INFLUENCE OF DIETARY COBALT ON PERFORMANCE, NUTRIENT DIGESTIBILITY AND RUMEN ACTIVITY IN LAMBS

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Abstract

The present study was conducted to study the effects of different inclusion levels of dietary cobalt (Co) on performance, nutrient digestibility and rumen activity in lambs. Twenty, 5.0 month-old weaned lambs (Egyptian native breed) were randomly divided into four groups which were fed with the basal diet containing 0.0486 mg Co/kg DM, the basal diet supplied, respectively, with 0.35, 0.70 and 1.00 mg Co/kg DM reagent grade CoSO4·7H2O for 100 days. In the end of experimental period, three lambs from each group were allocated to the individual metabolic cages to measure the effects of dietary Co on apparent nutrient digestibility. Samples of rumen content were taken once a week for seven consecutive weeks, two times daily (before feeding and 3 hours after feeding). Average daily gain and average daily feed intake for the fourth group were higher than control group (P<0.05). However, there was no difference in gain/feed. Supplemental 1.0 mg Co/kg DM resulted in highest digestibility of dry matter, organic matter, crude protein and crude fiber (P<0.05). Nitrogen free extract was higher for third group (0.7 mg Co/mg DM) than the other three groups (P<0.05). The pH values and ammonia nitrogen were low in supplemented groups than in the control. The concentrations of volatile fatty acids were increased after feeding and peaked at 3-h post-feeding in the third group. The number of total protozoa was significantly increased for third group than other groups. In conclusion, supplementation of the basal diet with 0.70-1.00 mg Co/kg DM (the total dietary Co level of 0.749 -1.049 mg/kg DM) in the basal diet enhanced growth performance and improved nutrient digestibility and rumen activity of lambs.

Key words: Cobalt, digestibility, volatile fatty acids, protozoa and lambs

INTRODUCTION

In ruminants, Vitamin B12 is produced from cobalt by microbes in the rumen. Hence, complete replacement of cobalt by Vitamin B12 in ruminant diets is not possible due to the positive effect of cobalt on the digestibility of the feed. 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. Vitamin B12 acts as a cofactor for protein and energy metabolism enzymes, namely methylmalonyl coenzyme A mutase and methionine synthase (Kennedy et al., 1992). Cobalt deficiency, therefore, impairs the energy and protein metabolism and then growth and development of the deficient animal, which can be defined as changes in the weight, shape and size of the body. Co deficiency affect on a number of important manifestations such as hepatic lipidosis (Johnson et al., 2004), changes in meat quality characteristics (Kadim et al., 2004) and differences in apparent nutrient digestibility (Kadim et al., 2003). The

currently recommended dietary levels of 0.1–0.2 mg Co/kg dry matter (DM) in sheep (NRC, 1985) are based on observations in grazing animals; recent findings however have demonstrated that 0.10 mg/kg DM of Co intake does not meet the rumen microbial requirements (Kisidayova et al., 2001 and Johnson et al., 2004). It was reported that 0.3–0.5 mg Co/kg DM enhanced ruminal microbial activity, fermentation and vitamin B12 synthesis (Singh and Chabra, 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 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.

MATERIALS AND METHODS

1. Animals and feeding management

The experiment was carried out at the Agricultural Experiment Station, Animal Nutrition Laboratory, Animal Production Dept., Faculty of Agriculture, Alexandria University, Alexandria, Egypt. Twenty, 5.0 month-old weaned lambs (Egyptian native breed), weighed 25±2.0 kg, were used in the present study for 100 days. Animal were divided into four similar groups (on the basis of body weight) and fed daily a basal diet of wheat straw + concentrate mixture without or with different levels of cobalt (0.0, 0.35, 0.70 and 1.00 mg Co/kg DM) reagent grade $CoSO_4 \cdot 7H_2O$ for 100 days. Chemical analysis of wheat straw and concentrate mixture is shown in Table 1. The animals were housed in pens. Feed were offered twice daily at 9:00 and 16:00 h. Drinking water was available all times. The quantity of feed offered, refused and feed intake was recorded throughout the feeding period. Animals were weighed every ten days.

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	86.15	77.02	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. Metabolism trial and collection of samples

A metabolism trial were conducted at the end of experimental feeding, lasted for 10 days in metabolism cages (i.e. 5 days adaptation in metabolic cages followed by 5 days of sample collection) with facility of quantitative collection of feces. Daily intake of feed and output of feces were recorded. Dry matter in feed and refusals was determined daily by drying at 80^{o}C. Samples of feed and refusals will take daily and compose until the end of collection period, dried at 80^{o}C for 24 h, ground through a 1 mm screen and used for chemical analysis. Daily faecal excretions will be collected every morning, weighed and recorded. Aliquots (10%) of the sample from each animal will be sampled daily. A portion of daily feces were dried for 24 h at 80^{o}C for DM determination. The remaining faecal samples were composed for each animal and kept in refrigeration.

3. Rumen fermentation

Rumen liquor samples were taken once a week for seven consecutive weeks, two times daily (before feeding and 3 hours after feeding) using a stomach tube according to the Langston University, animal care committee (Puchala et al., 2004). Ruminal pH was measured immediately after collecting the rumen fluid using a digital pH meter (Digital pH meter, SANTA ANA, CA. 927705 USA). Rumen liquor samples strained through four layers of cheese cloth and stored frozen pending analysis. Samples were analyzed for ammonia nitrogen and total volatile fatty acids according to Gips

and Markham, 1968 and Warner, 1964, respectively.

4. Chemical analysis

Feed and faecal Samples were milled through a 1 mm sieve for chemical analysis. Dry matter (DM) was determined by drying the samples at 80°C overnight and ash by igniting the samples in muffle furnace at 600°C 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. Chemical analysis was carried in duplicate. The chemical composition of wheat straw and concentrate is presented in Table 1.

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

1. Performance

Daily gain, feed intake and feed conversion of Rahmany lambs consumed tested ration with different levels of cobalt are presented in Table 2. The present results showed that average daily gain (ADG) and average dry matter intake (ADMI) were increased by supplementing Co. Also, there were significantly differences in gain/feed (G/F) (Table 2). Numerous studies have documented decreased intakes and/or gains in sheep fed Co deficient hay or barley diets, relative to those receiving Co supplementation (Kennedy et al., 1994a). Lambs fed the control diet, without Co supplementation, had a reduced appetite and gained less weight than the supplemented animals (Wang, et al., 2007). It was supported by Johnson et al. (2004) who reported that Omani goats fed with diet containing 0.12 mg Co/kg DM exhibited slower growth rate, dry ruffled hair coats and hepatic lipidosis. Tiffany et al. (2006) indicated that diets marginally deficient in Co adversely affect performance, and vitamin B12 status of finished steers. The major physiological effect of a Co-induced vitamin B12 deficiency is a progressive loss of appetite (Smith, 1997), which results in decreased gains, and in severe cases, weight loss, emaciation, and death (Underwood and Suttle, 1999). Work by Schwarz and colleagues (2000) determined that diets containing 0.07 mg Co/kg resulted in lower daily gains in German Simmental bulls than those containing at least 0.11 mg Co/kg. Their analysis of data, suggested that the optimum concentration of dietary Co for gain was 0.12 mg/kg DM. In the present study, lambs fed the control diet containing 0.0486 mg Co/kg DM, had lower gains than Co supplemented lambs. These results confirmed by the observations in steers (Tiffany, 2003). In contrast, Nagabhushana, et al. (2008) showed that average cumulative body weight, net gain in body weight or feed efficiency did not differ significantly between the groups with or without cobalt. TDN and DCP values of the experimental diets remained almost similar irrespective of dietary level of cobalt.

Table 2. Effects of dietary Co supplementation on daily gain, feed intake and feed conversion of Rahmany lambs (DM basis)

Items	Co supplemental levels (mg/kg DM)				L.S.D.
	0.0	0.35	0.70	1.0	
Animal No.	5	5	5	5	
Experimental period (d)	100	100	100	100	
Initial weight (kg)	24.8	25.2	23.4	24.0	
Final weight (kg)	35.0	36.1	34.3	37.1	
Total weight (kg)	10.2^{b}	10.9^{ab}	10.9^{ab}	13.1^{a}	2.072
Daily gain (kg)	0.102^{b}	0.109^{ab}	0.109^{ab}	0.131^{a}	0.023
Dry matter intake (kg)	0.778^{b}	0.781^{b}	0.814^{a}	0.796^{ab}	0.160
Gain/feed (kg/kg)	0.131^{b}	0.140^{b}	0.134^{b}	0.165^{a}	0.031

^{ab}Means within the same columns with different superscript are significantly different (P<0.05)

2. Digestibility

Digestibility of DM, OM, CP, NFE and CF were increased by supplementing cobalt (Table 3). The highest values of digestibility of DM, OM, CP, NEF, and CF were at the third and fourth level ($P<0.05$) (Table 3), but there were no differences in EE digestibility. In the present study, lambs fed the control diet containing 0.0486 mg Co/kg DM, had lower nutrient digestibility than Co supplemented lambs. The results in nutrient digestibility were consistent with the findings of Wang et al. (2007). Also, Kadim et al. (2003) demonstrated that low levels of dietary Co in goats resulted in lower apparent nutrient digestibility compared to goats supplemented with parenteral injections of vitamin B12. The increased digestibility of CF indicates the possible role of Co in fiber digestion. This may largely be due to the increase in activity of fiber digesting bacteria (Tomlinson and Socha, 2003). 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. In contrast, the results of Nagabhushana, et al. (2008) indicated that no significant effect was observed on the digestibility of dry matter, organic matter, crude protein, ether extract and fiber constituents like NDF, ADF, hemicellulose or cellulose by supplementation of 1 and 6 ppm Co to the diet of growing calves. Also, Balance of nutrients such as Nitrogen, Calcium and Phosphorus was similar and positive in all the treatment groups. Kisidayova et al., (2001) found that the elevated Co intake (2, 4 and 8 mg/kg DM) had no effects on degradability of DM, OM, NDF and ADF ($P>0.05$). Tiffany, (2003) showed that apparent DM digestibility of the control diet, without Co addition, did not differ from diets supplemented with Co. However, mixed ruminal cultures supplemented with 0.10 mg Co/kg had lower apparent digestibility than cultures fed diets supplemented with either 0.05 or 1.0 mg Co/kg DM. Other studies found that adding supplemental Co to diets of sheep, not deficient in vitamin B12, had no effect on digestibility (Smith and Marston, 1970).

Table (3): Effects of dietary cobalt (Co) supplementation on apparent nutrient digestibility coefficients[a] in lambs (DM basis)

Items	Co supplemental levels (mg/kg DM)				L.S.D.
	0.0	0.35	0.70	1.0	
DM	59.76[b]	62.38[b]	68.85[a]	70.21[a]	2.680
OM	67.34[b]	71.00[ab]	70.76[ab]	75.52[a]	5.017
CP	66.81[c]	70.02[b]	76.46[a]	76.86[a]	2.301
EE	83.70[a]	85.56[a]	82.33[a]	83.24[a]	3.624
CF	58.34[b]	58.00[b]	64.36[a]	66.76[a]	5.288
NFE	69.54[d]	73.10[c]	79.60[a]	76.76[b]	1.985

[a] DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; CF, crude fiber; NFE, nitrogen free extract.
[ab]Means within the same columns with different superscript are significantly different ($P<0.05$)

3. pH, Ammonia nitrogen, Volatile fatty acid and protozoa counts

The values of pH, total volatile fatty acids (VFA) and ammonia nitrogen (NH_3-N) concentrations and protozoa counts are presented in Table (4). pH values of rumen fluid for all groups before feeding was higher than post-feeding. The range of pH was 6.51 to 6.83 and 5.19 to 6.01 at 0 h and 3h of feeding, respectively. These values were higher than that observed by Tiffany, 2003 who used the higher percentage of concentrate that provides a rapidly fermentable substrate. Ruminal fluid pH was affected by Co supplementation ($P < 0.10$), however the effect was not consistent with increasing dietary additions of Co to the control diet (Table 4). In the present study, lambs fed the control diet containing 0.0486 mg Co/kg DM, had higher pH than Co supplemented lambs at 3 h after feeding. Tiffany (2003) found that ruminal fluid pH was similar between cultures

supplemented with 0.05 or 1.0 mg Co/kg DM, but tended ($P < 0.05$) to be higher in cultures supplemented with 0.10 mg Co/kg DM. Total VFA (meq/100 ml RL) increased after feeding compared to before feeding. Co addition was increased the levels of VFA and peaked at 3-h post-feeding for the third group (Table 4). The results was reported that 0.7–1.0 mg Co/kg DM enhanced ruminal microbial activity and fermentation. 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. Studies have documented the importance of Co and vitamin B12 for the production of propionate by some rumen microorganisms (Tanner and Wolfe, 1988 and Tiffany et al., 2006). The higher molar proportions of propionate in Co supplemented steers during the finishing phase may explain the higher plasma glucose concentrations observed in steers receiving supplemental Co (Tiffany, 2003). Protozoa counts in rumen fluid for all groups before feeding was lower than post-feeding (Table 4). The range of protozoa counts was 35.9 to 53.1 and 41.2 to 64.1 at 0 h and 3 h of feeding, respectively. The present study showed that protozoa counts were significantly increased by supplementing Co. The changes in the concentration of ammonia nitrogen in the rumen before and after feeding are shown in Table 4. The range of NH_3-N was 9.01 to 10.32 and 12.79 to 19.34 at 0 h and 3h of feeding, respectively. In the present study no significant effects of Co supplementation on NH_3-N at 0 h, while lambs fed the control diet, had higher NH_3-N than Co supplemented lambs at 3 h of feeding. In contrast ammonia was not greatly affected by Co supplementation (Tiffany, 2003).

Table 4. Effects of dietary Co supplementation on pH, VFA, NH_3-N and protozoa counts in rumen liquor of Rahmany lambs

Items	Co supplemental levels (mg/kg DM)				L.S.D.
	0.0	0.35	0.70	1.0	
pH					
Before feeding	6.51^{b}	6.78^{a}	6.83^{a}	6.79^{a}	0.179
After feeding (3 h)	6.01^{a}	5.19^{b}	5.70^{a}	5.79^{a}	0.342
VFA (meq/100 ml RL)					
Before feeding	10.67^{a}	11.53^{a}	14.62^{a}	13.38^{a}	5.232
After feeding (3 h)	13.14^{b}	12.13^{b}	22.04^{a}	17.00^{ab}	6.330
NH_3-N (mg/100 ml RL)					
Before feeding	9.01^{a}	9.84^{a}	10.32^{a}	10.01^{a}	3.541
After feeding (3 h)	19.34^{a}	12.79^{b}	14.41^{b}	13.31^{b}	4.612
Protozoa counts					
Before feeding	35.9^{b}	36.9^{b}	53.1^{a}	39.5^{a}	12.97
After feeding (3 h)	47.3^{b}	41.2^{b}	64.1^{a}	42.0^{a}	13.72

VFA, volatile fatty acids; NH3-N, ammonia nitrogen

^{ab}Means within the same columns with different superscript are significantly different ($P<0.05$)

CONCLUSIONS

Lambs fed the control diet containing 0.0486 mg Co/kg DM, had lower growth rate, dry matter intake, nutrient digestibility and rumen activity than Co supplemented lambs. The level of dietary Co for Rahmany lambs is recommended to be 0.7–1.0 mg Co/kg DM.

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