# EFFECT OF COBALT SUPPLEMENTATION ON GAS PRODUCTION MEASUREMENTS, ESTIMATED ENERGY VALUES AND MICROBIAL PROTEIN, *IN VITRO*

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**Abstract**

*In vitro gas production techniques simulate the rumen fermentation process and they have been used to evaluate the potential of feeds to supply nutrients to ruminants. Thus, effects of various levels of cobalt supplementation on gas production and rumen fermentation were investigated using an in vitro gas production technique. Ground samples (100 mg DM) of 70% wheat straw and 30% concentrate were incubated in 50 ml glass syringes with rumen fluid obtained from fistulated sheep fed berseem hay and commercial concentrate mixture twice a day. Cobalt (Co) was added at 0.00, 0.35, 0,70 and 1.0 mg/kg DM of feed. Cumulative gas production was recorded at 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation and the kinetics of gas production was described by using the equation Gas(t)= a + b (1- e-ct)). At 24 h, the gas production volume was highest for sample with the third level of cobalt (0.70 mg/ kg DM) ($P<0.05$) and greater for second and fourth levels of Co (0.35 and 1.0 mg/kg DM, respectively) ($P<0.05$) than sample without Co (control). Total gas production at 96 h and the maximum rate of gas production increased when Co was added to samples. Also, the results showed that there were significant differences ($p<0.05$) in metabolizable energy (ME) and net energy (NE), dry matter digestibility (DMD), organic matter digestibility (OMD), Short chain fatty acids (SCFA) and microbial protein. In an overall conclusion it seems that, the cobalt addition improved the rumen fermentation.*

**Key words**: Wheat straw, gas production, digestibility, energy value, microbial protein, sheep

## INTRODUCTION

The role of cobalt in carbohydrate digestion in the rumen has been clearly demonstrated. Some *in vivo* experiments have shown a positive influence of Co supplementation on the rumen utilization of forage diets (Lodochkina, 1983) and using the in sacco technique, Saxena and Ranjhan (1978) obtained with 0.1 mg/kg Co supplementation, an increased cellulose degradation with a straw based diet in calves. A few in vitro experiments have shown an increase in cellulolysis with a Co supply, such as the report of Durand and Kawashima (1980). McDonald and Suttle (1986) observed no effect of the Co supplementation of cobalt-deficient hay on digestibility parameters but the acetate/prppionate ratio was lowered by the Co deficiency. Also, it is well known that the quantity of B12 synthesized in the rumen depends on dietary Co level. It has been reported that low level of dietary Co can lead to vitamin B12 deficiencies clinically manifested as anemia, inappetence and poor production and biochemically characterized by decrease in the plasma concentration of vitamin B12, elevations in the plasma concentrations of methylmalonic acid (MMA) and homocysteine (HCY) in ruminants (Kennedy et al., 1990). It was reported that 0.3–0.5 mg Co/kg DM enhanced ruminal microbial activity, fermentation and vitamin B12 synthesis (Singh and Chhabra, 1995). In addition, a higher level of dietary Co has been suggested both in beef cattle (Stangl et al., 2000) and cows (Tomlinson and Socha, 2003) than the recommended data. In sheep, a supply of 0.5 mg/kg DM could be sufficient to

achieve both an optimal microbial activity and adequate vitamin B12 synthesis (Grace, 1989). However, goats have been described as being more resistant to low levels of dietary cobalt (Clark et al., 1986; Mburu et al., 1993). The present study was designed to evaluate the effects of dietary Co level on gas production measurements, estimated energy values and microbial protein, *in vitro*. In Egypt, the optimal dietary levels of Co in lambs have not been properly established, and the effects of dietary Co concentration on sheep nutrient digestibility, metabolic characteristics and ruminal microbial activity were not well understood. The objective of this study was, therefore, to investigate the effect of dietary Co level on performance, nutrient digestibility and rumen activity in lambs.

## MATERIAL AND METHOD

### *2.1. Animals and feeds*

Three fistulated Rahmany rams (Egyptian native breed), fed berseem hay and commercial concentrate mixture twice a day, were used for rumen liquor collection in order to application in gas production technique. The experimental samples including ration (70% wheat straw + 30% concentrate mixture) with different levels of cobalt (at 0.00, 0.35, 0.70 and 1.0 mg/kg DM of feed).

### *2.2. Chemical analysis*

Samples were milled through a 1 mm sieve for chemical analysis and gas production procedure. Dry matter (DM) was determined by drying the samples at 80oC overnight and ash by igniting the samples in muffle furnace at 600oC for 2 h. Organic matter (OM), ether extract (EE) and crude fiber (CF) following the procedure of AOAC (1990). Nitrogen free extract (NFE) was calculated as (100 – (CP + EE + CF + ash)). Nitrogen (N) content was measured by the kjeldahl method (AOAC, 1990). Crude protein (CP) was calculated as N * 6.25. All chemical analysis was carried out in duplicate. The chemical composition of wheat straw and concentrate used for *in vitro* gas production technique is presented in Table 1.

Table 1. Chemical composition (%) of concentrate mixture and wheat straw

| Items | DM | OM | CP | EE | CF | NFE | Ash |
|---|---|---|---|---|---|---|---|
| WS | 91.32 | 78.73 | 3.93 | 1.14 | 25.97 | 47.69 | 12.59 |
| C | 86.16 | 77.03 | 14.70 | 5.24 | 8.10 | 48.99 | 9.13 |

**WS**, wheat straw, **C**, concentrate, **DM**, dry matter, **OM**, organic matter, **CP**, crude protein, **EE**, ether extract, **CF**, crude fiber, **NFE**, nitrogen free extract.

### *2.3. In vitro gas production method*

*In vitro* gas production was undertaken according to Menke and steingass (1988). Rumen fluid was obtained before morning feeding from three fistulated Rahmany rams fed berseem hay and commercial concentrate mixture twice a day. The rumen fluid was filtered through four layers of cheese–cloth and flushed with CO2. The CO2-flushed rumen fluid was added (1:2, v/v) to the buffered mineral solution (Onodera and Henderson, 1980), which was maintained in a water bath at 39$^{o}$C, and combined. All laboratory handling of rumen fluid was carried out under a continuous flow of CO2. The 100 mg samples were accurately weighed in triplicate into calibrated glass syringes of 50 ml. The syringes were pre-warmed at 39$^{o}$C before injection of 15 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39$^{o}$C. All the syringes were gently shaken 30 min after the start of incubation and every one hour for the first 12 h of incubation thereafter five times daily. Readings of gas production were recorded before incubation (0) and 3, 6, 9, 12, 24, 48, 72 and 96 h after incubation. Total gas values were corrected for blank incubation which contains only rumen fluid. Cumulative gas production data were fitted to the model of Orskov and Mc Donald (1979)

$$Y = a + b\ (1\text{-}e\text{-}ct)$$

Where:

a = the gas production from the immediately soluble fraction (ml);

b = the gas production from the insoluble fraction (ml);

c = the gas production rate constant for the insoluble fraction (*b*);
t = the incubation time (h);
Y = the gas produced at time't'.

### *2.4. Estimation of energy values, organic matter digestibility, short chain fatty acids and microbial proteins*

The energy values and the percentages of organic matter digestibility of samples without or with different levels of cobalt can be calculated from the gas produced on incubation of 200 mg feed dry matter after 24 h of incubation with the levels of crude protein, ash and crude fat (Menke *et al*., 1979 and Menke and Steingass, 1988) as follows:

ME (MJ/kg DM) = 1.06 + 0.157GP + 0.084CP + 0.22CF – 0.081A

OMD (%) =14.88 + 0.889 GP + 0.45 CP + 0.0651A

Where:
ME is the metabolizable energy, OMD (%) is the percentage of organic matter digestibility, GP is the 24 h net gas production (ml/200 mg DM) after 24 h of incubation. CP, crude protein (%); CF, crude fat (%) and A, ash content (%).

NE (Mcal/Ib) = [2.2 + (0.0272 × Gas) + (0.057 × CP) + (0.149 × CF)] / 14.64

Where:
NE is the net energy; Gas, the net gas production in ml from one-gram dry sample after 24 h of incubation; CP, crude protein (%); CF, crude fat (%) then, net energy unit converted to be MJ/kg DM.
Short chain fatty acids (SCFA) were calculated according to Getachew et al. (2005) as follows:

SCFA= (-0.00425 + 0.0222 GP) × 100

Where: GP is 24 h net gas production (ml/200 mg DM).
Microbial protein was calculated as 19.3 g microbial nitrogen per kg OMD according to Czerkawski (1986).

### *2.5. Statistical Analysis*

Data were subjected to analysis of variance (ANOVA) using the General linear Model (GLM). Significant differences between individual means were identified using least significance difference (LSD) multiple range test (SAS, 2000).

## RESULTS AND DISCUSSION

***In vitro* gas production**: Cumulative gas production profiles, corrected for blank are shown in Figure 1. The cumulative volume of gas production increased with increasing time of incubation. There were significant differences between the substrates in terms of gas production at all incubation time. At all incubation times the gas production of supplemented substrates with Co were significantly higher ($P<0.05$) than those obtained from unsupplemented substrate. Total gas produced at 96 h of incubation (Fig. 1; Table 2) was highest for second level of Co ($P < 0.05$). Kinetics of gas production obtained from the exponential model is presented in Table 2. The gas production from the insoluble fraction (b) was highest for L2 ($P < 0.01$) and greater for L1 and L3 ($P<0.05$) than control. The gas production rate (c) of control and L1 was significantly lower ($P<0.05$) than those of L2 and L3. The present results have shown a positive influence of Co supplementation on the rumen activity and fermentation. The results in rumen activity were consistent with the findings of Lodochkina, (1983). Singh and Chhabra (1995) reported that 0.3–0.5 mg Co/kg DM enhanced ruminal microbial activity, fermentation and vitamin B12 synthesis. This is consistent with Komisarczuk-Bony and Durand (1991), who reported that 0.5 mg Co/kg DM is sufficient to achieve both an optimal microbial activity and adequate vitamin B12 synthesis. Incubation of feedstuff with buffered rumen fluid *in vitro*, the carbohydrates are fermented to short chain fatty acids (SCFA), gases, mainly CO2 and CH4, and microbial cells. Gas production is basically the result of fermentation of carbohydrates to acetate, propionate and butyrate (Steingass and Menke, 1986 and Hussein et al, 1994) and substantial changes in carbohydrate fractions or microbial activity was reflected by total gas produced (Deaville, and Givens, 2001). Nasser, (2010) showed that Co supplementation was increased the

digestion of fiber. This may largely be due to the increase in activity of fiber digesting bacteria (Tomlinson and Socha, 2003). The results of Saxena and Ranjhan (1978) showed that 0.1 mg/kg Co supplementation, increased cellulose degradation with a straw based diet in calves.

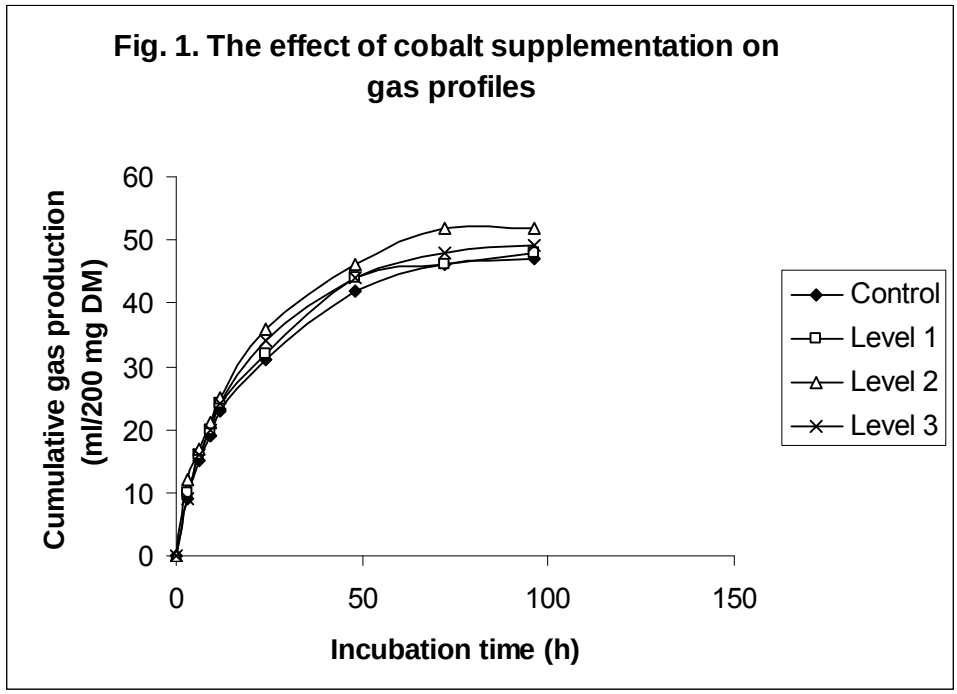


Table 2: Cumulative gas production volume and kinitecs parameters (ml/200 mg DM) at different incubation times for experimental rations (70% straw+30% concentrate)

| Items | 12 | 24 | 48 | 72 | 96 | a | b | c |
|---|---|---|---|---|---|---|---|---|
| Control | 22.5c | 31.0c | 40.5b | 46.0c | 46.5b | 3.796a | 43.148c | 0.041 |
| L1 | 24.0ab | 33.0b | 44.0a | 47.5bc | 49.5ab | 3.836a | 45.058b | 0.042 |
| L 2 | 24.5a | 35.0a | 44.5a | 50.0a | 50.5a | 4.621a | 46.503a | 0.046 |
| L 3 | 23.5a | 33.5b | 43.5a | 48.0b | 49.0ab | 3.439a | 45.491ab | 0.045 |

L1, level 1, L2, level 2, L3, level 3 of cobalt
abcMeans within the same columns with different superscript are significantly different (P<0.05)

**Energy contents and organic matter digestibility**

The predicted metabolizable energy (ME, MJ/kg DM), net energy (NE, MJ/kg DM), organic matter digestibility (OMD), microbial protein (mg/kg DM) and short chain fatty acids of tested substrates with or without Co are presented in Table 3. The present data showed that the ME and NE were highest (P<0.01) for L2 and greater for L1 and L3 (P<0.05) than for control. The values of ME and NE for L1 and L3 was significantly lower (P<0.05) than those of L2. ME was positively correlated with CP which is one of the limiting factors for microbial growth (Larbi *et al*., 1998). The results in table 3 showed that the OMD% and microbial protein were highest (P<0.01) for L2 and higher for L1 and L3 than for control. A close correlation between *in vitro* gas production and digestibility; the better correlation was achieved when the equation includes crude protein, crude fat and ash content (Menke and Steingass, 1988). *In vitro* gas production and *in vitro* apparent and

true degradability are highly correlated (Blümmel *et al*., 1990). Gas production is an indirect measure of substrate degradation and is a good predictor for the production of VFA, but it is not always positively related to microbial mass production. On the other hand, Blümmel *et al*., (1997) suggested that there is an inverse relationship between *in vitro* gas production and microbial biomass yield. Co addition was increased the levels of VFA compared with control. The results was reported that 0.7–1.0 mg Co/kg DM enhanced ruminal microbial activity and fermentation. These results confirmed by the observations in lambs (Nasser, 2010). In contrast, Tiffany (2003) found that total VFA and molar proportions of acetate, propionate, and isobutyrate, and acetate:propionate ratio were not affected by the addition of supplemental Co to the basal diet. However, molar proportions of butyrate, valerate, and isovalerate increased ($P < 0.05$) in response to supplemental Co. Also, McDonald and Suttle (1986) observed no effect of the Co supplementation of cobalt-deficient hay on digestibility parameters but the acetate/propionate ratio was lowered by the Co deficiency. In contrast, the results of Lodochkina, (1983) have shown a positive influence of Co supplementation on the rumen utilization of forage diets. Also, Saxena and Ranjhan (1978) obtained with 0.1 mg/kg Co supplementation, increased cellulose degradation with a straw based diet in calves.

Table 3: Metabolizable energy (ME), Net energy (NE), Organic matter digestibility (OMD), Microbial protein (MP) and Short chain fatty acids (SCFA)

| Items | **ME** (MJ/kg DM) | **NE** (MJ/kg DM) | **OMD** (%) | **MP** (g/kg OMD) | **SCFA** (mM) |
|---|---|---|---|---|---|
| Control | 7.60c | 5.18c | 49.01c | 59.24c | 68.4c |
| L1 | 7.89b | 5.34b | 50.87b | 61.36b | 72.8b |
| L2 | 8.20a | 5.51a | 52.65a | 63.51a | 77.3a |
| L3 | 7.97b | 5.38b | 51.29b | 61.87b | 73.9b |

L1, level 1, L2, level 2, L3, level 3 of cobalt
abcMeans within the same columns with different superscript are significantly different ($P<0.05$)

In conclusion, there are positive effects on *in vitro* gas production occurred more consistently when adding large amounts (0.7 or 1.0 mg/kg DM) of Co to cultures. Energy contents, VFA, MP and OMD were increased by adding Co.

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