Dietary Urea for Dairy Cattle. I. Relationship to Luteal Function

H. A. GARVERICK, 2 R. E. ERB, R. D. RANDEL,3 and M. D. CUNNINGHAM
Department of Animal Sciences, Purdue University
Lafayette, Indiana 47907

Abstract

Isonitrogenous complete mixed rations containing concentrates, corn silage, and ground corn cobs, differing only in source of supplemental nitrogen (Group I—soybean meal and Group II—urea), were fed to Holstein-Friesian heifers for a minimum of 60 days. The purpose was to study the effect of dietary urea on luteal function. Jugular blood and corpora lutea were collected on Day 12 after estrus. Average concentrations of progesterone, luteinizing hormone, corticosterone, and cortisol in blood plasma were approximately the same for both groups. Ten corpora lutea from Group I were heavier (P < .005) than the 12 from Group II (6.4 ± .6 g versus 4.3 ± .3 g). The corpora lutea from Group II were softer and more fragile than those from Group I, but there were no discernible histological differences. The contents and concentrations of progesterone and 20β-hydroxy-pregnene-3-one in unincubated corpora lutea were not significantly different between treatments. However, corpus luteum tissue from Group I as compared to Group II synthesized more progesterone (P < .05) during in vitro incubation in blood plasma (2 hr at 37 C) and contained more total progesterone (P < .05) after incubation. Source of jugular plasma substrate, either from Group I or Group II, had no significant effect on synthesis of progesterone. The relationship between differences in corpus luteum function due to ration and reproductive efficiency was not determined.

Received for publication May 17, 1971.

1 Journal Paper 4434, Purdue University Agricultural Experiment Station.

2 Fellow, Allied Chemical Foundation, New York City. Present address: Department of Dairy Husbandry, University of Missouri, Columbia 65201.

3 Present address: United States Department of Agriculture Range Livestock Experiment Station, Miles City, Montana 59301.

Introduction

Urea is used widely as a source of dietary nitrogen in cattle to replace plant proteins. Certain rumen microorganisms anabolize urea nitrogen via ammonia to protein for subsequent digestion and utilization by the cow. However, producers reluctantly incorporate urea into dairy rations because of inconclusive evidence that dietary urea may decrease reproductive efficiency (1, 6, 13, 20, 24, 28, 29).

Biological activity of luteinizing hormone (LH), the major luteotropic hormone in cattle (11, 16), is destroyed in vitro by substrates containing 6 M urea (30). Destruction is 50 to 70% in the presence of 1 M urea (30). It was theorized that breakdown products of urea caused inactivation of LH rather than urea (30). This suggests that an indirect inhibition of luteal function may occur if LH is partially inhibited in vivo in the presence of endogenous urea or endogenous breakdown products of urea.

The objective of the present study was to determine if dietary urea was associated with measurable differences in luteal function of dairy heifers. A brief report on part of the data has been published (8).

Materials and Methods

Fifteen Holstein-Friesian heifers, 12 to 18 months of age and weighing 270 to 550 kg, were divided into two groups equalized for age and body weight. Group I (seven heifers) was fed soybean meal and Group II (eight heifers) was fed urea (145 to 240 g/day for individuals) as sources of supplemental nitrogen. The two rations were isonitrogenous. Premix concentrate rations were prepared for each group (Table 1) for mixing with the roughage portion. The complete mixed total ration on a dry matter basis contained 22% premix, 50% corn silage, and 22% ground corn cobs. Average ad libitum feed consumption by individuals ranged from 6 to 9 kg of dry matter daily. Approximately 50% of the nitrogen in the total ration for Group II was from urea. Dietary urea, increased gradually for three weeks, was fed at the experimental level
TABLE 1. Ingredients of the premix concentrate rations.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Group</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>Soybean meal(^a)</td>
<td>74.40</td>
<td>........</td>
</tr>
<tr>
<td>Urea(^b)</td>
<td>........</td>
<td>10.94</td>
</tr>
<tr>
<td>Rolled shelled corn</td>
<td>15.35</td>
<td>78.81</td>
</tr>
<tr>
<td>Molasses</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Sodium chloride(^c)</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>.75</td>
<td>.75</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

\(^a\) 50\% Crude protein.
\(^b\) 281\% Crude protein equivalent.
\(^c\) Contained trace minerals.
\(^d\) Each kilogram of premix contained 16,875 IU of vitamin A and 3,000 IU of vitamin D.

Blood plasma was the substrate during incubation of CL tissue to determine if inhibitors of in vitro synthesis of progesterone were present. Equal amounts of blood plasma, collected at 7 AM, were combined from individuals within each group to make two pools of plasma. This was done prior to the day the first CL was excised. The fresh plasma of each pool was divided into aliquots prior to storage at -23 C until assayed for content of progesterone and 20β-hydroxy-pregnene-3-one (20β-ol). In addition, tissue from 10 of the CL, five from each from Groups I and II, was incubated for 2 hr in a Dubnoff metabolic shaker at 37 C under a stream of 95\% O\(_2\):5\% CO\(_2\). Approximately 150 mg of the minced tissue from each CL was incubated in duplicate in the presence of 5 ml of plasma from each of the three sources, namely, the animal's fresh plasma and stored (frozen) plasma from Groups I and II, respectively. After 2 hr the incubates were stored at -23 C until assayed for content of progesterone and 20β-ol.

Jugular plasma progesterone and 20β-ol were isolated and quantified by Harms and Malven's (12) procedures. Briefly described, the method included addition of progesterone-4-\(^{14}\)C to correct for procedural losses, homogenization, ether extraction, partial purification of the extract by two dimensional thin-layer chromatography, and quantification by gas liquid chromatography.

Jugular plasma progesterone, cortisol, and corticosterone were quantified in duplicate by the competitive protein binding technique described by Murphy (18) as modified by Randel et al. (21). Plasma LH was assayed in duplicate by double antibody radioimmunoassay (19).

Analysis of variance procedures as outlined by Steel and Torrie (27) were used to establish probabilities for significant differences between variables.

Results and Discussion

Plasma hormones. Average progesterone, luteinizing hormone, corticosterone, and cortisol in blood plasma were not significantly different between groups (Table 2) and were similar to other data for Day 12 of the estrous cycle (9).
UI%EA AND LUTEAL FUNCTION

TABLE 2. Average concentrations of hormones in jugular blood plasma from Groups I and II.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>14.4 ± 2.0a</td>
<td>15.1 ± 1.4</td>
</tr>
<tr>
<td>Luteinizing hormoneb</td>
<td>.46 ± .10</td>
<td>.46 ± .12</td>
</tr>
<tr>
<td>Corticosterone</td>
<td>2.3 ± .6</td>
<td>3.4 ± .9</td>
</tr>
<tr>
<td>Cortisol</td>
<td>3.8 ± 1.1</td>
<td>3.1 ± 1.0</td>
</tr>
</tbody>
</table>

a Standard error.
b Nanogram, National Institutes of Health luteinizing hormone, B6 per milliliter.

**Corpora lutea.** The CL from Group I averaged 6.4 ± .6 g as compared to 4.3 ± .3 g for Group II (P < .005). Only three of the 12 CL from Group II weighed more than 5 g as compared to 7 of the 10 CL from Group I. The Group II average of 4.3 g for Day 12 of the estrous cycle is lower than averages for several experiments summarized by Erb et al. (5). In comparison, the Group I average is slightly higher (5). Group II CL were softer, more fragile, and more difficult to express from the ovary because of the lack of firmness as compared to those from Group I. However, no differences in cellular structure (7) were detectable during histological examination. Moreover, there was no evidence in either group of structural changes indicative of regression as described by Foley and Greenstein (7).

The average concentrations of progesterone (nonsignificant) and 20β-ol (P < .10) were lower in CL from Group I than from Group II (Table 3). Average content of progesterone was higher (nonsignificant) for Group I because of larger CL as compared to Group II. The average content of 20β-ol was nearly the same because the concentration was about 100% higher for Group II than for Group I. The averages of 192 and 154 μg of progesterone per corpus luteum in Groups I and II are comparable to other data for a similar period of the estrous cycle as summarized by Erb et al. (5). Average 20β-ol was 11 and 15% of the total progestin as compared to 14% reported by Gomes et al. (10). Higher proportions of 20β-ol have been reported in CL during the late luteal phase (14) and during some stages of pregnancy (4). Such increases in proportion of 20β-ol to progesterone have been associated with maturity of CL in conjunction with changes in cellular structure (14) which are believed to be regressive (7).

Although dietary urea (Group II) was associated with decreased CL weight there was little evidence of delayed maturity since these CL contained slightly higher proportions of 20β-ol to progesterone than controls (Group I).

**Synthesis of progestins.** There were no significant differences between sources of plasma used as substrate and rate of synthesis of the two progestins (Table 4). Average luteal concentration and content of progesterone and total progestin were significantly (P < .025) higher in the plasma incubates than in unincubated luteal tissue (Table 4). Similarly compared, luteal concentration but not content of 20β-ol was increased by incubation (P < .025).

The average luteal concentrations of progesterone, 20β-ol, and total progestins were nonsignificantly different between groups before and after incubation (Table 4). The average content of progestone (P < .01) and total progestin (P < .05) was greater after incubation in luteal tissue from Group I as compared to Group II (Table 4). As calculated from Table 4, luteal tissue from Group I synthesized more progesterone (P < .05) and total progestin (P < .10). Luteal tissue from Group I synthesized an average of 146 μg of progesterone per CL as compared to 77 μg for Group II for respective increases of 70 and 48%. Similarly compared, total progestin during incubation increased 75% for Group I and 52% for Group II.

**TABLE 3. Average content of progestins in corpora lutea from Groups I and II.**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>192 ± 27a</td>
<td>31 ± 3</td>
</tr>
<tr>
<td>20β-olb</td>
<td>25 ± 10</td>
<td>3 ± 1</td>
</tr>
</tbody>
</table>

a Standard error.
b 20β-hydroxy-pregnene-3-one.
Table 4. Relationship of sources of luteal tissue and plasma to amounts of progestins in luteal tissue following in vitro incubation.

<table>
<thead>
<tr>
<th>Source of blood plasma</th>
<th>Group I</th>
<th></th>
<th>Group II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(μg/g)</td>
<td>(μg/CL)</td>
<td>(μg/g)</td>
<td>(μg/CL)</td>
</tr>
<tr>
<td>Progesterone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonea</td>
<td>32 ± 7c</td>
<td>210 ± 49</td>
<td>40 ± 3</td>
<td>160 ± 11</td>
</tr>
<tr>
<td>Unfrozenb</td>
<td>60 ± 19d</td>
<td>356 ± 65d</td>
<td>58 ± 11</td>
<td>232 ± 45</td>
</tr>
<tr>
<td>Frozen, Group I</td>
<td>56 ± 10</td>
<td>379 ± 99</td>
<td>60 ± 6</td>
<td>242 ± 29</td>
</tr>
<tr>
<td>Frozen, Group II</td>
<td>50 ± 3</td>
<td>334 ± 49</td>
<td>59 ± 4</td>
<td>236 ± 24</td>
</tr>
<tr>
<td>Combined incubates</td>
<td>55 ± 7</td>
<td>357 ± 40</td>
<td>59 ± 4</td>
<td>237 ± 18</td>
</tr>
<tr>
<td>20β-hydroxy-pregnene-3-one</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>5 ± 2</td>
<td>33 ± 18</td>
<td>9 ± 2</td>
<td>37 ± 8</td>
</tr>
<tr>
<td>Unfrozen</td>
<td>10 ± 2</td>
<td>67 ± 18</td>
<td>11 ± 1</td>
<td>45 ± 4</td>
</tr>
<tr>
<td>Frozen, Group I</td>
<td>11 ± 4</td>
<td>80 ± 40</td>
<td>16 ± 2</td>
<td>61 ± 8</td>
</tr>
<tr>
<td>Frozen, Group II</td>
<td>9 ± 2</td>
<td>62 ± 24</td>
<td>18 ± 5</td>
<td>73 ± 22</td>
</tr>
<tr>
<td>Combined incubates</td>
<td>10 ± 2</td>
<td>69 ± 16</td>
<td>15 ± 2</td>
<td>60 ± 8</td>
</tr>
<tr>
<td>Total progestin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>37 ± 8</td>
<td>243 ± 67</td>
<td>49 ± 3</td>
<td>197 ± 16</td>
</tr>
<tr>
<td>Unfrozen</td>
<td>70 ± 21</td>
<td>423 ± 79</td>
<td>69 ± 11</td>
<td>277 ± 45</td>
</tr>
<tr>
<td>Frozen, Group I</td>
<td>67 ± 13</td>
<td>459 ± 138</td>
<td>76 ± 6</td>
<td>303 ± 33</td>
</tr>
<tr>
<td>Frozen, Group II</td>
<td>59 ± 5</td>
<td>396 ± 71</td>
<td>77 ± 8</td>
<td>308 ± 42</td>
</tr>
<tr>
<td>Combined incubates</td>
<td>65 ± 8</td>
<td>426 ± 54</td>
<td>74 ± 5</td>
<td>297 ± 22</td>
</tr>
</tbody>
</table>

a Unincubated.  
b Blood plasma from animal contributing corpus luteum tissue.  
c Standard error.  
d Smallest corpus luteum had the highest rate of synthesis (82 μg/g).

General discussion. Two differences in luteal function, associated with including urea in the ration (Group II), were reduced weight of CL (P < .005) at 12 days after estrus and decreased synthesis of progestins, especially progesterone (P < .05), during in vitro incubation. Inhibition of CL growth as noted 12 days after estrus infers suboptimum stimulation from LH during the early phases of the estrous cycle since LH is considered the major luteotropic hormone in cattle (11, 16).

Results from incubation of luteal tissue in blood plasma of heifers on the two diets to detect the presence of possible inhibitors were inconclusive. Such inhibitors, if present in blood plasma, probably are not urea or ammonia because these levels are similar when diets containing natural protein or urea at nontoxic levels are compared (28, 29). There was more synthesis (nonsignificant) of progesterone when luteal tissue from Group I was incubated in frozen plasma from Group I as compared to incubating the same tissue in unfrozen plasma or in frozen plasma from Group II. Though the amounts synthesized were less in Group II as compared to Group I the trends were similar to Group I.

Anti-luteinizing hormone reduced corpus luteum weight and content of progesterone when administered to heifers on Days 2 to 6 of the estrous cycle, but concentration of progesterone in the CL was unchanged (26). A similar effect was observed when anti-LH was administered to hysterectomized heifers (2). If luteal function is inhibited as the result of feeding urea, differences in LH and progesterone in blood plasma should be detectable. However, levels of the two hormones were similar in both groups (Table 2). Failure to detect differences between groups in progesterone in peripheral plasma on Day 12 of the estrous cycle may be inconclusive since differences in rates of secretion, metabolism, and excretion could result in maintenance of similar concentrations of progesterone. Although LH produced large increases in bovine luteal synthesis of progesterone in vitro (15), increases in concentration of progesterone in peripheral blood plasma were detected only about 50% of the time when LH was injected in vivo (25).
Urea and Luteal Function

Toxic levels of urea in the diet alter electrocardiogram and respiratory patterns in ruminants (3, 22) which are not detected when nontoxic levels are fed (23). The daily intake of urea by Group II apparently was not stressful since levels of cortisol and corticosterone were similar to Group I.

Previous studies on dietary urea for dairy cattle generally have been short term and multidirectional. Dietary urea had no adverse effect on estrous cycles (13, 24, 28) or services per conception (1, 13) in some experiments and adverse effects in other experiments (6, 20, 28, 29). Oltjen (20) reported that dietary urea which furnished nearly 100% of the dietary nitrogen in purified diets was associated with shortened estrous cycles, increased services per conception, longer gestations, increased rates of abortion and decreased birthweight of calves. Virtanen (28, 29) has reported increased services per conception for dairy heifers receiving 99% of their dietary nitrogen from urea and ammonium salts. Including vitamin E in the diet was ineffective for improving conception rates (29).

The present study indicates that luteal growth and capacity to synthesize progesterone in vitro may be reduced when 50% of the supplemental nitrogen from urea is substituted for plant protein in the diets of heifers for 60 or more days. However, design of this experiment precludes evaluation of effects of the differences on reproductive efficiency. Since the relationships between differences in luteal weight and capacity of functional tissue to synthesize progesterone in vivo and reproductive efficiency are unknown, no adverse effects on reproduction due to dietary urea can be ascertained from this study.

Acknowledgments

The authors thank Dr. L. E. Reichert, Jr., Emory University, Atlanta, Georgia for providing purified bovine luteinizing hormone, Dr. G. D. Niswender, University of Michigan, Ann Arbor for providing anti-bovine luteinizing hormone antiserum and the Allied Chemical Corporation, New York City for partial financial support.

References

(17) Moody, E. L., and W. Hansel. 1969. LH stimulated progesterone biosynthesis in...


