Early Lactational Response to Supplemental Protein by Dairy Cows Fed Grass-Legume Forage

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ABSTRACT
A total of 24 primiparous and 46 multiparous Holstein cows were in two trials to determine the influence of concentration of dietary protein on performance during early lactation. Treatments were 13.5 or 16.5% dietary crude protein for the first 12 wk of lactation. Complete mixed diets of grass-legume forage and concentrates were fed for ad libitum consumption. Treatment patterns repeated across trials. Yields of milk and 3.5% fat-corrected milk, intake of dry matter, and returns over feed costs were improved by feeding the diet with 16.5% crude protein. Percent milk fat, body weight change, and reproductive performance were unaffected by treatment. The results demonstrate benefit, during early lactation, from supplementing protein to grass-legume based diets containing 13.5% crude protein. Performance responses to increased concentrations of dietary crude protein during early lactation appear greater for multiparous than primiparous cows.

INTRODUCTION
Numerous studies have measured responses by dairy cows to increased concentrations of dietary crude protein (CP) during early lactation. Substantial benefit usually has resulted from adding protein to diets with less than 13 to 14% CP (2, 3, 6, 12, 16). Both positive (2, 3, 9, 12, 13, 15, 19) and negative (6, 8) responses have resulted from adding protein to diets with more than 13 to 14% CP. However, only Edwards et al. (6) fed legume-forage based diets. Results from studies comparing responses of primiparous and multiparous cows to increased concentrations of dietary CP are conflicting. Multiparous cows have been more responsive to additional protein than primiparous cows in some studies (5, 12, 15, 16) and no different in others (4, 20). This research was to determine relative performance responses of primiparous and multiparous cows in early lactation to protein supplementation of grass-legume based diets.

MATERIALS AND METHODS

General
Two lactation trials evaluated effects of concentration of dietary CP on performance in early lactation. Experimental procedures were similar in both trials. Two weeks before predicted date of parturition, cows were blocked by parity and season of calving and assigned randomly to treatments, which were compared during wk 1 through 12 postpartum. Treatments were 13.5% low (LP) or 16.5% high (HP) dietary CP.

Animals and Rations
Thirty Holstein cows (12 primiparous and 18 multiparous) were in Trial 1 and 40 Holstein cows (12 primiparous and 28 multiparous) were in Trial 2. Eight extra multiparous cows were assigned to HP in Trial 2 for use in a subsequent experiment later in lactation. Rations in Trial 1 contained 50% concentrate, and those in Trial 2 contained 55% concentrate (dry matter). Dry matter (DM) of forages was measured weekly to maintain desired proportions of concentrate. Desired concentrations of dietary CP were achieved by replacing cereal grain with soybean meal (SBM) in Trial 1 or cottonseed meal (CSM) in Trial 2 (Table 1). Soybean meal averaged 50% CP (DM) with 14.8% of total...
nitrogen (N) soluble in .15 M NaCl solution and 7.7% insoluble in acid detergent solution. Cottonseed meal contained 48.7% CP with 17.4% of total N soluble in .15 M NaCl and 2.7% insoluble in acid detergent solution. Animals were managed and complete mixed rations prepared and fed as described in (17).

**Measurements and Analysis**

Feed offered was recorded twice daily and ors once daily. Samples of feeds were collected weekly and dried at 60°C. Samples from 4 wk were composited and analyzed as in (17). Milk yields were recorded twice daily and composite samples of morning and evening milk were taken twice weekly and analyzed for fat and protein as in (17). Animals were weighed once weekly, and changes in liveweight were calculated by regressing weight on week of lactation. Number of days open and services per conception were recorded for each cow.

**Data Analysis**

Least squares analysis of variance was for unequal subclass numbers (10). The model for individual trials included main effects of parity, protein treatment, season of calving (winter vs summer), and interaction of parity x treatment. Because the same treatment pattern occurred repeatedly, trials also were combined.

**RESULTS**

**Ration Composition**

Mean concentrations of ration nutrients (calculated from laboratory analysis and consumption data) are in Table 2. Concentrations of DM, acid detergent fiber (ADF), and neutral detergent fiber (NDF) were uniform across rations within trials. Ration concentrations of CP were as planned except that ration HP in Trial 2 was .9 percentage units greater than intended. Thus, the increment between protein treatments was greater in Trial 2 than Trial 1. All rations contained high proportions of total N as soluble N and acid detergent insoluble N (ADIN), reflecting high proportions of these nitrogenous fractions in the hay-crop forage. Alfalfa hay averaged 44.3% soluble N and 8.8% ADIN. Alfalfa-grass silage averaged 59.6% soluble N and 14% ADIN. Estimated net energy for lactation (NEₐ), calculated from National Research Council (NRC) tabular data (14), averaged 1.57 Mcal/kg DM in Trial 1 and 1.59 Mcal/kg DM in Trial 2.

**Milk Yield**

Mean daily yields of milk and 3.5% fat-corrected milk (FCM) are in Table 3, and weekly FCM means of pooled trials are in Figure 1A. Concentration of dietary CP tended

**TABLE 1. Ingredient proportions in complete rations.**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Trial 1 (%)</th>
<th>Trial 2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alfalfa-grass silage</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Barley</td>
<td>44</td>
<td>36</td>
</tr>
<tr>
<td>Shelled corn</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>. . .</td>
<td>8</td>
</tr>
<tr>
<td>Cottonseed meal</td>
<td>. . .</td>
<td>. . .</td>
</tr>
<tr>
<td>Beet pulp</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mineral</td>
<td>1²</td>
<td>1²</td>
</tr>
</tbody>
</table>

³ Containing 67% monosodium phosphate and 33% trace mineral salt (guaranteed analysis: 98% NaCl, .35% Zn, .28% Mn, .18% Fe, .04% Cu, .01% I, .01% Co).

³ Containing 35.7% dicalcium phosphate, 35.7% trace mineral salt (guaranteed analysis: 98% NaCl, 3.5% Zn, .28% Mn, .18% Fe, .04% Cu, .01% I, .01% Co), and 28.6% Dynamite (22% S, 18% K, 11% Mg).
to influence milk production positively. In each trial, yields of milk and FCM of both primiparous and multiparous (●) cows fed 13.5 (---) or 16.5% (--•--) dietary crude protein (CP) during early lactation.

Figure 1. Yield of milk, intake of dry matter (DM), and change in body weight of primiparous (○) and multiparous (●) cows fed 13.5 (---) or 16.5% (--•--) dietary crude protein (CP) during early lactation.

Table 2. Nutrient composition of complete rations.†

<table>
<thead>
<tr>
<th>Component</th>
<th>Trial 1 Low CP</th>
<th>Trial 1 High CP</th>
<th>Trial 2 Low CP</th>
<th>Trial 2 High CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM, %</td>
<td>54.0</td>
<td>53.6</td>
<td>49.9</td>
<td>52.1</td>
</tr>
<tr>
<td>CP, % DM</td>
<td>13.6</td>
<td>16.4</td>
<td>13.8</td>
<td>17.4</td>
</tr>
<tr>
<td>Acid detergent fiber, % DM</td>
<td>27.5</td>
<td>27.5</td>
<td>29.3</td>
<td>30.2</td>
</tr>
<tr>
<td>Neutral detergent fiber, % DM</td>
<td>49.4</td>
<td>49.2</td>
<td>45.5</td>
<td>43.5</td>
</tr>
<tr>
<td>Acid detergent insoluble N, % total N</td>
<td>10.1</td>
<td>10.2</td>
<td>11.7</td>
<td>9.8</td>
</tr>
<tr>
<td>NaCl soluble N, % total N</td>
<td>41.1</td>
<td>37.4</td>
<td>41.2</td>
<td>36.0</td>
</tr>
</tbody>
</table>

†CP = Crude protein; DM = dry matter, N = nitrogen.
### TABLE 3. Least square means for yield and composition of milk.\(^1\)

<table>
<thead>
<tr>
<th>Item</th>
<th>Trial 1</th>
<th></th>
<th>Trial 2</th>
<th></th>
<th>Pooled trials</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Low CP</td>
<td>High CP</td>
<td>SE</td>
<td>Low CP</td>
<td>High CP</td>
<td>SE</td>
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<tr>
<td>No. animals</td>
<td>6</td>
<td>6</td>
<td></td>
<td>6</td>
<td>6</td>
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<tr>
<td>Milk yield, kg/day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actual milk</td>
<td>22.0</td>
<td>24.3</td>
<td>1.4</td>
<td>21.0</td>
<td>22.5</td>
<td>.9</td>
</tr>
<tr>
<td>3.5% FCM</td>
<td>20.5</td>
<td>22.9</td>
<td>1.3</td>
<td>21.2</td>
<td>23.0</td>
<td>1.2</td>
</tr>
<tr>
<td>Milk composition, %</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Fat</td>
<td>3.1</td>
<td>3.2</td>
<td>.1</td>
<td>3.6</td>
<td>3.6</td>
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<td>Protein</td>
<td>3.4</td>
<td>3.3</td>
<td>.1</td>
<td>3.2</td>
<td>3.2</td>
<td>.1</td>
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<tr>
<td>Heifers</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. animals</td>
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<td>9</td>
<td></td>
<td>10</td>
<td>18</td>
<td></td>
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<tr>
<td>Milk yield, kg/day</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actual milk</td>
<td>29.3</td>
<td>30.2</td>
<td>1.1</td>
<td>27.4(^a)</td>
<td>32.2(^b)</td>
<td>.6</td>
</tr>
<tr>
<td>3.5% FCM</td>
<td>28.0</td>
<td>29.2</td>
<td>1.0</td>
<td>28.6(^a)</td>
<td>32.1(^b)</td>
<td>.8</td>
</tr>
<tr>
<td>Milk composition, %</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>3.2</td>
<td>3.3</td>
<td>.1</td>
<td>3.8</td>
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<td>.1</td>
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<tr>
<td>Protein</td>
<td>3.3</td>
<td>3.3</td>
<td>.1</td>
<td>3.3(^a)</td>
<td>3.0(^b)</td>
<td>.1</td>
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<tr>
<td>No. animals</td>
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<tr>
<td>Milk yield, kg/day</td>
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<td>3.5% FCM</td>
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<td>Protein</td>
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</tbody>
</table>

\(^a,b\) Means within parity-trial subclasses with unlike means differ (P<.05).

\(^1\) CP = crude protein; FCM = fat-corrected milk.
### TABLE 4. Least square means for feed intake and body weight change.  

<table>
<thead>
<tr>
<th></th>
<th>Trial 1</th>
<th></th>
<th>Trial 2</th>
<th></th>
<th>Pooled trials</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low CP</td>
<td>High CP</td>
<td>SE</td>
<td>Low CP</td>
<td>High CP</td>
<td>SE</td>
<td>Low CP</td>
<td>High CP</td>
<td>SE</td>
</tr>
<tr>
<td><strong>Heifers</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intake</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, kg/day</td>
<td>15.5</td>
<td>17.0</td>
<td>.5</td>
<td>15.1</td>
<td>18.8</td>
<td>.9</td>
<td>15.2</td>
<td>17.9</td>
<td>.5</td>
</tr>
<tr>
<td>NE\textsubscript{L}, Mcal/day</td>
<td>24.3</td>
<td>26.7</td>
<td>.7</td>
<td>24.0</td>
<td>30.0</td>
<td>1.4</td>
<td>24.1</td>
<td>28.4</td>
<td>.8</td>
</tr>
<tr>
<td>CP, kg/day</td>
<td>2.11\textsuperscript{a}</td>
<td>2.82\textsuperscript{b}</td>
<td>.07</td>
<td>2.05\textsuperscript{a}</td>
<td>3.25\textsuperscript{b}</td>
<td>.16</td>
<td>2.09</td>
<td>3.04</td>
<td>.09</td>
</tr>
<tr>
<td><strong>Body weight, kg</strong></td>
<td>522</td>
<td>503</td>
<td>17</td>
<td>534</td>
<td>555</td>
<td>13</td>
<td>528</td>
<td>529</td>
<td>10</td>
</tr>
<tr>
<td><strong>Body weight change, kg/day</strong></td>
<td>.07</td>
<td>.13</td>
<td>.17</td>
<td>.34</td>
<td>.26</td>
<td>.19</td>
<td>.14</td>
<td>.29</td>
<td>.13</td>
</tr>
<tr>
<td><strong>Cows</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Intake</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM, kg/day</td>
<td>17.7\textsuperscript{a}</td>
<td>19.4\textsuperscript{b}</td>
<td>.4</td>
<td>17.9\textsuperscript{a}</td>
<td>20.7\textsuperscript{b}</td>
<td>.6</td>
<td>17.9</td>
<td>20.1</td>
<td>.5</td>
</tr>
<tr>
<td>NE\textsubscript{L}, Mcal/day</td>
<td>27.9\textsuperscript{a}</td>
<td>30.5\textsuperscript{b}</td>
<td>.6</td>
<td>28.6\textsuperscript{a}</td>
<td>32.9\textsuperscript{b}</td>
<td>.9</td>
<td>28.2</td>
<td>31.8</td>
<td>.6</td>
</tr>
<tr>
<td>CP, kg/day</td>
<td>2.44\textsuperscript{a}</td>
<td>3.18\textsuperscript{b}</td>
<td>.06</td>
<td>2.49\textsuperscript{a}</td>
<td>3.64\textsuperscript{b}</td>
<td>.11</td>
<td>2.46</td>
<td>3.38</td>
<td>.07</td>
</tr>
<tr>
<td><strong>Body weight, kg</strong></td>
<td>635</td>
<td>615</td>
<td>14</td>
<td>617</td>
<td>618</td>
<td>9</td>
<td>626</td>
<td>617</td>
<td>8</td>
</tr>
<tr>
<td><strong>Body weight change, kg/day</strong></td>
<td>-.25</td>
<td>-.43</td>
<td>.14</td>
<td>-.54</td>
<td>-.09</td>
<td>.13</td>
<td>-.40</td>
<td>-.27</td>
<td>.10</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b} Means within parity-trial subclasses with unlike superscripts differ (P<.05).

\textsuperscript{1} CP = crude protein; DM = dry matter; NE\textsubscript{L} = net energy for lactation.
percent of NRC requirement (14), are in Figure 2. Even though concentrations of these nutrients were held constant within rations, their intakes increased with advancing week of lactation because of improved intakes of DM (Figure 1B). Animals in both age groups consumed less CP than needed during most of the first 12 wk postpartum when fed a 13.5% CP diet for ad libitum consumption. Increasing concentration of dietary CP to 16.5% resulted in intakes of CP that exceeded requirement by 1 wk postpartum for primiparous cows and 4 wk postpartum for multiparous cows (Figure 2A). Animals in all age-treatment groups consumed less than suggested allowance for energy for several weeks following calving. Increasing concentration of dietary CP from 13.5 to 16.5% reduced length and magnitude of the postpartum deficit of energy (Figure 2B).

Body Weight Change

Across trials body weights averaged 528 kg for primiparous and 622 kg for multiparous cows (Table 4). Animals in all age-treatment groups lost weight during early lactation (Figure 1C). Heifers lost less (P<.05) weight and tended to reach minimum body weight sooner postpartum than older cows. Primiparous cows weighed more and multiparous cows less at 12 wk than at 1 wk postpartum. Body weight change was unaffected (P>.05) by treatment; however, animals in both age groups on HP tended to lose less weight postpartum and weighed more at 12 wk postpartum than those on LP.

Reproductive Performance

Reproductive performance was not related to concentration of dietary CP. Average days open was 108 and average number of services per conception was 1.9.

### Table 5. Economic evaluation of feeding 13.5 or 16.5% dietary crude protein (CP) during early lactation.

<table>
<thead>
<tr>
<th>Item</th>
<th>Primiparous cows</th>
<th>Multiparous cows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13.5% CP</td>
<td>16.5% CP</td>
</tr>
<tr>
<td>No. animals</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>DM intake, kg/day&lt;sup&gt;1&lt;/sup&gt;</td>
<td>15.2</td>
<td>17.9</td>
</tr>
<tr>
<td>Feed cost, $/day&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.73</td>
<td>2.26</td>
</tr>
<tr>
<td>3.5% FCM yield, kg/day</td>
<td>20.8</td>
<td>22.9</td>
</tr>
<tr>
<td>Returns, $/day&lt;sup&gt;3&lt;/sup&gt;</td>
<td>6.03</td>
<td>6.64</td>
</tr>
<tr>
<td>Returns above feed cost, $/day</td>
<td>4.30</td>
<td>4.38</td>
</tr>
</tbody>
</table>

<sup>1</sup> DM = dry matter; FCM = fat-corrected milk.

<sup>2</sup> Feed costs ($/ton DM): silage, 73; hay, 83; cereal grain, 128; and protein supplement, 233.

<sup>3</sup> Milk price ($29/100 kg).
Economic Evaluation

Averages of intake and production from pooled trials were used to evaluate economics of adding supplemental protein to a diet containing 13.5% CP (Table 5). Feed costs were higher for animals fed the diet with 16.5% CP, because it cost more per kilogram and was consumed in greater amounts than the diet with 13.5% CP. Animals fed the 16.5% CP diet had higher returns than those fed the 13.5% CP diet. Returns above feed costs were greater for the diet with 16.5% CP. Economic benefit from adding supplemental protein to a 13.5% CP diet was greater for multiparous than primiparous cows.

DISCUSSION

Level of Protein

Milk yield during early lactation was improved by increasing concentration of dietary CP from 13.5 to 16.5%. Lactational response was probably due to an improved intake of energy. On average, animals fed 16.5% dietary CP consumed 15% more \( (P<.01) \) calculated NE\(_1\) and produced 10% more \( (P<.02) \) milk than animals fed 13.5%. Others \( (2, 3, 5, 6, 12, 13, 16, 20) \) observed an improved intake of energy and a concomitant improvement of milk yield from increased concentration of dietary CP. Little or no milk response has been observed when there was no change of energy intake \( (5, 6, 7, 12, 16, 18) \).

Milk composition of primiparous cows was unaffected by concentration of dietary CP. Percent fat in milk of multiparous cows also was unaffected, but percent protein was reduced \( (P<.02) \). We are unaware of other results indicating reduced protein content in milk from increasing concentration of dietary CP. Results of studies \( (3, 6, 7, 9, 16, 18, 19) \) comparing similar concentrations of CP during early lactation indicate no relationship \( (P>.05) \) between concentration of CP in the diet and concentrations of protein or fat in milk.

Our results and those of others \( (1, 6, 8, 17, 20) \) suggest no relationship \( (P>.05) \) between concentration of dietary CP and reproductive performance. In contrast, Jordan and Swanson \( (11) \) reported that reproductive efficiency decreased \( (P<.05) \) with increasing concentration of dietary CP.

Age of Animal

Results from studies measuring relative responses of milk yield by primiparous and multiparous cows to increased concentrations of dietary CP during early lactation are conflicting. In our study, milk yields of cows in both age groups were improved by increasing concentration of dietary CP from 13.5 to 16.5%. These results and those of others \( (4, 20) \) indicate no interaction between parity and concentration of dietary CP on milk yield. In contrast, results of studies \( (5, 12, 15, 16) \) during early lactation indicate that milk production of multiparous but not primiparous cows is improved significantly by increasing concentration of dietary CP.

Results of this and other studies \( (5, 12, 15, 16, 20) \) were combined to examine further relative responses of primiparous and multiparous cows to supplemental protein during early lactation. The merged data gave 66 primiparous and 113 multiparous cows on 10 comparisons of two percents of dietary protein. Added protein improved milk yields by cows of both age groups in 9 of 10 comparisons. However, milk responses were arithmetically greater for cows than heifers in 8 of 10 comparisons. Average responses, per percentage unit of added CP, were 1.2 kg for multiparous and .3 kg for primiparous cows. Thus, it appears that multi-
parous cows are more responsive than primi-
parous cows to supplemental protein.

The merged data also were used to compare
milk yield response to protein added to low vs.
high protein diets. Comparisons were grouped
into classes by midpoint of CP compared (<
14% CP vs. > 14% CP). Milk responses per
percentage unit of supplemental CP were
greater when added to low protein than to high
protein diets (Figure 3). The decrement in milk
response for high vs. low protein diets was
greater for multiparous than for primiparous
cows.

Source of Protein

Source of protein may influence response to
added dietary CP during early lactation. How-
ever, firm conclusions about the relative values
of CSM and SBM are not possible, because
protein source is confounded with trial.

The increment between protein treatments
was not uniform across trials, so responses were
expressed as kilograms per percentage unit of
added CP. Across age groups, daily intake of
DM increased .6 kg per percentage unit of
added CP from SBM (Trial 1) and .9 kg per
percentage unit of CP from CSM (Trial 2). The
apparently greater DM intake response for CSM
than SBM is consistent with results of other
experiments comparing similar concentrations
of dietary CP. Roffler and Thacker (17) observed
that DM intake increased .8 kg per percentage
unit of added CP from CSM but only .1 kg per
percentage unit of added CP from SBM. Sim-
ilarly, Van Horn et al. (19) observed DM intake
responses per percentage unit of added CP of .3
kg for CSM but only .1 kg for SBM.

The effect of protein source on milk yield
appears to be less consistent than on DM
intake. Daily yield of milk rose .6 kg per
percentage unit when concentration of dietary
CP was increased from 13.5 to 16.5% with SBM
(Trial 1) and .9 kg per percentage unit with
CSM (Trial 2). Similarly, Van Horn et al. (19)
observed a greater response of milk yield from
CSM than SBM (.4 vs. 0 kg per percentage unit of
added CP). In contrast, Roffler and Thacker
(17) observed less response of milk yield from
added CSM than SBM (.7 vs. 1.2 kg per per-
centage unit of added CP).

CONCLUSION

Production results and economic considera-
tions suggest that cows in early lactation fed
diets of concentrate and grass-legume forage for
ad libitum consumption benefit when con-
centration of dietary CP is increased above
13.5% with preformed protein. Intakes of
energy and protein fall short of NRC standards
(14) for several weeks postpartum when con-
centration of dietary CP is 13.5%. Increasing
dietary CP to 16.5% reduces the length and
magnitude of the postpartum deficits of energy
and protein. Performance response and eco-
nomic benefit from increasing dietary CP from
13.5 to 16.5% appear greater for multiparous
than for primiparous cows.

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