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Effects of cobalt and copper supplementation on vitamin B_{12} status and blood parameters in lambs*

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ABSTRACT

The experiment was conducted to investigate the effects of cobalt (Co) and copper (Cu) supplementation on vitamin B_{12} status and blood parameters in lambs. Sixteen lambs were assigned randomly to control group which was fed with the basal diet containing 0.086 mg Co/kg DM and 6.09 mg Cu/kg DM and the Co-, Cu- and Co-Cu groups, supplementation of 0.03 mg Co/kg DM and 10 mg Cu/kg DM, separately and in combination to the control diet. The Co- and Co-Cu supplemented lambs showed higher vitamin B_{12} concentrations in both ruminal fluid and plasma, and lower methylmalonic acid in plasma compared with other groups ($P<0.01$). No difference was observed in plasma glucose between treatments ($P>0.05$). Haeme-depending blood parameters were enhanced by Co or Cu supplementation. It was concluded that vitamin B_{12} production by rumen microorganism was accounted for by cobalt intake with no collaborate effects of Co plus Cu supplied in the diet. Co or Cu supplementation has positive effects on blood parameters.

KEY WORDS: cobalt, copper, vitamin B_{12}, blood parameter, lamb

INTRODUCTION

Cobalt (Co) is required for the synthesis of vitamin B_{12} by rumen microorganisms. Vitamin B_{12} acts as a co-factor for methylmalonyl-CoA mutase and methionine synthase which are important for gluconeogenesis and methionine synthesis. It has been reported that low level of dietary Co can lead to vitamin B_{12} deficiencies clinically manifested as anaemia, inappetence and poor production and

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biochemically characterized by decrease in the plasma concentration of vitamin B_{12}, elevations in the plasma concentrations of methylmalonic acid (MMA) and methionine in ruminants (Kennedy et al., 1990). Copper (Cu) is required for the activity of enzymes associated with iron (Fe) metabolism, normal red blood cell formation and wool pigmentation. Early studies showed that the supplementation of Co plus Cu had positive effects on microbial population, rumen fermentation and digestion of low quality forages in calves and heifers (Saxena and Ranjhan, 1980; Lopez-Guisa and Satter, 1992). Moreover, addition of Co and Cu had an additive effect on weight gains of dairy calves (Maro and Kategile, 1980). However, the effects of Co and Cu on vitamin B_{12} synthesis and haematopoiesis in ruminants have not been reported. Therefore, the present study was designed to evaluate the effects of dietary Co and Cu alone or in combination on vitamin B_{12} and haeme-depending blood parameters in sheep.

MATERIAL AND METHODS

Animals, diets and procedures

Sixteen wether lambs (Poll Dorset×Small Tailed Han sheep) with an average body weight of 22.60±0.62 kg were used in the experiment. Prior to the study, all of the lambs were fitted with ruminal cannulas and fed a Chinese wild rye-grass hay (*Aneurolepidium Chinese*) based diet for three weeks. Lambs were then weighed and randomly assigned to one of four treatments using a completely randomized block design. The treatment groups are: the control group which was fed with the basal diet containing 0.086 mg Co/kg DM and 6.09 mg Cu/k g DM. The Co-, Cu- and Co-Cu supplemented groups, supplementation of 0.03 mg Co/kg DM and 10 mg Cu/kg DM, separately and in combination to the control diet. The basal diet was

Table 1. Ingredient and composition of the basal diet fed to the lambs, % of DM

Feed ingredients		Chemical composition	
Chinese wild rye-grass hay	50.0	Organic matter	92.03
Maize	27.9	Crude protein	13.96
Wheat bran	12.0	Neutral detergent fibre	39.80
Soyabean meal	7.0	Acid detergent fibre	23.76
Cottonseed meal	2.0	Ca	0.34
Limestone	0.2	P	0.34
Salt	0.6	Metabolizable energy, MJ/kg DM [2]	8.92
Premix [1]	0.3	Zn, mg/kg DM	38.56

[1] provided per kg of premix, mg: Zn 8400, Mn 6000, Fe 4000, I 260, Se 20; IU: vit. A 500,000, vit. D 85,000, vit. E 5,000

[2] metabolizable energy was calculated according to the National Standard, NY/T 816-2004

formulated to meet the nutrient requirements of 100 g daily gain with the exception of Co or Cu for lambs (NRC, 1975). The Co or Cu was added gradually to the premix, in forms of $CoSO_4 \cdot 7H_2O$ or $CuSO_4 \cdot 5H_2O$ as the experimental design. The ingredient and compositions of the basal diet are shown in Table 1.

The lambs were housed in individual pens in an open-sided barn. Each animal was fed 900 g per day. The Chinese wild rye-grass hay was offered once a day, and the concentrate was supplied in two equal portions at 08.00 and 18.00. All animals had free access to water containing undetectable concentration of Co or Cu. The experiment lasted for 70 days.

At the end of the experiment, 12 h after the last feeding, the blood was collected from each lamb into heparinized vacutainers, centrifugated at 4°C for 10 min at 1100 *g*, and then stored at -70°C for determination of vitamin B_{12}, MMA and glucose. The whole blood sample was collected into tubes containing Na-EDTA and analysed for packed cell volume (PCV), haemoglobin (HB) and red blood cell (RBC). Ruminal fluid was collected and strained through four layers of cheese-cloth, and then 10 ml of the filtrate was immediately frozen at -70°C for analysis of vitamin B_{12}.

Chemical analyses

Co, Cu and Fe concentrations in feed were determined using an atomic absorption spectrophotometer (Model 5100, HGA-600 Graphite Furnace, Perkin-Elmer, Norwalk, CT) by measuring absorbance at 240.7, 248.3 and 324.8 nm. Vitamin B_{12} concentration was analysed using a competitive binding radio-immunoassay kit, in which the non-specific vitamin B_{12}-binding R-protein was removed by affinity chromatography (ICN, Costa Mesa, CA, USA). Plasma MMA concentration was determined by a modified GC method (Model 6890, Agilent Technologies, Wilmington, DE) as described by McMurray et al. (1986), while plasma glucose by Sigma glucose diagnostic kit; PCV, HB and RBC by automated chemical analyser (TECHNICON RA-1000, Technicon Instruments Corp., USA).

Statistical analysis

Data were statistically analysed using the GLM procedure of SAS version 8 (SAS Inc., Cary, NC). Duncan's multiple range tests were used to detect the statistical significance between treatment groups.

RESULTS

Vitamin B_{12} concentration in ruminal fluid was significantly increased in lambs supplemented Co alone and Co plus Cu when compared with other lambs ($P<0.01$; Table 2). The results of plasma vitamin B_{12} showed the similar pattern with that of ruminal fluid. By contrast, plasma MMA concentration was significantly higher in control and Cu supplemented lambs than those of other animals ($P<0.01$). The plasma glucose did not differ between all treatment groups ($P<0.05$).

Table 2. Vitamin B_{12} and plasma metabolic parameters of lambs fed different Co and Cu diets

Item	Group				SEM	P-value
	control	Co	Cu	Co-Cu		
Ruminal vitamin B_{12}, nmol/l	4.76^{cC}	34.13^{aA}	4.81^{cC}	37.51^{aA}	0.669	P<0.01
Plasma vitamin B_{12}, pmol/l	349.5^{cC}	1688.0^{aA}	348.5^{cC}	1694.0^{aA}	69.83	P<0.01
Plasma methylmalonic acid, µmol/l	5.93^{aA}	2.69^{cC}	5.68^{aA}	3.03^{cC}	0.236	P<0.01
Plasma glucose, mmol/l	3.59	3.61	3.61	3.71	0.016	NS

means within a row with different lowercase letters differ ($P<0.05$) and values followed by different capital letters within each row are significantly different ($P<0.01$). SEM - standard error of the mean

The supplemented groups showed the improved concentrations of RBC, PCV and HB and lowered the plasma Fe concentration when compared with control ($P>0.05$; Table 3).

Table 3. Haeme-depending blood parameters of lambs fed different Co and Cu diets

Item	Group				SEM	P-value
	control	Co	Cu	Co-Cu		
Red blood cell, 10^{12}/l	10.14^{b}	10.88^{a}	10.96^{a}	11.31^{a}	0.201	P<0.05
Packed cell volume, %	31.74^{b}	32.55^{ab}	33.21^{a}	34.03^{a}	0.478	P<0.05
Haemoglobin, g/l	96.83^{b}	98.75^{ab}	103.16^{a}	106.25^{a}	1.869	P<0.05
Iron, µmol/l	31.82^{a}	29.32^{ab}	28.72^{b}	28.69^{b}	0.662	P<0.05

means within a row with different lowercase letters differ ($P<0.05$). SEM - standard error of the mean

DISCUSSION

Vitamin B_{12} concentration in ruminal fluid showed that vitamin B_{12} production by rumen microorganism was accounted for by cobalt intake with no collaborate effects by supplementation of cobalt and copper in combination. It was reported that the greatest amount of ingested Co may occur in form of ions in rumen, and only 3 to 13% of the ingested Co are incorporated into corrinoid compound and converted to vitamin B_{12} by the rumen microorganism (McDowell, 2003).

The results in the present study showed that the Co availability incorporated into vitamin B_{12} was not enhanced by Cu addition. The similar trend in plasma vitamin B_{12} concentrations was ascribed to variations in ruminal vitamin B_{12} concentration.

MMA has been early proposed as a diagnostic tool for determining Co deficiency. The decreased plasma MMA concentrations in Co- and Co-Cu groups in the present study showed the improved activity of methylmalonyl CoA mutase, and thus suggesting the increased vitamin B_{12} synthesis by supplementation of Co alone or Co plus Cu. The result was in agreement with vitamin B_{12} concentrations in both ruminal fluid and plasma.

It was documented that glucose was mainly formed by gluconeogenesis activated by methylmalonyl CoA mutase. The changed activity of this enzyme was reflected in the present study, however, findings revealed that the glucose formation was not influenced by treatments. It was speculated that the time we took blood samples for glucose determination is somewhat early since an alteration in plasma glucose concentration was a late manifestation of Co-deficiency (Kennedy et al., 1990).

Vitamin B_{12} has an important function in erythropoiesis. The lowered values in blood RBC, HB and PCV of control animals were in accord with previous studies with goats (Mburu et al., 1993). However, the improved results in supplemented groups indicated that Co and Cu supplementation separately or in combination favoured the blood production. The result was supported in calves (Saxena and Ranjhan, 1978), which might be explained by the collaborative interactions between Co or Cu and Fe. Co has a direct effect on blood production by enhancing Fe available for marrow. Moreover, Cu is required for blood cell formation by allowing normal Fe absorption and release. The decreased Fe concentration in plasma was resulted from Fe efficient use by marrow for blood cell formation. Interactions of Co with Zn and Cu were also reported in rats (Roschberg and Kappas, 1989). Whether the supplementation of Co, Cu or Co-Cu affects the status of Zn in lambs needs to be determined. Cu may protect tissues from oxidant stress *via* two distinct pathways, one involving impaired Fe metabolism, the other a Cu-Zn superoxide dismutase enzyme. The improved Fe metabolism suggested that Co, Cu and Co-Cu supplementation may have a negative influence on antioxidant status in lambs.

CONCLUSIONS

It was concluded from this study that vitamin B_{12} production by rumen microorganism was accounted for by cobalt intake with no collaborate effects by Co plus Cu supplied in diet. Supplementation of cobalt and copper separately or in combination has positive effects on haeme-depending blood parameters.

REFERENCES

Kennedy D. G., Cannavan A., Molloy A., Harty F.O., Taylor S.M., Kennedy S., Blanchflower W. J., 1990. Methylmalonyl-CoA mutase (EC5.4.99.2) and methionine synthase (EC2.1.1.13) in the tissues of cobalt-vitamin B_{12} deficient sheep. Brit. J. Nutr. 64, 721-732

Lopez-Guisa J.M., Satter L.D., 1992. Effect of copper and cobalt addition on digestion and growth in heifers fed diets containing alfalfa silage or corn crop residues. J. Dairy Sci. 75, 247-256

Maro J.K., Kategile J.A., 1980. Studies on copper and cobalt in dairy calves. Brit. J. Nutr. 44, 25-31

Mburu J.N., Kamau M.Z., Badamana M.S., 1993. Changes in serum levels of vitaminB_{12}, feed intake, liveweight and hematological parameters in cobalt deficient small east African goats. Int. J. Vitam. Nutr. Res. 63, 135-139

McDowell L.R., 2003. Minerals in Animal and Human Nutrition. 2nd Edition. Elsevier Science B. V., Amsterdam (The Netherlands), pp. 277-303

McMurray C.H., Blanchflower W.J., Rice D.A., McLoughlin M., 1986. Sensitive and specific gas chromatographic method for the determination of methymalonic acid in the plasma and urine of ruminants. J. Chromatogr. 378, 201-207

NRC, 1975. Nutrient Requirements of Sheep. 5th revised Edition. National Academy of Sciences. Washington, DC

Roschberg D.W., Kappas A., 1989. Trace metal interactions in vivo: Inorganic cobalt enhances urinary copper excretion without producing zincuresis in rats. J. Nutr. 119, 1259-1268

Saxena K.K., Ranjhan S.K., 1978. Effect of cobalt and copper supplementation, separately and in combination, on the digestibility of organic nutrients and mineral balances in Hariana calves. Indian J. Anim. Sci. 48, 566-711

Saxena K.K., Ranjhan S.K., 1980. Effect of cobalt and copper supplementation on the ruminal pH, total nitrogen, ammonia and TVFA concentration in Hariana calves. Indian J. Anim. Sci. 50, 21-25