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Effects of Dietary Copper on Ruminal Fermentation, Nutrient Digestibility and Fibre Characteristics in Cashmere Goats*

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ABSTRACT : Thirty-six 1.5 year-old Inner Mongolian White Cashmere wether goats (body weight 28.14±1.33 kg) were used to determine the effects of dietary copper (Cu) concentration on ruminal fermentation, nutrient digestibility and cashmere fibre characteristics. Wethers were fed a basal diet (containing 7.46 mg Cu/kg DM) that was supplemented with either 0 (control), 10, 20 or 30 mg Cu/kg DM. To ensure full consumption, animals were fed restrictedly with 0.75 kg feed (DM) in two equal allotments per day. The results indicated that, (1) supplemental 10 mg Cu/kg DM in the basal diet significantly ($p<0.05$) decreased ruminal fluid pH value and total VFA concentrations were significantly ($p<0.05$) increased on all Cu treatment groups. (2) Cu supplementation had no influence on DM intake and digestibility of DM, CP and ADF ($p>0.05$); however, NDF digestibility of groups supplemented with 10 and 20 mg Cu/kg DM were significantly higher than that of the control group ($p<0.05$). Apparent absorption and retention of copper were decreased with increasing level of supplementation. (3) 20 mg Cu/kg DM treatment significantly ($p<0.05$) improved cashmere growth rate, but cashmere diameter was not affected by Cu supplementation ($p>0.05$). In conclusion, supplementation of cashmere goats with Cu at the rate of 10 to 20 mg/kg DM in the basal diet resulted in some changed rumen fermentation and was beneficial for NDF digestibility, while supplementation of 20 mg Cu/kg DM improved cashmere growth. Collectively, the optimal supplemental Cu level for cashmere goats during the fibre growing period was 20 mg/kg DM (a total dietary Cu level of 27.46 mg/kg DM). (**Key Words** : Copper, Rumen Fermentation, Nutrient Digestibility, Cashmere Fibre Characteristics)

INTRODUCTION

Copper (Cu) is an essential mineral element in ruminants with deficiency and toxicity occurring frequently in many parts of the world. Nutritional Cu requirement of goats, sheep, and beef cattle are 8-10 mg/kg DM (NRC, 1981), 5 mg/kg DM (NRC, 1975), and 10 mg/kg DM (NRC, 1996), respectively. Species differences in susceptibility to Cu overload are high: sheep are extremely intolerant and cattle and goats less intolerant of excess Cu (Underwood and Suttle, 1999). Sheep suffering from Cu deficiency produce "steely" or "stringy" wool which is lacking in crimp, tensile strength, affinity for dyes, and elasticity. Depigmentation of wool has been noted as a sign of severe deficiency (NRC, 1975). While Cu deficiency has been reported in dairy (Khan et al., 2007) and range (Ramirez et al., 2004) goats, its effect on growth of cashmere fibre is unknown. China is the largest producer of cashmere, with yields accounting for 50% the world's total production (Bai et al., 2006). The Inner Mongolian White Cashmere Goat (IMWG) and Liao Ning White Cashmere Goat are two of the major cashmere goat breeds in China.

Some studies suggested that dietary Cu might affect ruminal fermentation (Essig, 1972; Saxena and Ranjhan, 1980; Saxena et al., 1980), nutrient digestibility (Reddy and Mahadevan, 1976) and performance in cattle (Ward and Spears, 1997; Engle and Spears, 2000a; Engle et al., 2000; Dorton et al., 2006). However, research with Cu supplementation in cattle has produced conflicting results. Reddy and Mahadevan (1976) reported that Cu supplementation (daily intake of 43 to 62 mg) significantly reduced CP digestibility in lactating cows. However, Saxena and Ranjhan (1978) indicated that digestibility of

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Table 1. Ingredients and chemical composition of basal diet[1] (% as-fed basis)

Ingredient	
Hay	70.00
Corn	16.00
Wheat bran	7.20
Soybean meal	5.40
Limestone	0.16
Dicalcium phosphate	0.23
Salt	1.00
Vitamin premix [2]	0.01
Chemical composition	
Metabolizable energy (ME, MJ/kg)	8.40
Crude protein (CP)	9.65
Dry matter (DM)	90.58
Neutral detergent fiber (NDF)	54.19
Acid detergent fiber (ADF)	31.56
Calcium (Ca)	0.47
Phosphorus (P)	0.31
Cu mg/kg DM [2]	7.46

[1] Basal diet contained 7.46 mg Cu/kg DM.

[2] Contained per kilogram of premix: 54,000,000 IU of vitamin A, 10,800,000 IU of vitamin D, and 18,000 IU of vitamin E.

DM and CP in calves was not affected by dietary Cu. Copper supplementation did not affect performance during the growing phase in Angus steers (Engle and Spears, 2000a) nor during the finishing phase in Simmental steers (Engle and Spears, 2001). Similar results were observed in Angus and Simmental heifers (Mullis et al., 2003).

Limited research has evaluated the effects of Cu supplementation on productive performance in the goat. Luginbuhl et al. (2000) found that DMI and ADG for 0, 10, and 30 mg supplemental Cu/kg DM in growing meat goats were not affected by dietary Cu level. However, Solaiman et al. (2006) found that average daily feed intake (F) decreased (linear; p = 0.05), and the ratio of average daily gain: F increased (quadratic; p = 0.02) in goat kids fed 0, 100 and 200 mg Cu/d (Basal diet contained 13.8 mg Cu/kg DM). Also, more recently Mondal and Biswas (2007) found that Cu supplementation at the level of 10, 20 and 30 mg Cu/kg DM in the basal diet containing 5.7 mg Cu/kg DM improved the performance and nutrient utilization in goats.

The optimal dietary levels of Cu in cashmere goats have not been established, and the effects of dietary Cu concentration on nutrient fermentation, metabolism and cashmere performance are not understood. The objectives of this study were to determine the effects of dietary Cu on ruminal fermentation, nutrient digestibility and fibre characteristics in IMWG.

MATERIALS AND METHODS

Animals

Thirty-six 1.5 year-old IMWG wethers (body weight 28.14±1.33 kg) were stratified by weight and randomly assigned to the following treatments; 0 (control), 10, 20 or 30 mg Cu/kg DM with the basal diet containing 7.46 mg Cu/kg DM.

Diets and feeding

All cashmere goats were fed the basal diet for 14 days (d), and were then gradually switched to the experimental diets. The basal diet was formulated to meet or exceed all nutrient requirements for goats with the exception of Cu (NRC, 1981) (Table 1). Cu was added as $CuSO_4 \cdot 5H_2O$ to the premix using fine maize flour as a carrier and mixed with concentrate. To ensure full consumption, animals were fed restrictedly with 0.75 kg feed (DM) at 06:00 and 18:00 in two equal allotments per day, but were allowed to drink water *ad libitum* (contained less than 0.01 mg Cu /kg). The experiment lasted for 60 days.

Sample collection

On d 20 of the experiment, four goats from each treatment with similar feed intakes were used to collect ruminal fluid using a stomach tube, 2 h after feeding. Approximately 80 ml of ruminal contents was sampled and transferred to a prewarmed (approximately 39°C) insulated container, before pH was measured at the laboratory. The ruminal contents were then strained through four layers of gauze prior to acidification of 10 ml of ruminal fluid with 1 ml of a 25% (wt/vol) metaphosphoric acid solution. Samples were stored at -20°C to allow determination of volatile fatty acids (VFA). On d 35, all goats were allocated to metabolism crates to study the effects of dietary Cu on apparent nutrient digestibility and apparent Cu absorption

Table 2. Effects of dietary copper level on ruminal fermentation of cashmere goats

Parameter	Cu supplemental levels (mg Cu/kg DM)				SEM	p-value		
	0	10	20	30		Linear	Quadratic	Cubic
Ruminal pH	6.13[a]	5.95[b]	6.08[a]	6.07[a]	0.032	0.493	0.039	0.248
Volatile fatty acid concentration (mmol/L)								
Acetate	28.48[b]	34.75[a]	35.80[a]	32.06[ab]	1.039	0.213	0.015	0.043
Propionate	9.73	10.99	11.54	10.59	0.314	0.276	0.111	0.226
Butyrate	5.71	6.79	6.00	6.95	0.203	0.105	0.276	0.069
Total	43.92[b]	52.53[a]	53.34[a]	49.61[a]	1.348	0.142	0.015	0.040

Means in the same row with different superscripts differ significantly ($p<0.05$).

SEM = Standard error of the mean.

and retention. After a five-day acclimation period total feces and urine were collected for five days for each wether. Feed intakes were recorded daily. Fecal and urinary samples were collected daily (approximately 10% of the daily output), then added sulphuric acid to prevent nitrogen loss and were immediately labeled and frozen at -20°C pending analysis.

At the beginning of the study a 10 cm^2 patch of white fibres, located over the right scapular, was dyed black. On day 60 fibres were removed using a conventional hair clipper (Shahjalal et al., 1992).

Analytical procedures

Ruminal fluid VFA concentrations were determined by GLC (Varian Instruments Model 3800, Wolnut Greek, CA) using a Nikol fused-silica column (15 m, 0.53 mm i.d., and 0.50 um film thickness; Supelco, Bellefonte, PA). The oven temperature program began with an initial temperature of 80°C, followed by an increase of 20°C/min to 140°C, which was held for 2 min. Temperature was then increased to 175°C, where it was held for 1 min to flush the column. Fecal samples were later thawed at room temperature and dried at 60°C for 48 h in a forced-air drying oven. Urine samples were thawed at room temperature. Fecal and urinary samples were mixed thoroughly for each animal. Feeds and the dried fecal samples were then ground to pass a 1 mm screen. The dry matter (DM) and nitrogen (N) of feeds and fecal samples were determined according to AOAC (1990) and neutral detergent fiber (NDF) and acid detergent fiber (ADF) by the method of Van Soest et al. (1991). Cu concentrations of the feed, feces and cashmere fibre were analyzed by atomic absorption spectrophotometry (Model 5100, HGA-600 Graphite Furnace; Perkin-Elmer, USA). Approximately 0.5 g of sample was weighed and dry-ashed for 12 h at 550°C. The dry-ashed sample was then dissolved in 0.6 mol/L hydrochloric acid and brought to 50 ml in a volumetric flask with deionized water. Urine samples were diluted with deionized water until within the detectable range of the assay. The diluted mixture was vortexed vigorously and then analyzed. Cashmere fibre samples were washed by soaking overnight in a detergent solution, rinsed thoroughly in deionized water and then dried at 80°C. Length of cashmere was estimated using a scaled ruler. Diameter of cashmere was measured on a random sample of 100 fibres as described by Shahjalal et al. (1992).

Statistical analysis

Data were analyzed using the GLM procedure of SAS (1988). Duncan's multiple range tests were used to detect the statistical significance between different treatments. The nature of response to incremental additions of Cu was analyzed by polynomial contrasts. The model included linear, quadratic and cubic contrasts for effects of supplemental Cu.

RESULTS AND DISCUSSION

Effects of dietary Cu on ruminal fermentation

The results indicated supplementation of the basal diet with 10 mg Cu/kg DM significantly ($p<0.05$) decreased rumen fluid pH when compared with other treatment groups. However, total VFA concentrations were significantly ($p<0.05$) increased on all Cu supplemented treatments. The molar concentrations of acetate was significantly ($p<0.05$) higher in groups supplemented with 10 and 20 mg Cu/kg DM compared with the control, but were not different from those in the treatment group supplemented with 30 mg Cu/kg DM ($p>0.05$). The molar concentration of propionate and butyrate were not significantly affected by the supplements ($p>0.05$). These results suggest that 10 and 20 mg Cu/kg DM treatments had a positive influence on rumen fermentation. These findings were consistent with those of Saxena and Ranjhan (1980) who reported that rumen pH was significantly depressed, but that total VFA concentrations were significantly increased by supplementation of 6 mg Cu/kg DM in a basal diet (containing 6 mg Cu/kg DM) fed to Hariana calves. In addition, Saxena et al. (1980) also indicated that acetic acid concentration was significantly greater in Hariana calves fed the supplemented diet than in control animals, but propionic acid and butyric acid concentrations were not significantly affected by the supplements. By contrast, Angus steers (Essig et al., 1972) fed a high concentrate diet with much higher concentrations of Cu (4.4 g of $CuSO4 \cdot 5H_2O$ per 100 kg BW) via a bolus had decreased acetic, propionic, butyric and total VFA concentrations ($p<0.05$). Engle and Spears (2000b) also reported that ruminal fluid pH, acetic, propionic, butyric and total VFA concentrations in Angus steers were not affected by supplementation of 10 or 20 mg Cu/kg DM in the basal diet (containing 4.9 mg Cu/kg DM). These experiments in cattle indicated that high levels of Cu may be detrimental to rumen fermentation (Essig et al., 1972), but at lower levels may be beneficial (Saxena and Ranjhan 1980; Saxena et al., 1980). Based on the present study in cashmere goats it is suggested that addition of 10 or 20 mg Cu/kg DM to the basal diet resulted in some change in rumen fermentation.

Effect of dietary Cu on DMI and nutrient digestibility

Cu supplementation had no influence on DMI and digestibility of DM, CP and ADF ($p>0.05$) (Table 3), but NDF digestibility in groups supplemented with 10 and 20 mg Cu/kg DM was significantly higher than that of the control group ($p<0.05$).

A number of experiments have indicted that low levels of dietary Cu do not change DMI, but much higher levels

Table 3. Effects of dietary copper level on DMI and nutrient digestibility of cashmere goats

Items	Cu supplemental levels (mg Cu/kg DM)				SEM	p-value		
	0	10	20	30		Linear	Quadratic	Cubic
DM intake (DMI, g/d)	736.88	745.42	750.00	736.88	3.704	0.898	0.374	0.569
DM digestibility (%)	57.41	61.80	61.14	59.79	0.841	0.415	0.163	0.284
CP digestibility (%)	62.21	65.95	63.98	65.01	0.739	0.355	0.453	0.354
NDF digestibility (%)	50.34[b]	56.72[a]	57.16[a]	54.11[ab]	1.159	0.277	0.041	0.121
ADF digestibility (%)	48.37	53.28	53.16	50.09	0.991	0.594	0.102	0.226

Means in the same row with different superscripts differ significantly ($p<0.05$).
SEM = Standard error of the mean.

Table 4. Effects of dietary copper level on copper apparent absorption and retention of cashmere goats

Items	Cu supplemental levels (mg Cu/kg DM)				SEM	P-value		
	0	10	20	30		Linear	Quadratic	Cubic
Cu intake (mg/d)	5.48[d]	13.06[c]	20.60[b]	27.98[a]	2.530	0.000	0.000	0.000
Fecal Cu (mg/d)	4.81[d]	11.61[c]	19.40[b]	26.70[a]	2.481	0.000	0.000	0.000
Urinary Cu (mg/d)	0.12	0.06	0.09	0.10	0.013	0.844	0.532	0.624
Cu retention (mg/d)	0.55[b]	1.39[a]	1.11[a]	1.18[a]	0.099	0.059	0.008	0.024
Cu apparent absorption rate (%)	12.18[a]	11.09[a]	5.83[b]	4.59[b]	1.149	0.001	0.006	0.011
Cu apparent retention rate (%)	10.07[a]	10.61[a]	5.40[b]	4.24[b]	1.024	0.005	0.021	0.023

Means in the same row with different superscripts differ significantly ($p<0.05$).
SEM = Standard error of the mean.

Table 5. Effects of dietary copper level on growth and fibre characteristics of cashmere goats

Items	Cu supplemental levels (mg Cu/kg DM)				SEM	p-value		
	0	10	20	30		Linear	Quadratic	Cubic
Initial weight (kg)	28.27	28.68	27.45	28.17	0.38	0.676	0.906	0.678
Final weight (kg)	30.02	30.57	30.00	29.77	0.34	0.688	0.807	0.689
ADG (g/d)	29.17[b]	31.39[b]	42.50[a]	26.67[b]	1.95	0.847	0.048	0.944
Cashmere length (cm)	2.33[b]	2.38[b]	2.60[a]	2.37[b]	0.035	0.290	0.041	0.000
Cashmere growth rate (mm/d)	0.39[b]	0.40[b]	0.44[a]	0.40[b]	0.006	0.299	0.035	0.000
Cashmere diameter (μm)	14.28	15.34	15.92	14.33	0.316	0.810	0.099	0.185
Cashmere copper (mg/kg)	8.88	8.34	8.70	9.18	0.274	0.631	0.615	0.800

Means in the same row with different superscripts differ significantly ($p<0.05$).
SEM = Standard error of the mean.

may depress intake (Zervas et al., 1990; Luginbuhl et al., 2000; Solaiman et al., 2006). An unchanged DMI in the current study showed that the supplemental level of Cu did not depress the feed intake. Furthermore, it was reported that supplementing 20 or 30 mg Cu/kg DM in the basal diet would cause Cu toxicity in sheep, manifested by excess salivation, mild anemia and slight growth depression (Zervas et al., 1990; Luginbuhl et al., 2000; Solaiman et al., 2001). None of these signs occurred in goats in the present experiment suggesting that cashmere goats are more resistant to Cu toxicity than sheep.

Limited research has been done on the effects of dietary Cu on nutrient digestibility in sheep and goats, but some information is available for cattle in which high levels of dietary Cu may reduce digestibility. For example, Reddy and Mahadevan (1976) reported that Cu supplementation (daily intake of 43 to 62 mg) significantly reduced digestibility of CP in lactating cows. However, Saxena and Ranjhan (1978) indicated that digestibility of DM and CP was not affected by dietary Cu in Hariana calves fed a basal diet (6.0 mg Cu/kg DM) supplemented with 6 mg Cu/kg DM, although the digestibility of crude fibre was increased. Also, more recently Engle and Spears (2000b) found that *in vitro* DM digestibility was not affected by dietary Cu in Angus steers on a basal diet (4.9 mg Cu/kg DM) supplemented with 10 or 20 mg Cu/kg DM. The present study with goats suggested that the digestibility of DM, CP and ADF was not affected by Cu supplementation, whereas NDF digestibility was improved by supplemental 10 and 20 mg Cu/kg DM. The improved NDF digestibility might be due to Cu-enhanced ruminal fermentation (Engle and Spears, 2000a). Differences observed between these studies for nutrient digestibility might be caused by environmental, genetic and dietary factors including Cu concentration in the basal diet and level and duration of Cu supplementation (Engle and Spears, 2001). Interactions of Cu with other elements such as Mo, S and Zn may also be implicated (Suttle, 1991; Yadav et al., 2004).

Effects of dietary Cu on Cu apparent absorption and retention

Apparent absorption and retention are general indicators of the amount of a specific nutrient absorbed from the gastrointestinal tract and retained in the animal's body, respectively (Vierboom et al., 2003). Fecal Cu increased with increasing Cu intake with the highest concentration found in the 30 mg Cu/kg DM supplementation group (Table 4). However, there was no difference in the concentration of Cu excreted in the urine between all treatment groups (p>0.05). Cu retention was improved by Cu supplementation (p<0.05), but did not differ between supplemented groups (p>0.05). This result concurs with the suggestion that the major route of excretion for Cu is via the feces, with smaller amounts being lost via the urine (Chopra and Harjit, 1989; Vierboom et al., 2003). Fecal Cu excretion is the major mode by which animals fed high dietary Cu sustain normal Cu metabolism without incurring Cu toxicity (Underwood and Suttle, 1999).

Effect of dietary Cu on growth and cashmere performance

There was no difference in final weight between all groups (p>0.05), but the ADG was highest in animals supplied with 20 mg Cu/kg DM (p<0.05). The present results indicated that length and growth rate of the cashmere fibre was significantly higher in the 20 mg Cu/kg DM treated group compared with other treatment groups (p<0.05). There were no differences in fibre diameter and cashmere Cu concentration between treatments (p>0.05). No other studies, as far as we are aware, have examined the effects of dietary Cu on cashmere performance. Based on the present results, Cu supplementation at the level of 20 mg/kg DM (a total dietary level of 27.46 mg Cu/kg DM) seemed to be beneficial for cashmere growth.

CONCLUSION

Findings from this study revealed that IMWG fed the basal diet, containing 7.46 Cu mg/kg DM, showed impaired rumen fermentation, lower nutrient digestibility and slower cashmere growth rate compared with Cu supplemented wethers. Supplementation with 10 to 20 mg Cu/kg DM enhanced rumen fermentation and improved nutrient digestibility whereas supplementation with 20 mg Cu increased cashmere growth. It was concluded that the optimal supplemental Cu level for IMWG during the cashmere growing period was 20 mg/kg DM (a total dietary Cu level of 27.46 mg/kg DM).

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