The influence of an increased cobalt supply on ruminal parameters and microbial vitamin B₁₂ synthesis in the rumen of dairy cows

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The aim of the study was to examine the effects of an elevated dietary cobalt supply to dairy cows on rumen fermentation parameters and microbial vitamin B₁₂ synthesis in the rumen. Five lactating dairy cows fitted with a ruminal and a duodenal cannula were subsequently fed either a ration containing only the native cobalt content (0.17 mg Co/kg DM) or a ration supplemented with cobalt sulphate (0.29 mg Co/kg DM). The pH-value, the ammonia concentration as well as the concentration and the molar proportions of short chain fatty acids in the rumen were not significantly influenced by feeding the ration with the higher cobalt content. While there was no difference in microbial protein flow, the cobalamin flow at the duodenum was significantly elevated in supplemented animals (3.67 ± 0.69 vs. 8.63 ± 2.22 mg B₁₂/d). The efficiency of cobalt utilisation for ruminal vitamin B₁₂ synthesis was calculated to be 7.1 ± 1.3% for the unsupplemented and 9.5 ± 2.4% for the supplemented ration. Further investigation has to prove if there are any benefits for cows resulting from the elevated cobalamin synthesis measured, caused by feeding higher amounts of dietary cobalt.

Keywords: cobalt; vitamin B₁₂ synthesis; ruminal parameters; dairy cows

1. Introduction

Cobalt (Co) is known to be an essential trace element for humans and animals, but there are differences between species in which form cobalt has to be supplied to the diet. While most animals (with the exception of animals with caecotrophy or coprophagy) require cobalt in form of vitamin B₁₂ (cobalamin), adult ruminants need cobalt only as trace element, because of the ability of rumen microbes to synthesise vitamin B₁₂ in the presence of cobalt (McDowell 2003). The recommendations for cobalt requirement in dairy cows vary between 0.10 mg/kg DM (NRC 2001) and 0.20 mg/kg DM (GfE 2001).

Vitamin B₁₂ synthesis is accomplished by only a few species of micro-organisms such as some strains of bacteria, blue algae and yeast (Friedrich 1987; Smith 1997). Therefore, in higher organisms such as plants and animals vitamin B₁₂ synthesis is not possible and ruminants are entirely dependent on its microbial synthesis in the rumen. In young ruminants up to the age of 6–8 weeks the rumen is neither completely developed nor ready for vitamin B₁₂ synthesis yet (McDowell 2000). Therefore, pre-ruminant calves and lambs require a dietary source of cobalamin, such as colostrum, milk or milk replacer.
Among the multitude of bacteria-strains in the rumen, only a few of them are able to synthesise vitamin B$_{12}$ and vitamin B$_{12}$ analogues (Dryden et al. 1962). For example *Propionibacterium shermanii* is one of the best cobalamin synthesisers in the rumen, which is also used in the industry to produce vitamin B$_{12}$ (Friedrich 1987).

The amount of synthesised cobalamin depends on the composition of the ration (relation of roughage to concentrate; fibre content) and the dry matter intake (Smith and Marston 1970; Walker and Elliot 1972; Hedrich et al. 1973). This might be one reason for changes in microbial population patterns while feeding higher proportions of concentrate (Walker and Elliot 1972; Halpin et al. 1984). Nevertheless, the most important factor for the production of vitamin B$_{12}$ seems to be the concentration of cobalt in the diet. But only little is known about the efficiency of cobalamin production in the rumen. While Smith and Marston (1970) found that Co was converted to cobalamin more efficiently in Co-depleted sheep, Singh and Chhabra (1995) demonstrated for cattle a positive correlation between the amount of dietary cobalt and the vitamin B$_{12}$ content in the ruminal fluid. Because of these contrary results the following investigation in lactating dairy cows was conducted to test the influence of an increased cobalt supply on microbial vitamin B$_{12}$ synthesis as well as on rumen parameters like pH value, short chain fatty acid concentrations and their molar proportions, ammonia concentration, and microbial protein synthesis.

2. Material and methods
2.1. Experimental design and procedure

Five lactating dairy cows (mean body weight 678 ± 45 kg; mean milk yield 19.6 ± 3.7 kg, mean FCM 23.7 ± 4.4 kg), fitted with large rumen cannulae (inner diameter: 100 mm) and T-shaped cannulae (inner diameter: 20 mm) in the proximal duodenum close to the pylorus, were allotted to an incomplete cross-over design.

Animals’ ration consisted of grass silage (10 kg DM per animal and day), 3 kg concentrate I (composition per kg: 350 g soybean meal, 200 g barley, 200 g wheat, 210 g dried beet pulp, 20 g soybean oil, 20 g minerals) and 1 kg concentrate II (composition per kg: 305 g soybean meal, 174 g barley, 174 g wheat, 180 g dried beet pulp, 17 g soybean oil, 150 g minerals) with or without a cobalt supplement. The mineral premix contained per kg: 160 g Ca, 80 g P, 100 g Na, 30 g Mg, 700,000 IE vitamin A; 80,000 IE vitamin D$_{3}$, 400 mg vitamin E, 950 mg Fe, 770 mg Cu, 4000 mg Mn, 6000 mg Zn, 50 mg I and 50 mg Se. With the exception of cobalt, minerals were fed according to the recommendations of GfE (2001). Nutrient composition of feedstuffs is shown in Table 1. For the experimental group cobalt was added as cobalt-sulphate (CoSO$_{4}$·7 H$_{2}$O) to concentrate II.

The experiment comprised two periods of 21 days each, where an unsupplemented ration and a ration supplemented with cobalt were fed subsequently. After an adaptation period of 12 days, for determination of the pH value and the ammonia concentration, ruminal fluid was collected every 30 min (starting 30 min after feeding – until 300 min after feeding) through rumen cannulae using a hand vacuum pump. For analysis of short chain fatty acids in ruminal fluid, on three subsequent days samples were taken at 3 h after the start of feeding.

After 16 days of adaptation to the ration, duodenal chyme was collected applying a method published by Rohr et al. (1979). Chromium oxide mixed with wheat flour (1:4) was used as a flux marker according to the method described by Ørskov et al. (1971). The marker was applicated into the rumen in two portions per day (in total 100 g/d) starting 10 days before sampling of duodenal chyme. One day before the start of sampling and during the collection period, marker was given in four portions per day.
Four samples of 100 ml each were collected from the duodenal T-shaped cannula every two hours on five days running. The sample with the lowest pH was immediately frozen and pooled on a daily basis for analysis of dry matter and nutrient contents. The sample with the second lowest pH was also frozen immediately and pooled on the basis of the whole collecting period (five days). These samples were used to determine the cobalt and vitamin B12 content in duodenal chyme. In comparative studies, this spot-sampling procedure has shown only small differences in flow as compared to total collection (Rohr et al. 1984).

2.2. Analysis

Analysis of dry matter and nutrient content in feedstuffs and duodenal chyme were performed according to the official German standard procedures (VDLUFA 2004). ADF and NDF were expressed on an ash free basis (ADFom and NDFom). Kjeldahl nitrogen was analysed in thawed chyme samples whereas all other analyses were performed by using freeze-dried and ground duodenal digesta samples.

A modified Conway-method was used to measure ammonia-N in the digesta (Voigt and Steger 1967). Short chain fatty acids in ruminal fluid were determined by gas chromatography (Hewlett Packard 5580, Avondale, PA, USA) as described by Geissler et al. (1976).

The amounts of microbial N in duodenal contents were estimated using the near-infrared reflectance spectroscopy (NIRS, Lebzien and Paul 1997). Lebzien and Paul (1997) calibrated and validated this method using samples from duodenally fistulated cows receiving diets consisting of a wide range of basic feedstuffs and concentrates. Forage-to-concentrate ratio varied between 100:0 and 39:61, digestible OM intakes ranged from 4.7–13.3 kg per day and concentrates were given twice to four times daily. The portion of microbial nitrogen in the duodenal samples was estimated by 15N measurements and NIRS. No effect of ration composition on the precision of the NIRS method could be stated.

The Cr2O3 in marker and digesta samples was determined by atomic absorption spectrometry according to Williams et al. (1962).

Cobalt content in the duodenal chyme was measured in the pooled samples (5 d of sampling). For the determination, chyme was dried, ashed at 550°C and afterwards taken

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### Table 1. Nutrient composition and Co-content of feedstuffs used in the experiment.

<table>
<thead>
<tr>
<th></th>
<th>Dry matter [g/kg]</th>
<th>Organic matter [g/kg DM]</th>
<th>Ash [mg/kg DM]</th>
<th>Crude protein [g/kg]</th>
<th>Ether extract [g/kg]</th>
<th>NDF</th>
<th>ADF</th>
<th>Co [mg/kg DM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grass silage</td>
<td>626</td>
<td>892</td>
<td>108</td>
<td>209</td>
<td>38</td>
<td>532</td>
<td>274</td>
<td>0.09</td>
</tr>
<tr>
<td>Concentrate I</td>
<td>886</td>
<td>942</td>
<td>58</td>
<td>240</td>
<td>24</td>
<td>210</td>
<td>96</td>
<td>0.31</td>
</tr>
<tr>
<td>Concentrate II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without Co*</td>
<td>881</td>
<td>834</td>
<td>166</td>
<td>221</td>
<td>14</td>
<td>199</td>
<td>88</td>
<td>0.62</td>
</tr>
<tr>
<td>With Co</td>
<td>883</td>
<td>828</td>
<td>172</td>
<td>212</td>
<td>12</td>
<td>201</td>
<td>82</td>
<td>2.56</td>
</tr>
<tr>
<td>Whole ration</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without Co</td>
<td>700</td>
<td>899</td>
<td>101</td>
<td>217</td>
<td>33</td>
<td>439</td>
<td>223</td>
<td>0.17</td>
</tr>
<tr>
<td>With Co</td>
<td>700</td>
<td>898</td>
<td>102</td>
<td>216</td>
<td>33</td>
<td>439</td>
<td>222</td>
<td>0.29</td>
</tr>
</tbody>
</table>

*Only native Co-content.
up with 10 ml HCl and again with 10 ml HNO₃. Measurement was done by ICP-Analysis (ICP-MS; isotope mass 59).

Vitamin B₁₂ concentration was analysed in fresh and also in dried duodenal chyme. For the measurement in fresh chyme, a commercial test kit (SimulTRAC-SNB Radioassay Kit Vitamin B₁₂) was used. For determination of cobalamin in dried chyme, the pooled samples were frozen, ground (Ø 0.5 mm) and analysed by using a commercial test system which was usually established for food samples analysis (r-biopharm, RIDASCREEN® Vitamin B₁₂, No. R2101). The test system is based on an antigen-antibody reaction and was modified for dried chyme.

2.3. Statistical analysis

Data are reported as means and standard deviation. Means were compared using Tukey’s multiple comparison test. All analysis were performed by use of computer software SAS (version 6.12 for windows). Values of $p < 0.05$ were considered to be significant.

3. Results

During the experimental period all cows consumed the whole ration of 13.5 kg DM (12.2 kg OM) per day without any refusals. Except one cow with a mastitis catarrhalis (Romke) the animals remained healthy. Mean daily milk yield amounted to 17.3 ± 2.7 kg (19.6 ± 3.7 kg FCM) in the first period and to 16.3 ± 3.4 kg (18.6 ± 5.1 kg FCM) in the second period without any differences between treatments. Milk fat, protein and lactose concentrations as well were not affected by cobalt treatments.

The control ration (unsupplemented) contained 0.17 mg Co/kg DM and the supplemented ration 0.29 mg Co/kg DM, corresponding to a daily cobalt intake of 2.27 mg (controls) and 3.97 mg (supplemented), respectively. The cobalt content in feedstuffs is shown in Table 1.

3.1. Rumen parameters

The pH-values in the rumen of unsupplemented and supplemented animals for the first 300 min after feeding are shown in Figure 1. Although the pH values in the ruminal fluid

Figure 1. pH-value in ruminal fluid of control group and Co-supplemented cows in dependence of time after feeding (Means ± SD; $n = 5$).
of supplemented cows were slightly higher than in controls, the differences between treatments were not significant. With both treatments the pH-values were lowest 90 min after the start of feeding.

Corresponding to the pH-value measured in ruminal fluid, the concentration of short chain fatty acids in the rumen of supplemented cows were numerically ($p > 0.05$) lower than in the rumen of unsupplemented cows. Nevertheless, no differences between treatments could be detected with regard to the molar proportions of short chain fatty acids (Table 2).

Regarding to cobalt content in the ration there was no significant effect on ammonia-concentration in ruminal fluid. Directly after feeding, microbial degradation of protein and non protein nitrogen started, indicated by increased ammonia concentration (Figure 2). For both treatments, the highest concentration of ammonia was measured 150–180 min after the beginning of feeding, Figure 2.

Table 2. Concentrations of short chain fatty acids and their molar proportions in the ruminal fluid of control and supplemented animals 3 h after feeding (means ± SD, $n = 5$).

<table>
<thead>
<tr>
<th></th>
<th>Control (0.17 mg Co/kg DM)</th>
<th>Co-supplemented (0.29 mg Co/kg DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of short chain fatty acids [mmol/l]</td>
<td>120 ± 10</td>
<td>110 ± 12</td>
</tr>
<tr>
<td>Short chain fatty acids [molar proportions, %]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetic acid (C2)</td>
<td>65.9 ± 0.3</td>
<td>65.8 ± 0.5</td>
</tr>
<tr>
<td>Propionic acid (C3)</td>
<td>17.4 ± 0.7</td>
<td>17.4 ± 1.0</td>
</tr>
<tr>
<td>Iso-butyric acid (C4iso)</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>Butyric acid (C4)</td>
<td>12.9 ± 0.4</td>
<td>12.9 ± 0.3</td>
</tr>
<tr>
<td>Iso-valeric acid (C5iso)</td>
<td>1.4 ± 0.5</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>Valeric acid (C5)</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>C2:C3-ratio</td>
<td>3.79 ± 0.18</td>
<td>3.78 ± 0.24</td>
</tr>
</tbody>
</table>

Figure 2. Ammonia concentration in ruminal fluid of control group and Co-supplemented cows in dependence of time after feeding (Means ± SD; $n = 5$).
3.2. Microbial protein synthesis in the rumen

Vitamin B₁₂ is an important factor for growing bacteria (especially for some strains of propionibacteria). Therefore, a deficiency of cobalamin may decrease microbial protein synthesis in the rumen. On the other hand, an elevated supply may lead to higher microbial protein synthesis. Therefore, effects of an elevated cobalt supply on several parameters of microbial protein synthesis were tested.

Data for microbial protein (MP) synthesis, endogenous protein and utilisable crude protein \( \text{uCP} = (\text{NAN} \cdot 6.25) - \text{endogenous protein} \) are presented in Table 3. Although nutrients flow at the duodenum of supplemented animals seemed to be slightly lower than in controls, the differences did not prove to be significant. Therefore, increased dietary cobalt did not influence efficiency of microbial protein synthesis per kg fermented OM.

3.3. Concentrations of cobalt and vitamin B₁₂ in duodenal chyme

To get some information about the influence of cobalt supply on ruminal vitamin B₁₂ synthesis the daily cobalt intake and the flow of cobalt and vitamin B₁₂ at the duodenum have to be related (Table 4). In Table 4 the recovery rate of cobalt (% of intake) is shown as well.

Table 3. Nutrient flow at proximal duodenum after feeding cows an unsupplemented or a Co-supplemented ration \( (n = 5) \).

<table>
<thead>
<tr>
<th>nutrient</th>
<th>Control ( (0.17 \text{ mg Co/kg DM}) )</th>
<th>Co-supplemented ( (0.29 \text{ mg Co/kg DM}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (DM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[kg/d]</td>
<td>7.59 ± 0.78</td>
<td>7.53 ± 0.62</td>
</tr>
<tr>
<td>[% of intake]</td>
<td>56.1 ± 5.7</td>
<td>55.6 ± 4.6</td>
</tr>
<tr>
<td>Crude protein (CP)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[g/d]</td>
<td>2270 ± 0.29</td>
<td>2180 ± 0.23</td>
</tr>
<tr>
<td>[% of intake]</td>
<td>77.5 ± 10.4</td>
<td>74.8 ± 7.6</td>
</tr>
<tr>
<td>Organic matter (OM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[kg/d]</td>
<td>5.69 ± 0.55</td>
<td>5.68 ± 0.43</td>
</tr>
<tr>
<td>[% of intake]</td>
<td>46.7 ± 4.9</td>
<td>46.8 ± 3.6</td>
</tr>
<tr>
<td>Fermented OM#</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[kg/d]</td>
<td>9.25 ± 0.09</td>
<td>9.10 ± 0.19</td>
</tr>
<tr>
<td>[% of OM-intake]</td>
<td>75.9 ± 0.8</td>
<td>75.0 ± 1.6</td>
</tr>
<tr>
<td>Microbial N [% of non-ammonia-N]</td>
<td>67.5 ± 5.9</td>
<td>67.5 ± 5.2</td>
</tr>
<tr>
<td>Microbial protein</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[g/d]</td>
<td>1463 ± 281</td>
<td>1400 ± 238</td>
</tr>
<tr>
<td>[g/kg fermented OM]</td>
<td>158 ± 31</td>
<td>154 ± 26</td>
</tr>
<tr>
<td>[g/MJ ME]</td>
<td>10.2 ± 1.94</td>
<td>9.79 ± 1.63</td>
</tr>
<tr>
<td>Endogenous protein* [g/d]</td>
<td>171 ± 17.5</td>
<td>169 ± 13.8</td>
</tr>
<tr>
<td>Utilisable crude protein (uCP)° [g/d]</td>
<td>2239 ± 301</td>
<td>2148 ± 219</td>
</tr>
</tbody>
</table>

\( ^* \)Fermented OM = OM-intake – (OM-flow at the duodenum – microbial OM); Microbial OM = 11.8 \cdot \text{microbial N} (\text{Schafft 1983}); \( ^\circ \)22.5 g/kg DM flow at the duodenum (Brandt et al. 1980); \( ^\circ \)Utilisable crude protein = CP-flow at the duodenum – endogenous protein.
Although there were numerically differences between cobalt flows at the duodenum in individual animals, the mean recovery rate was about 93.4 ± 4.3% when feeding the control ration and 85.8 ± 7.2% when the supplemented ration was given. The vitamin B12 molecule contains about 4.35% cobalt. If all the cobalt in the ration would be incorporated into cobalamin, the calculated vitamin B12 flow at the duodenum would amount to 52.1 ± 0.1 mg/d for controls and to 91.4 ± 0.1 mg/d for supplemented animals (Table 4). In fact, only small portions of cobalt were used for cobalamin synthesis. In our experiment, in controls only 7.1 ± 1.3% of the cobalt at the duodenum was bound in vitamin B12. After supplementation with cobalt, the efficiency of cobalt utilisation for vitamin B12 production was calculated to be 9.5 ± 2.4%. This difference proved to be statistically significant.

The results show significantly higher amounts of vitamin B12 reaching the duodenum when the ration was supplemented with Co (8.63 ± 2.22 mg B12/d) as compared to controls (3.67 ± 0.69 mg B12/d), although the differences between individuals were considerable.

Finally the vitamin B12 synthesis expressed as milligrams per mg Co in duodenal fluid was higher while feeding the supplemented ration (0.29 mg Co/kg DM).

4. Discussion
The aim of the present experiment was to study the influence of an elevated cobalt supply on rumen fermentation parameters and microbial vitamin B12 synthesis in dairy cows.

Unfortunately the experimental design was no crossover arrangement. But also in literature there are no data, how long cobalt supply is influencing the cobalt content in liver and in the whole body and the vitamin B12-synthesis in the rumen. There is also no information available, how long the washing period between the two treatments has to be in this case. Therefore, the study was designed without a mutual alteration of the treatment in the second experimental period, to avoid influences from the first period.

Effects of cobalt deficiency on microbial population pattern are well described. In accordance with Gall et al. (1949), who reported marked alterations in the numbers of
bacteria and in the type of bacteria strains in the rumen of cobalt deficient sheep, Singh and Chhabra (1995) found significant higher bacterial counts in supplemented animals. Changes in ruminal microbial species might result in changes in molar proportions of short chain fatty acids, but Marston et al. (1972) and Kisídáková et al. (2001) did not find any effect of cobalt supply on molar portions of short chain fatty acids in ruminal fluid. These results correspond to the present investigation, in which the cobalt content in the ration did not affect ruminal parameters. The ruminal parameters measured in the present study were found to be within the physiological range, leading to the conclusion that the ruminal conditions did not impair microbial fermentation and vitamin B12 synthesis. Unfortunately in the present study no investigations concerning microbial population pattern could be conducted.

Cobalt and vitamin B12 are known to be a growing factor for some strains of bacteria (Stárka 1968, Friedrich 1987). Therefore, under cobalt deficiency microbial protein synthesis might be reduced. In the present experiment no difference between treatments concerning microbial protein synthesis could be observed, even for controls the cobalt supply was higher than 0.1 mg Co/kg DM. In both treatments the proportion of microbial N as percentage of non-ammonia-N (see Table 3) was with slightly lower than reported by Lebzien and Paul (1997) (69.3%; range 50.3–82.3%), who measured parameters of microbial protein synthesis in more than 150 samples of duodenal chyme in dairy cows. Data for microbial nitrogen in relation to organic matter fermented in the rumen were described to be in between 63–338 g MP per kg fermented organic matter (Stern et al. 1994, Lebzien and Voigt 1999, National Research Council [NRC] 2001). In both groups of this experiment mean values measured are slightly lower than reported earlier, but were always within the range given in the literature.

Gall et al. (1949) reported that protein synthesis is reduced in cobalt deficient ruminants (<0.10 mg Co/kg DM) because of changes in microbial population. In the present experiment no changes in microbial protein synthesis were detected, but the Co-supply in the control group was much higher than 0.10 mg/kg DM. So, in this case an extra oral supply with cobalt has no influence on microbial protein synthesis. Nevertheless, until now, less is known about the microbial vitamin B12 synthesis in the rumen and possible influences, which might reduce or enhance this synthesis. For example, some microbes synthesise cobalamin, while others have a large requirement for vitamin B12 as a growth factor (Stárka 1968). If there is not enough vitamin B12 for these microbes, it is possible that the protein synthesis is reduced.

In the present study an increase of oral supply of cobalt from 0.17 mg/kg DM to 0.29 mg/kg DM resulted in significant higher vitamin B12 concentrations in duodenal chyme of fistulated dairy cows. These finding correspond to results of Smith and Marston (1970), who also found an elevated vitamin B12 concentration in ruminal fluid of sheep after feeding a Co supplemented ration. Investigations of Singh and Chhabra (1995) showed a linear relationship between the cobalt content in the ration and the vitamin B12 concentration in ruminal fluid, while feeding rations containing 0.38–1.89 mg Co per day. But no linear effect could be detected when the dietary Co-level increased further. A reason for this might be a higher synthesis of cobalamin analogues while feeding highly Co-supplemented rations.

On the other hand, if the cobalt supply is sufficient, other factors like the composition of the diet (roughage content, relation between roughage and concentrates) and the dry matter intake can limit the vitamin B12 production in the rumen (Hayes et al. 1966; Smith and Marston 1970; Walker and Elliot 1972; Hedrich et al. 1973; Halpin et al. 1984).
Schwab et al. (2006) concluded that an increase of dietary forage content decrease the apparent synthesis of cobalamin in the rumen. In the present investigation these influences can be excluded, because dry matter intake and diet composition were the same. The only difference between treatments was the cobalt content in the ration (0.17 vs. 0.29 mg/kg DM).

Feeding the ration with higher cobalt content resulted in significant higher vitamin B₁₂ synthesis in all animals (Table 4). Therefore, the most important factor for ruminal vitamin B₁₂ synthesis seems to be the cobalt content in the ration. Nevertheless, individual factors and former supply with cobalt have to be taken into account (see SD values in Table 4).

Assuming a mean recovery rate of 89.6% (Table 4), the efficiency of cobalt utilisation for ruminal vitamin B₁₂ synthesis was calculated to be 7.1 ± 1.3% for the unsupplemented and 9.5 ± 2.4% for the supplemented ration. These values are within the range of sheep (3–15%) reported by Smith and Marston (1970). Whereas these authors observed a more efficient use of cobalt in cobalt deprived animals (3% efficiency of cobalt utilisation) in comparison to supplemented sheep (15% efficiency of cobalt utilisation), the present study showed contrary results. A reason for this discrepancy maybe the different bioavailability of the chemical compounds used in the two experiments (Co chloride vs. Co sulphate). Another explanation might be a higher Co-requirement because of an increased microbial growth in deprived animals.

The average recovery rate of cobalt at the duodenum was about 89.6% (Table 4). This value is in between the range given in the literature (Monroe et al. 1952; Smith and Marston 1970; Looney et al. 1976; Uden et al. 1980). Cobalt is not absorbed from the rumen (Underwood and Suttle 1999). But it might be possible, that small amounts of cobalt (as cobalamin) are already absorbed in the upper part of the duodenum before the cannula. On the other hand, it is possible that analytical methods are not sensitive enough.

In former experiments given in the literature the vitamin B₁₂-synthesis and the vitamin B₁₂-flow was based on the cobalt intake instead of the recovery rate in the duodenum. For better comparison of the data, the vitamin B₁₂ synthesis and the flow in the present experiment was also based on the total cobalt intake. Depending on the composition of the diet, the flow of vitamin B₁₂ to the duodenum is reported to be 0.6–10 mg/d (Steinberg and Klünter 1995) and 7.5–12.3 mg/d (Zinn et al. 1987). Regarding to NRC (2001), the duodenal vitamin B₁₂-flow amounts to about 70 mg/d. Assuming a requirement of 0.10 mg Co/kg DM (NRC 2001) and a daily intake of 25 kg DM in dairy cows, the maximal vitamin B₁₂ production would amount to 57.5 mg/g (100% conversion of cobalt to vitamin B₁₂). This value does not correspond to the data for vitamin B₁₂-flow published by the NRC (2001). As can be seen in Table 4, own data for vitamin B₁₂ flow to the small intestine amounted to 3.67 ± 0.69 mg/d in controls and 8.63 ± 2.22 mg/d in supplemented cows.

In the literature only little information about the relation between dietary cobalt supply and vitamin B₁₂ synthesis in the rumen is available (Elliot et al. 1971; Sutton and Elliot 1972; Hedrich et al. 1973; Schwab et al. 2006). As the cobalt content in the ration increased, the measured cobalamin content in duodenal fluid increased, too. Although the authors fed different levels of cobalt in the ration (0.04–2.1 mg Co/kg DM), the vitamin B₁₂ production per mg Cobalt did not differ extremely (0.4–3.7; see Figure 3). For example, Schwab et al. (2006) fed a ration containing almost 10-times more cobalt than in the present experiment, but the mean vitamin B₁₂ production (mg B₁₂/mg Co) was only doubled. Reasons for this are not quite clear, but it seems that the vitamin B₁₂ synthesis cannot be increased unlimited as a result of limited bacterial capacity for
cobalamin production. On the other hand it is known, that micro-organisms in the rumen produce vitamin B$_{12}$ analogues (cobamines), which have no vitamin B$_{12}$ activity when the cobalt content in the ration increases (Gawthorne 1970). Nevertheless, as shown in Figure 3, the results derived from the recent experiment can be compared to former investigations.

5. Conclusions

It can be concluded that an elevated dietary cobalt supply (0.29 mg Co/kg DM) had no influence on ruminal parameters such as pH-value, short chain fatty acids and microbial protein synthesis in comparison to the native cobalt content in the ration (0.17 mg Co/kg DM). Nevertheless, feeding 0.29 mg Co/kg DM resulted in higher amounts of vitamin B$_{12}$ in the duodenal digesta, although the individual differences were considerable. Further studies have to reveal if this affects the vitamin B$_{12}$ concentration in the serum, liver and milk of dairy cows.

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References


