Effect of Supplementing Rumen Protected Amino Acids on the Milk Protein Content of Cows Fed Calcium Soap of Fatty Acids

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Supplementing the diet of dairy cows with calcium soap of fatty acids (CS) is common in Kyushu, the southernmost major island of Japan, to alleviate the decrease in milk yields and milk fat (MF) contents during hot summers. The increase in milk yields and MF contents caused by supplementing 330 g CS/day averaged 0.25 kg/day and 0.30%, respectively, whereas a slight reduction in the milk protein (MP) content was observed3,8,10,11). This reduction in MP content is probably due to the dilution effect, because, commonly, the rate of increase in milk yield exceeds that of the MP yield.

SNF content, in which MP is one of major constituents, is a concern of Kyushu farmers because milk price is based partly on the SNF content1. Therefore, it is necessary to find measures that will prevent the reduction in the MP content when dairy cows are fed CS.

An insufficient supply of high protein feed seems to cause a reduction in the MP content, especially when the cow has a deficiency in the essential amino acids that form milk protein. Some papers\cite{5,8,15} reported that supplementing the cows’ diet with rumen protected essential amino acids, such as Met and Lys could elevate the MP content.

The object of this study was to examine whether the reduction in the MP content of cows fed CS could be alleviated by supplementing their diet with rumen protected amino acids (RPAA).

**Materials and Methods**

**CS and RPAA:** The CSs prepared from palm oil and tallow were obtained from Taiyo Yushi Co., Ltd. (Yokohama) and Nippon Soda Co., Ltd. (Tokyo), respectively. Their fatty acid compositions were as follows; myristic, 1.0, 6.3%, palmitic, 40.1, 27.4%, stearic, 5.3, 14.1%, oleic, 39.3, 49.6%, linoleic, 8.8, 2.5% and others, 5.1, 0.1% respectively.

RPAA obtained from Nippon Soda Co., Ltd. was mixed as follows; The Met (DL-methionine) was mixed into the CS powder of tallow, while the Lys (L-lysine monohydrochloride) was mixed into the CS powder of palm oil. The mixtures were then firmly cast into a cylindrical shape (2.1 mm diameter, 3.0 mm length). The former contained 85.5% CS prepared from tallow, 12.8% Met and 1.7% binder while the latter contained 83.3% CS from palm oil and 16.7% Lys. The ruminal protection and postruminal release of Met in the former were 97 and 84% respectively, 62 and 44% respectively for Lys. These values were determined by Nippon Soda Co., Ltd. In this Experiment, RPAA is defined as Met and Lys only, excluding CS by which the amino acids are protected.

**Experimental procedures:** Experiments 1, 2 and 3 were conducted from late July through early September, from early July through mid August and from mid July through late August at Saga, Okinawa Prefectural Livestock Experiment Station and Kyushu National Agricultural Experiment Station, respectively.

Nine primiparous (average body weight, 461 kg, Experiment 1), 6 multiparous (569 kg, Experiment 2) and 6 multiparous (631 kg, Experiment 3) lactating Holstein cows were used in a triplicated 3 x 3 or a replicated 3 x 3 Latin square designed experiment using 14 day periods. Data collected from the three stations were analyzed using a combination of a Latin square design and a block design\cite{18}. The cows were in early lactation in Experiment 1 and in mid lactation in Experiments 2 and 3. All cows received three treatments; the first was a conventional basal diet(C), the second was an addition of 225 g CS to the basal diet(T 1, 104 g CS from tallow and 121 g CS from palm oil in addition to the basal diet) and the third was T 1 plus RPAA (T 2, 104 g CS from tallow 121 g CS from palm oil, 17 g Met and 24 g Lys in addition to the basal diet).

**Rations:** In Experiment 1, the basal diet was a total mixed ration consisting of 29.7% Italian ryegrass silage, 6.8% alfalfa haycube, 6.8% beet pulp, 6.8% rice straw, 21.6% formula feed for lactation, 5.4% barley and 6.8% soybean meal (on a fresh basis), which met the nutrient requirements for maintenance and milk yield according to Japanese Feeding Standard for dairy cattle (JFS).

In Experiment 2, the basal diet consisted of 5.5–9.8 kg of formula feed for lactation, 2.5–3.0 kg of beet pulp, 3.0 kg of barley, 1.0 kg of alfalfa haycube and 14.6–17.1 kg of guineagrass silage (on a fresh basis), which met the nutrient requirements for maintenance and milk yield according to JFS.

In Experiment 3, cows were offered 7.2–9.1 kg of formula feed for lactation, 2.0–3.0 kg of beet pulp, 1.7–1.8 kg of soybean meal and 1.0 kg of alfalfa haycube to meet or exceed slightly the nutrient requirements for milk yield based on JFS. In addition, corn haylage was fed ad
Milk Protein Respond to RPAA Supplement

Prior to feeding of the diets, the CS or CS plus RPAA was fully mixed with the formula feed or total mixed ration.

In all Experiments, the crude protein and TDN content (of the diets) were 15–17% and 67–69% of DM, respectively. Especially in Experiment 3, those contents were corrected by adding soybean meal to the formula feed, after first predicting the roughage intakes and crude protein contents.

In Experiments 1 and 2, dry matter intake was determined for the last 5 days of each treatment.

In all Experiments, body weights were determined on the 11th and 13th day of each treatment.

Analysis of milk samples: In all Experiments, milk yield was recorded daily and milk samples were collected at morning and evening milking times for the last 5 days of each treatment. The analysis of the milk composition and milk fatty acid composition were performed according to the previous paper2), except for the analysis of the milk composition in Experiment 3 where a Foss Milko-scan 133B (Foss Electric, Hillerod, Denmark) was used.

Results and Discussion

There was no problem with the palatability of CS and CS plus RPAA as the diets containing the supplements (1.3 to 1.8% of the dietary DM) were almost completely ingested by the cows.

Dry matter intakes, which ranged from 14.6 to 14.8 kg/day and from 17.9 to 18.6 kg/day in Experiments 1 and 2, respectively, were unaffected by treatments.

No significant changes in body weights were observed.

SCHAUFF and CLARK16) reported that CS could comprise up to 6 percentage of the dietary DM without deleterious effects on ruminal fermentation and digestibilities of most nutrients. The authors also demonstrated that the metabolite contents in the blood serum of cows were unaffected by supplementing up to 420 g CS/day3).

Cows consuming the diet containing CS or CS plus RPAA produced more milk than those consuming the conventional diet (p<0.05). Our papers1,4) and other papers11,17) have demonstrated that supplementing CS to cows increased their energy intake and subsequently their milk yields, although the increased milk yield amounts varied with different researchers. In this Experiment, the increase in milk yield reached 1.1 kg/day when cows were given 225 g CS/cow/day (Table 1).

There was no difference in milk yield between T1 and T2. This fact indicates that the milk yield was unaffected by supplementing RPAA to diet containing CS. Some papers7,12,14) also reported that supplementing RPAA has no effect on milk yields.

The MP yield was greatest in the cows fed a diet CS plus RPAA (p<0.01). MP yields increased by 20 g and 40 g/day for T1 and T2, respectively, compared with that for C. There was a significant difference between T1 and T2 (p<0.05).

The MP content tended to decrease for T1 and increase for T2 compared with that for C, and it was 0.11 percentage unit higher for T2 than for T1 (p<0.05) although no significant difference was found between T2 and C. The SNF content was lower for T1 than for either C or T2 (p<0.05). The major reason for the decrease in the SNF content is thought to be due to the decrease in the MP content.

Some studies3,4,10,17) have demonstrated that after fat was added to the cows’ diets, the MP yield increased while its content decreased. Commonly, the addition of fat to diets causes MP to be reduced by 0.1 to 0.2 percentage units. In this Experiment, the MP content tended to decrease by 0.06 percentage unit after the conventional diets were supplemented with CS.

As shown in Table 1, the increasing rate of milk yield resulting from the addition of CS
Table 1. Effects of supplementing rumen protected amino acids to the dairy cows fed calcium soap of fatty acids on milk yield and milk composition

<table>
<thead>
<tr>
<th>Production</th>
<th>Control</th>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>l.s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk (kg/day)</td>
<td>21.0(^b)</td>
<td>22.1(^a)</td>
<td>22.0(^b)</td>
<td>0.9</td>
</tr>
<tr>
<td>Fat (g/day)</td>
<td>726(^b)</td>
<td>788(^a)</td>
<td>802(^a)</td>
<td>29</td>
</tr>
<tr>
<td>Protein (g/day)</td>
<td>650(^b)</td>
<td>670(^a)</td>
<td>690(^b)</td>
<td>15</td>
</tr>
<tr>
<td>Milk composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td>3.47(^b)</td>
<td>3.59(^b)</td>
<td>3.66(^a)</td>
<td>0.17</td>
</tr>
<tr>
<td>Protein</td>
<td>3.08(^b)</td>
<td>3.02(^b)</td>
<td>3.13(^a)</td>
<td>0.09</td>
</tr>
<tr>
<td>SNF</td>
<td>8.66(^a)</td>
<td>8.56(^b)</td>
<td>8.63(^b)</td>
<td>0.07</td>
</tr>
<tr>
<td>Total solids</td>
<td>12.13</td>
<td>12.15</td>
<td>12.29</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Milk fatty acid composition (%)

<table>
<thead>
<tr>
<th></th>
<th>C4-C14 group</th>
<th>C16 group</th>
<th>C18 group</th>
<th>l.s.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4-C14</td>
<td>27.1(^b)</td>
<td>24.3(^a)</td>
<td>25.2(^a)</td>
<td>1.7</td>
</tr>
<tr>
<td>C16</td>
<td>33.5</td>
<td>32.7</td>
<td>32.9</td>
<td>1.8</td>
</tr>
<tr>
<td>C18</td>
<td>29.9(^b)</td>
<td>34.9(^a)</td>
<td>34.0(^a)</td>
<td>2.8</td>
</tr>
</tbody>
</table>

Milk fatty acids production (g)

<table>
<thead>
<tr>
<th></th>
<th>C4-C14 group</th>
<th>C16 group</th>
<th>C18 group</th>
<th>Body weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4-C14</td>
<td>82</td>
<td>81</td>
<td>83</td>
<td>557</td>
</tr>
<tr>
<td>C16</td>
<td>217</td>
<td>226</td>
<td>227</td>
<td>552</td>
</tr>
<tr>
<td>C18</td>
<td>55(^b)</td>
<td>66(^a)</td>
<td>66(^a)</td>
<td>552</td>
</tr>
<tr>
<td>C4-C14</td>
<td>127(^b)</td>
<td>166(^a)</td>
<td>162(^a)</td>
<td>248(^a)</td>
</tr>
<tr>
<td>C16</td>
<td>13(^a)</td>
<td>15(^b)</td>
<td>16(^a)</td>
<td>11</td>
</tr>
<tr>
<td>C18</td>
<td>182</td>
<td>176</td>
<td>187</td>
<td>11</td>
</tr>
<tr>
<td>C15-C14</td>
<td>227</td>
<td>236</td>
<td>236</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>199(^b)</td>
<td>251(^a)</td>
<td>248(^a)</td>
<td>11</td>
</tr>
</tbody>
</table>

\(^ab\) Numbers with different superscripts in the same row differ (p<0.05).

\(^1\) The sum of total fatty acids on a chromatogram, calculated as 100%.

was 5.0% [(22.1 kg - 21.0 kg) × 100 / 22.1 kg = 5.0%], whereas the rate of MP was 3.0% [(670 g - 650 g) × 100 / 670 g = 3.0%], that is, the reason for the decrease on the MP content was due to the former being in excess of the latter (the dilution effect). However, the depression in the MP content can be prevented by the addition of RPAA to lactation diets containing CS because the rate of increase in MP yield [(690 g - 650 g) × 100 / 690 g = 5.8%] exceeded that of the milk yield [(22.0 kg - 21.0 kg) × 100 / 22.0 kg = 4.5%]. Some papers\(^8,15\) also reported that supplementing the cows' diet with rumen protected essential amino acids, such as Met and Lys could elevate the MP content.

The MF yield and its content increased (p<0.01 and p<0.05) after supplementing CS plus RPAA, and the MF yield also increased (p<0.01) with the CS addition, although the increase in MF content was not significant. When cows received diets containing CS plus RPAA, about one third of the ingested CS seemed to transfer into milk fat (Table 1).

The MF yield tended to be higher for T2 than for T1, although the difference was not significant. This fact was consistent with the
results of other papers⁵,⁶, suggesting that supplementing RPAA have the advantage of being able to stimulate the fat metabolism of mammary gland⁶,¹²), where ingested CS is converted into MF.

Among the milk fatty acid groups, the C₁₈ group content increased (p<0.01 or P<0.05) by supplementing CS or CS plus RPAA, whereas the C₄-C₁₄ group decreased (p<0.05) and the C₁₆ group tended to decrease.

Among the milk fatty acids, C₁₈:₁ predominantly increased (p<0.01) and C₁₈:₀ also increased (p<0.05), whereas C₁₆:₀ remained almost unchanged. These results suggest that C₁₈:₁, of which CS (prepared from palm oil and tallow) is a rich source, transferred effectively into MF.

According to GRUMMER⁹), the C₁₈ group content increased with the addition of fat to the diet, whereas C₁₆ group decreased relatively, because de novo synthesis of C₁₆:₀ was suppressed in the mammary secretory tissue. In addition, the milk fatty acid composition is largely dependent on a variety of fat which has been added to the diet.

In conclusion, to prevent a detrimental effect on the MP content following the supplementation of CS, RPAA was added to the lactation diets containing CS. This had the advantage of improving MP content and hence the SNF content, in comparison to the supplementation of CS alone.

References