FM Suhartati and Wardhana Suryapratama/Animal Production. 18(2):59-65, May 2016
Accredited by DGHE No. 81/DIKTI/Kep./2011. ISSN 1411-2027

The Improvement of Rumen Fermentation Products Through *In-Vitro* Supplementation of Mg and Co Minerals

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Abstract. A study has been conducted to examine the effect of Mg and Co supplementation on rumen fermentation products. The study was conducted in an in vitro experiment, applying completely randomized design, 3x3 factorial. The first factor was three levels of Mg (0; 7.5 and 15.0 mM), and three levels of Co (0; 0.03 and 0.06 mM) as the second factor, total 9 treatments each repeated three times, comprising 27 experimental units all together. In vitro incubation lasted for 4 hours. Variables measured were the concentrations of VFA, N-NH_3 and protein synthesis of microbial rumen. Data were subject to analysis of variance and orthogonal polynomials test. The results showed an interaction effect between Mg and Co on the concentration of VFA, N-NH_3 and protein synthesis of microbial rumen. The increasing supplementation of Mg at 0.06 mM Co increased VFA concentration; the highest concentration of N-NH_3 was achieved by rumen fluid supplemented with 15.0 mM of Mg and 0.03 mM of Co. The highest protein synthesis of microbial rumen was achieved by the rumen fluid supplemented with 8.18 mM of Mg with no supplementation of Co.

Keywords: Mg, Co, VFA, N-NH_3 and microbial protein synthesis

Abstrak. Penelitian dilakukan untuk menguji pengaruh suplementasi Mg dan Co terhadap produk fermentasi rumen. Menggunakan metode eksperimen secara *in vitro*, rancangan acak lengkap, pola faktorial 3x3. Faktor pertama terdiri dari tiga taraf Mg (0, 7,5 , dan 15 mM), dan faktor kedua terdiri dari tiga taraf Co (0, 0,03 dan 0,06 mM), sehingga terdapat 9 macam perlakuan, setiap perlakuan diulang tiga kali, sehingga terdapat 27 unit percobaan. Inkubasi *in vitro* berlangsung selama 4 jam. Variabel yang diukur meliputi konsentrasi VFA, N-NH3 dan sintesis protein mikroba rumen. Data yang diperoleh dianalisis menggunakan analisis ragam dan uji orthogonal polinomial. Hasil penelitian menunjukkan bahwa terdapat pengaruh interaksi antara Mg dan Co terhadap konsentrai VFA, N-NH3 dan sintesis protein mikroba rumen. Semakin meningkat suplementasi Mg pada 0,06 mM Co, konsentrasi VFA juga semakin meningkat, konsentrasi N-NH3 tertinggi dicapai oleh cairan rumen yang mendapat suplementasi 15 mM Mg dan 0,03 mM Co, sedangkan sintesis protein mikroba rumen tertinggi dicapai oleh cairan rumen yang mendapat suplementasi Mg 8,18 mM tanpa suplementasi Co.

Kata Kunci: Mg, Co, VFA, N-NH3 dan sintesis protein mikroba

Introduction

The success of a livestock business is determined by several factors, among which is feeding. Quality ruminant feed is determined by the availability of feed protein that is able to contribute to microbial proliferation in rumen and capable of supplying the feed protein in the intestine. Given the important role of microbial rumen as a major supplier of protein for livestock, it required some efforts to maximize rumen microbial production by optimizing the conducive rumen environmental conditions (Ginting, 2005). In fact, Indonesian farmers, especially in rural areas, use agricultural waste as forage for their livestock, thus making it less optimal for supporting the development of the microbial rumen as the source of protein for the host animal. On the condition of waste-based local feed and crop residues, chances of disharmony between proteins rapidly dissolved and degraded and energy supply which is easily and quickly fermented are very large. Action that can be done to get this balance is by providing growth factors, among others, Mg and Co (Ginting, 2005).

Based on the description that has been elaborated, it is necessary to conduct a study to assess the effect of Mg and Co supplementation on the concentration of VFA and NH_3, and protein synthesis of microbial rumen.

Materials and Method

Rumen fluid of beef cattle was immediately taken after the animals was slaughtered in slaughter house of Tambaksari was used in the study. The basal feed consisted of grass and concentrate with a proportion of 40:60, plus 15% (based on concentrate DM) of *Moringa oleifera* leaf flour. The concentrate consisted of rice bran, cassava waste (*onggok*), coconut cake, grinded corn and mineral mix. *Moringa oleifera* leaf was dried by air drying method, which is dried at room temperature (25 ± 4 °C) for seven days, then smoothed in a blender (Gyamfi et al., 2011). Nutrient content of concentrate, elephant grass, and *Moringa oleifera* is listed in Table 1.

The method used is an in vitro experiment (Tilley and Terry, 1963), applying completely randomized design (CRD), 3x3 factorial. Mg supplementation (0.0 mM, 7.5 mM and 15 mM) and Co supplementation (0.0 mM, 0.03 mM and 0.06 mM) were the first and second factors; thus there were nine treatments and each was repeated three times, making up all together 27 experimental units. The treatments were P1 = basal feed; P2 = P1 + 0.0 mM Mg + 0.03 mM Co; P3 = P1 + 0.0 mM Mg + 0.06 mM Co; P4 = P1 + 7.5 mM Mg + 0.00 mM Co; P5 = P1 + 7.5 mM Mg + 0.03 mM Co; P6 = P1 + 7.5 mM Mg + 0.06 mM Co; P7 = P1 + 15 mM Mg + 0, 00 mM Co; P8 = P1 + 15 mM Mg + 0.03 mM Co and P9 = P1 + 15 mM Mg + 0.06 mM Co.

Parameters measured included (1) The concentration of total VFA of rumen fluid (using the vapor distillation method, according to the General Laboratory Procedures, 1966); (2) The concentration of N-$NH_3$of rumen fluid (using Micro Diffusion Conway, according to the General Laboratory Procedures, 1966); (3) Protein Synthesis of Rumen Microbial (Zinn and Owens, 1995).

Results and Discussion

Mean VFA obtained in the current study (Table 2) is much higher than 37.23 - 38.13 mM (Ozturk et al., 2010) and similar to 70-104 mM (Hindratiningrum et al., 2011). Mean VFA results of the current study were higher than 70-130 mM of normal concentration of VFA in cattle rumen (Khampa and Wanapat, 2006). Such discrepancy may occur because the VFA production depends on the chemical composition and structure of the feed materials as well as the ratioof forage and concentrate ration (Suwandyastuti and Rimbawanto, 2011). The high concentration of VFA can be used as a measure of the energy availability in the form of ATP (Suryapratama, 1999), despite the high concentration of VFA can also be due to the lack of VFA absorption of in vitro experiments. The high VFA results obtained in the current study illustrate that the activity of fermentation in the rumen is quick.

Table 1. Nutrient content of concentrate, Elephant grass and *Moringa oleifera* leaves flour

Nutrient	Feed Source		
	Concentrate	Elephant grass	*Moringa oleifera*
Dry Matter (% DM)	89.69	96.41	93.41
Protein (% DM)	13.25	11.50	20.36
Fat (% DM)	8.55	4.59	9.37
Crude Fibre (% DM)	11.32	24.31	4.14
Ash (% DN)	7.04	15.27	9.62
NFE*(% DM)	60.25	44.33	56.50

*NFE: Nitrogen-Free Extract

Table 2. Concentration of VFA, N-NH_3 and protein synthesis of cow microbial rumen

Treatments tested	VFA (mM)	N-NH_3 (mM)	Microbial Protein Synthesis (mg/20 ml)
P_1	206.67±5.77	10.67±1.15	45.63±4.16
P_2	193.33±5.77	11.67±1.15	60.30±4.58
P_3	113.33±5.77	10.33±1.15	385.63±9.29
P_4	100.00±0.00	10.67±0.58	432.30±8.19
P_5	250.00±0.00	10.00±0.00	166.63±10.79
P_6	173.33±5.77	11.00±0.00	179.97±16.29
P_7	213.33±5.77	14.00±1.00	164.00±23.81
P_8	216.67±5.77	14.67±0.58	235.00±8.54
P_9	260.00±10.0	12.33±0.58	146.00± 9.85

P1 = basal feed. P2 = P1 + 0.0 mM Mg + 0.03 mM Co. P3 = P1 + 0.0 mM Mg + 0.06 mM Co. P4 = P1 + 7.5 mM Mg + 0.00 mM Co. P5 = P1 + 7.5 mM Mg + 0.03 mM Co. P 6 = P1 + 7.5 mM Mg + 0.06 mM Co. P7 = P1 + 15 mM Mg + 0.00 mM Co. P8 = P1 + 15 mM Mg + 0.03 mM Co. P9 = P1 + 15 mM Mg + 0.06 mM Co.

The average of N-NH_3 of the current study (Table 2) is in the normal range required by microbes to digest the feed maximally. Paengkoum et al. (2006) stated that, the concentration of N-NH_3 microbial rumen needed to digest feed maximally is within 5-20 mg/dL, which is the equivalent to 3.57-14.28 mM. The results achieved in the current study is higher than the results of Hindratiningrum et al. (2011) which is 6.67-7.82 mM. Such discrepancy may occur due to the production of N-NH_3 which is highly affected by several factors, including feed, mainly depending on the composition and properties of feed protein (Suwandyastuti and Rimbawanto, 2011).

Based on the analysis of variance, it is known that the concentration of VFA, N-NH_3 and microbial protein synthesis are affected by the interaction between Mg and Co supplementation ($P < 0.01$). Increasing Mg supplementation at 0.06 mM Co, also increased VFA concentration (Figure 1), thus the highest concentration of VFA was achieved by rumen fluid supplemented with 15 mM Mg and 0.06 mM Co. This does not hold with the concentration of N-NH_3, the highest concentration is achieved by rumen fluid supplemented with 15 mM Mg and 0.03 mM Co ($P<0.01$). The high concentration is not necessarily due to the resulting high production, but could due to the available N-NH_3 which has not been yet optimally utilized by microbial rumen. This fact is supported by Hindratiningrum et al. (2011) that the ammonia results from feed protein degradation in the rumen will be used as the primary nitrogen source for microbial protein synthesis.

Figure 2 illustrates that the interaction between Mg and Co resulted in the highest N-NH_3, i.e. at 15 mM Mg and at 0.03 mM Co levels; although both supplemented with 0.06 mM and with no Co supplementation resulted in the increasing response. By contrast, the highest production of rumen microbial protein synthesis was achieved by the rumen fluid with no supplementation of Co. The quadratic response follows the equation $Y = 45.63 + 95.22x - 5.821x^2$, with coefficient of determination (R^2) of 0.99; P (8.18; 434.97) (Figure 3), which implies that the highest microbial protein production i.e. 434.97 mg/20 ml is achieved at 8.18 mM Mg supplementation.

If the concentration of VFA, N-NH_3 and rumen microbial protein synthesis supplemented Mg with no Co supplementation combined into a single image (Figure 4), it is clear that at the time of VFA and N-NH_3decrease, it is followed by an increase in rumen microbial protein synthesis. The highest rumen microbial protein synthesis was achieved at 8.18 mM Mg, whereas the lowest VFA and N-NH_3were reached at 7.37 mM and 7.33 mM of Mg, respectively. This proves that

the decrease in the concentration of VFA and N-$NH_3$was because they have been utilized for rumen microbial protein synthesis. Baldwin and Allison (1983) explained that the growth or protein synthesis by rumen microorganisms needs carbohydrates and protein as the main nutrients substance to supply the needs of ammonia, energy and carbon atoms (Figure 5). Ammonia and energy must be available simultaneously for rumen microbial protein synthesis (Zadeh et al., 2013). This is in agreement with the statement of Orskov (1988) that there is a link between fermentation process and microbial protein production. The results obtained from the anaerobic fermentation of carbohydrates in the form of VFA then combine with Nitrogen into microbial cells.

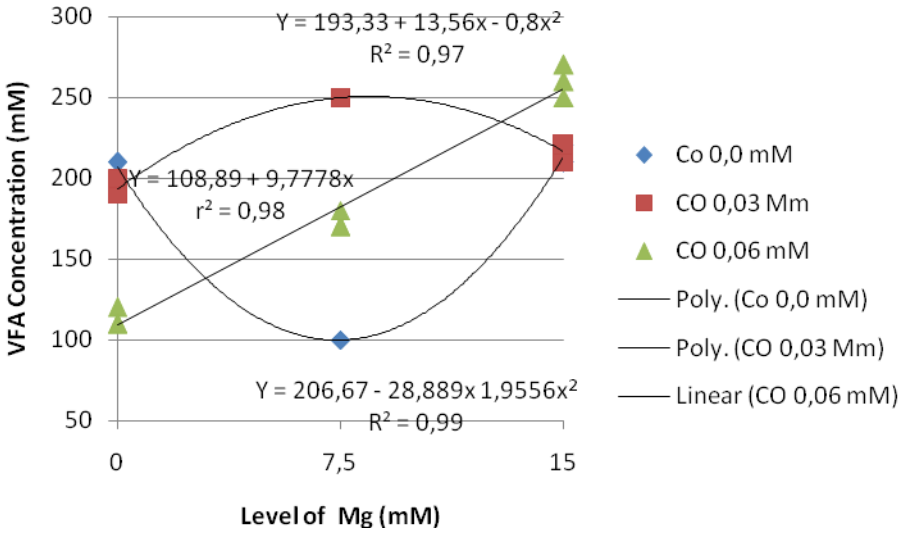

Figure 1. Interaction effect between Mg and Co on concentration of VFA rumen

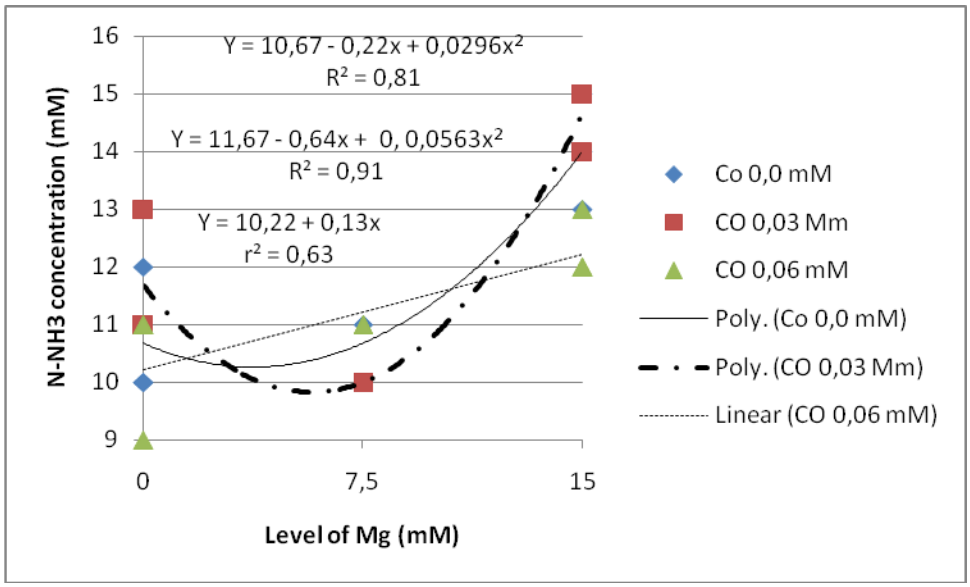

Figure 2. Interaction effect between Mg and Co on concentrations of N-NH_3 rumen

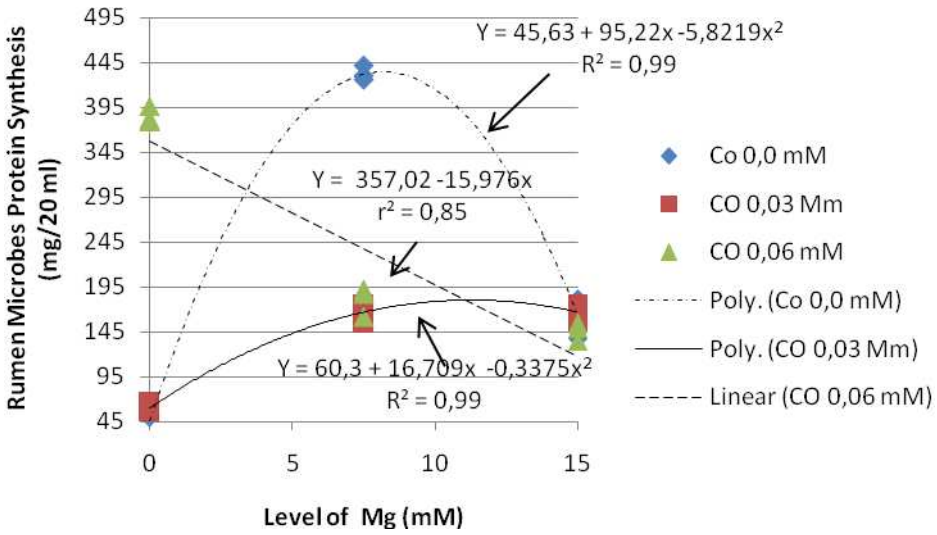

Figure 3. Interaction effect between Mg and Co on rumen microbes protein synthesis

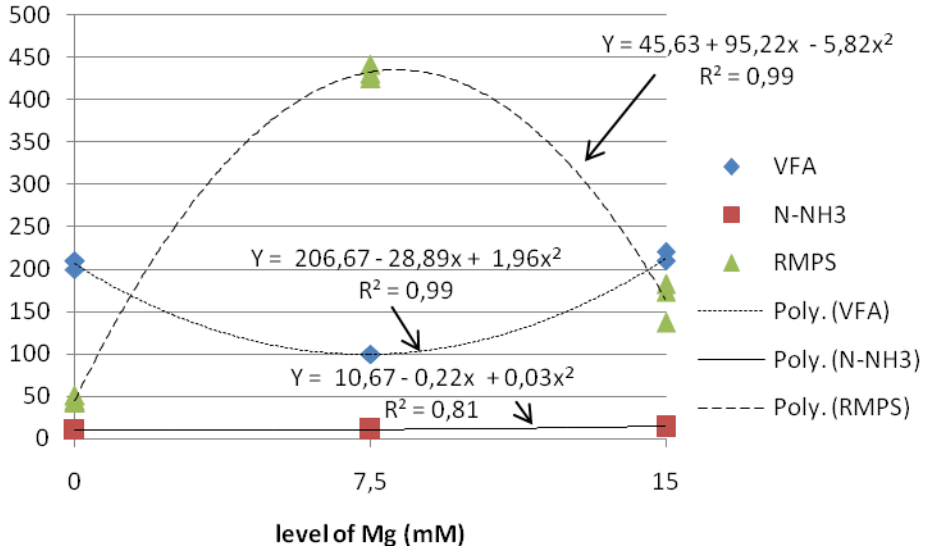

Figure 4. Effect of Mg supplementation on the concentration of VFA, N-NH_3 and rumen microbial protein synthesis

Adenosine triphosphate (ATP) produced during the fermentation of hexose or pentose is a source of energy for growth and basic living of microbes. Some co-factors required for the efficient synthesis of microbial protein among others are mineral elements e.g. S, Mg, Co and branched-chain fatty acids and amino acids (Ginting, 2005). Co acts as a stimulator which is capable of increasing the growth and sellulolytic activity (Talib et al., 2000).

Result of the current study proves that once Mg exceeds the level of 8.18 mM, microbial protein synthesis begins to decline; it is presumably because there is imbalance between the degradation of protein and energy. Widyobroto et al (2007) argued that the volatile fatty acids and NH_3 is the result of carbohydrate and protein degradation by microbial rumen. The speed of carbohydrate degradation which is matched with the speed

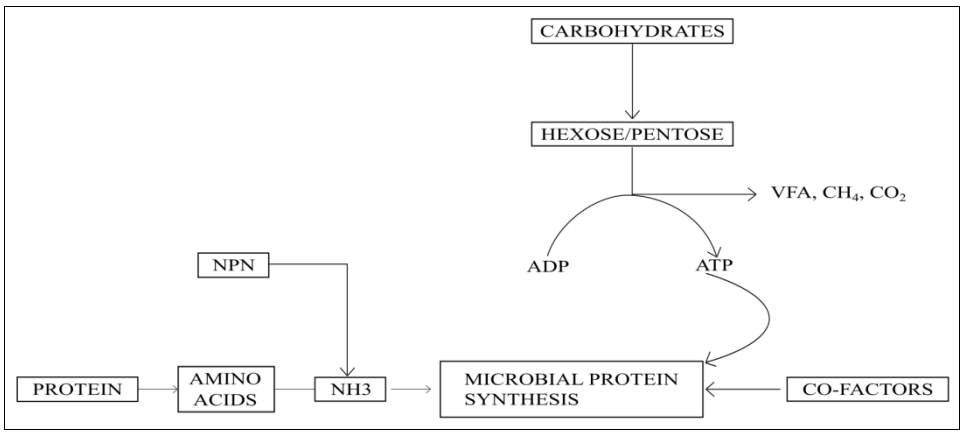

Figure 5. Flow chart of relationship between carbohydrates and nitrogen substance in microbial cells synthesis process (Baldwin and Alison, 1983)

of protein degradation will increase the efficiency of microbial protein synthesis. In addition, an excess of Mg will be poisoning, so that the increasing Mg level will decrease the rumen microbial protein synthesis.

It similarly happens to the Co, the addition of Co of 0.03 mM and 0.06 mM would produce lower microbial protein synthesis than that of with no Co supplementation. The microorganisms in the rumen utilize Co to compose vitamin B12 (Dwipartha et al., 2014) and as stimulator which can improve the growth and cellulolytic activity (Talib et al., 2000). Because forages for ruminants feed absorbs Co element from soil, thus it makes the livestock excessively consume Cobalt (Dwipartha et al., 2014). Although in vitro experiments did not use experimental animals, the tested feed also contains 40% grass. The high Co element in the feed will negatively affect rumen microbial protein synthesis.

Conclusions

The more supplementation level of Mg at 0.06 mM Co, the more increased VFA concentration, and the highest N-NH_3 concentration is achieved by rumen fluid supplemented with 15 mM Mg and 0.03 mM Co, while the highest rumen microbial protein synthesis is achieved by the rumen fluid supplemented with 8.18 mM Mg with no Co supplementation.

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