Wayamba Journal of Animal Science – ISSN: 2012-578X; 2022, Volume 14, P 1904 – P 1912
First Published: October 06, 2022; Number 1663826596

EFFECT OF DIETARY COBALT SUPPLEMENTATION ON THE INTAKE, PERFORMANCE, WHOLE TRACT DIGESTIBILITY AND BLOOD METABOLITES IN DAIRY COWS FED TROPICAL FORAGES

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ABSTRACT

Dietary Co is essential to ruminal vitamin B_{12} formation by microbes. The majority of research related to dietary Co was based on temperate forages and feeding systems. Therefore, it is vital to study the effect of Co supplementation on tropical forages and feeding systems. Fifteen dairy cows (Jersey) weighing 283 ± 30.9 kg were allocated separately to three dietary treatments in a 3 × 3 Latin Square Design with 3 periods of 28 days duration, with 21 days of adaptation followed by 7 days of sampling. The dietary treatments (mg supplementary Co/kg DM) were: 0 (Control: CON), 0.2 (COL) and 0.4 (COH). The cows were fed a basal forage mix (75% Guinea grass; *Panicum maximum* and 25% of CO3; *Pennisetum purpureum*) *ad libitum*. Animals were individually stall tied and machine milked at 0600 and 1600 hours (h), and feed intake and milk yield were recorded daily during the sampling week. Faecal samples were collected during the final five days of the sampling period, and blood samples were collected by jugular venepuncture. Dry matter (DM) intake increased ($P<0.05$) with increasing dietary concentration of Co. In contrast, there was no effect ($P>0.05$) of dietary treatment on milk yield or fat content, with mean values of 4.01 kg/d and 41.3 g/kg, respectively. Similarly, there was no effect ($P>0.05$) of dietary treatment on whole tract digestibility of DM, N, or fibre. Mean plasma mineral concentrations of Co, Fe, Zn, Cu, Mn, and Se were 0.42, 36.9, 13.6, 15.5, 3.50, and 2.29 mol/L, respectively. Plasma glucose and vitamin B_{12} levels were not affected by the treatments and had mean values of 65.5 mg/dL and 746 pmol/L, respectively. In conclusion, the addition of Co increased intake, but did not alter performance, whole tract digestibility, plasma mineral levels, and glucose and vitamin B_{12} contents in Jersey cows managed and fed under tropical conditions.

Keywords: Cobalt, Dairy cow, Digestibility, Feed intake; Performance, Vitamin B_{12}

1. INTRODUCTION

Dietary Cobalt (Co) is essential for vitamin B_{12} synthesis by rumen microbes (Tiffany *et al.,* 2003). Low dietary Co concentrations can decrease microbial biosynthesis of vitamin B_{12} in the rumen which limits the availability for both microbial growth and metabolism by the host animal (Suttle, 2010). Mills (1981) reported that higher dietary concentrations of Co increased ruminal vitamin B_{12} synthesis, and Tiffany et al. (2006) reported that ruminal vitamin B_{12} synthesis increased *in vitro* when the dietary Co concentration was increased from 0.1 to 1.0 mg/kg DM. Similarly, several researchers have also reported that the vitamin B_{12} content of milk increased with the supplementation of Co in the diet of lactating dairy cows (O'Halloran *et al.,* 1961; Quick *et al.,* 1988). In contrast, NRC (2001) recommended that 0.11 mg Co/kg DM was sufficient for dairy cow metabolism and milk production.

Some microbes utilize vitamin B_{12} in pathways associated with propionate metabolism (Chen and Wolin, 1979), whereas others use vitamin B_{12} as an important growth factor (Tanner and Wolfe, 1988). About 13% of dietary Co is incorporated into vitamin B_{12} depending on the quantity (Marston et al., 1961; Somers and Gawthorne, 1969) and nature (Sutton and Elliot, 1972) of the diet, and Smith and Marston (1970) reported that about 5% of vitamin B_{12} leaving

the rumen is absorbed in the small intestine. Some studies have also suggested that Co may improve fibre digestion in the rumen by acting as a divalent cation (Lopez-Guisa and Satter, 1992). The effectiveness of supplemental Co on animal health and performance is, however, dependent on the biological availability of Co and vitamin B_{12} not only to ruminal micro-organisms but ultimately to the host animal (Ammerman *et al.,* 1982). The current Co recommendations for dairy cows are based on experiments that were conducted predominately on sheep (Smith and Marston, 1970; Hedrich *et al.,* 1973; Bigger *et al.,* 1976). However, most of these early estimations of the minimum Co requirement lacked a sufficient range of dietary Co concentrations to provide a valid statistical analysis, or have been obtained from animals producing well below industry standards (Stangle *et al.,* 2000. Also, most of these studies were undertaken using temperate forages, and studies conducted using tropical forages fed to animals managed under tropical conditions are lacking. The hypothesis was that added dietary cobalt will improve vitamin B_{12} status, diet digestibility, and performance in lactating dairy cows when fed tropical forages and the objectives of the current study were to evaluate the effects of Co supplementation on the production performance, diet digestibility, and vitamin B_{12} status of dairy cows fed tropical forages and managed under tropical conditions.

2. MATERIALS AND METHODS

2.1. Animals, Experimental Design and Procedures

Ethical clearance for this study was obtained from the Faculty Ethics Review Committee, Faculty of Livestock, Fisheries, and Nutrition, Wayamba University of Sri Lanka, and Senate Research and Higher Degree Committee.

The study was undertaken at the Galpokuna farm of the National Livestock Development Board of Sri Lanka (Latitude: 7º 27' 53N, Longitude: 79º 59' 25E) from April 2017 to July 2017. Fifteen Jersey cows (4 multiparous and 11 primiparous) were on average 104 ± 19 days post calving, yielding 5.84 ± 1.72 kg/day of milk and weighing 284 ± 32 kg with a body condition score of 2.74 ± 0.17 (Ferguson et al., 1994) were randomly allocated to one of the three dietary treatments in a 3 × 3 Latin square design based on recordings of production performance obtained in the week prior to the start of the study. Each period consisted of 21 days of adaptation to the dietary treatments, followed by 7 days of sampling.

All cows were fed the same basal forage mix [75% of Guinea grass (*Panicum maximum*) and 25% of CO3 (*Pennisetum purpureum*) DM basis] *ad libitum,* and the dry matter intake (DMI) was calculated as the difference between the daily amount offered and daily refusals. The cows were supplemented with 200 g of ground standard dairy concentrate feed (Table 1) as a carrier once daily to supply the following treatments:

CON : No added Co

COL : Additional 0.2 mg of added Co/kg total DM

COH : Additional 0.4 mg of added Co/kg total DM

Before each feeding, the concentrates were mixed well to avoid the sedimentation of Co in the feed.

Table 1. Ingredient composition of cattle feed

Ingredient	Inclusion level (g/kg DM)
Maize	250
Coconut poonac	150
Rice bran	100
Dhal kernel	150
Palm kernel	150
Black gram	100
Soya bean meal	50
Cattle premix	50

In addition, all cows received dairy cow concentrate feed (1.8 kg/day) during milking in two equal meals. Cows were housed and tied throughout the study in an open-air house that had a separate feeding alley.

On the second and sixth day of each week two forage samples were collected; one sample was oven dried at 105 ºC to determine the DM and the ratio of the forages adjusted to the desired level, whilst the second sample was stored at -20 ºC for subsequent analysis. Animals were machine milked using a single bucket milking machine (BMS1-DVP170, De Laval, Sweden) twice daily at approximately 0600 and 1600 h. During the 7-day sampling period, milk production was recorded at each milking, and samples were obtained during the morning and evening milking for constituent analysis. Blood samples were collected over four days during the final week of each period by venepuncture at four different times (0700, 0900, 1100, and 1300 h). The plasma was separated by centrifugation (Orto Alresa Digicen 21, Spain) at 1300 × *g* for 15 Minutes (min), and the plasma was pipetted off using disposable pipettes and stored in Eppendorf tubes at -18 ºC (BD19B, Haier, China) until analysis. Faecal grab samples were taken from all cows over five days in the 4th week of each period with two samplings at 1000 h and 1600 h. Samples were stored at -20 ºC before analysis. Animals were weighed and body conditions were scored during the week before the study beginning and on the final day of each sampling period (Ferguson *et al.,* 1994).

2.2. Chemical Analysis

Chemical analysis was carried out at the laboratory of the Department of Livestock and Avian Sciences, Wayamba University of Sri Lanka. The DM content of the diets and faecal samples was determined according to the Association of Official Analytical Chemists (AOAC, 2012). Samples of dried feed and

faecal samples were analysed according to AOAC (2012) for ash (942.05) and crude protein (CP; 988.05) using a Gerhardt (UK) system. Ether extract was determined by solvent extraction using a fat analyser (SDR148, Velp, Italy), neutral detergent fibre (NDF) content according to the method of Van Soest et al. (1991), and acid detergent fibre (ADF) content according to Goering and Van Soest, (1970). Whole tract digestibility was calculated using the method described by Van Keulen and Young (1977) using acid insoluble ash as an internal marker. Frozen plasma was thoroughly defrosted at room temperature and vortexed prior to analysis. Then, 100 µL of thawed plasma was micro pipetted into a 50 mL volumetric flask and made up to 50 mL with deionized water. Prior to analysis stock mineral solutions were prepared to obtain a calibration curve for each individual element. Blood, feed and forage samples were then analysed by atomic absorption spectrophotometry (Thermo Scientific, UK). During feed and forage mineral analysis, initially, a 0.5 g of dried and ground (using a mixture grinder, Model SL 4 MIXGR- N and sieved using a 0.5 mm sieve) forage mixture and cattle feed samples were accurately weighed into the digestion tubes with the use of an analytical balance. Then 10 mL of conc. HNO_3 (69%) (Sigma-Aldrich) was added into the digestion tubes and left 10 minutes before being sealed properly. After that, all samples were digested for 1 h and 15 min at 200 ºC using a MARS 6 microwave digestion unit (Model-MARS 6, USA, 240/50 Digested samples were filtered through filter papers (Whatman 1- pore size: 11 µm) into a 50 mL volumetric flask and then filled up to the mark with deionized water. The procedure was conducted in triplicate for each sample. Finally, all the samples were transferred into 50 mL falcon tubes, sealed with lids, and stored in a refrigerator at 5 ºC until analysed.Ca, Mg, Na, K, P, Fe, Cu, Zn, Co, and Mn concentrations in digested samples were analysed using an ICE 3000 series Thermo Scientific Atomic Absorption Spectrometer.

Plasma glucose was measured using a glucose meter (Freestyle optimum glucose meter, UK). Plasma samples were analysed for vitamin B_{12} by Durdans Laboratory, Colombo 10, Sri Lanka.

2.3. Statistical Analysis

Milk yield parameters, live weight, body condition score, and blood parameters were analysed (at CL 95%) by ANOVA as a Latin Square Design in Minitab ver. 18.

Table 2. Chemical composition and mineral concentration of the forage mix and cattle feed

Chemical analysis	Forage mix	Cattle feed
DM, g/kg	176	907
OM, g/kg DM	946	944
Ash, g/kg DM	53.4	55.8
CP, g/kg DM	93.4	162
Fat, g/kg DM	34.6	47.1
NDF, g/kg DM	686	413
ADF, g/kg DM	441	271
Hemicellulose, g/kg DM	244	143
Macro minerals, g/kg DM		
Na	0.28	3.18
Mg	0.72	1.13
P	3.02	5.32
K	8.33	12.5
Ca	1.21	1.70
Micro minerals, mg/kg DM		
Mn	59.7	109
Fe	63.0	127
Co	0.03	0.04
Cu	5.78	12.6
Zn	12.9	31.0

3. RESULTS

3.1. Forage Mixture and Cattle Feed Analysis

The chemical composition and mineral concentration of the forage mix and dairy cow concentrate feed are provided in Table 2. The DM, OM, CP, fat and ash

content of the lactating cattle feed was higher than the forage mixture. In contrast, the NDF, ADF, and hemicellulose content (determined as NDF – ADF content) was higher in the forage mix. The macro mineral (Na, Mg, K, P, and Ca) and micro minerals (Mn, Fe, Co, Cu, and Zn) were higher in the lactating cattle feed than in the lactating forage mix.

3.2. Performance and Digestibility

Animals receiving the high dietary Co content (COH) had a higher (P<0.05) DMI than those receiving CON or COL (Table 3). However, treatment had no effect (P>0.05) on BCS, BW or milk yield. Similarly, dietary treatment did not affect (P>0.05) milk fat, protein, or solid non-fat (SNF) content. There was an effect of treatment on organic matter, nitrogen, NDF and ADF intake, which were higher (P<0.05) in cows when COH than in the Control, with COL being intermediate. Similarly, the amount of DM, OM, NDF, and hemicellulose digested was higher (P < 0.05) in cows when fed COH than COL or the Control. However, the digestibility of DM, OM, ADF, NDF, and nitrogen were not affected (P>0.05) by treatment, with mean values of 0.72, 0.74, 0.47, and 0.67 kg/kg respectively.

3.3. Effect of Treatment on Plasma Mineral, Glucose and Vitamin B_{12} Concentration

There was no effect (P > 0.05) of dietary treatment on plasma mineral concentration with mean values of 1.31, 1.04, 3.02, 3.76 mmol/L for Ca, Mg, P, K, respectively, and 0.42, 36.9, 13.7, 15.5, 3.50, and 2.29 µmol/L for plasma Co, Fe, Zn, Cu, Mn or Se, respectively (Table 4). Further, there was no effect of treatment (p=0.127) on plasma glucose concentration, with a mean value of 3.66 mmol/L (Fig. 1.), or plasma vitamin B_{12} concentration, with mean values of 769, 701, and 770 pmol/L for cows fed CON, COL or COH, respectively (Fig. 2.).

4. DISCUSSION

4.1. Performance

Kincaid and Socha (2007) reported no effect of dietary Co concentration (0.15, 0.89, or 1.71 mg/kg DM) on milk yield or composition in early lactation dairy cows. Similarly, Kincaid et al. (2003) reported that dietary supplementation with Co did not affect the BCS, DMI, or live weight of dairy cows. Similarly, Weerathilake et al. (2018) observed no effect of dietary Co or vitamin B_{12} supplementation or injection of vitamin B_{12} on DMI, BCS or live weight in high-yielding Holstein dairy cows. Akins et al. (2013) also did not observe an effect of Co supplementation on DMI, BCS, body weight, or milk yield in lactating cows. However, there was an effect of dietary Co supplementation on DMI in the current study, with the intake of cows when supplemented with 0.4 mg Co/kg DM being 0.46 kg DM/d higher than when they received the control. The mean milk production in Sri Lanka was reported as 2 kg/cow/day in 2009 and it increased to 3.6 kg/cow/day in the year 2014 (DAPH, 2010, 2015). In contrast to the current study, previous studies have contained considerably higher concentrations of Co in the basal ration, which often exceeded the 0.11 mg/kg DM recommended by NRC (2001) whereas in the current study the level in the control diet was only 0.031 mg/kg DM. The lower milk yield of the dairy cows used in the current study may also have reduced the requirement for vitamin B_{12} for the metabolism of propionate and methionine and may explain the lack of response in milk performance.

4.2. Whole Tract Digestibility

Increasing the dietary Co concentration from a sub-optimal level of 0.086 to 0.336, 0.586, 0.836, or 1.086

Table 3. Mean dry matter intake, body condition score, body weight, milk yield and milk composition of low yielding dairy cows fed CON=control diet, COL= control diet with 0.2 mg Co /kg DM and COH= control diet with 0.4 mg Co /kg DM

Parameter	CON	COL	COH	s.e.d	Significance
DMI, kg/d	8.59[a]	8.83[b]	9.05[c]	0.038	0.0001
Body weight, kg	284	286	288	4.783	0.952
BCS	2.78	2.75	2.78	0.017	0.677
Milk yield, kg/d	3.99	4.03	4.20	0.179	0.882
Milk composition, g/kg					
Fat	42.1	38.8	42.9	0.814	0.636
Protein	36.9	34.7	36.3	0.509	0.184
SNF	90.2	88.5	90.2	0.639	0.452
Yield, kg/d					
Fat	0.16	0.15	0.17	0.007	0.502
Protein	0.15	0.14	0.15	0.006	0.63
SNF	0.36	0.36	0.38	0.016	0.787

Values that do not share a common superscript within the raw are not different at P > 0.05

Table 4. Mean digestibility of dry matter digestibility, organic matter, crude protein, neutral detergent fibre, acid detergent fibre and hemicellulose of low yielding dairy cows fed CON = control diet, COL = control diet with 0.2 mg Co /kg DM or COH = control diet with 0.4 mg Co /kg DM

	CON	COL	COH	s.e.d	Significance
Dry matter (kg/day)					
Intake	8.59[a]	8.83[b]	9.05[c]	0.038	<0.001
Faecal output	3.43	3.58	3.60	0.041	0.165
Digested	5.16[a]	5.25[b]	5.45[c]	0.046	0.034
Digestibility (kg/kg)	0.60	0.59	0.60	0.030	0.779
Organic matter (kg/day)					
Intake	8.13[a]	8.36[b]	8.57[c]	0.036	0.001
Faecal output	3.17[a]	3.31[b]	3.33[c]	0.038	0.035
Digested	4.96	5.05	5.23	0.043	0.109
Digestibility (kg/kg)	0.60	0.60	0.61	0.004	0.139
Nitrogen (g/day)					
Intake	14.45[a]	14.80[b]	15.12[c]	0.054	0.001
Faecal output	6.04	6.31	6.35	0.072	0.165
Digested	8.41	8.48	8.76	0.075	0.141
Digestibility (kg/kg)	0.58	0.57	0.57	0.004	0.760
NDF (kg/day)					
Intake	5.36[a]	5.52[b]	5.67[c]	0.116	<0.001
Faecal output	1.50	1.56	1.58	0.119	0.165
Digested	3.86[a]	3.96[b]	4.09[c]	0.150	<0.001
Digestibility (kg/kg)	0.72	0.72	0.72	0.021	0.767
ADF (kg/day)					
Intake	3.48[a]	3.59[b]	3.68[c]	0.075	<0.001
Faecal output	2.01	2.10	2.11	0.159	0.165
Digested	1.47	1.49	1.57	0.163	0.217
Digestibility (kg/kg)	0.42	0.41	0.43	0.044	0.767
Hemicellulose (kg/day)					
Intake	1.91[a]	1.97[b]	2.03[c]	0.042	<0.001
Faecal output	0.51	0.53	0.54	0.041	0.165
Digested	1.40[a]	1.44[b]	1.49[c]	0.052	<0.001
Digestibility (kg/kg)	0.73	0.73	0.73	0.020	0.765

mg Co/kg DM has been shown to increase whole tract digestibility quadratically in sheep, being highest at 0.586 mg/kg DM (Wang et al., 2007). Castagnino et al. (2016) reported that vitamin B_{12} is an essential growth factor for efficient microbial metabolism, with the ruminal synthesis of vitamin B_{12} and subsequent flow to the duodenum being significantly related to the rate of microbial protein synthesis in the rumen. This has been suggested to improve fibre digestion in the rumen (Lopez-Guisa and Satter, 1992). Further, Kadim et al (2003) reported that low levels (0.038 to 0.063 mg/kg DM) of dietary Co in goats resulted in a lower apparent nutrient digestibility coefficient compared to those supplemented with vitamin B_{12}. In contrast, Kisidayova et al (2001) reported that increased Co intake (0.2, 0.4 or 0.8 mg/kg DM) had

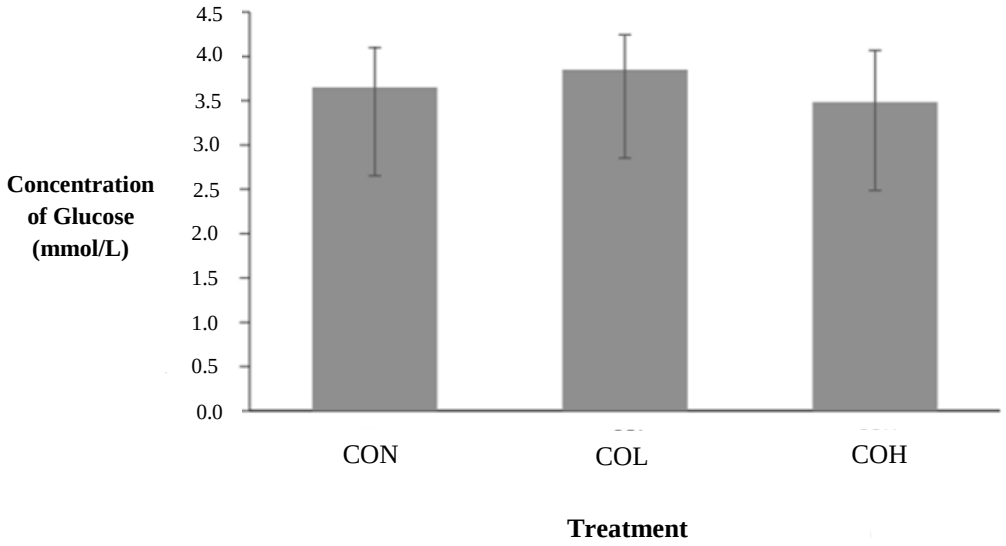

Figure 1. Mean plasma glucose concentration in low yielding dairy cows fed CON = control diet, COL = control diet with 0.2 mg Co/kg DM or COH = control diet with 0.4 mg Co/kg DM. Error bars represents the standard errors.

no effect on the digestibility of DM, OM, NDF, or ADF. In the current study, there was also no effect ($P>0.05$) of Co supplementation on DM, OM, NDF, ADF, or nitrogen digestibility, a finding in agreement with that reported by Weerathilake et al (2018) in high-yielding, Holstein dairy cows managed under temperate conditions in the United Kingdom, although the total amount of NDF and hemicellulose digested was increased, mainly due to the effects of Co on increasing DM intake.

4.3. Plasma Mineral Concentration

The mean plasma concentrations of Mg, P, Fe, Zn, and Cu are similar to those reported by Weerathilake et al. (2018), whereas plasma Co and Se were higher in the current study (Table 5). In contrast, the plasma concentration of Ca in the current study was lower than the value observed by Valldecabres et al. (2021). Results from the current study showed that there was no effect of total dietary Co concentrations of 0.03, 0.23, or 0.43 mg/kg DM on the plasma concentration of minerals (Ca, Mg, P, K, Co, Fe, Zn, Cu, and Se). In agreement, Kincaid et al (2003) reported that there was no effect of dietary Co supplementation on serum or whole blood concentrations of Co, Cu, Zn, and Fe, whilst plasma concentrations of Co were increased following supplementation with 0.2 mg Co/kg DM or injection of vitamin B_{12}, but not by feeding vitamin B_{12} in the study of Weerathilake et al. (2018).

4.4. Plasma Glucose

Plasma glucose concentration was not affected in the current study, possibly due to the adequate plasma concentration of vitamin B_{12}, and that plasma glucose concentration is under strong homeostatic control. Akins et al. (2013) also reported that there was no effect of Co supplementation on plasma glucose concentrations with a mean value of 60.4 mg/dL, which is similar to the 65.5 mg/dL reported here. Further, Weerathilake et al. (2018) reported a similar value (61.02 mg/dL for the plasma glucose) which was unresponsive to dietary supplementation of Co or vitamin B_{12}, or injection of vitamin B_{12}.

4.5. Plasma Vitamin B_{12}

The mean plasma concentration of vitamin B_{12} in cows fed any of the dietary treatments in the current study of 747 pmol/L study was well above the threshold of 150 pmol/L reported by Duplessis et al. (2017) above which there is little further benefit to milk performance in dairy cows. Akins et al. (2013) also reported there was no effect of dietary addition of Co on plasma vitamin B_{12} concentration. Similarly, Kincaid et al. (2003) reported that there was no effect of treatment on serum vitamin B_{12} in dairy cows with a mean value of 1.86 ng/mL for Co supplementation levels of 0.37, 0 68, or 1.26 mg/kg DM respectively. The findings of current study are therefore in agreement and showed no effect of dietary Co concentrations of 0.03, 0.23 or 0.43 mg/kg DM respectively, despite the control diet being predicted to be deficient in Co according to NRC (2001). In contrast, Wang et al. demonstrated that plasma vitamin B_{12} concentrations of 202, 1688, 2225 and 2386 pmol/L for Co supplementation levels 0.086, 0.336, 0.836 and 1.086 mg/kg DM respectively in lambs.

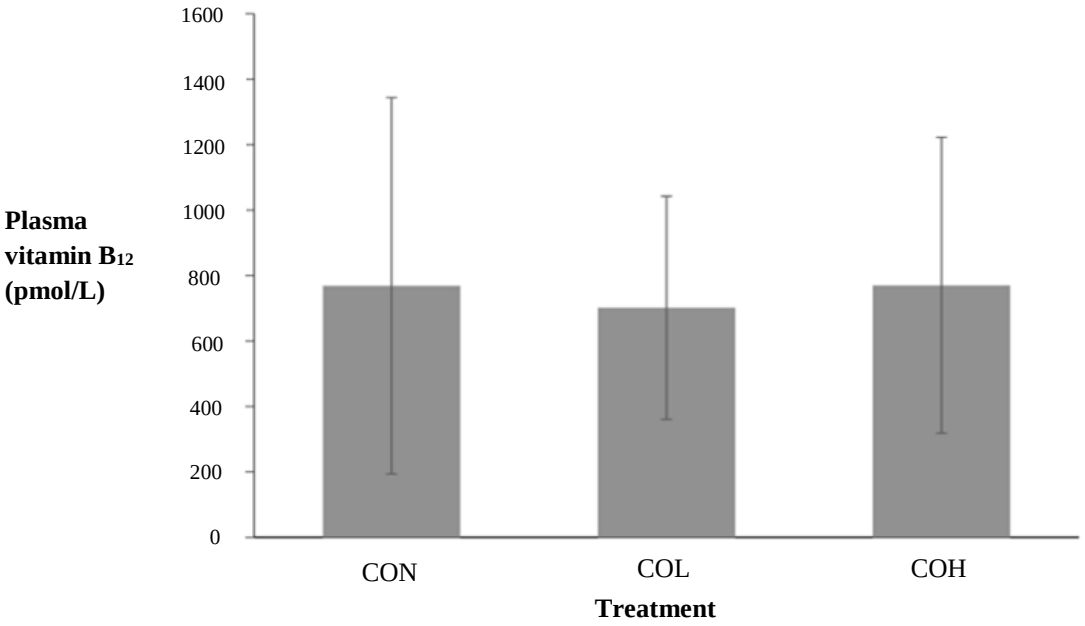

Figure 2. Mean plasma vitamin B_{12} level of low yielding dairy cows fed a control diet (CON), control diet with 0.2 mg Co/kg DM (COL) or the control diet with 0.4 mg Co/kg DM (COH). Error bars represent the standard errors.

Table 5. Mean plasma mineral concentration of low yielding dairy cows fed CON = control diet, COL = control diet with 0.2 mg Co/kg DM or COH = control diet with 0.4 mg Co/kg DM

Plasma mineral	CON	COL	COH	s.e.d	Significance
Ca mmol/L	1.30	1.17	1.46	0.062	0.154
Mg mmol/L	1.08	1.00	1.05	0.074	0.914
P mmol/L	3.04	3.05	2.98	0.024	0.453
K mmol/L	4.10	3.69	3.50	0.232	0.573
Co µmol/L	0.38	0.42	0.45	0.021	0.381
Fe µmol/L	38.9	37.5	34.4	2.470	0.757
Zn µmol/L	14.3	13.8	12.9	0.393	0.388
Cu µmol/L	16.2	14.8	15.6	0.320	0.192
Mn µmol/L	3.56	3.47	3.49	0.023	0.322
Se µmol/L	2.18	2.36	2.33	0.156	0.890

5. CONCLUSION

It is concluded that the addition of Co to the diet increases intake and the quantity of fibre digested in the whole tract, but does not affect performance, whole tract digestibility, plasma minerals, glucose or vitamin B_{12} concentration in low-yielding dairy cows managed and fed under tropical conditions. However, the short-term nature of the current study did not permit changes in live weight or BCS to be accurately determined, and a longer-term study is required to determine whether the increase in intake and the amount of fibre digested could be translated into a change in body energy reserves.

Acknowledgement

The authors would like to acknowledge National Livestock Development Board for the support given in providing the study location and University Grant Commission Research Grant for providing financial assistance for this study.

Funding

"This research received The University Grant Commission Research Grant"

Conflict of interest

The authors declare no conflict of interest.

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