In Vitro Gas Production and Dry Matter Degradability of Diets Consumed by Goats with or Without Copper and Zinc Supplementation

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Abstract An in vitro gas production technique was used to evaluate the effects of copper and zinc supplementation on the amount and rate of gas production, dry matter degradability (IVDMD), utilization of metabolizable energy (ME), and ruminal fermentation patterns using rumen fluid from four Boer male goats as inoculum. The goats were fed twice daily at 07:00 and 19:00 h a total mixed ration containing 10.3 and 22.5 mg/kg DM of Cu and Zn, respectively. This diet was incubated in vitro for 96 h with four treatments being: control, Cu (21.7), Zn (5.6), and Cu–Zn (21.7 and 5.6) which was provided as a mineral premix. Data were analyzed as a completely randomized design. Rates of gas production (RGP) at 4 (RGP_{4h}) and 6 h (RGP_{6h}) and gas production (GP) at 24 (GP_{24h}) and 48 h (GP_{48h}) differed ($p<0.01$) among treatments. An addition of Cu increased the RGP_{4h}, RGP_{6h}, GP_{24h}, and GP_{48h} ($p<0.0001$). The Cu treatment had the highest IVDMD and control the lowest ($p<0.05$), and the Cu treatment was the highest values of ME and SCFA. The addition of Cu to the in vitro ruminal fermentation increased gas production and efficiency of energy use.

Keywords Copper · Zinc · In vitro degradability · Gas production · Goats

Abbreviations

ADF	Acid detergent fiber
B	Potential gas production

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CP	Crude protein
DM	Dry matter
GP	Gas production
IVGP	In vitro gas production
IVDMD	In vitro DM degradability
K	Fractional rate of gas production
L	Lag time
ME	Metabolizable energy
NDF	Neutral detergent fiber
RGP	Rate of gas production
SCFA	Short chain fatty acids

Introduction

In vitro gas production is used to measure fermentation properties of ruminant feedstuffs [1, 2] wherein the gas produced is primarily the result of carbohydrate fermentation, including starch, cellulose, and hemicelluloses, to volatile fatty acids (VFA). The volatile fatty acids produced are mainly acetate, propionate, and butyrate and, to a lesser extent, succinate, format, lactate, and ethanol. The gases include carbon dioxide (CO_2), methane, and traces of hydrogen [3, 4]. Trace mineral elements such as copper (Cu) and zinc (Zn) are involved in many enzyme systems and may affect metabolic utilization of major dietary nutrients such as proteins and carbohydrates [5]. While severe Zn deficiency is uncommon in ruminants, symptoms related to marginal Zn deficiencies may occur more often than expected [6]. Moreover, grazing ruminants in many parts of the world often experience Cu deficiency or toxicity, which may affect growth and digestibility of nutrients [7, 8]. Research on the effects of adding Cu to ruminant diets on the digestibility of nutrients is scarce and, where available, results are contradictory. For example, adding 10, 20, and 30 mg Cu/kg dry matter (DM) to ruminant diets did not affect digestibility of DM, crude protein (CP), and acid detergent fiber (ADF) in Cashmere goats [9, 10], while the intra-ruminal administration of boluses containing 8.65 mg Cu/kg DM reduced apparent digestibility of neutral detergent fiber (NDF) and CP in growing heifers [11]. Research to determine the effects of Zn supplementation on digestibility of nutrients is also still limited and inconclusive [12–14]. Supplementation of 1 g/day with organic Zn in diets fed to dairy goats increased the digestibility of DM, organic matter, and CP [15]. It is noteworthy that the effects of adding Cu or Zn to diets on carbohydrate fermentation and nutrient metabolism are not yet fully understood. Thus, our objective was to evaluate effects of Cu and Zn supplementation on in vitro gas production, DM digestibility, energy metabolism, and ruminal fermentation.

Material and Methods

Experimental Diets and Treatments

A total mixed ration (TMR) was prepared to meet the nutritional requirements of growing male Boer goats weighing about 40 kg and gaining 300 g/day. Each day, the goats were fed 1.1 kg/head of the diet, except for Cu and Zn, which were added in different concentrations from one of four mineral premixes (Table 1) formulated to meet the requirements for Cu (35 mg/day) and Zn (31 mg/day) [16]. The concentration of trace mineral elements in the

Table 1 Ingredients and chemical composition of the basal diet

Ingredient	Dry matter basis (%)
Avena sativa	46.06
Medicago sativa	11.97
Heliocarpus velutinus	2.99
Guazuma ulmifolia	6.98
Corn grain	9.21
Sorghum grain	9.21
Soybean meal	5.03
Molasses cane	5.03
Urea 46% N	1.00
Mineral premix[a]	2.52
Chemical composition[b]	
Crude protein	14.74
Neutral detergent fiber	48.21
Acid detergent fiber	26.42
Hemicellulose	21.79
Ash	10.43
Organic matter	89.57
Cu (mg/kg DM)	10.30
Zn (mg/kg DM)	22.58

[a] Contained per kilogram of mineral premix: 19.60 g/kg of calcium, 22.10 g/kg of sulfur, 4 mg/kg of cobalt, 15.93 mg/kg of iodine, 15.49 mg/kg of selenium; Cu–Zn treatment: 860.73 mg/kg of copper, 224.07 mg/kg of zinc; Cu treatment: 860.73 mg/kg of copper, 0 mg/kg of zinc; Zn treatment: 0 mg/kg of copper, 224.07 mg/kg of zinc; control: 0 mg/kg of copper, 0 mg/kg of zinc

[b] Analyzed values

treatment premixes was: Cu–Zn treatment 1, 860.73 mg Cu/kg, 224.07 mg Zn/kg; Cu treatment 2, 860.73 mg Cu/kg, 0 mg Zn/kg; Zn treatment 3, 0 mg Cu/kg, 224.07 mg Zn/kg; and the control, 0 mg Cu/kg, 0 mg Zn/kg.

In Vitro Gas Production

The in vitro gas production determination used ruminal fluid obtained pre-feeding (i.e., 07:00 h) from four growing male Boer goats aged 6 months and weighing 20 kg. Using a stomach tube, the ruminal fluid was obtained from multiple sites in the rumen and it was then strained through two layers of muslin cloth and kept at 39°C under a continuous stream of CO_2. The rumen donor goats were fed with a 68:32 (DM) forage-to-concentrate diet containing 10.3 mg Cu/kg DM and 22.6 mg Zn/kg DM. The goats were also supplemented with 2.5% of a mineral and vitamin premix (Núcleo Max Plus, Nupropec, S. A. de C.V., Mexico). The goats were fed twice daily at 07:00 and 19:00 h and had free access to clean drinking water.

In vitro gas production was determined according to Theodorou et al. [17] with the modifications of Mauricio et al. [18] using 160 ml serum bottles with a rubber stopper cap sealer. To each bottle, 1±0.002 g of sample was added followed by 90 ml of buffer solution which was previously saturated with CO_2. Lastly, 10 ml of ruminal fluid was added to each bottle before incubation at 39°C to record gas production with a pressure transducer fitted with a microprocessor (HD 8804, Delta Ohm, Italy). Samples were incubated in triplicate and cumulative gas production was monitored at 2, 4, 6, 8, 10, 12, 15, 19, 24, 30, 36, 48, 72, and 96 h post-incubation. Three bottles without a substrate were used as blanks to correct for gas produced from the rumen contents.

Calculations

The gas production data (in milliliters per gram DM) were fitted according to the model of France et al. [19] as:

$$A = b \times \left[1 - e^{-k(t-L)}\right]$$

Where: A is the volume of gas production at time t; b the asymptotic gas production (in milliliters per gram DM); k is the rate of gas production (per hour) from the slowly fermentable feed fraction b; and time lag (L) is the discrete lag time prior to gas production. The rate of gas production (RGP) at 4 and 6 h was calculated from recorded volumes of gas produced before and after these times. For example, RGP at 4 h was calculated as:

$$\mathrm{RGP}_{4h}[(\mathrm{ml/g\ DM})/\mathrm{h}] = \frac{(\text{volume of gas produced at 6 h} - \text{volume of gas produced at 2 h})}{4 \times \text{sample weight(in grams)}}$$

In vitro DM digestibility (IVDMD) was determined after 96 h of incubation by recovery of the undigested fraction by filtration of the residue using sintered glass crucibles (1, 100- to 160-μm pore size). Recovered residues were washed under running water and subsequently dried at 100°C for 24 h. Metabolizable energy (ME, in millijoules per kilograms DM) was estimating according to Menke and Steingass [20] as:

$$\mathrm{ME} = 2.20 + 0.136\ \mathrm{IVGP}_{24}(\mathrm{ml}/0.5\ \mathrm{g\,DM}) + 0.057\ \mathrm{CP}(\%\ \mathrm{DM})$$

where: IVGP_{24} was 24 h gas volume and CP (percent DM) is the content of dietary CP.

Short chain fatty acids (SCFA, in millimoles per gram DM) were estimated from the relationship between gas production at 24 h and SCFA using the equation of Getachew et al. [21] as:

$$\mathrm{SCFA} = -0.00425 + 0.0222(\text{ml gas at 24 h})$$

Statistical Analysis

The in vitro ruminal fermentation parameters, gas production parameters, in vitro DM digestibility, and ME were analyzed as a completely randomized design. In the case of significance ($p<0.05$) among treatments, Tukey's test was used to separate means [22].

Results

The effect of adding Cu and Zn to a TMR for goats on in vitro gas production, IVDMD, ME, and the production of SCFA is in Table 2 and Fig. 1. The rate of gas production after 4 and 6 h of incubation and gas production after 24 and 48 h were higher ($p<0.05$) for the treatment to which Cu was added, followed by the control; while Cu–Zn and Zn treatments showed the lowest values. Asymptotic gas production (b) was higher ($p=0.0068$) for diets supplemented with trace mineral elements in individual forms when compared to the control. There were no differences among treatments in the fractional rate of gas production (k). Diets supplemented with Cu and Zn (Cu–Zn) or Cu alone had a longer ($p=0.01$) L than the Zn treatment and the control. The treatment to which a combination of Cu and Zn (Cu–Zn) increased IVDMD ($p=0.038$) versus all others. Means for ME and SCFA were

Table 2 In vitro ruminal fermentation parameters, gas production parameters, in vitro dry matter degradability, metabolizable energy, and short-chain fatty acids production of the basal diet (Cu, 10.3 mg/kg DM; Zn, 22. 6 mg/kg DM) consumed by goats with or without copper and zinc addition (Cu, 21.7 mg/kg DM; Zn, 5.6 mg/kg DM)

Item	Cu–Zn	Cu	Zn	Control	SEM	Probability
RGP_{4h}	4.48c	9.34a	4.35c	6.49b	0.63	<0.0001
RGP_{6h}	3.65c	9.06a	3.20c	4.77b	0.73	0.0001
GP_{24h}	67.34c	165.48a	56.86c	88.17b	12.96	<0.0001
GP_{48h}	137.28b, c	231.78a	111.29c	149.40b	13.83	<0.0001
b (ml/g DM)	270.63a, b	287.90a	285.95a	238.33b	6.84	0.0068
k (/h)	0.014	0.037	0.038	0.020	0.007	0.5688
L (h)	1.21a	1.30a	0.77a, b	0.41b	0.12	0.0103
IVDMD (g/kg DM)	731.38a	725.05a, b	720.05a, b	703.72b	3.89	0.0384
ME (MJ/kg DM)	15.00c	21.68a	14.29c	16.42b	0.88	<0.0001
SCFA (mmol/g DM)	0.75c	1.84a	0.64c	0.98b	0.14	<0.0001

Means in the same row with different letters differ ($p<0.05$)

DM dry matter, RGP_{4h} rate of gas production (in milliliters per gram DM per hour at 4 and 6 h), *GP* gas production (in milliliters per gram at 24 and 48 h), *b* asymptotic gas production (in milliliters per gram DM), *k* fractional rate of gas production (per hour), *L* lag phase (hour); IVDMD, in vitro dry matter degradability (in grams per kilograms DM), *ME* metabolizable energy (in megajoules per kilogram DM), *SCFA* short chain fatty acid (millimole per grams DM), *SEM* standard error of the mean

higher ($p<0.05$) in diets supplemented with Cu followed by the control diet. Lowest values were in the treatment diets supplemented with a combination of Cu and Zn (Cu–Zn) and Zn alone.

Discussion and Conclusion

Research information in the literature on the effects of addition of trace minerals to diets on rumen fermentation and digestibility is limited and, in most cases, inconclusive. Studies of

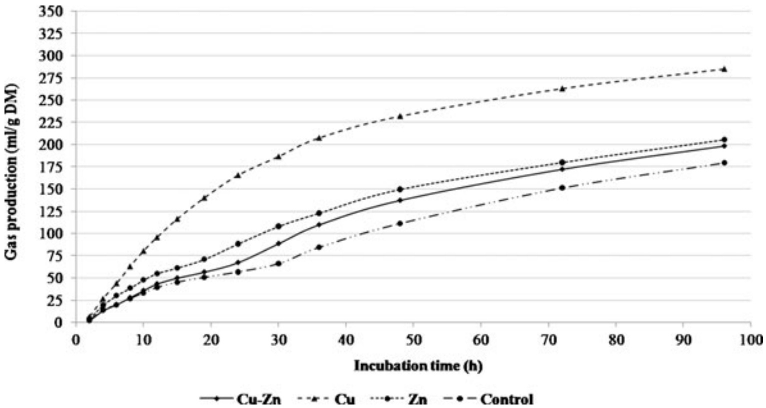

Fig. 1 Gas production profiles of the diets of goats supplemented with Cu–Zn (21.7 mg Cu/kg DM–5.6 mg Zn/kg DM), Cu (21.7 mg Cu/kg DM), Zn (5.6 mg Zn/kg DM), and without supplementation (control)

Engle and Spears [23] indicated that IVDMD was not affected when Angus steers were fed a basal diet supplemented with 10 or 20 mg Cu/kg DM intake. In another study, supplementing goats with 10, 20, and 30 mg Cu/kg DM did not affect the digestibility of DM, CP, and ADF, while NDF digestibility was reduced with the addition of 30 mg Cu/kg DM [10, 24, 25]. Zhang et al. [9] concluded that the digestibility of DM and CP was not affected by Cu supplementation at levels of 10, 20, and 30 mg/kg DM of a basal diet containing 7.38 mg Cu/kg DM consumed by Cashmere goats, but NDF and ADF digestibility was increased by the addition of 10 mg Cu/kg DM intake. The improvement in NDF digestibility could be due to increased ruminal fermentation as a result of Cu supplementation [23], which may be supported by findings that supplementation of basal diets of growing lambs with various concentrations of mineral elements resulted in the increased digestibility of DM and CP [8]. Copper supplementation affects the overall proportion of ruminal VFA [25], although supplementation with Cu (100 and 200 mg/day) did not affect VFA proportions [26]. These results, and those in our study, suggest that the addition of Cu to the diet can positively influence ruminal microbial fermentation [27, 28]. Solaiman et al. [26] hypothesized that Cu supplementation increases the efficiency of fermentation by decreasing the number of protozoa in the rumen. This is contrary to findings by Arthington [11] who reported that there may be a decrease in apparent digestibility of nutrients from forage in cattle supplemented with an intra-ruminal bolus containing Cu due to its toxic effects on rumen microorganisms. It is noteworthy that in vitro studies have indicated that Cu concentrations above 21 mg/ml could reduce fermentation activities and growth of some bacterial species, which could result in reduced animal productivity [23]. It is worth noting that these results do not agree with results obtained in some in vivo studies, which may be due to the nature of the microbial action that may be responsible for the formation of insoluble Cu compounds that are not normally found in feeds consumed by ruminants [27]. This may also due to Cu interactions with mineral elements such as Mo, S, and Zn which may also be involved [8, 29]. These results suggest that Cu supplementation could increase the use of feedstuffs consumed by ruminants when dietary Cu levels are within NRC recommendations [16].

It has been reported that when the dietary concentration of Zn in the supplement is increased, the digestibility of cellulose decreased by 31% [30]. This may be because Zn tends to selectively inhibit growth, or metabolic activities, of rumen microbes [12]. Salama Ahmed et al. [15] and Jia et al. [13, 14] found no differences in the digestibility of DM, CP, NDF, and ADF in goats supplemented with Zn, which does not agree with our findings that Zn supplementation increased IVDMD compared to the control. Moreover, adding Zn in combination with Cu, and separately, decreased IVDMD. These results are, however, consistent with those of Kathirvelan and Balakrishnan [31] who reported that supplementing 10 mg Zn/kg DM decreased IVDMD by 12.7% and NDF digestibility in paddy straw by 13.8% after 48 h of in vitro incubation. The addition of Zn supplements in cattle affects ruminal fermentation by changing the proportion of VFA in rumen fluid, with these changes being most pronounced during the first hour after consumption [32]. Indeed, IVDMD decreased linearly when “prairie hay” was supplemented with five concentrations of Zn [12] and incubated in vitro for 24 h. This was accompanied by the lowest RGP and GP in the diet supplemented with Zn alone or in combination with Cu. This effect could be due to the inhibitory effect of Zn on the digestion of fiber by rumen microbes [33]. Attachment of cellulolytic bacteria to fiber is a very important initiation in cellulose fermentation [34]. Therefore, the time of colonization of cellulose by cellulolytic bacteria is an important determinant in cellulose digestion [35]. In this way, a decrease in the concentration of cellulase can reduce the rate of cellulose digestion in the rumen [33], indicating that Cu and

Zn may affect microbial cell attachment to cellulose particles, and/or that the presence of these elements in the medium of inoculation can inactivate soluble proteins including enzymes in ruminal liquor [33]. The time of colonization in this study is consistent with the concept that Cu and Zn can reduce the cellulolytic activity of bacteria in the inoculation medium. It should be noted that the lack of Zn availability to bacteria can inhibit their multiplication in addition to affecting the ability of bacteria to attach to plant cell walls [5]. Indeed, it was found that IVDMD was higher in treatments to which Zn was added in comparison with the control. As the major carbohydrate in most forages is cellulose, its fermentation by ruminal microbes provides ME to the animal [35], this suggests that much of the increased ME in the diet supplemented with Cu may be a result of increased IVDMD, possibly due to the increased activity of rumen microorganisms [26]. In conclusion, the addition of Cu in diets for goats modifies in vitro ruminal fermentation by increasing gas production, IVDMD, ME, and SCFA production.

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