VITAMIN B\textsubscript{12} AND MONENSIN EFFECTS ON PERFORMANCE, LIVER AND SERUM VITAMIN B\textsubscript{12} CONCENTRATIONS AND ACTIVITY OF PROPIONATE METABOLIZING HEPATIC ENZYMES IN FEEDLOT LAMBS\textsuperscript{1,2}

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ABSTRACT

Monensin in ruminant diets increases production of propionic acid. We have tested the hypothesis that propionic acid may be elevated to such an extent by monensin that it cannot be optimally metabolized by the methyl malonyl-CoA pathway requiring vitamin B\textsubscript{12} (B\textsubscript{12}) in the liver. Thus, the effects of weekly B\textsubscript{12} injections (10 mg/head \textsuperscript{1-wk\textsuperscript{-1}}, intramuscularly) with and without dietary monensin (25 mg/kg diet) on average daily gain (ADG), dry matter intake (DMI), feed to gain ratio (F/G), liver and serum B\textsubscript{12} concentrations and liver activity of propionate metabolizing enzymes were examined in an 84-d trial. Sixteen lambs (27.5 kg average initial wt) were assigned randomly to one of four treatments in a factorial arrangement: monensin plus B\textsubscript{12}, monensin without B\textsubscript{12}, no monensin plus B\textsubscript{12}, and no monensin without B\textsubscript{12}. Lambs were fed an 80% concentrate diet and slaughtered at the end of the trial. Liver samples were obtained by biopsy on d 0 and at slaughter on d 84 to determine activity of propionate metabolizing enzymes and B\textsubscript{12} concentrations. Serum samples were taken on d 0, 28, 56 and 84 to determine serum B\textsubscript{12} concentration. Neither monensin nor B\textsubscript{12} affected (P>.10) ADG, DMI or F/G. Lambs receiving B\textsubscript{12} had higher (P<.01) serum B\textsubscript{12} concentrations, but this was not reflected (P>.10) in higher liver B\textsubscript{12} concentrations. No difference (P>.10) in liver propionate metabolizing activity among treatments was detected; however, monensin decreased (P<.05) fumarate and malate formation by liver homogenates. Liver B\textsubscript{12} concentrations were highly correlated with endogenous propionate metabolizing activity at d 0 (r = .73, P<.01) and d 84 (r = .51, P<.05). Results suggest no advantage to providing supplemental B\textsubscript{12} to lambs fed monensin-supplemented, high-concentrate diets. (Key Words: Vitamin B\textsubscript{12}, Monensin, Methylmalonyl-CoA Mutase, Sheep, Performance.)

Introduction

Monensin is a carboxylic ionophore used widely in the cattle feeding industry. A primary effect of monensin is an increased molar proportion of ruminal propionate (Richardson et al., 1976) resulting from increased propionate production (Prange et al., 1978).

Effective liver metabolism of propionate depends on activity of propionyl-CoA synthetase, propionyl-CoA carboxylase, methylmalonyl-CoA racemase and methylmalonyl-CoA mutase, which sequentially convert propionate to succinyl-CoA to enter the Krebs cycle and gluconeogenic pathways (Flavin and Ochoa, 1957). Vitamin B\textsubscript{12} (B\textsubscript{12}) is a cofactor in the methylmalonyl-CoA mutase (2-methylmalonyl-CoA-CoA-carbonyl mutase, EC 5.4.99.2) catalyzed conversion of methylmalonyl-CoA to succinyl-CoA (Stadtman, 1971). This reaction becomes limiting in severe cobalt or B\textsubscript{12} deficiencies (Gurnani et al., 1960; Marston et al., 1961). Limited evidence suggests liver propionate metabolism may be affected by B\textsubscript{12} status even in the absence of a B\textsubscript{12} deficiency condition and before clinical deficiency symptoms appear (Somers, 1969; Rickard and Elliot, 1982). Furthermore, feeding high-concentrate, low-roughage diets decreases ruminal synthesis

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of true B\textsubscript{12} (i.e., 5,6-dimethylbenzimidazole cobamide cyanide) and increases synthesis of B\textsubscript{12} analogs that do not perform the coenzyme function of true B\textsubscript{12} in mammals (Sutton and Elliot, 1972).

High-concentrate diets supplemented with monensin may decrease liver B\textsubscript{12} status by enhanced ruminal propionate production, coupled with less true B\textsubscript{12} synthesis. The following study examined the effect of monensin and B\textsubscript{12} supplementation on growth, feed intake, feed efficiency, serum and liver B\textsubscript{12} concentrations and liver propionate metabolizing activity in feedlot lambs.

**Experimental Procedure**

**Animals and Sampling.** Sixteen Suffolk wether lambs (27.5 kg average wt) were randomly allocated to treatments in an 84-d feedlot trial with a 2 x 2 factorial arrangement of treatments. Treatments included (four lambs/treatment): monensin plus B\textsubscript{12}, monensin without B\textsubscript{12}, no monensin plus B\textsubscript{12} and no monensin without B\textsubscript{12}. The trial was divided into three 28-d periods, and all lambs were slaughtered at the end of the trial on d 84. Carcasses from monensin-fed lambs were destroyed. Lambs were housed outdoors in shaded, individual pens (3 m x 1.5 m) with free access to water and feed. A 60% concentrate diet was fed in a 17-d adaptation period that began immediately after lambs were weaned. An 80% concentrate, pelleted diet was fed throughout the 84-d trial period (table 1). Monensin was fed at the rate of 25 mg/kg diet dry matter, and intramuscular B\textsubscript{12} injections (10 mg) were given once/week. Sham injections of 2 ml of aqueous Na\textsubscript{Cl} (.9% w/v) were given once/week to lambs receiving no B\textsubscript{12}. Vitamin B\textsubscript{12} injectable solution was prepared using crystalline B\textsubscript{12}\textsuperscript{6} dissolved in aqueous Na\textsubscript{Cl} (.9% w/v).

Individual feed intake were recorded daily, and unshrunk animal weights were obtained at d 0 and at the end of each period on d 28, 56 and 84. Feed samples were obtained daily, and composited weekly for analysis (table 1) by standard procedures (AOAC, 1980). Blood samples for B\textsubscript{12} analysis were collected in 10-ml non-heparinized serum separator tubes via jugular puncture of each lamb on d 0, 28, 56 and 84, and centrifuged at 1,000 x g for 15 min. Serum was stored frozen in glass vials protected from light until analyzed.

Liver samples for B\textsubscript{12} and enzyme analyses were obtained from each lamb on d 0 by making a 5-cm lateral incision on the right side of the abdomen, just below the rib cage, and removing a 1.5-g sample from the right lobe of the liver. A local anesthetic (1% lidocaine-HCl) was administered before making the incision. Lambs were given antibiotics (procaine penicillin G plus dihydrostreptomycin) for 4 d postsurgery. Liver samples were obtained again from each lamb on d 84 by removing a 1.5-g sample from the right lobe of the liver about 8 min after slaughter. Liver samples were transported to the laboratory in ice-cold (3 to 5 C) .25 M sucrose solution and prepared for B\textsubscript{12} and enzyme analyses using a procedure similar to Mathias and Elliot (1967). Crude homogenates of liver were prepared by homogenizing 1 g fresh liver for 2 min in 10 ml of a hypotonic solution consisting of .04 M Tris-HCl buffer (trihydroxymethylaminomethane) and 1.0 mM cysteine, pH 7.4, in a Potter-Elvehjem glass homogenizer immersed in ice (3 to 5 C) with

![Sigma Chemical Co., St. Louis, MO.](https://example.com/sigma.png)

**Table 1. Composition and Analysis of the Pelleted Diet\textsuperscript{a}**

<table>
<thead>
<tr>
<th>Ingredient\textsuperscript{b}</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrated alfalfa meal (1-00-022)</td>
<td>20</td>
</tr>
<tr>
<td>Ground corn grain (4-02-933)</td>
<td>63.1</td>
</tr>
<tr>
<td>Hominy feed (4-02-887)</td>
<td>1</td>
</tr>
<tr>
<td>Molasses (4-04-696)</td>
<td>5</td>
</tr>
<tr>
<td>Cottonseed meal (5-01-621)</td>
<td>8.9</td>
</tr>
<tr>
<td>Ground limestone (6-02-632)</td>
<td>1.3</td>
</tr>
<tr>
<td>Trace mineralized salt\textsuperscript{c}</td>
<td>.5</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>.25</td>
</tr>
<tr>
<td>Chemical composition</td>
<td></td>
</tr>
<tr>
<td>Dry matter</td>
<td>89.3</td>
</tr>
<tr>
<td>Ash\textsuperscript{b}</td>
<td>6.5</td>
</tr>
<tr>
<td>Crude protein\textsuperscript{b}</td>
<td>14.1</td>
</tr>
<tr>
<td>Acid detergent fiber\textsuperscript{b}</td>
<td>10.7</td>
</tr>
<tr>
<td>Acid detergent lignin\textsuperscript{b}</td>
<td>3.1</td>
</tr>
<tr>
<td>Cobalt\textsuperscript{b}</td>
<td>.25\textsuperscript{d}</td>
</tr>
</tbody>
</table>

\textsuperscript{6}Sigma Chemical Co., St. Louis, MO.

\textsuperscript{a}Contains vitamin A at 638 IU/kg, oxytetracycline at 24 mg/kg and monensin (when included) at 25 mg/kg of dietary dry matter.

\textsuperscript{b}Dry-matter basis.

\textsuperscript{c}Contains 94.5% NaCl and a maximum of .007% I, .24% Mn, .24% Fe, .05% Mg, .032% Cu, .011% Co and .032% Zn.

\textsuperscript{d}mg/kg.
the polyethylene pestle driven at 1,200 rpm. Homogenates were centrifuged (600 x g, 15 min, 5 C) to remove nuclei and cell debris. Supernatant fractions were decanted and volumes recorded to the nearest .1 ml to enable calculation of liver B12 concentrations. Aliquots of crude liver homogenates were diluted 1:50 with deionized water to determine protein (Lowry et al., 1951) and B12 content.

Liver and Serum B12 Analysis. Liver and serum B12 concentrations were determined with a competitive protein-binding assay7 adapted from other procedures (Rothenberg, 1963, 1968; Lau et al., 1965; Mortensen, 1972; Ithakissios et al., 1980; Houts and Carney, 1981) that utilized [57Co] B12 as tracer. Purified porcine intrinsic factor was used as the B12 binding protein, which measures only true B12, because R-proteins capable of binding cobalamin analogs (Kolhouse et al., 1978) have been eliminated from the preparation. Separation of bound and unbound B12 was accomplished by adsorption of the unbound fraction on hemoglobin-coated charcoal. Twenty-five microliters of serum and 50 /al of the 1:50 dilution of crude liver homogenate were diluted to a final volume of .2 ml with the zero standard reagent (protein-based buffer). Vitamin B12 concentrations were quantified in a gamma counter8 by counting [14C] B12 tracer in the unbound fraction. Validation of the assay yielded within and between assay coefficients of variation of 3.0% (n = 5) and 5.9% (n = 3), respectively. When 2 ng B12 were added to 1 ml of wether serum containing 4.8 -+ .3 ng endogenous B12/ml (n = 15), 6.7 -+ .1 ng/ml were recovered (i.e., 99.5% of added B12). Addition of 5 ng B12 to 1 ml of wether serum (n = 15) resulted in a recovery of 107.5%.

Enzyme Assay. The assay to measure activity of propionate metabolizing enzymes was adapted from other procedures (Flavin and Ochoa, 1957; Smith and Monty, 1959; Marston et al., 1961; Smith and Osborne-White, 1965; Smith et al., 1965; Mathias and Elliot, 1967). Dialyzed liver homogenates were prepared with a procedure similar to Flavin and Ochoa (1957). Dialysis was conducted at 3 C with a 40 mM Tris·HCl and 1 mM cysteine, pH 7.4 buffer. Crude liver homogenates were dialyzed for 6 h against a 100-fold excess of buffer maintained at 3 C with fresh buffer supplied 1 and 3 h after beginning dialysis. Dialyzed homogenates were stored, protected from light, at 3 C until assayed for enzyme activity, about 48 h after obtaining liver samples. The assay used [14C] NaHCO39 as substrate in a reaction mixture10 (1 ml final volume) that contained Tris·HCl buffer, pH 7.5, 100 µmol; MgCl2, 10 µmol; ATP (disodium salt·3 H2O), 5 µmol; propionyl-CoA (dilithium salt·1.5 H2O), 1 µmol; reduced glutathione (GSH; free acid), 5 µmol; 5,6-dimethylbenzimidazole cobamide coenzyme B12, 6.33 x 10-3 µmol (when included in the reaction mixture); ATP-generating system [10 units of pyruvate kinase activity plus 10 µmol of the tricyclohexylammonium salt of phosphoenolpyruvate (PEP)], made fresh by adding to the working solution of GSH in 100 mM Tris·HCl, pH 7.0; [14C] NaHCO3, 10 µmol (specific activity .36 µCi/10 µmol); and .5 ml dialyzed liver homogenate (average 10 mg protein/ml). Final pH of the reaction mixture was 7.0.

Incubations were conducted in duplicate in sealed 15 x 48-mm glass vials in a water bath at 30 C for 30 min. The gas phase was air. The assay was validated without coenzyme B12 in the reaction mixture and product formation was linear over time until approximately 190 nmol had accumulated [i.e., product formation was linear up to 30 min at 30 C with .5 ml dialyzed liver homogenate (about 5.0 mg protein)]. The reaction was started by addition of [14C] NaHCO3 to the incubation medium after a 10-min thermal equilibration period. Incubations were terminated and CoA-thio esters hydrolyzed by addition of .2 ml 2 N KOH. Reaction vials were placed in a water bath (95 C) for 10 min to ensure complete hydrolysis of CoA-thio esters. Contents of each reaction vial were transferred to 16 x 125-mm polystyrene screw-cap culture tubes with rinsing of reaction vials using .25 ml/vial of carrier acid solution containing 60 µg of fumarate, methylmalonate, succinate, malate and malonate in 40 mM Tris·HCl, pH 7.5. The

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7 No-Boll vitamin B12 Assay Kit No. RP-1600. Micromedic Systems, Inc., Horsham, PA.
8 Packard Auto-Gamma 500C gamma counter. Packard Instruments, Downers Grove, IL.
9 [14C]-NaHCO3 obtained from New England Nuclear, Boston, MA.
10 All biochemicals obtained from Sigma Chemical Co., St. Louis, MO.
11 A unit of activity will convert 1.0 µmol PEP to pyruvate/min at pH 7.7 and 37 C. Calculations were made from the enzyme specific activity assay supplied by Sigma Chemical Co., St. Louis, MO.
reaction mixtures were deproteinized by adding .5 ml of 4 N HCl to each 16 x 125-mm tube, which was placed under a hood overnight in a desiccator containing filter paper soaked with 2 N NaOH to trap evolved $^{14}$CO$_2$. Precipitated protein was removed by centrifuging at 600 x g for 15 min. Supernatant fractions were transferred to 20 x 150-mm glass culture tubes.

Enzyme reaction products were extracted from the aqueous phase by shaking vigorously for 10 min with 10 ml of ethylacetate extraction vial and repeating once. After each extraction, the aqueous phase was frozen (1 h at -20 C) and the ethylacetate phase was poured into 16 x 125-mm glass tubes. Ethylacetate was then evaporated under N$_2$ at 75 C until approximately 3 ml remained, which was transferred to 12 x 75-mm glass tubes and evaporated to dryness. Dried residue was dissolved in 2 ml of 100% ethanol and 50 #t spotted on 9.5 x 22-cm strips of Whatman No. 1 chromatography paper under a stream of warm air. Before chromatographing, paper was equilibrated with the polar stationary phase of the solvent (deionized water) by spraying chromatograms with a light mist until the paper was slightly moist. Ascending chromatography was conducted for 5 h at 25 C in isoamylalcohol saturated with 4 N formate (Flavin and Ochoa, 1957) after pH adjustment to 3.5 using NH$_4$OH. Acids were located with .04% (w/v) bromophenol blue indicator in 95% ethanol, pH 6.7, and were identified by comparing $R_f$ values with a standard chromatographed simultaneously with samples. Mean (n = 20) $R_f$ values for acids in this solvent system were: fumarate, .74; methylmalonate, .60; succinate, .48; malonate, .34 and malate, .19. Two unidentified spots frequently appeared on chromatograms with $R_f$ values of .11 and .04; however, these spots contained no radioactivity. Spots were cut out, including 10 mm of paper beyond the visible spot, and placed in liquid scintillation vials. Acids were eluted off the paper by adding 1 ml of 40 mM Tris-HCl buffer, pH 7.5, to each scintillation vial and agitating with a vortex mixer. Ten milliliters of Aquasol$^{13}$ counting fluid was added to each vial, and vials were counted for 10 min in a liquid scintillation spectrophotometer$^{14}$.

Counting efficiency was determined by preparing a quench correction curve from a set of quenched standards using the external standard sample channels ratio method. A set of quench correction standards was prepared for both sample and $[^{14}$C]NaHCO$_3$ total counts vials according to the procedure of Neame and Homewood (1974) using $[7,^{14}$C] benzoic acid certified calibration standard$^{13}$. Radioactive label was present in fumarate, malate, succinate, methylmalonate and malonate (negligible). Net disintegrations/min (DPM) in each acid were determined by correcting for dilution factors, quench and differential extraction of each acid in the extraction step. Enzyme reaction vials containing $^{14}$C-labeled standards of fumarate, succinate, malate and methylmalonate were taken through the extraction step to determine extraction efficiency and procedural losses. The nmol formed/min of succinate, fumarate and malate was summed to obtain total product formation/min, and liver propionate metabolizing activity was expressed as nmol total product (Sum) formed/min$^{-1}$ x mg protein$^{-1}$. Essentially, the collective activity of the three methylmalonyl-CoA pathway enzymes was measured.

When counting the $[^{14}$C]NaHCO$_3$ total counts vials to determine total net DPM added to each reaction vial, an alkaline mixture of Aquasol counting fluid, pH 14, was prepared (50 #l of 2 M NaOH/10 ml Aquasol) by a procedure adapted from Huskisson and Ward (1980). Alkaline counting fluid was allowed to stand until the chemiluminescent reaction (Benson, 1976) had gone to completion and counts were near background (approximately 1 wk).

Statistical Analysis. Analysis of variance was performed using the General Linear Model procedures of the Statistical Analysis System (SAS, 1982). The model for lamb gain, intake and feed efficiency data for the entire 84-d trial included effects for monensin, B$_{12}$ and monensin x B$_{12}$ interaction. Serum and liver B$_{12}$ concentrations were analyzed assuming a split-plot design with d 0, 28, 56 and 84 in the subplot for serum B$_{12}$ and d 0 and 84 in the subplot for liver B$_{12}$. A B$_{12}$ x sampling day interaction (P<.05) was noted for serum B$_{12}$;
therefore, serum B12 concentrations on d 0, 28, 56 and 84 were analyzed using a model with effects for monensin, B12 and monensin × B12 interactions. In addition, a day effect (P<.01) was noted for liver B12 concentrations, which were then analyzed within d 0 and 84.

Liver propionate metabolizing activity without excess coenzyme B12 added to the reaction mixture (i.e., endogenous activity) was initially analyzed with a model including day effects; however, a treatment × day interaction (P<.01) was noted, so enzyme activity was analyzed within d 0 and 84. A monensin × B12 interaction (P<.05) was noted for enzyme activity for d 0 liver samples; therefore, enzyme activity was analyzed by a covariate analysis where the change in enzyme specific activity between d 0 and 84 was calculated by subtracting d 0 values from corresponding d 84 values. Monensin, B12 and monensin × B12 interaction terms were included in the model for covariate analysis. Enzyme activity with coenzyme B12 added at saturating levels (i.e., total activity) is not reported because the enzyme assay was validated without coenzyme B12 added to the reaction mixture. Correlation coefficients between liver and serum B12 concentrations, liver B12 concentrations and enzyme activity, and enzyme activity and lamb gain, intake and feed efficiency pooled across treatments were determined (SAS, 1982). Least-squares means are reported because one lamb from the monensin without B12 treatment suffered a rectal prolapse in period 3 and was euthanized.

Results and Discussion

Neither monensin nor B12 affected (P>.10) DMI, ADG or F/G (table 2). Average daily gain by lambs fed monensin-supplemented, high-concentrate diets has varied, with reports of improvements in ADG (Nockels et al., 1978); although others have reported monensin had no effect on ADG (Joyner et al., 1979). In addition, monensin effects on DMI and F/G appear to be less consistent and more variable in feedlot lambs than in cattle. Joyner et al. (1979) and Calhoun et al. (1979) reported monensin decreased feed intake and improved F/G, whereas Nockels et al. (1978) reported no difference in DMI or F/G in monensin-fed lambs compared with control lambs. In the present study, B12 injections tended (P>.10) to decrease F/G (table 2); however, results suggest animal performance in general was not enhanced by providing supplemental B12.
Additional feeding trials (Daugherty et al., 1982) also showed no effect of monensin or B$_{12}$ on performance of lambs fed a 60% concentrate diet with the same treatments as in the present study. In those trials, B$_{12}$ was injected at a dosage of 100 µg/wk. Moreover, ruminal pH, ammonia and total volatile fatty acid concentrations were not different among treatments in those earlier trials (Daugherty, 1984).

No monensin X B$_{12}$ interactions (P> .10) were observed for serum B$_{12}$ concentrations; therefore, main effect means are shown in table 3. Neither monensin or B$_{12}$ had an effect on serum B$_{12}$ levels at d 0, as these samples were taken before treatments were applied. Mean serum B$_{12}$ concentration on d 0 across all treatments was about 1.2 ng/ml (table 3). Monensin had no effect (P> .05) on serum B$_{12}$ concentrations at d 28, 56 or 84, whereas lambs receiving B$_{12}$ injections (10 mg·head$^{-1}$·wk$^{-1}$) had consistently higher (P< .01 on d 28 and 84; P< .06 on d 56) serum B$_{12}$ concentrations throughout the trial. Serum B$_{12}$ concentrations of lambs in this trial are reasonably close to values reported by others (Dawbarn et al., 1957; Marston, 1970).

Main effect means for liver and crude liver homogenate B$_{12}$ concentrations at d 0 and 84 are shown in table 4. No differences (P> .10) among treatments were noted in liver B$_{12}$ concentrations at d 0. Values averaged approximately .37 µg B$_{12}$/g liver (wet wt). Liver B$_{12}$ concentrations increased in all lambs to an average of about .66 µg/g (wet wt) across all treatments at d 84, however, no difference (P> .10) in liver B$_{12}$ concentrations among treatments was noted at d 84, although lambs receiving B$_{12}$ had numerically higher liver B$_{12}$ concentrations at d 84 (.71 vs .61 µg/g for the B$_{12}$-supplemented and no-B$_{12}$ groups, respectively). The dosage of B$_{12}$ supplied approximately 100 times the minimum weekly requirement for maintaining animal growth (Marston, 1970). Lambs receiving B$_{12}$ had higher serum B$_{12}$ concentrations but not greater liver B$_{12}$ storage, suggesting that most of the supplemental B$_{12}$ was excreted. The average .66 µg B$_{12}$/g liver (wet wt) across treatments attained after 84 d on feed (table 4) is about one-half the maximum liver B$_{12}$ concentration reported for ruminants fed high-roughage diets (Marston, 1970; Walker and Elliot, 1972; Rickard et al., 1975). High-concentrate diets decrease ruminal synthesis of

### Table 3. Effects of Dietary, Monensin and Intramuscular Vitamin B$_{12}$ on Serum Vitamin B$_{12}$ Concentrations at Various Times in the Feeding Trial

<table>
<thead>
<tr>
<th>Days on trial</th>
<th>Treatment $^a$</th>
<th>Vitamin B$_{12}$, ng/ml</th>
<th>Treatment $^a$</th>
<th>Vitamin B$_{12}$, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1.18</td>
<td>25</td>
<td>1.26</td>
</tr>
<tr>
<td>28</td>
<td>0</td>
<td>4.18</td>
<td>25</td>
<td>4.64</td>
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<tr>
<td>56</td>
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<td>84</td>
<td>0</td>
<td>7.26</td>
<td>25</td>
<td>8.21</td>
</tr>
</tbody>
</table>

$^a$ See table 2 footnote a for description of treatments.

$^b$ Standard error of least-squares means, calculated from common factorial analysis of variance.

$^c$ The vitamin B$_{12}$ assay used measured only true vitamin B$_{12}$ as opposed to total serum B$_{12}$ levels which includes vitamin B$_{12}$ analogs.

$^d$ Means in same row within a main effect with different superscripts differ (P< .01).

$^e$ Means in the same row within a main effect with different superscripts differ (P< .06).
TABLE 4. EFFECTS OF DIETARY MONENSIN AND INTRAMUSCULAR VITAMIN B<sub>12</sub> ON LIVER CONCENTRATIONS OF VITAMIN B<sub>12</sub> AT VARIOUS TIMES IN THE FEEDING TRIAL

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Vitamin B&lt;sub&gt;12&lt;/sub&gt; concentration (true B&lt;sub&gt;12&lt;/sub&gt;)</th>
<th>Day 0</th>
<th>Day 84</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver&lt;sup&gt;a&lt;/sup&gt;, Crude liver extract&lt;sup&gt;b&lt;/sup&gt;, Crude liver extract&lt;sup&gt;b&lt;/sup&gt;,</td>
<td>µg/g</td>
<td>µg/g</td>
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<tr>
<td>Monensin, mg/kg</td>
<td>µg/g ng/ml ng/mg protein</td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td>.39 40.1 3.55</td>
<td>.63 64.1 6.29</td>
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<tr>
<td>25</td>
<td>.36 37.6 3.35</td>
<td>.69 69.9 6.98</td>
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<td>Vitamin B&lt;sub&gt;12&lt;/sub&gt;, mg/wk</td>
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<td>0</td>
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<td>.61 61.1 6.25</td>
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</tr>
<tr>
<td>10</td>
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<td>.71 73.0 7.03</td>
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</tr>
<tr>
<td>SE&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.03 2.9 .27</td>
<td>.07 6.8 .61</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>µg vitamin B<sub>12</sub>/g liver wet weight.

<sup>b</sup>Crude liver extract = undialyzed nuclear-free homogenate.

<sup>c</sup>See table 2, footnote a for description of treatments.

<sup>d</sup>Standard error of least-squares means, calculated from common factorial analysis of variance.
true B\textsubscript{12} and increase B\textsubscript{12} analog production (Sutton and Elliot, 1972; Bigger et al., 1976), and result in decreased liver storage of true B\textsubscript{12} (Walker and Elliot, 1972), which may account for the lower liver B\textsubscript{12} concentrations of lambs in our trial compared with ruminants fed high-roughage diets.

Serum and liver B\textsubscript{12} concentrations were positively correlated at d 84 ($r = .67$, $P<.01$), which agrees with values reported for sheep (Marston, 1970; Rickard et al., 1975). Liver and serum B\textsubscript{12} concentrations were not highly correlated ($r = .40$; $P>.10$) at d 0, which is similar to a report by Wilson et al. (1967; $r = .34$) in lactating dairy cattle. Significant correlations between serum and liver B\textsubscript{12} concentrations reported in the literature, in some cases but not in others, is not surprising because diets and conditions under which the experiments were conducted varied widely. The work of Marston (1970) indicates serum B\textsubscript{12} concentrations reach a relatively steady state when liver B\textsubscript{12} concentrations reach their maximum level under the conditions of the experiment, and reflect a balance between such factors as ruminal production, absorption from the gut, tissue uptake and utilization. The poor correlation between serum and liver B\textsubscript{12} concentrations at d 0 in the present study may indicate that a steady-state condition had not been reached.

No monensin $\times$ B\textsubscript{12} interaction or difference in liver propionate metabolizing activity (table 5) among treatments was observed by d 84 for liver samples without coenzyme B\textsubscript{12} added to the reaction mixture. The average specific activity across all treatments was about .77 nmol-min\textsuperscript{-1}.mg protein\textsuperscript{-1} (column labeled “Sum” in table 5). Activity was defined as the sum of the fumarate, malate and succinate formed (nmol-min\textsuperscript{-1}.mg protein\textsuperscript{-1}). A monensin $\times$ B\textsubscript{12} interaction ($P<.05$) was noted, however, for enzyme activity in d 0 liver samples, with the no-monensin plus B\textsubscript{12} group having a higher ($P<.05$) enzyme activity than other treatments (.3 vs <.3 nmol-min\textsuperscript{-1}.mg protein\textsuperscript{-1} for other treatments; table 5). This difference was obviously not related to treatments because liver samples on d 0 were taken before treatments had begun.

Because of the difference in enzyme activity on d 0, activity was analyzed by a covariate analysis by calculating the change in enzyme activity between d 0 and 84. No monensin $\times$ B\textsubscript{12} interaction ($P>.10$) on enzyme activity was noted in the covariate analysis; therefore, main effect means for change in enzyme activity over time are reported in table 6. No difference ($P>.10$) in enzyme activity among treatments was observed, with an average change in propionate metabolizing activity of about .5 nmol-min\textsuperscript{-1}.mg protein\textsuperscript{-1} across all treatments (row labeled “Sum” in table 6). Assays for methylmalonyl-CoA mutase activity without coenzyme B\textsubscript{12} added to the reaction mixture reflect the amount of endogenous holoenzyme in the tissue (Peters and Elliot, 1984). The observation in the present study that no differences among treatments in endogenous activity were apparent reflects the fact that no differences were detected in liver B\textsubscript{12} concentrations among treatments. Peters and Elliot (1984) reported differences ($P<.05$) in endogenous methylmalonyl-CoA mutase holoenzyme in liver preparations (.41, 1.65 and 2.64 $\mu$mol/g tissue\textsuperscript{-1}.min\textsuperscript{-1}) from young lambs with liver B\textsubscript{12} concentrations of .07, 1.5 and 2.7 $\mu$g/g (wt wt), respectively, with the large differences in liver B\textsubscript{12} concentrations probably accounting for differences in enzyme activity.

The amount of fumarate and malate formed was decreased by approximately 50% in monensin-fed lambs compared with lambs receiving no monensin (table 6). This effect was also noted in assays where coenzyme B\textsubscript{12} was added to the reaction mixture (data not shown). These results suggest monensin had an inhibitory effect on one or more Krebs cycle enzymes, possibly by reducing the amount of enzyme protein present in the cell. Monensin is known to inhibit protein synthesis in mammalian cells (Alonso et al., 1979) by decreasing the intracellular K+ concentration which, in turn, decreases protein and DNA synthesis (Lubin, 1967; Cahn and Lubin, 1978). Carrasco and Smith (1976) observed the concentration of monovalent ions affected in vitro translation of several different messenger ribonucleic acid (mRNA) molecules. Translation of some mRNA is stimulated while, simultaneously, translation of other mRNA is strongly inhibited, resulting in increased and decreased protein synthesis, respectively. Thus, a mechanism could exist by which monensin apparently had no effect on propionate metabolizing enzymes, but may have affected one or more Krebs cycle enzymes. Herberg et al. (1978) reported liver monensin concentrations of .59 $\mu$g/g (approximately $9 \times 10^{-7}$ M) in steers sacrificed 12 h after the last dose of
TABLE 5. EFFECTS OF DIETARY MONENSIN AND INTRAMUSCULAR VITAMIN B<sub>12</sub> ON LIVER PROPIONATE METABOLIZING ACTIVITY ON DAYS 0 AND 84 OF THE FEEDING PERIOD (SIMPLE EFFECT MEANS)

<table>
<thead>
<tr>
<th>Treatment&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Liver propionate metabolizing activity&lt;sup&gt;a&lt;/sup&gt; (nmol·min&lt;sup&gt;-1&lt;/sup&gt;·mg protein&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Day 0</th>
<th>Day 84</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Fum</td>
<td>Suc</td>
</tr>
<tr>
<td>0 mg/kg monensin, 0 mg/wk B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>1.20</td>
<td>.015&lt;sup&gt;d&lt;/sup&gt;</td>
<td>.16&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>0 mg/kg monensin, 10 mg/wk B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>1.49</td>
<td>.029&lt;sup&gt;e&lt;/sup&gt;</td>
<td>.29&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>25 mg/kg monensin, 0 mg/wk B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>1.43</td>
<td>.027&lt;sup&gt;de&lt;/sup&gt;</td>
<td>.18&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>25 mg/kg monensin, 10 mg/wk B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>1.26</td>
<td>.020&lt;sup&gt;de&lt;/sup&gt;</td>
<td>.18&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>SE&lt;sup&gt;f&lt;/sup&gt;</td>
<td>.13</td>
<td>.004&lt;sup&gt;de&lt;/sup&gt;</td>
<td>.03</td>
</tr>
</tbody>
</table>

<sup>a</sup>Enzyme activity was determined without coenzyme B<sub>12</sub> added to the reaction mixture.

<sup>b</sup>See table 2, footnote a for description of treatments.

<sup>c</sup>Abbreviations are MM = methylmalonate, Fum = fumarate, Suc = succinate, Mal = malate, Sum = nmol·min<sup>-1</sup>·mg protein<sup>-1</sup> of fumarate + malate + succinate.

<sup>d</sup>,<sup>e</sup>Means in same column that do not have a common superscript differ (P<.05).

<sup>f</sup>Standard error of least squares means, calculated from common factorial analysis of variance. At d 0, n = 4. At d 84, n = 3 for monensin without B<sub>12</sub> treatment; n = 4 for other treatments.
<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Monensin, mg/kg</th>
<th>0</th>
<th>25</th>
<th>Vitamin B₁₂, mg/wk</th>
<th>0</th>
<th>10</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td>.53</td>
<td>.48</td>
<td></td>
<td>.49</td>
<td>.51</td>
<td>.08</td>
<td></td>
</tr>
<tr>
<td>Fumarate</td>
<td>.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.01&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td>.02</td>
<td>.02</td>
<td>.004</td>
<td></td>
</tr>
<tr>
<td>Malate</td>
<td>.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>.04&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
<td>.06</td>
<td>.04</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>Succinate</td>
<td>.43</td>
<td>.42</td>
<td></td>
<td>.40</td>
<td>.45</td>
<td>.08</td>
<td></td>
</tr>
<tr>
<td>Methylmalonate</td>
<td>.63</td>
<td>.73</td>
<td></td>
<td>.81</td>
<td>.54</td>
<td>.10</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>See table 2, footnote a for description of treatments.

<sup>b</sup>Change in activity = activity at d 84 - activity at d 0 in assays without coenzyme B₁₂ added to the reaction mixture.

<sup>c</sup>Standard error of least-squares means, calculated from common factorial analysis of variance.

<sup>d</sup>Sum = change in specific activity of fumarate + malate + succinate.

<sup>e,f</sup>Means in same row within a main effect with different superscripts differ (P<.05).
monensin. Alonso et al. (1979) reported monensin at $1 \times 10^{-5}$ M resulted in greater than 99% inhibition of protein synthesis in Ehrlich ascites cells. The apparent inhibitory effect of monensin in our trial must be interpreted with caution because the enzyme assay was not designed to measure activity of Krebs cycle enzymes. Furthermore, it is questionable whether monensin accumulates in liver in concentrations great enough to affect host physiology (Donoho, 1984). Endogenous propionate metabolizing activity was correlated with liver $\text{B}_{12}$ concentrations at d 0 and 84, with correlation coefficients of .73 ($P<.01$) and .51 ($P<.05$) for d 0 and d 84 liver samples, respectively. These values are in agreement with, although lower in magnitude than the correlation coefficient of .90 ($P<.05$) between liver endogenous mutase activity/gram wet tissue and liver $\text{B}_{12}$ concentrations reported by Peters and Elliot (1984); however, values were reported on a per gram of wet tissue basis rather than per milligram protein in the incubation medium, as in our study. No significant ($P>.10$) correlations were noted between the change in enzyme activity without added coenzyme $\text{B}_{12}$ between d 0 and 84 and DMI ($r = .04$), ADG ($r = -.26$) or F/G ($r = .27$).

In general, data from these trials suggest that there is no advantage to providing supplemental $\text{B}_{12}$ to lambs fed monensin-supplemented, high-concentrate diets containing adequate Co. Little research has been conducted on the possible effects of monensin on the host animal; however, the decrease in the amount of fumarate and malate formed in the enzyme assays in liver samples from monensin-fed lambs warrants further research.

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