) and Ca level. Dietary treatments, namely: R0 (Ration with DCAB+14); R1 (Ration with DCAB+14 + Cr 3ppm,); R2 (Ration with DCAB 0 + Ca); R3 (Ration with DCAB 0 + Cr 3 ppm + Ca), were allocated in twenty four of 1.5-2 years old Garut rams in a randomized block design. The results showed that Cr supplementation in rations containing different levels of Ca did not affect feed intake, body weight gain, and dry matter digestibility, but reduced the absorption of Cr and Ca of the low Ca diet. Supplementation of Cr had no effect on Cr, Ca, Zn, and Mg status in blood and semen of the rams. Level of Cr intake had negative correlation with Ca absorption and positive correlation with blood Cr levels. There is a positive relationship between level of Ca intake with Ca and Mg absorption and blood Ca and Zn levels. Intake of Cr and Ca was not related to the semen Cr and Ca levels.

Key words: chromium, calcium, kation-anion balance, Garut rams

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INTRODUCTION

The benefits of Cr have been known for 50 years. However, the dietary requirements of livestock for chromium have not been defined (Suttle, 2010). Chromium in the form of glucose tolerance factor (GTF) is known to play a role in blood flow by increasing the potential of insulin activity and therefore the entry of glucose (NRC, 2001) required in the metabolism of fat and protein synthesis in cells (Pollard *et al*., 2001; Suttle, 2010). The possible roles of Cr include its participation in maintaining the stability of the structure of proteins and nucleic acids and playing a role in reproduction because it is required for growth and development of the fetus (Lindemann, 2004; Pechova & Pavlata, 2007).

Chromium deficiency depresses nucleic acid synthesis and decreases spermatozoa count and fertility in rodents (Anderson & Polansky, 1981). On the other hand, high levels of Cr reduce the production of spermatozoa (Skandhan *et al*., 2005), alter semen quality and reproductive hormones as well as decrease the number and morphology of normal spermatozoa (Kumar, 2008).

Minerals interact each other which result in toxicity or deficiency. Excessive nutrient levels can be diagnosed and prevented, otherwise deficiency is often difficult to be controlled and anticipated because an element has different functional roles. Mineral interactions occur in the digestive tract, so that the consumption of minerals simultaneously leads to competition in absorption. Intake of high Cr reduces retention of Co, Fe, and Mn (Luseba, 2005; Gropper *et al*., 2009). In contrast, reduced absorption of Cr may occur if Ca is given in the form of calcium carbonate and antacids (Mateljan, 2010), high dietary Zn, V and Fe (Krejpcio, 2001), and Cr bound to phytic acid (Krejpcio, 2001; Mateljan, 2010).

Cations (Na^+ and K^+) and anions (Cl^- and SO_4^-) have a major influence on metabolic processes in the body and acid-base values. Cation and anion levels are expressed by dietary cation-anion balance (DCAB) (Chan *et al*., 2005). Values of DCAB affect ration consumption, Ca metabolism (Tauriainen, 2001), Mg levels in plasma and semen (Hidayat, 2009) and amino acid metabolism (Mowat, 2008).

The objective of this study was to evaluate the utilization of minerals and growth of Garut rams fed ration supplemented with Cr and different dietary cation anion balance (DCAB) and Ca level.

MATERIALS AND METHODS

Feeding Experiment

Feed consisted of corn hay, yellow corn, rice bran, coconut meal, soybean meal, corn oil, urea, $ZnSO_4$, $CaCO_3$, and $CaCl_2$. Chromium was given in the form of organic Cr resulted from yeast fermentation on soybean substrate. Rations were formulated isoprotein and isocalory according to the recommendations of NRC (1985) (Table 1). Dietary treatments, namely: R0 (ration with DCAB+14); R1 (Ration with DCAB+14 + Cr); R2 (Ration with DCAB 0 + Ca,); R3 (Ration with DCAB 0 + Cr + Ca), were allocated into twenty four 1.5-2 years old Garut rams in a randomized block design.

Animal Rearing Experiment

Forty-four rams (mean 1.5 years old with body weight 32.02±3.71 kg) were used in this experiment. Ram was grouped according to their body weight. Rams were were maintained for 3 months in individual cages in the Laboratory of Meat and Work Animal Nutrition, Faculty of Animal Science, Bogor Agricultural University.

In the first 21 days, rams were adapted to the conditions of management, the cages environment and feedstuffs. Furthermore, on day 22 to day 96 rams were fed dietary treatment. During the experimental, rams were fed at around 7:00 and 14:00 o'clock. Drinking water was provided *ad libitum*.

Sampling and Analysis

A total fecal collection to measure the value of nutrient digestibility and mineral absorption were performed on day 35 to day 42. Feces collected as much

Table 1. Ingredient and chemical composition of the rations

Ingredient/nutrient	Ration			
	R0	R1	R2	R3
Feed composition (% of DM basal ration)				
Corn straw	35	35	35	35
Rice bran	21.5	21.5	21.5	21.5
Yellow corn	19.65	19.65	19.65	19.65
Soybean meal	13.6	13.6	13.6	13.6
Coconut cake meal	8	8	8	8
Urea	0.25	0.25	0.25	0.25
Corn oil	2	2	2	2
Content of nutrient (%)[1]				
Dry matter	90.31	90.31	90.41	90.41
Ash	7.4	7.4	8.1	8.1
Crude protein	13.5	13.5	13.5	13.5
Crude fat	7.5	7.5	7.7	7.5
Crude fiber	17.5	17.5	17.5	17.5
Gross energy (Kcal/kg)	3263	3263	3263	3263
Content of mineral[2]				
Cr (ppm)	5.59	8.59	5.59	8.59
Ca (%)	0.21	0.21	0.42	0.42
Zn (ppm)	139.12	139.12	139.12	139.12
Mg (%)	1.46	1.46	1.46	1.46
DCAB (meq)	14	14	0	0

Note: R0= Basal ration, R1= R0 + Cr, R2= R0 + Ca, R3= R2 + Cr (addition of minerals on the R1, R2, and R3 by calculation).
[1]The results of proximate analysis laboratory in PAU (2008) and Laboratorium of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University (2009); [2]The results of mineral analysis in Dairy Nutrition Laboratory, Faculty of Animal Science, Bogor Agricultural University and Balitnak (2009)

as 10% for analysis of mineral content. Consumption of each ram was measured daily and rams were weighed every week. Feeding treatments were continued for 49 days (one period of spermatogenesis). On day 50 was performed collection of rams semen, and then levels of the mineral in semen were analyzed. On day 53 blood sampling was done to determine the mineral status of the blood. Analysis of dry matter and nutrient content of feed and feces were done using the method of proximate (AOAC, 2002). Minerals analysis (Cr, Mg, Zn, and Ca) of feed, feces, blood, and semen were done by wet ashes and measured by atomic absorption spectrophotometer (AAS) Varian Spectra AA220 series (Carry & Allaway, 1971). The data obtained were tabulated and analyzed by analysis of variance. Analysis followed by Tukey test to determine differences between treatments.

RESULTS AND DISCUSSION

Feed Intake and Average Daily Gain of Garut Ram

Supplementation of 3 ppm Cr in the ration did not affect dry matter intake and average daily gain Garut sheep males (Table 2, Figure 1 & 2). Previous research reports showed that Cr supplementation of 0.3 ppm in rations containing 707-858 mg Cr/kg increased feed intake and average daily gain of transport-stressed lamb (Kraidees *et al*., 2009) and not stressed lamb (Yan *et al*., 2008). While the study of Mathius *et al*., (2005), 4 ppm Cr supplementation in sheep rations did not affect consumption and average daily gain. Chromium content of the ration in this experiment is thought to be sufficient and still in the safe range so it did not affect the consumption of rations and growth. Chromium needs to be increased in conditions such as motoric activity or stress, transport, and infection when the loss of Cr in the urine increases (NRC, 2007).

Minerals Absorption in Male Sheep Rations

Chromium supplementation in lamb rations significantly decreased absorption of Cr (Table 3). Absorption values at R1 and R3 rations supplemented 3 ppm Cr decreased 19.25% and 30.01% compared to supplemented rations without Cr. Chromium absorption in the digestive tract is affected by the form of Cr ration. Absorption of inorganic Cr is lower than the Cr binding with amino acids or other ligands that bind to inorganic Cr (Gropper *et al*., 2009). Impairment of absorption of Cr on rations R1 and R3 indicated that endogenous Cr

Tabel 2. Dry matter intake (DM), Cr, Ca, Zn, Mg, and average daily gain (ADG) of Garut ram

Variables	Ration			
	R0	R1	R2	R3
Intake				
DM (g/d)	873.00±176.00	865.00±145.00	922.00±167.00	1,088.00±167.00
DM (% of BW)	2.64± 0.40	2.63± 0.45	2.83± 0.29	3.02± 0.59
Cr (mg)	4.16± 0.80[a]	6.63± 1.18[b]	4.56± 0.80[a]	7.97± 1.50[b]
Ca (g)	1.57± 0.30[a]	1.63± 0.29[a]	3.46± 0.61[b]	3.93± 0.74[b]
Zn (mg)	103.61± 19.86	107.50± 19.21	113.48± 19.95	129.12± 22.33
Mg (g)	10.84± 2.08	11.25± 2.01	11.88± 2.09	13.52± 2.55
ADG (g)	114.00± 70.00	102.00± 32.00	116.00± 25.00	109.00± 64.00

Means in the same row with different superscript differ significantly (P<0.05).

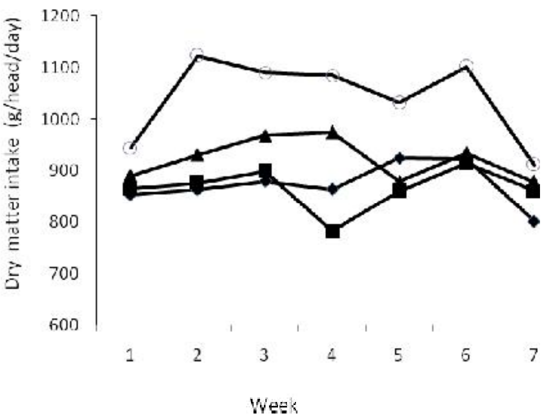

Figure 1. Dry matter intake of Garut ram for seven weeks fed R0 (-◆-), R1 (-■-), R2 (-▲-), and R3 (-○-).

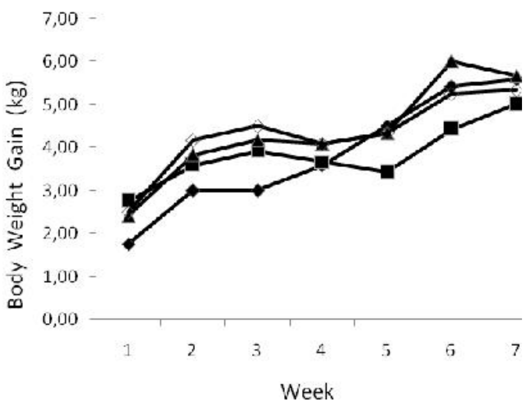

Figure 2. Body weight gain of Garut ram for seven weeks fed R0 (-◆-), R1 (-■-), R2 (-▲-), and R3 (-○-).

in the basal feed (R0) is more easily absorbed than Cr of fermented products. Although Cr of ration R1 and R3 comes from the fermentation by fungi which is a method of organic Cr production, but most of Cr are still in the form of inorganic Cr as only 12.01% of Cr in the form of the protein (Agustin *et al.*, 2010). Chromium mineral in the ration R0 and R2 is a natural Cr feed in organic form (Vincent, 1999). Absorption of inorganic Cr is only 2%-3% (Anderson & Kozlovsky, 1985) and lower than the organic form of about 10%-25% (NRC, 1997). There was a negative relationship between consumption of Cr with absorption of Cr (R= 0.58) (Table 4). In addition, higher consumption of Cr caused lower absorption of Cr (Anderson & Kozlovsky, 1985).

Chromium supplementation in the ration reduced the absorption of Ca at low Ca ration (R1). This means that Cr supplementation on low Ca rations exacerbated Ca absorption. While on R2 and R3 rations contain enough Ca, Cr supplementation did not affect absorption of Ca. Absorption of Ca in the digestive tract requires Calbindin which is a Ca-binding protein (CaBP) to enter into the membrane of the digestive tract. Molecule of CaBP has a high affinity for Ca (Georgievskii, 1982). However, a higher affinity to the other minerals affects the bond between Ca and Calbindin in the intestine (Suttle, 2010). Based on the period of the periodic table of elements, Cr has greater affinity than Ca, so that Cr is easier bound to the protein compounds such as CaBP. In addition there are similarities between the mechanism of action and structure of Calbindin and low molecular-weight chromium binding substance (LMWCr) (Vincent, 1999) which would lead to competition between minerals (Lehninger *et al*., 2004). It is therefore supposed that low absorption of Ca in R1 due to the competition between the two minerals to bind CaBP.

Ca absorption of R2 and R3 ration was higher than Ca absorption of R0 and R1 ration. This was also caused by DCAB differences. The Low DCAB in rations R2 and R3 stimulates increased Ca absorption from the gut. According to Ramberg *et al.*, (2009), DCAB reduction enhances renal production of 1.25-$(OH)_2D_3$, the active metabolite of vitamin D, increases the responsiveness of target tissue receptors to 1,25-$(OH)_2D_3$ and increases both the efficiency of Ca absorption and mobilization of calcium from bone.

Absorption of Zn was not affected by Cr supplementation at different levels of Ca rations. Zinc absorption of sheep rations was in a fairly narrow range of between 30.21 to 35.58 ppm. The value of mineral absorption of Mg in the ration of sheep was not influenced by Cr supplementation at different levels of Ca. But there was a negative relationship between consumption of Ca with Mg absorption values (R = 0.43, P<0.05). High levels of Ca intake may reduce Mg absorption in the digestive tract (Gropper *et al*., 2009).

The value of Cr mineral absorption (Table 4) had a negative relationship with the consumption of Cr (R= 0.58). The higher Cr consumption reduced the absorption of Cr. Any increase in one unit of Cr consumption will reduce absorption of Cr 6.4%. Blood Cr values also had a positive relationship with consumption of Cr, the higher the consumption of Cr it increased blood Cr, while Cr consumption was not associated with levels of Cr in semen. The value of Ca absorption related to

Table 3. Means absorption of Cr, Ca, Zn, and Mg Garut ram rations (%)

Variables	Ration			
	R0	R1	R2	R3
Cr	68.52± 9.86[a]	19.25± 7.72[b]	58.03±12.34[a]	30.01±16.31[b]
Ca	78.11± 5.93[a]	70.10±10.24[b]	82.53± 8.09[a]	84.16± 5.54[a]
Zn	32.47±15.44	30.21± 8.37	35.58± 7.65	31.17± 9.84
Mg	73.05± 7.95	71.25±11.89	65.82± 5.44	61.75± 6.59

Means in the same row with different superscript differ significantly (P<0.05).

Table 4. Correlation and regression of mineral intake with absorption and mineral status in Garut ram blood and semen

Variables	R	Significance	Regression equation
Cr intake–Absorption of Cr	-0.58	0.080	Y= 84.691 - 6.390X
Cr intake–Cr blood	0.42	0.067	Y= 0.022 + 0.016X
Ca intake–Absorption of Ca	0.48	0.020	Y= 69.46 + 3.58X
Ca intake–Absorption of Mg	-0.45	0.030	Y= 77.01 - 3.41X
Ca intake–Ca blood	0.56	0.005	Y= 30.30 + 2.84X
Ca intake –Zn blood	0.46	0.024	Y= 4.65 + 0.221X
Zn intake Zn–Zn blood	0.52	0.009	Y= 3.695 + 0.14X
Mg intake–Ca blood	0.43	0.043	Y= 24.87 + 1.101X
Mg intake–Zn blood	0.52	0.009	Y= 3.696 + 0.13X

consumption of Ca. The higher consumption of Ca increased absorption of Ca. Meanwhile Ca consumption had negative relationship with Mg absorption due to competition in the digestive tract. So that high Ca consumption would reduce Mg absorption. Status of Ca and Zn in blood was associated with Ca consumption. The higher consumption of Ca increased blood Ca and Zn but not increased value of Ca in semen. Thus, Cr and Ca status in blood can be used as indicators of the adequacy of Cr and Ca in the ration, while Cr and Ca in semen does not show Cr and Ca in the ration.

Mineral status of Male Sheep Semen and Blood

Supplementation of Cr at different levels of Ca did not affect Cr status of semen and blood. However, in conditions sufficient rations with Ca (R3), mean Cr content of semen was higher than the low-Ca ration (R1). Status of sheep blood Cr ranged between 0.13 and 0.22 ppm. The status of blood Cr was within the range of normal levels of 0.01 to 0.3 ppm (NRC, 1997). The value of semen Cr was not affected by Cr supplementation in the ration and not correlated with the consumption of Cr. This means that the Cr status of semen was more influenced by other factors, such as antagonism minerals, hormones, vitamins, disease, and stress (NRC, 1997: Watts, 1989).

Status of Ca in the semen varied from 30.15 to 90.53 ppm (Table 5). This shows that semen Ca balance is not as tight as in the homeostasis of blood minerals. Supplementation of Cr and decreased DCAB did not affect the status of blood Ca. The levels of blood Ca within the range of 32.19 to 42.05 ppm, means that the Ca status of sheep in marginal conditions. The range of normal blood Ca is 40-60 ppm (Georgievskii *et al*., 1982). Even though blood Ca concentration is maintained within narrow limits by calcitonin and parathyroid hormone (suttle, 2010), the levels of blood Ca could reach a value of 112.5 to 118.1 ppm (Ratchford *et al*., 2001; Stojkovic, 2009). Garut sheep response to Cr supplementation under conditions of high Ca is expected to be more positive than the low-Ca conditions.

Supplementation of Cr in the ration did not affect blood Zn of Garut sheep. The concentration of blood Zn ranged from 5 to 5.45 ppm (Table 5), but still within the normal range 4-6 ppm (Suttle, 2010). Experimental sheep rations containing 139.12 ppm Zn was higher than Zn ration of Ratchford *et al*. (2001) which contained 43.7 ppm Zn. However Zn Garut sheep blood Zn levels lower than blood Zn of sheep reported by Ratchford *et al*. (2001) which was 9.1 ppm. Low levels of blood Zn describe, there are many factors that limit the absorption of Zn. Factors that limit the absorption of Zn and Zn status in blood is interaction of minerals, adequacy of Zn in ration, vitamins, and chelating agent (Gropper *et al*., 2009).

Supplementation of Cr on ration containing low and sufficient Ca did not affect the status of blood Mg. The levels of blood Mg of male Garut sheep ranged from 66.31 to 83.96 ppm. The value of blood Mg of Garut sheep can be categorized high. Ratchford *et al*. (2001) reported that sheep blood Mg levels in the range of 22.5 to 23.1 ppm, as well as Stojkovic (2009) reported sheep blood Mg levels reached 26.1 ppm. High levels of Mg in the blood was caused by high level of Mg ration. According to Georgievskii *et al.* (1982), status of blood Mg is a function of Mg content in the ration, the higher Mg content of the ration will increase blood Mg. This means that under conditions of good status of Mg, blood Mg levels were not affected by Cr supplementation.

Nutrient Digestibility of Rations

Digestibility of dry matter, organic matter, protein, fat, and crude fiber was not affected by Cr supplementation in rams ration (Table 6). Supplementation of Cr did not affect digestibility of dry matter and organic matter rations. Supplementation of Cr did not affect digestion in the rumen and post-rumen. Supplementation of Cr-pikolinat in sheep rations did not affect body weight gain and number of rumen protozoa (Dallago *et al*., 2011). Yan *et al*. (2008) and Yan *et al*. (2010) stated that providing as much as 0.4 ppm Cr in sheep ration enhanced growth, improve the utilization of glucose and

Table 5. Means status of Cr, Ca, Zn, dan Mg Garut ram semen and blood (ppm)

Variables	Ration			
	R0	R1	R2	R3
Semen				
Cr	0.22± 0.02	0.37± 0.14	0.42± 0.28	0.52± 0.36
Ca	70.51±107.45	30.15±31.01	92.53± 74.44	50.57±60.59
Zn	32.87± 6.23	37.26± 6.23	36.77± 6.23	33.15± 6.83
Mg	225.00± 71.00	263.00±68.00	233.00±131.00	186.00±61.00
Blood				
Cr	0.13± 0.06	0.14± 0.06	0.18± 0.07	0.22± 0.06
Ca	37.43± 5.07	32.19± 5.39	39.96± 4.79	42.05± 4.87
Zn	5.00± 0.23	5.15± 0.66	5.45± 0.55	5.35± 0.57
Mg	74.21± 9.27	83.96±13.62	66.31± 8.45	78.06±40.05

Table 6. Means digestibility coefficients of nutrient and digestible energy (DE) of ram ration

Variables	Ration			
	R0	R1	R2	R3
DM (%)	63.24± 5.70	61.22± 3.30	63.61± 4.62	60.16± 6.42
OM (%)	65.20± 2.77	64.75± 3.00	66.91± 4.29	65.15± 4.07
Crude protein (%)	60.08± 3.10	59.43± 1.86	61.48± 4.79	59.78± 5.19
Crude fat (%)	96.50± 1.53	94.50± 2.30	94.35± 2.68	93.04± 1.22
Crude Fiber (%)	32.78± 3.87	30.70± 8.14	34.68± 11.19	31.48± 15.19
DE (Kcal/kg)	1,263 ±247	1,432 ±268	1,518 ±419	1,704 ±462

energy metabolism. Mackie *et al*. (2002) suggested that microbial activity in the digestive tract greatly affected the digestibility. Supplementation of Cr had little effect on rumen function (Besong *et al*., 2001). This means that the ration of Cr supplementation did not affect rumen microbial activity.

CONCLUSION

Supplementation of Cr at different levels of Ca and DCAB in Garut rams rations did not affect feed intake, nutrient digestibility of ration and body weight gain. Supplementation of Cr reduced absorption of Cr and Ca of low-Ca ration. Cr supplementation did not affect the status of Cr, Ca, Zn, and Mg Garut sheep blood and semen. Cr consumption had a negative correlation with the absorption of Cr and positive correlation with Cr levels in the blood. There is a positive relationship between consumption of Ca with the absorption of Ca and Mg and with Ca and Zn levels in the blood. Intake of Cr and Ca was not related to the semen Cr and Ca levels.

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