Lambs were fed a basal purified diet low in nickel (60 ppb) or the basal diet supplemented with 5 ppm of nickel to determine if rumen bacterial urease was a nickel-requiring enzyme. Two collection periods with lambs fed a diet in which all the nitrogen was supplied as preformed protein (casein) indicated that ruminal urease activity was much lower in lambs fed the low nickel diet. When 1% urea was added to the basal diet, urease activity increased slightly with both treatments; however, bacterial urease activity was still much higher in the lambs receiving 5 ppm of nickel. Ruminal volatile fatty acids were not influenced by dietary nickel. Ruminal urease requires nickel for maximal activity.

EXPERIMENTAL PROCEDURES

Twelve wether lambs initially averaging 28.6 kg were allotted randomly to either a basal purified diet formulated to be low in nickel (Table 1) or the basal diet plus 5 ppm of nickel. The basal diet contained approximately 60 ppb of nickel as determined by atomic absorption spectrophotometry (Perkin-Elmer-306). Nickel was added to the control diet as NiCl₂·6H₂O. All known essential trace elements for the rat with the exception of nickel were provided by the mineral mix (Table 2).

Lambs were maintained in wooden metabolism crates with plastic feeders and waterers to help prevent nickel contamination. Animals were fed twice daily at 6% of metabolic body weight throughout the first two collection periods. Distilled water was offered ad libitum throughout the experiment. After the lambs had been on their respective diets for 60 days, rumen samples were collected for determination of urease activity (designated as period 1). Two weeks later samples were collected from the same lambs (period 2) and again assayed for urease. Rumen samples were collected 2 h after morning feeding by stomach tube.

On day 90 of the experiment, urea was incorporated into the diet at .5%. After 5 days urea was increased to 1% of the diet for an additional 5 days. At the end of the 10-day adjustment rumen samples were collected for determination of urease activity and volatile fatty acid (VFA) concentrations. All lambs were restricted to 600 g of feed per day in two equal portions during the urea feeding period.

Urease was assayed by a modification of the method of Cook (5). Rumen samples were diluted in 50 mM phosphate buffer pH 7.2, and assays were incubated at 37 C for 7 min. Ammonia was determined by the procedure of Chany and Marbach (4). Protein was deter-
### TABLE 1. Composition of the basal diet.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dried skim milk</td>
<td>38.24</td>
</tr>
<tr>
<td>Solka flocculent</td>
<td>5.00</td>
</tr>
<tr>
<td>Glucose</td>
<td>12.00</td>
</tr>
<tr>
<td>Starch</td>
<td>40.66</td>
</tr>
<tr>
<td>Corn oil</td>
<td>3.00</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>.95</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>.10</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>.05</td>
</tr>
</tbody>
</table>

- aLand O’Lakes, Inc., Minneapolis, MN. Assayed 1.7% K, 1.3% Ca, and 1.1% P.
- bBrown Company, Chicago, IL.
- CMallinckrodt, Inc., St. Louis, MO.
- dA. E. Staley Manufacturing Company, Decatur, IL.
- eExpresses as units or mg/lb. Vitamin A acetate, 1,500,000 units; Vitamin D3, 150,000 units; D-α-tocopherol acetate, 10,000 units; Riboflavin, 500 mg; D-Pantothenic acid, 2,750 mg; Niacin, 7500 mg; Choline chloride, 75,000 mg; Vitamin B12, 8 mg.
- fAureo-10 (Chlortetracycline).

### TABLE 2. Composition of mineral mix used in the basal diet.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/kg diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>5.0000</td>
</tr>
<tr>
<td>MgSO4</td>
<td>3.5000</td>
</tr>
<tr>
<td>FeSO4 · 7H2O</td>
<td>.3000</td>
</tr>
<tr>
<td>CuSO4 · 5H2O</td>
<td>.0300</td>
</tr>
<tr>
<td>KI</td>
<td>.0262</td>
</tr>
<tr>
<td>MnSO4 · H2O</td>
<td>.0590</td>
</tr>
<tr>
<td>Na4 MoO4 · 2H2O</td>
<td>.0030</td>
</tr>
<tr>
<td>CoCl2 · 6H2O</td>
<td>.0038</td>
</tr>
<tr>
<td>Na2 SeO3</td>
<td>.0002</td>
</tr>
<tr>
<td>CrCl3 · 6H2O</td>
<td>.0020</td>
</tr>
<tr>
<td>NaF</td>
<td>.0022</td>
</tr>
<tr>
<td>ZnO</td>
<td>.0620</td>
</tr>
<tr>
<td>SnCl2 · 2H2O</td>
<td>.0038</td>
</tr>
<tr>
<td>Na2SiO3 · 9H2O</td>
<td>.5000</td>
</tr>
<tr>
<td>NH4 VO3</td>
<td>.0005</td>
</tr>
</tbody>
</table>

### RESULTS AND DISCUSSION

Urease activity was reduced in lambs maintained on low nickel diets (Table 3) (P<.001). There was no difference between the two collection periods. In period 1 urease was not detected in the rumen of two lambs on the low nickel diet, and in period 2 no urease was detected in the rumen of one of the low nickel lambs. Since dietary protein was supplied exclusively from dry skim milk, the only urea available to rumen microorganisms would be that which was recycled via way of the saliva (13) and urea that diffused from the blood stream across the rumen wall (8).

When urea was incorporated into the diet, urease activity was increased (Table 4) for both treatments as compared to the two earlier periods (P<.05 for low nickel group and P<.10 for nickel supplemented group). However, the difference between treatments was still highly significant whether expressed as μM NH3-N liberated per ml of rumen fluid (P<.005) or per mg of protein (P<.001).

Ammonia concentrations in the rumen are in Table 5. Since casein and urea are degraded rapidly to ammonia, rumen ammonia was high for both treatments. The lower rumen ammonia in the low nickel lambs in period 3 may reflect the lower urease activity in this group as compared to the nickel supplemented lambs. However, rumen ammonia was not affected significantly by treatment at any time.

In contrast to previous studies (2, 3, 14) urease activity increased slightly as a result of urea supplementation. This could be attributed to the lower rumen ammonia concentrations.
TABLE 4. Influence of dietary nickel on ruminal urease in lambs fed a urea containing diet.

<table>
<thead>
<tr>
<th></th>
<th>Low Ni</th>
<th>5 ppm</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>μM NH₃-N/min/ml rumen fluid</td>
<td>1.52ᵃ</td>
<td>7.93ᵇ</td>
<td>.72</td>
</tr>
<tr>
<td>μM NH₃-N/min/mg protein</td>
<td>.126ᶜ</td>
<td>.502ᵈ</td>
<td>.028</td>
</tr>
</tbody>
</table>

ᵃᵇ Means in the same line bearing different superscripts differ (P<.005).
ᶜᵈ Means in the same line bearing different superscripts differ (P<.001).

during this period (Table 5). During 30 min-incubations, ammonia concentrations ranging from .04 M to .4 M had no inhibitory effect on purified rumen urease (11). John et al. (9) found that urease production by a ureolytic strain of *Selenomonas ruminantium* was greatly depressed when the bacteria were grown on a high urea or ammonia medium. They suggested that ammonia repressed the synthesis of urease (9). Chalupa et al. (3) noted a reduction in ruminal urease activity in sheep fed urea.

Houpt (8) observed that the net transport of nitrogen from the blood stream into the rumen was increased by as much as 13 fold when no attempt was made to remove or inhibit bacterial urease in rumen epithelium. Since the ammonia molecule is smaller and penetrates cell membranes much faster than the urea molecule, it has been suggested that urease in the rumen epithelium facilitates transport of urea nitrogen into the rumen from the blood (8). Chalupa et al. (3) found that in urea-fed sheep decreased ureolytic activity in the rumen mucosa was associated with the lowered rumen bacterial urease activity. Therefore, the low urease activity may have influenced the recycling of nitrogen via this route which is of major importance during periods of low nitrogen intake.

Ruminal VFA's are in Table 6. No significant differences were observed in total VFA concentrations or molar proportions of the individual acids measured suggesting that rumen fermentation was not drastically affected by 5 ppm of dietary nickel. Earlier in vitro work (12) with rumen microorganisms indicated that nickel was extremely inhibitory at low concentrations. Spears et al. (20) found no depression in total digestibility when a low nickel diet was supplemented with 5 ppm of nickel. Digestibility results were similar when steers were fed much higher dietary nickel (17).

Ruminal urease has a nickel requirement, and the differences between treatments were not due to a decrease in the rumen microbial population. The increased activity could have been due to a direct activation of the enzyme by nickel or nickel could be an essential component of the enzyme as was found with

TABLE 5. Influence of dietary nickel on rumen ammonia concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Low Ni</th>
<th>5 ppm Ni</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period 1ᵃ</td>
<td>48.3</td>
<td>45.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Period 2ᵃ</td>
<td>48.9</td>
<td>48.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Period 3ᵃ</td>
<td>21.2</td>
<td>30.5</td>
<td>3.5</td>
</tr>
</tbody>
</table>

ᵃ mg NH₃-N/100 ml rumen fluid.

TABLE 6. Influence of dietary nickel on rumen volatile fatty acids.

<table>
<thead>
<tr>
<th></th>
<th>Low Ni</th>
<th>5 ppm Ni</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetateᵇ</td>
<td>51.3</td>
<td>52.7</td>
<td>.9</td>
</tr>
<tr>
<td>Propionateᵇ</td>
<td>32.4</td>
<td>31.0</td>
<td>.8</td>
</tr>
<tr>
<td>Butyrateᵇ</td>
<td>9.9</td>
<td>10.8</td>
<td>.5</td>
</tr>
<tr>
<td>Valerateᵇ</td>
<td>6.4</td>
<td>5.6</td>
<td>.2</td>
</tr>
<tr>
<td>Total VFA'sᶜ</td>
<td>114.7</td>
<td>112.3</td>
<td>4.5</td>
</tr>
</tbody>
</table>

ᵃ Results from period 3.
ᵇ Molar percent.
ᶜ μM/ml rumen fluid.
the jack-bean urease (7). The bacterial enzyme also must be quite specific for nickel since all other known essential trace elements for the mammalian system were supplied in the diet by the mineral mix (Table 2). It cannot be determined from the present study if the effect of nickel on rumen urease is due to a decrease in the number of ureolytic bacteria in the rumen or if these species are present but have a defective enzyme.

ACKNOWLEDGMENTS

We are indebted to R. B. Hespell for helpful discussions and advice. C. J. S. was supported in part by NSF Grant ENG-7420777 and U.S. Department of Agriculture Grant 35-331.

REFERENCES