Monitoring Nutritional Status of Dairy Cows in Taiwan Using Milk Protein and Milk Urea Nitrogen**

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ABSTRACT: The climate and marketing system of raw milk in Taiwan create problems in balance feeding of protein and energy in lactating cows in Taiwan. Level of urea nitrogen both in bulk milk and serum reflects ruminal protein degradation and post-ruminal protein provision, whereas milk protein concentration responds to dietary energy intake and bacterial protein production in the rumen. Establishment of a range of reference standards in milk protein and urea nitrogen levels can be applied as a noninvasive economical feeding guide to monitor the balance of protein and energy intake. Standard reference levels of 3.0% milk protein and 11-17 mg/dL milk urea nitrogen (MUN) were established. Level of milk protein below 3.0% is regarded as indicating inadequate dietary energy whereas MUN below or above the range is regarded as a deficiency or surplus in dietary protein. Results from analysis of bulk milk samples collected from 174 dairy herds over Taiwan showed that only one quarter (25.29%) of the herds received a balanced intake of protein and energy, 33.33% adequate protein with energy inadequate, 22.99% herds in protein surplus with energy inadequate, 10.35% herds in protein surplus with energy adequate, 4.6% protein deficiency with energy adequate, and 3.45% herds with both protein and energy inadequate. Energy inadequate herds accounted for 60% of the total dairy herds in Taiwan with 56% adequate, 38% surplus and 6% inadequate in protein. In comparing milk sampled from bulk milk on different seasons from Lee-Kang area in the southern Taiwan, the concentrations of milk fat and milk protein were significantly higher in the cool season (February) than in the warm season (August) (p<0.05), whereas the urea nitrogen in the milk was significantly lower in the cool season than in the warm season (p<0.05). This indicated that lactating cows had excess protein and/or inadequate energy intake in the warm season. It appears that major problem feeding in lactating cows is energy intake shortage, especially during the warm season in Taiwan. (Asian-Aus. J. Anim. Sci. 2000, Vol. 13, No. 12 : 1667-1673)

Key Words: Dairy Cows, Milk Protein, Milk Urea Nitrogen

INTRODUCTION

There is a high ambient temperature averaging above 21°C and a high mean relative humidity of 76.3% in Taiwan (21.8-25.6 °N). The long warm season casts eight months, from April to November, with the ambient temperature above 26°C. During this warm season, cultivated tropical forages are in rapid growth with various maturity stages within a plant. Forage quality is readily over-mature and lignified without on time harvesting (Hsu, 1984). In addition to climate and forages, the raw milk marketing system is also implicated in the inappropriate feeding strategy in dairy farming. Since fresh milk consumption is seasonal and reach its peak during the warm season, the quota and price of raw milk paid to farmers varies according to the season. With less than 37% to more than 63% of the annual milk yield quoted for the cool and warm seasons, respectively, the price paid to producers differs from NT $13.24/kg in the cool season to $18.73-20.73/kg in the warm season according to fat (3.4%), density (1.0300-1.0309) and temperature of the raw milk (15°C) (Taiwan Provincial Bulletin, 1991). In order to take advantage of the seasonal raw milk pricing policy, dairy farmers attempt to maximize milk yield during the warm season by concentrate feeding. This leads to a protein and energy feed imbalance. Consequently, milk protein concentration has gradually declined over the past decade according to the dairy herd improvement report (DHI). Milk protein declined from 3.21% in 1992 to 3.16% in 1994 (Chang et al., 1997), and to 3.04% in 1995 (Huang et al., 1995). Milk protein is at present not accounted for in the milk price. Milk pricing is based on the solids-non-fat and impact milk density (reject if <1.0280).

Milk protein reflects the balance of dietary protein and energy intake (Roseler et al., 1993; Sato, 1998). High protein intake generally increases the urea nitrogen concentration in serum (BUN) and in the milk (MUN) (Baker et al., 1995). Dietary energy intake also affects the urea level in blood and milk (Coulon and Remond, 1991). Blood urea can be recycled into the rumen for bacterial protein synthesis or excreted via the urine. High urea nitrogen concentration, however, depresses dairy cow reproduction efficiency (Ferguson et al., 1993; Butler et al.,

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MUN indicates protein intake (Quaife, 1994; Roenfeldt, 1996) and milk protein concentration reflects energy intake in lactating dairy cows (Coulon and Remond, 1991; Sato, 1998). Using milk protein and urea concentrations in either blood or milk to monitor dietary energy and protein intake in dairy cows has obtained increased interest in Europe (Kirchgesner et al., 1986; Nagel, 1994), the United States (Hutjens and Barmore, 1995) and Japan (Ougi, 1994). Determination of urea nitrogen content in bulk milk can also be used to monitor the feeding status of lactating cows (Ropstad and Refsdal, 1987) since the urea content is highly correlated to blood urea levels (Refsdal, 1983). This study analyzed the protein and urea nitrogen content in bulk milk to monitor the feeding status of lactating cows and derive a reliable tool to improve dairy farming.

**MATERIALS AND METHODS**

**Materials**

Bulk milk samples were collected from 174 dairy herds of the contract dairy farms of three milk processing plants (Kuang-Chuan, President and Wei-Chuan) and two special dairy farming zones, Lee-Kang (Pingtung County), and Lio-Yin (Tainan County). Samples were collected during the warm season from April to November 1995 with mean temperature of 21°C and above. To study the seasonal effect on milk constituents, additional samples were taken from 30 Lee-Kang herds in different seasons. The mean ambient temperature was 20.9°C, ranging from 17.8-24.7°C during the cool season in February. The warm season mean temperature was 29.6°C with a range of 26.8-33.1°C in August. A 200 ml sample was collected from each bulk milk tank and stored at 4°C for analysis.

**Analysis**

Milk fat, protein and solids-non-fat contents were analyzed according to the AOAC (1984) using infrared apparatus (Milko-scan 104, Foss Electric, Denmark). Milk samples were treated with 3% trichloroacetic acid (TCA) and centrifuged. The supernatant was then analyzed for urea nitrogen according the method of Crocker (1967) using a Sigma diagnostics kit #535 (Sigma Chemical Co., USA), reading at 540 nm.

**Standard reference for milk protein and urea nitrogen**

From an extensive literature review, a standard reference level of 3.0% milk protein and 11-17 mg/dL milk urea nitrogen (MUN) was established. A milk protein level below 3.0% was regarded as diet-energy inadequate, whereas MUN below or above the range was regarded as a deficiency or surplus in dietary protein.

**Statistical analysis**

The data are presented as means and standard errors. Variance was analyzed using Statistical Analysis System (1985) with General Linear Model (GLM). Means were compared using Duncan’s New Multiple Range Test (Steel and Torrie, 1960).

**RESULTS AND DISCUSSION**

**Standard milk protein and urea nitrogen references for monitoring dietary protein and energy intake**

Evaluation of protein and energy balance in a forage-based diet using the level of milk protein and urea nitrogen has been established in many countries. The standard range in Germany for milk protein is 3.2 to 3.6%, and 7.0 to 14.0 mg/dL for milk urea nitrogen. (Nagel, 1994). In the United States, the reference range from 3.0 to 3.2% for milk protein and 11 to 17 mg/dL for milk urea nitrogen (Hutjens and Barmore, 1995). In Japan, there is also a reference which ranges from 8-20 mg/dL in serum urea nitrogen, and 3.0% milk protein (Ougi, 1994).

The percentage of milk protein is affected by not only dietary conditions but also genetic history, and genetics affects the percentage of milk protein stronger than fat. In view of the difference in breeding history between Taiwan and other countries, three-percent milk protein should be considered a reasonable reference in that country. Since the milk fat is set at 3.5% in Taiwan and the percentage ratio of protein to fat in milk is 0.85 to 0.88 under normal nutritional status for lactating cows (Mahanna, 1995). Cannon and Sniffen (1983) also suggested that a level of 3.5% milk fat and 3.0% milk protein could be a standard reference.

The reference values for BUN and MUN in dairy cattle are presented in table 1. The BUN concentration threshold for normal reproductive functioning is 19 mg/dL for cows (Quaife, 1994; Butler, 1998). Cows with a BUN level greater than 20 mg/dL (Ferguson et al., 1993; Stevensen, 1994) or greater than 18 mg/dL MUN fail to conceive after receiving insemination (Sato et al., 1996). The standard MUN reference value of 11 to 17 mg/dL recommended by Hutjens and Barmore (1995) agrees with the Ferguson et al. (1988) suggestion of 13 to 20 mg/dL serum urea nitrogen since MUN is 85% of the BUN concentration (DePeters and Ferguson, 1992; Roseler et al., 1993). This MUN value of 11 to 17 mg/dL also agrees with the 18 mg/dL value of Sato et al. (1996), 10 to 16 mg/dL of Jonker et al. (1999) and 12 to 18 mg/dL of Roenfeldt (1996). Therefore the standard reference for MUN was recommended as 11 to 17 mg/dL for this
Table 1. Reference values of BUN and MUN contents in dairy cattle

<table>
<thead>
<tr>
<th>Reference value (mg/dl)</th>
<th>Author</th>
<th>Year</th>
<th>Nation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-12</td>
<td>Bartley et al.</td>
<td>1976</td>
<td>USA</td>
</tr>
<tr>
<td>≥ 20</td>
<td>Oltnor and Wiktorsson</td>
<td>1983</td>
<td>Sweden</td>
</tr>
<tr>
<td>10.0-15.5</td>
<td>Ferguson et al.</td>
<td>1988</td>
<td>USA</td>
</tr>
<tr>
<td>13-15</td>
<td>Sommer</td>
<td>1991</td>
<td>Germany</td>
</tr>
<tr>
<td>12.7-14.2</td>
<td>Sato et al.</td>
<td>1992</td>
<td>Japan</td>
</tr>
<tr>
<td>15-25</td>
<td>Ferguson et al.</td>
<td>1993</td>
<td>USA</td>
</tr>
<tr>
<td>≤ 19</td>
<td>Roseler et al.</td>
<td>1993</td>
<td>USA</td>
</tr>
<tr>
<td>12.7-14.2</td>
<td>Gustafsson and Carlsson</td>
<td>1993</td>
<td>Sweden</td>
</tr>
<tr>
<td>15-25</td>
<td>Wenninger and Distl</td>
<td>1994</td>
<td>Germany</td>
</tr>
<tr>
<td>≤ 20</td>
<td>Quailfe</td>
<td>1994</td>
<td>USA</td>
</tr>
<tr>
<td>12-17, ≤ 20</td>
<td>Stevenson</td>
<td>1994</td>
<td>USA</td>
</tr>
<tr>
<td>8-20</td>
<td>Nagel</td>
<td>1994</td>
<td>Germany</td>
</tr>
<tr>
<td>15.1-15.6</td>
<td>Ougi</td>
<td>1994</td>
<td>Japan</td>
</tr>
<tr>
<td>11-17</td>
<td>Baker et al.</td>
<td>1995</td>
<td>USA</td>
</tr>
<tr>
<td>12-16</td>
<td>Hutjes and Barmore</td>
<td>1995</td>
<td>USA</td>
</tr>
<tr>
<td>10-18</td>
<td>Harris</td>
<td>1995</td>
<td>USA</td>
</tr>
<tr>
<td>≤ 19</td>
<td>Butler et al.</td>
<td>1996</td>
<td>USA</td>
</tr>
<tr>
<td>12-18</td>
<td>Roenfeldt</td>
<td>1996</td>
<td>USA</td>
</tr>
<tr>
<td>≤ 18</td>
<td>Sato et al.</td>
<td>1996</td>
<td>Japan</td>
</tr>
<tr>
<td>14.2±3.6</td>
<td>Garcia and Linn</td>
<td>1997</td>
<td>USA</td>
</tr>
<tr>
<td>8-22</td>
<td>Moore</td>
<td>1997</td>
<td>USA</td>
</tr>
<tr>
<td>≤ 19</td>
<td>Butler</td>
<td>1998</td>
<td>USA</td>
</tr>
<tr>
<td>10-16</td>
<td>Jonker et al.</td>
<td>1999</td>
<td>USA</td>
</tr>
</tbody>
</table>

A MUN level less than 11 mg/dL reflects an inadequate protein intake whereas more than 17 mg/dL indicates an excess protein intake. Broderick et al. (1997), using data from 35 trials with 482 lactating cows, fed 106 diets in the US and obtained a very strong relationship between MUN and dietary protein for % dry matter ($r^2=0.839$) and per unit NE_L ($r^2=0.833-0.878$). Based on their equations, the standard MUN reference can be interpreted as inadequate protein feeding below 16.66% DM or 104.1 g/Mcal NE_L and excesses feeding higher than 18.27% DM or 114.8 g/Mcal NE_L. It appears that MUN can be utilized as an economical noninvasive indicator to monitor the protein status of a dairy herd.

Roseler et al. (1993) suggested that protein intake elevates plasma and milk urea nitrogen, and a negative relationship exists between energy intake and plasma urea nitrogen. Decreased excess levels of protein in an energy deficient diet still results in a high MUN level because protein can be used for energy in an energy deficient diet. Refsdal et al. (1985) suggested energy intake must be considered while using MUN in evaluating protein intake. Oltnor and Wiktorsson (1983) demonstrated that MUN concentrations altered only slightly when the amount of ingested protein decreased or increased as long as the ratio between protein and energy was held constant. Because milk protein responds to the energy supply level of the dairy cows (Coulon and Remond, 1991), it can be used as an indicator for energy intake in a dairy herd. It appears that milk protein greater than 3.0% with an 11 to 17 mg/dL range for MUN should be the standard reference for lactating cows in Taiwan.

Application of the standard reference for MUN and milk protein as a model to monitor dietary energy and protein balance in lactating cows was referred to by Kirchgesner et al. (1986), Nagel (1994) and Ougi (1994). They believed that MUN and milk protein could be used as a monitor for the protein and energy intake of dairy herds. Cattle producing milk that contains a level of MUN and milk protein within the ranges of the standard reference values of 11-17 mg/dL. MUN and 3.0% milk protein is regarded as indicating a balanced protein and energy intake. Using the NRC feeding standard diet for dairy cattle in the United States, Baker et al. (1995) obtained milk contents of 3.1 to 3.2% protein and 15.1 to 15.6 mg/dL urea nitrogen. Sato et al. (1992) derived a mean MUN value of 13 to 15 mg/dL using the Japanese feeding standard for a dairy herd. Oltnor and Wiktorsson (1983) obtained a MUN level of 14.15 mg/dL using the Swedish feeding standard. These research figures were obtained from herds fed protein and energy balanced diets.
Milk protein contents less than 3.0% are regarded as an energy deficiency in dairy feeding. Less than 3.0% milk protein and <11 mg/dL MUN, indicates that the herd was fed inadequately in both protein and energy. With <3.0% milk protein and >17 mg/dL MUN may reflect a protein surplus with inadequate energy intake. This kind of prediction agrees with Nagel (1994) that normal MUN levels with below 3.0% milk protein may indicate a deficient energy intake in a dairy herd. Conversely, milk protein greater than 3.0% indicates an adequate energy intake. Protein intake may be inadequate with adequate energy intake when MUN is below 11 mg/dL, or protein intake may be excessive when the MUN level is greater than 17 mg/dL, as shown in table 2.

**Evaluation of dairy cows feeding status in Taiwan**

The milk protein and urea nitrogen data from the 174 herds' milk samples collected throughout Taiwan were classified according to the standard references in table 2 and presented in table 3. Results showed that only one quarter (25.29%) of the herds received a balanced intake of protein and energy, 33.33% adequate protein and inadequate energy, 22.99% a protein surplus and inadequate energy, 10.35% of the herds with a protein surplus and adequate energy, 4.6% with a protein deficiency with adequate energy, and 3.45% of the herds had both inadequate protein and energy. Energy inadequate herds accounted for 60% of the total dairy herds in Taiwan with 56% protein adequate, 38% surplus and 6% protein inadequate. Monitoring energy intake in dairy feeding through milk protein has positive meaning (Oltner et al., 1985; Roseler et al., 1993) when milk protein is substituted for milk fat as the major factor determining the price of raw milk (Gusafsson and Palmquist, 1993). Dairy farmers in Taiwan have neglected milk protein value since it is not a factor in milk pricing. Milk protein content has therefore declined from 3.26 to 3.04% while the milk fat has been consistent at 3.67 to 3.74% for the past decade. Therefore monitoring milk protein is essential from a nutritional management point of view. From this study, the milk protein only ranged from 2.77% to 3.18% and was lower than the lowest level classified by Nagel (1994) in which milk protein was classified into three grades, i.e., <3.20%, 3.20 - 3.60% and >3.60%. Milk protein was classified into only two categories by Ougi (1994), i.e., <3.0% and ≥3.0%.

In evaluating dairy feeding through monitoring MUN and milk protein in 167 samples with nine categories based on the standard reference for MUN and milk protein, Nagel (1994) found in dairy herds in Germany that inadequate energy intake was 49% with a 20% excessive energy intake. He also indicated that 31% of the herds were fed appropriate levels of both protein and energy. The dairy herds with proper feeding of both energy and protein, 31%, was much higher than only 25% of the herds in Taiwan. Dairy herds with low energy intake in Germany were also far below the 60% dairy herds in Taiwan. This severe shortage of energy intake in lactating cows in Taiwan may not only be attributed to the tropical climate and quality of the tropical forages. The feeding strategy for premium raw milk revenue and improper nutritional management may accentuate this problem.

**Table 2. The feeding status of dairy cattle evaluated by milk protein and urea nitrogen (MUN) contents in Taiwan**

<table>
<thead>
<tr>
<th>Milk protein (%)</th>
<th>Low MUN (&lt;11 mg/dl)</th>
<th>Optimal MUN (11-17 mg/dl)</th>
<th>High MUN (&gt;17 mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3.0</td>
<td>Protein and energy</td>
<td>Protein in balance and</td>
<td>Protein surplus and</td>
</tr>
<tr>
<td></td>
<td>deficiency</td>
<td>energy deficiency</td>
<td>energy deficiency</td>
</tr>
<tr>
<td>≥ 3.0</td>
<td>Protein deficiency</td>
<td>Protein in balance or</td>
<td>Protein surplus and</td>
</tr>
<tr>
<td></td>
<td>and energy in balance or slight surplus</td>
<td>balance</td>
<td>energy in balance or slight deficiency</td>
</tr>
</tbody>
</table>

**Table 3. The evaluation of the feeding status of dairy cattle in Taiwan with milk protein % and urea nitrogen (MUN) contents**

<table>
<thead>
<tr>
<th>Class of evaluation</th>
<th>Protein (%)</th>
<th>MUN (mg/dl)</th>
<th>Number of samples</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Protein and energy deficiency</td>
<td>2.86±0.09</td>
<td>10.13±0.39</td>
<td>6</td>
<td>3.45</td>
</tr>
<tr>
<td>2. Protein in balance and energy deficiency</td>
<td>2.89±0.07</td>
<td>14.42±1.76</td>
<td>58</td>
<td>33.33</td>
</tr>
<tr>
<td>3. Protein surplus and energy deficiency</td>
<td>2.87±0.08</td>
<td>20.18±2.51</td>
<td>40</td>
<td>22.99</td>
</tr>
<tr>
<td>4. Protein deficiency and energy in balance</td>
<td>3.05±0.30</td>
<td>9.50±1.47</td>
<td>8</td>
<td>4.60</td>
</tr>
<tr>
<td>5. Protein and energy in balance</td>
<td>3.08±0.07</td>
<td>14.29±1.71</td>
<td>44</td>
<td>25.29</td>
</tr>
<tr>
<td>6. Protein surplus and energy in balance</td>
<td>3.08±0.10</td>
<td>19.54±2.12</td>
<td>18</td>
<td>10.35</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>174</td>
<td>100.00</td>
</tr>
</tbody>
</table>
Table 4. Effects of cool and warm season on milk composition (n=30)

<table>
<thead>
<tr>
<th>Season</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Urea nitrogen (mg/dl)</th>
<th>SNF (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cool</td>
<td>3.94±0.15a</td>
<td>3.12±0.11a</td>
<td>15.15±0.62b</td>
<td>8.67±0.12b</td>
</tr>
<tr>
<td>Warm</td>
<td>3.68±0.22b</td>
<td>2.86±0.12b</td>
<td>19.18±1.94a</td>
<td>8.44±0.15b</td>
</tr>
</tbody>
</table>

* SNF: solids-not-fat.
ab Data with different superscripts in the same column differ significantly (p<0.05).

Balanced protein and energy intake in dairy feeding is the major management problem in dairy operations (Refsdal et al., 1985). Under the circumstance that dairy farmers do not have information on the ingredient composition, feed formulation and cattle intake, taking a sample from the bulk milk for MUN and milk protein analysis to monitor the protein and energy intake balance may provide alternative information for farmers and extension workers to improve the dairy operation. Based on this information, extension workers may focus on the direction to analyze the problems of a herd or individual cows for different milk yields and dry matter intake. In practice, this information may also provide a trace for evaluating improvement.

Effect of season on milk protein and urea nitrogen

In order to study the effect of different seasons, warm and cool, on milk constituents and hence the feeding strategy by dairy farmers in Taiwan, the bulk milks of thirty herds from the Lee-Kang area in southern Taiwan were sampled in the cool (February) and warm seasons (August) for analysis of milk fat, solids-non-fat, protein and urea nitrogen. The results of the analyzed milk constituents are presented in table 4.

The concentration of milk fat and milk protein was significantly higher in the cool than in the warm season (p<0.05). The urea nitrogen in the milk was significantly lower in the cool season than in the warm season (p<0.05). This indicated that the lactating cows had a surplus of protein and/or inadequate energy intake in the warm season in this area. In the survey of milk constituents and raw milk quality, Lee and Chen (1985) and Lee et al. (1987) also demonstrated a lower milk fat, protein and solids-non-fat in the summer than in the winter milk. The milk was found to contain more urea nitrogen in the summer than in the winter, i.e., 5.4±1.9 vs. 4.5±0.9 mmol/L (Refsdal et al., 1985), 5.24 vs. 3.57, 7.82 vs. 5.01 mmol/L (Rajcevic et al., 1996) and 16.3 vs. 14.0-14.2 mg/dL (Ferguson et al., 1997), again indicating a problem with the protein and energy intake balance. The coefficient of variance for MUN showed a higher value in the warm than the cool season, i.e., 10.1 vs. 4.1% in this study, and 35.2 vs. 20.0% in Refsdal et al. (1985). Ferguson et al. (1997) also indicated a higher standard deviation in summer (3.45 mg/dL) than in the winter, spring and fall milk (2.88, 3.16 and 3.05 mg/dL), reflecting a greater variation in feeding lactating cows in the warm than in the cool season. It appears that the major problem in lactating cow feeding is energy intake shortage and the problem accentuates during the warm season in Taiwan.

Low reproductive efficiency in dairy cows during the warm season in Taiwan includes low fertility and a long calving interval (Fung et al., 1981; Fung and Shiea, 1981). This may not simply be attributed to uterus conditions, survivability of fertilized ovum (Lewis et al., 1984; Putney et al., 1989) or the hormonal system (Lee et al., 1994) in the high ambient temperature of the warm season. The high blood urea level from the relatively high protein to the elevated urea concentration in the fluid in the urogenital tract may also impair reproductive performance (Carroll et al., 1988; Ferguson and Chalupa, 1989). This high blood urea level also reflects a high MUN (>17 mg/dL).

CONCLUSION

The standard reference for MUN and milk protein as a model to monitor dietary energy and protein balance in lactating cows is practically applicable in Taiwan. From the monitoring results we observed that energy intake shortage is the major problem in feeding lactating cows. The problem is accentuated during the warm season in Taiwan. The low reproductive efficiency in the cows may also be attributed to improper surplus protein feeding with deficient energy during the warm season in Taiwan.

ACKNOWLEDGMENT

Milk samples in this study were collected from the milk plants of Kuang-Chuan, President and Wei-Chuan milk companies as well as the milk collecting station of Lee-Kang. All of the assistance from these said parties is acknowledged. Special thanks go to President Company assisting in analyzing milk composition.

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