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Roles of Dietary Cobalt and Administration of Mixed Rumen Bacteria in Regulating Hematological Parameters of Pre-weaning Twin Lambs

T. Adelina[a,b,*,§], A. Boediono[c,#], I. G. Permana[d,#], T. R. Wiradarya[b,§], D. Evvyernie[d,#], & T. Toharmat[d,#]
[a]Major Program of Animal Nutrition and Feed Science, Graduate School, Bogor Agricultural University
[b] Study Program of Animal Science, Faculty of Agriculture and Animal Science,
State Islamic University of Sultan Syarif Kasim Riau
[§] Jln. H.R. Soebrantas Km 16 Panam, Pekanbaru 28293, Indonesia
[c]Department of Anatomy, Physiology, and Pharmacology, Faculty of Veterinary Medicine,
Bogor Agricultural University
[d]Department of Nutrition and Feed Technology, Faculty of Animal Science, Bogor Agricultural University
[#]Jln. Agatis, Kampus IPB Darmaga, Bogor 16680, Indonesia
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ABSTRACT

Cobalt (Co) is required by rumen microorganism for vitamin B_{12} synthesis. Vitamin B_{12} is an important cofactor for methionine synthesis and gluconeogenesis. In young ruminants up to 6–8 wk old, the rumen has not been completely developed and rumen microorganisms are not ready to supply vitamin B_{12}. The aim of this research was to determine the potency of mixed rumen bacteria and dietary supplementation of Co and its effect on plasma glucose, blood minerals (Co, Fe, and Zn) concentrations, and hematology of pre-weaning twin lambs. Twelve one month-old local twin lambs were assigned to 4 groups in a randomized complete block design. Lambs were fed cow milk at 10% body weight, adjusted weekly for 80 d. Mixed rumen bacteria were offered at 15 mL/d ($8.295x10^{10}$ cfu). Dietary treatments were: 1) basal diet (Control), 2) basal diet + 1 mg/kg DM cyanocobalamin ($VitB_{12}$) and 3) basal diet + 1 mg/kg DM of Co + administration of 15 mL mixed rumen bacteria (CoBac). There were no treatment effects on neither plasma glucose and blood mineral concentrations nor hematological profiles. This study demonstrated that pre-weaning twin lambs are not responsive to supplementation of Co and administration of mixed rumen bacteria.

Key words: blood, cobalt, lamb, rumen bacteria, vitamin B_{12}

ABSTRAK

Kobalt (Co) dibutuhkan mikrob rumen untuk mensintesis vitamin B_{12}. Vitamin B_{12} berperan dalam sintesis metionin dan glukoneogenesis. Ruminansia berumur di bawah 6-8 minggu belum dapat mensintesis vitamin B_{12} karena rumen belum berkembang optimal. Tujuan penelitian adalah menggali informasi mengenai potensi campuran bakteri rumen dan Co dalam mengatur glukosa plasma, mineral (Co, Fe, dan Zn) darah, serta hematologi pada anak domba kembar prasapih. Sebanyak 12 ekor anak domba lokal kembar prasapih umur 1 bulan dibagi dalam 4 kelompok dengan rancangan acak kelompok (RAK). Anak domba mendapat susu sapi 10% bobot badan. Campuran bakteri rumen diberikan 15 mL/h ($8,295x10^{10}$ cfu). Perlakuan terdiri atas: 1) ransum basal (kontrol), 2) ransum basal + suplementasi vitamin B_{12} sintetis 1 mg/kg BK ($VitB_{12}$), dan 3) ransum basal + Co 1 mg/kg BK + 15 mL campuran bakteri rumen (CoBac). Glukosa plasma, mineral Co, Fe, dan Zn serta hematologi tidak berbeda antar perlakuan. Pemberian vitamin B_{12} maupun CoBac tidak mengubah konsentrasi glukosa plasma, status mineral Co, Fe dan Zn serta mempertahankan hematologi normal pada domba kembar prasapih. Penelitian ini memperlihatkan bahwa anak domba kembar prasapih tidak responsif terhadap suplementasi Co dan campuran bakteri rumen.

Kata kunci: darah, kobalt, domba, bakteri rumen, vitamin B_{12}

*Corresponding author:
E-mail: trianiadelina@yahoo.com

INTRODUCTION

Twin born lambs tend to have a low birth weight that causes a trouble in regulating body temperature and are more susceptible to infection, which may increase risk for a high mortality rate. Improvement of nutrient intake and immune system status by dietary supplementation of Co or vitamin B_{12} increased body weight of ruminant (Yousuf *et al*., 2009; Rusmana *et al*., 2002; Tiffany & Spears, 2005).

Rumen microbes require Co for a corrin ring structure during vitamin B_{12} synthesis. Vitamin B_{12} is essential as a cofactor for methylmalonyl-CoA mutase and methionine synthase on gluconeogenesis and methionine synthesis. A general function of vitamin B_{12} is to promote red blood cell synthesis and to maintain nervous system integrity (Hershko *et al*., 2006; Hvas & Nexo, 2006; Mc Dowell, 2003). Deficiency in Co decreases microbial rumen population, limits the amount of available vitamin B_{12} to microbes and host animal (Tiffany *et al*., 2003) and results in reduce intake and average daily gain (Wang *et al*., 2007). Hershko *et al*. (2006) also showed that vitamin B_{12} deficiency may be preceded by Fe deficiency.

Ruminant have the ability to synthesize vitamin B_{12} if they are supplied by an adequate dietary Co and have a rumen that function normally. Rumen becomes functional for synthesis of vitamin B_{12} at 6-8 wk of age (McDowell, 2003). Supplementation of vitamin B_{12} in the ration is needed by lambs because rumen is not completely developed. Therefore, vitamin B_{12} supplementation is necessary, especially to promote adaptation to fermentation products like propionate, which will be used as a source of energy through gluconeogenesis and lambs survival.

The aim of this research was to determine the potency of mixed rumen bacteria and dietary supplementation of Co and its effect on plasma glucose, blood mineral (Co, Fe and Zn) concentrations and hematological parameters of pre-weaning twin lambs.

MATERIALS AND METHODS

General

Twelve one month-old local twin lambs were assigned into three groups of four lambs in a randomized complete block design. The basal diet consisted of corn meal, pollard, soybean meal, molases, mix mineral dan crude palm oil (CPO) and was formulated to meet the nutrients requirement of lambs (NRC, 2007). Total basal crude protein diet was 20.78% DM and TDN was 83.56% DM (Table 1). The lambs had *ad libitum* access to feed and water during experimental period. Lambs were fed cow milk at 10% body weight, adjusted weekly for 80 days and fed in the morning and afternoon. Mixed rumen bacteria was offered at 15 mL/d ($8.295x10^{10}$ cfu) after morning milk feeding. Mixed rumen bacteria that used in this reseach was a mixture of 6 rumen bacteria isolates grown in Brain Heart Infusion medium with a 6 hours incubation period. Lambs were housed in individual metabolize cages of 115 x 57 x 175 cm^3, and each cage was equipped with a feeder and a water trough. Treatments consisted of : 1) basal diet (control), 2) basal diet + 1 mg/kg DM cyanocobalamin (vitB_{12}), and 3) basal diet + 1 mg/kg DM of Co + administration of 15 mL mixed rumen bacteria (CoBac). The ingredient and composition of the basal diet are shown in Table 1.

Data Collections and Sample Analysis

Blood samples were collected on day 53 of the experimental period. Blood samples were collected 4 h after feeding, via jugular venipuncture into heparinized tubes for determination of plasma glucose and blood mineral (Co, Fe, and Zn) concentrations and hematological parameters. Whole blood concentrations of Co, Fe, and Zn were determined by atomic absorption spectrophotometry (Shimadzu 25000) according to AOAC (2003). Plasma glucose was determined by GOD-PAP Methods (HUMAN, Human Gesellschaft fur Biochemica und Diagnostica mbH, Germany). The hematological variables including packed cell volume (PVC), red blood cell (RBC), white blood cell (WBC), and the differentiation of WBC (neutrophils, lymphocytes, monocytes and eusinophils) were determined according to Sastrasdipraja *et al*. (1989) and Hemoglobin (Hb) was determined by Mercktest.

Data were analyzed as a randomized complete block design according to the method described by Steel & Torrie (1993). Data were presented as means ± standard error.

RESULTS AND DISCUSSION

Initial and final body weights in this study were similar among treatments (Table 2). The lambs were in

Table 1. Feed ingredient composition of basal diet offered to pre-weaning twin lambs (% DM)

Ingredients	%	DM	Ash	OM	CP	CF	EE	NFE	TDN
Corn meal	45.00	39.78	0.44	44.56	4.39	0.58	1.98	37.62	38.44
Pollard	15.00	13.36	0.76	14.24	2.32	1.24	0.57	10.11	11.51
Soybean meal	30.00	26.41	2.03	27.97	13.98	0.95	0.47	12.57	28.46
Molases	8.53	4.88	0.64	7.89	0.09	0.00	0.08	7.71	5.16
Mix mineral	0.47	0.47	0.47	-	-	-	-	-	-
CPO	1.00	1.00	-	1.00	-	-	1.00	-	-
T O T A L	100.00	85.91	4.34	95.66	20.78	2.77	4.09	68.01	83.56

a similar physiological condition. In the period of milk feeding, DM intake (concentrate and milk) of lambs were not differences among treatments. The same DM intake resulted in the same final body weight and body weight gain of twin lambs.

Plasma glucose and whole blood mineral (Co, Fe, and Zn) concentrations are shown in Table 2. The mean of plasma glucose concentration in pre-weaning lambs at 67 days old was 65.65±9.21 mg/dL. Plasma glucose of lambs in pre-weaning period were not affected by vitB_{12} and CoBac treatments (P>0.05). However, plasma glucose concentration in the present study was related to DM intake of twin lambs. The same glucose plasma concentration was likely the result of the same feed intake among group of lambs in the treatments (Table 2). The same DM intake and glucose plasma concentration was the reason for the same final body weight of twin lambs (Table 2).

Glucose plasma concentration in the present study was in contrary to the report of Wang *et al*. (2007). They reported that dietary supplementation at 0.25, 0.50, 0.75, and 1.00 mg Co/kg DM in weaned lambs of Chinese Poll Dorset x Small Tailed Han fed basal diet containing 0.086 mg increased glucose plasma concentration. Bishehsari *et al*. (2010) reported that the study in Mehraban male lambs fed a basal diet supplied with 0, 0.25, 0.5, 0.75, and 1 mg Co/kg DM for 70 d showed a significant increase in plasma glucose concentration at all level of Co supplementation on day 68 of the experiment period. Tiffany & Spears (2005) also indicated that addition of Co to basal diet increased plasma glucose concentration of steers.

The data indicated that supplementation vitamin B_{12} and CoBac to twin lambs at level of 1 mg cyanocobalamin/kg DM and 1 mg Co/kg DM + mixed rumen bacteria was not sufficient to increased the availability of glucose precursor supplied by rumen and gluconeogenesis. Burgess *et al*. (2009) explained that vitamin B_{12} stimulated the activity of methylmalonyl CoA mutase and methionine synthase in gluconeogenesis. The result indicated that to meet energy requirement, pre-weaning twin lambs need higher supplementation of vitamin B_{12} or Co.

Plasma glucose concentrations in this study were higher than adult sheep. Astuti *et al*. (2011) noted that blood glucose from sheep fed tropical browse plants was 37.5-59.49 mg/dL. Poore *et al*. (2007) stated that normal concentration of plasma glucose in sheep was 57.6 mg/dL. However Abdelrahman (2010) reported the higher serum glucose concentration, that was 185 mg/dL, in Awassi lambs weaned at 60 d old and supplemented with direct-fed microbial. Udum *et al*. (2008) also reported that plasma glucose concentration from lambs with different feeding regimens was 107.2 mg/dL. Wang *et al.* (2010) noted that plasma glucose from lambs fed with supplemental 0.3 mg Co/kg DM was 64.98 mg/dL.

Blood Co, Fe, and Zn concentrations of pre-weaning twin lambs did not differ among treatments (Table 2). Blood Co, Fe, and Zn were unlikely as indicators for determination of vitamin B_{12} status in lambs. Data also showed that Co supplementation did not interfere Fe and Zn status. Even though blood Co concentration of lambs were slightly above the normal range, but the levels were in the safe range. Abdelrahman (2012) reported that Co and Zn serum concentrations of growing lambs ranged between 0.09-0.16 ppm and 0.97-2.68 ppm. Antunovic *et al*. (2012) noted that blood Fe concentration from lambs ranged from 1,641.90 to 2,083.65 ppm. The blood Co status indicated that dietary 1 mg Co/kg DM was sufficient to meet the requirement of lambs.

Hematological of pre-weaning twin lambs are presented in Table 3. The hematological variables including Hb, PVC, RBC, WBC, and the differentiation of WBC (neutrophils, lymphocytes, monocytes and eusinophils) during pre-weaning period were not affected by VitB_{12} and CoBac treatments. The result indicated that the dietary supplementation did not influence hematological parameter.

The PVC, RBC and Hb values in the present study were higher than the previous studies. It assumed that level vitamin B_{12} and Co in this study was already sufficient to promote red blood cells formation in pre-weaning twin lambs. Wang *et al*. (2009) reported that PVC,

Table 2. Body weight, intake, plasma glucose and whole blood mineral in pre-weaning twin lambs

Parameter	Treatments		
	Control	VitB12	CoBac
Initial body weight (kg)	4.65± 2.40	4.53± 0.94	4.83± 1.69
Final body weight (kg)	7.38± 3.46	7.45± 0.66	7.88± 1.54
Average daily gain (kg)	90.83±47.09	97.50±23.15	101.67±17.53
Intake (g/kg $BW^{0.75}$) :			
Dry matter :	52.77± 5.55	48.37± 5.55	53.66± 5.55
Concentrate	32.11± 6.35	29.03± 6.35	34.20± 6.35
Milk	20.66± 1.12	19.34± 1.12	19.46± 1.21
Plasma glucose (mg/dL)	70.29± 5.49	59.66± 5.49	67.01± 4.46
Co (ppm)	0.11± 0.05	0.27± 0.05	0.22± 0.04
Fe (ppm)	463.89±32.25	515.69±32.25	538.49±26.21
Zn (ppm)	1.32± 0.40	2.18± 0.40	2.56± 0.33

Table 3. Blood hematological of pre-weaning twin lambs

Parameter	Treatments		
	Control	VitB12	CoBac
Hb (g%)	9.52±1.04	12.72±1.04	12.48± 0.84
PVC (%)	26.88±3.65	36.63±3.65	34.13± 2.97
RBC (106/mm3)	12.87±1.15	13.81±1.15	14.86± 0.93
WBC (103/mm3)	13.97±3.13	10.71±3.13	8.60± 2.55
WBC differentiation (%):			
Lymphocytes	57.92±15.58	42.52±15.58	66.00±12.66
Neutrophils	34.48±17.01	48.88±17.01	26.25±13.82
Monocytes	4.31± 0.77	1.91± 0.77	3.25± 0.63
Eusinophils	3.30± 1.99	6.70± 1.99	4.50± 1.62

Note: PVC= packed cell volume; RBC= red blood cell; WBC= white blood cell.

RBC and Hb of lambs offered diet supplemented with 1 mg Co /kg DM diet were 33.88%; 11.01x10^6/mm^3 and 9.68%-10.48 g%, respectively. Marca *et al*. (1996) reported that blood Hb of lambs injected with 1 mg cyanocobalamin during the first week of life was 9.1%-11.8 g%.

PVC, RBC, and Hb in the present study were contrast with Johnson *et al*. (2004). They reported that weaned goats fed with dietary 0.12 mg Co/kg DM and bi-monthly subcutaneous injections of 2000 µg of hydroxycobalamin exhibited declined in erythrocyte counts, hemoglobin and PVC. Johnson *et al*. (2010) also reported that dietary 0.1 mg Co/kg DM and bimonthly subcutaneous injections on 2000 µg hydroxycobalamin resulted in an early impairment of phagocytic function.

Hemoglobin values obtained in this study were related to blood Fe concentration (Table 2). Fe is required for hemoglobin synthesis in the bone marrow (Moosavian *et al*., 2010). The result indicated that dietary vitamin B_{12} and Co had no negative effect on Fe and were sufficient to maintain normal level of RBC and Hb of twin lambs.

WBC and WBC differentiation including lymphocytes, neutrophils, monocytes and eusinophils on preweaning twin lambs were not affected by vitB_{12} and CoBac treatments. WBC of the lambs in the control treatment were slightly higher than the normal range, while the lambs in vitB_{12} and CoBac had the normal level of WBC. Goats (Al-Habsi *et al*., 2007) and preweaning calves offered with rumen bacteria concortium (Prihantoro *et al*., 2013) in both sufficient and deficient Co had the same WBC values. Lack of effect of treatments on blood cells may indicate that supplementation vitamin B_{12} and Co as 1 mg/kg DM did not influence WBC and WBC differentiation.

Hematological parameter including Hb, PVC, RBC, and WBC differentiation in pre-weaning twin lambs period were in the normal range. Kramer & Byers (2011) reported the normal range of RBC, Hb and PVC were 9-15x10^6/mm^3; 9-15 g%; and 27%-45%, respectively. Normal range for WBC and its components are WBC 4-12x10^3, lymphocytes 40%-55%, neutrophils 10%-50%, monocytes 0%-6%, and eusinofil 0%-10%, but lambs within 3 mo of ages, lymphocytes represent 70%-80% of the total WBC population.

CONCLUSION

Dietary supplementation of vitamin B_{12} and Co accompanied by the administration of mixed rumen bacteria do not influence weight gain, plasma glucose concentration, blood Co, Fe, and Zn concentration and maintaining normal hematological parameters of pre-weaning twin lambs. This study demonstrated that pre-weaning twin lambs are not responsive to supplementation of Co and administration of mixed rumen bacteria.

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