ABSTRACT

The hypothesis that addition of proline may save dietary protein for milk production was tested by administration of proline to two goats through a duodenal cannula. Because proline is synthesized by the mammary gland in vitro from arginine, the effect of proline supplementation on arginine uptake by the gland was tested in vivo. Arginine uptake, calculated from arterio-venous difference, dropped significantly in both animals, especially in the morning 1 h after milking, when a low-protein diet was fed. Milk production and total nitrogen in milk were not affected significantly by proline supplementation. A trend was toward decrease of milk orotic acid and an increase of milk fat due to proline supplementation.

INTRODUCTION

Most of the digestible protein in rations fed to lactating ruminants is used for milk production. Because the main milk protein synthesized in the mammary gland is casein, the supply of amino acids to the gland should meet the requirement for casein synthesis. Most of the work on this subject deals with essential amino acids (8). Several of them have been proposed as limiting, including phenylalanine, methionine, lysine, threonine (4, 12), and others. Some nonessential amino acids required for milk protein production are synthesized in the gland from several essential ones, including arginine (10) and the branch-chained amino acids (10, 18). Ornithine is taken up by the gland, although it is not a constituent of proteins (16). Arginine is extracted from the blood in amounts greatly exceeding the need for protein synthesis (1, 9, 13) and undergoes extensive catabolism in the gland. Part of the arginine and ornithine is converted to spermidine, connected with RNA synthesis (11), but about 20% of these amino acids is used for proline synthesis (2, 17). Proline, a nonessential amino acid, is one of the most abundant amino acids in casein, constituting about 12%. Proteins of conventional feeds and of microbial proteins of the rumen contain generally from 3 to 5% proline. Because conversion of arginine to proline is accompanied by a substantial loss of nitrogen, it is possible that the nitrogen supplied to the dairy cow may be reduced, especially at peak lactation, if additional proline is protected to prevent microbial degradation in the rumen. Halfpenny et al. (5) found low proline concentrations in the plasma of lactating dairy cows and assumed that proline may be lacking for efficient synthesis of milk protein.

The influence of proline infusion into the duodenum on arginine uptake by the mammary gland and on nitrogen utilization for synthesis of milk protein was measured in lactating goats.

MATERIALS AND METHODS

Animals

Two Saanen goats in mid lactation were fitted with duodenal cannulae, and their carotid arteries were exposed by the following technique. Prior to anaesthesia, food was withheld for 24 h. Ketamine hydrochloride, dose 3 mg/kg, was injected rapidly into the jugular vein as a preanesthetic (15). Endotracheal tube was inserted blindly into the trachea and connected to a closed circuit anaesthetic apparatus. A skin incision of about 10 cm was made parallel to the jugular vein in the left middle third of the neck. The skin then was freed of its subcutis, and the muscles underneath...
were separated bluntly. The carotid artery was freed from its sheath and the vagus nerve for a length of ~ 8 cm. After its small branches were ligated, the free carotid artery was brought under the skin. A Marlex mesh (Davol, US), 6 cm long by 3 cm wide, was sutured to the sterno-cephalicus muscle with braided Marlex sutures. The carotid artery then was placed on it. This rigid mesh forced the artery to remain close to and directly under the skin. The artery could be palpated and the pulse felt easily. Blood was taken by direct percutaneous puncture, sometimes several times a day during the experiment. The carotid artery has been functioning without any difficulty for more than 2 yr.

**Diets**

The goats received a daily ration of 1.25 kg pellets and .25 kg chopped alfalfa hay twice daily. In the preliminary and the first experiment, the pellets were made from a concentrate and constituted 83% of the diet, providing 11.9% crude protein; in the second experiment, half of the concentrate pellets were replaced by corn pellets, and the crude protein given as concentrate was dropped to 9.6% (Table 1). Hay supplied 2.6% crude protein in both experiments.

**Chemical Analysis**

Total nitrogen was measured after digestion of duplicate aliquots and nesslerization (6).

**Experimental Procedures**

Experimental diets were fed first for a 1-wk adaptation. Thereafter saline (control) or a solution of proline in saline were infused continuously into the duodenum by peristaltic pump. The goats were milked and fed at 0800 and at 1800.

In a preliminary experiment 260 ml of 1% proline solution was infused daily for 4 days. In the first and the second experiments, the concentration of proline was raised to 2%, and 400 ml was infused daily for 9 days. In each experiment samples of blood, milk, and urine were taken during the last 2 days of infusion. A control period of the same duration as the experimental period preceded each proline infusion.

Blood was sampled at 0900 and 1300 from the carotid artery and the subcutaneous abdominal vein. Plasma ultrafiltrates were analyzed for amino acids in a Technicon TSM Amino Acid Analyzer. Uptake by the mammary gland was calculated from arterio-venous differences;

---

**TABLE 1. Composition of diets in Experiments 1 and 2.**

<table>
<thead>
<tr>
<th>Pellets&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Hay</th>
<th>Corn pellets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1 (high protein)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83</td>
<td>17</td>
</tr>
<tr>
<td>Experiment 2 (low protein)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>41.5</td>
<td>17</td>
</tr>
<tr>
<td>Chemical composition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter</td>
<td>87.8</td>
<td>90.1</td>
</tr>
<tr>
<td>Crude protein</td>
<td>14.3</td>
<td>15.3</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>8.9</td>
<td>27.3</td>
</tr>
<tr>
<td>Crude fat</td>
<td>3.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Ash</td>
<td>5.5</td>
<td>8.4</td>
</tr>
</tbody>
</table>

<sup>a</sup>The pellets contained (%): soybean oil meal, 14.0; corn, 56.0; alfalfa hay, 30.0; di-calcium phosphate, .1; A and D vitamins, .02.

<sup>b</sup>The crude protein content of the diets was 14.5% in Experiment 1 and 12.2% in Experiment 2.
mammary blood flow was estimated from the regression equation of Kronfeld et al. (7).

**RESULTS AND DISCUSSION**

No differences were significant between control and proline-supplemented animals in the preliminary experiment, probably because of the low proline concentration and short infusion time.

Results of the first experiment (Table 2) show a reduction of total nitrogen output in urine and of orotic acid output in milk after proline infusion, but differences were not significant. Milk fat percentage increased significantly from 2.8 to 3.2.
The second experiment was 3 wk after the first. Characteristics measured returned to control 1 wk after completion of the first experiment. Lowering the protein content of the ration in the second experiment (Table 3) accentuated the effect of proline infusion; there was a significant increase of percent milk protein and of total protein excreted in milk. Fat percentage rose to 4.0 as compared with 2.8 in the control. The difference was highly significant. Less orotic acid was in milk after proline addition.

The effect of proline on excretion of orotic acid should be tested in cows, because goats excrete relatively small amounts of orotic acid in milk. It is possible that in cows orotic acid is synthesized as the end product of ammonia detoxification, because the mammary gland lacks the complete urea cycle (2, 10).

Uptake of arginine was not significantly diminished by proline supplementation in the first experiment with high protein in the diet but dropped to about half when proline was added to the low protein diet in the second experiment (Table 4). Uptake of arginine was measured twice daily, in the morning after milking and in the afternoon. In the morning, arginine uptake by the gland without proline addition was always much higher than with proline addition. In one goat arginine uptake dropped by 30% and in the other by 60% because of proline supply.

In the afternoon, arginine uptake (and probably milk protein synthesis) was low in the first goat. Again, addition of proline lowered the arginine uptake substantially. In the second goat there was an active arginine uptake in the afternoon and it was lowered by proline supply; the small difference of arginine uptake with and without proline indicates that also in this case arginine might not be used for milk protein synthesis, in contrast to the morning activity. There are substantial differences of uptake measured at different times during the day (8). The arteriovenous difference depends on the activity of the gland for either protein synthesis or for other purposes at the time of measurement.

It could be calculated roughly that about 20% of the arginine was saved, due to proline treatment, in accordance with the data obtained in vitro by Verbeke et al. (17). These authors found that proline in the sheep udder is synthesized from both ornithine and arginine in an amount approaching 20%.

The experiments will be repeated with the goats at peak lactation, and if encouraging results are obtained again, with lactating cows.

ACKNOWLEDGMENTS

Amino acid analysis was in the Division of Animal Nutrition, The Volcani Center, under the supervision of Z. Harduf and B. Iosif. The.

---

**TABLE 4. Arterio-venous difference (A–V) and uptake of arginine by the lactating mammary gland of two goats in Experiment 2.**

<table>
<thead>
<tr>
<th>Goat no.</th>
<th>Treatment</th>
<th>Time (h)</th>
<th>A – V (mg/liter)</th>
<th>Uptake (mg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>SD</td>
</tr>
<tr>
<td>1</td>
<td>–Proline</td>
<td>0900</td>
<td>8.88</td>
<td>.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1300</td>
<td>2.97</td>
<td>.4</td>
</tr>
<tr>
<td></td>
<td>+Proline</td>
<td>0900</td>
<td>7.08</td>
<td>.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1300</td>
<td>.29</td>
<td>.1</td>
</tr>
<tr>
<td>2</td>
<td>–Proline</td>
<td>0900</td>
<td>6.76</td>
<td>.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1300</td>
<td>8.87</td>
<td>.9</td>
</tr>
<tr>
<td></td>
<td>+Proline</td>
<td>0900</td>
<td>2.83</td>
<td>.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1300</td>
<td>8.01</td>
<td>.4</td>
</tr>
</tbody>
</table>

*aMeasured twice daily during 2 consecutive days.

*Difference from relevant control (–proline) significant at P<.05 (paired t test).
skillful assistance of I. Zukerman (veterinary surgery) is acknowledged gratefully. The authors thank M. Meidler and S. Krasner for handling blood samples and for analysis of feeds.

This research was supported by a grant from the United States-Israel (Binational) Agricultural Research and Development Fund (BARD).

REFERENCES


