Changes in and correlations between some serum constituents and milk components from early to late lactation in a dairy herd with subclinical production disorders

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Summary

Changes in some serum constituents and milk components and correlations between them, were used for defining the reason(s) of suboptimal milk composition in a dairy herd with a history of low protein tests during summers. Four groups of 8 adult Holsteins, 20-70, 70-110, 110-150 and 150-210 days in milk (DIM), under heat stress, with similar feeding and management practices were sampled for blood and milk four times on a 10 day interval. Milk volume, percentages of milk fat and protein and levels of serum glucose, total protein, albumin and BUN were determined. Milk volume decreased as DIM increased (P<0.05). Milk fat showed normal changes, but milk protein was similarly low in all groups (P>0.05), showing probable shortages in energy and/or protein intake. Serum parameters showed no significant differences among groups (P>0.05). Weak positive correlations were found between serum total protein and milk components after 150 DIM (maximum correlation with milk fat, r = 0.61). It is concluded that shortage in intake of energy and/or protein, which may result in lowered milk protein, may not be differentiated by measuring serum glucose, total protein, albumin and BUN and milk components. More detailed experiments on serum and milk are necessary for defining the problem properly.

Key words: Dairy cow, Milk components, Serum chemistry, Subclinical production disorders

Introduction

Subclinical diseases or nutritional imbalances may be contributing to suboptimal productivity (Carlson, 2002) which may cause great economic losses. Sequential assessment of animal responses in subjects such as milk composition and milk production is critical for defining and resolving subclinical production disorders before they become too costly, but this is a difficult and complicated task (Herdt, 2000; Carlson, 2002). Serum chemistry profiles are useful for assessing nutritional status of dairy cows and they are generally reserved for problem situations (Herdt, 2000). Although diagnosing clinical diseases in individual animals by serum chemistry is a routine practice in veterinary medicine, application of these tests for detecting reasons of poor nutriture and poor performance in herd-level in clinically normal animals, poses a more difficult situation different from diagnosing nutritional diseases (Moore, 1997a, b; Herdt, 2000). The difficulty arises when interpreting test results from multiple clinically normal animals (Moore, 1997b). Selecting the tests to be run for detecting subclinical problems depends on their costs, ease of sampling and experimental methods and their worth to illuminate a problem correctly (Herdt, 2000). The present study was conducted in a commercial dairy farm with a history of suboptimal milk composition during summers to determine the probable value of milk components and some serum constituents for defining the cause(s) of the problem.

Materials and Methods

Animals, diet and environment

The study was done in adult Holstein-
Friesian cows, in a dairy farm, located in Marvdasht area, Iran. Adult cows with daily production of 35 kg or more and/or a body condition score less than 2.75 (using a 5-point scale with quarter point divisions) were kept in the high-string regardless of their stage of lactation and were fed similarly. Thirty-two clinically healthy cows of this group, in their second lactation or later, at various stages of their lactation cycle, were chosen for this study and were divided into 4 groups according to their days in milk (DIM) and their physiological status. The cows in the first group (EL) were in early lactation (20-70 DIM) and were considered to be in negative energy balance (NEB). The second group (ML) was in mid lactation (70-110 DIM) and was considered to be in balance of energy or still in negative energy balance. The third group (ML2) was also in mid lactation (110-150 DIM) and theoretically was in slight balance of energy and finally, the forth group (LL) was in late lactation (150-210 DIM) with positive energy balance. The diet of the cows consisted of 43.25% (11.4 kg DM) forage (alfalfa hay, fresh alfalfa, corn silage) and 56.75% (15 kg DM) concentrates (ground barley, beet pulp, wheat bran, cottonseed meal, whole cottonseed, mineral/vitamin supplements and sodium bicarbonate) and was fed in 9 meals per day. Chemically, the diet composed of 1.61 kcal/kg net energy for lactation, 16.54% crude protein, 36.89% NDF, 35.30% NSC, 0.90% calcium and 0.55% phosphorus. The study was completed during July and August when cows were subjected to maximum daily temperatures up to 40°C (104°F) and had mean rectal temperatures of 39.2°C and respiratory rates up to 80 min⁻¹ at 11 a.m. The cows were cooled under showers at least two times per day at 1100 and 1500 and were milked three times at 0500, 1300 and 2100. Thus, all cows under study were heat stressed, managed similarly and receiving an isocaloric, isonitrogenous diet regardless of their physiological status. The cows were fed the same ration and subjected to high ambient temperatures for at least one month before the beginning of the experiments early in July. An approximate estimate of feed refusal was recorded every day.

Samplings and experiments
During a period of 30 days, four blood samples were collected every 10 days from the coccygeal vein in vacumm tubes containing no anticoagulant. Sampling was done at about 0600, after morning milking and prior to feeding. Being transferred to the laboratory within less than two hours, the samples were centrifuged at 3000x g for 20 minutes and serum glucose was measured immediately (ortho-toluidine method). Then the aliquots were stored at -20°C until they were analyzed for serum total protein (biuret method), albumin (bromocresol green method) and urea nitrogen (BUN, diacetylmonoxine method) (Sonnenwirth and Jarett, 1980). Composite milk samples of individual cows were also collected four times, on 10 day intervals at each milking time, when the volume of milk was also recorded. Beginning at 1300, samples were collected and were refrigerated until they were transferred to the laboratory early in the next morning. Samples were first analyzed for their fat percentages using Gerber’s method (Horwitz, 1975) and the remainder was stored at -20°C. Within less than 2 months, the samples were thawed at room temperature to be analyzed for crude protein and non-protein nitrogen (NPN) percentages as Kjeldhal N x 6.38 (Horwitz, 1975). For measuring NPN, 5 ml of 12% trichloroacetic acid was added to 5 ml of milk in order to precipitate true protein of milk. Then, the mixture was centrifuged at 3000 x g for 20 minutes and NPN was measured in the supernatant. Percentage of true protein was calculated in 24hrs milk from the values obtained as above.

Statistical analysis
Comparison among groups was done with one-way ANOVA and Duncan’s multiple range tests using the pool data of each group. Correlations between blood and milk parameters were determined by bivariate Pearson’s test using pool data of all groups and separate data of each group. The SPSS statistical software was used for data analysis.

Results
Milk volume differed significantly
among groups (P<0.05). Milk fat percentages tended to increase as DIM progressed, approaching significance level (P<0.05) between groups I and IV (P = 0.055) (Fig. 1). Percentages of crude protein and milk parameters in separate groups and in sum of all groups (Table 1). An average of 1.2 kg DM day⁻¹ (mainly corn silage) was not consumed.

**Discussion**

The rate of decline in milk volume appeared to be somewhat higher than the normal rate in adult dairy cows, which may be 6% per month after peak lactation (Muller, 1992). This could be due to reduced feed intake (mainly corn silage) and other drastic effects of heat stress (see below). Reduction in feed intake may appear as a result of heat stress during which cows consume less feed (McDowell et al., 1976). Greater heat increment has been associated with higher acetate production in the rumen of cows fed high forage diets (Tyrrell et al., 1979). Thus cows often voluntarily limit their forage consumption during hot weather, even to the extent of drastically shifting acetate to propionate ratios and lowering butterfat content of milk (Huber, 1996). In addition, maintenance requirements of lactating dairy cows increase by about 30% if ambient temperatures are raised from 25 to 40°C for 6 h d⁻¹ (Huber, 1996). Accordingly, as diet used in the present study was not adjusted, it was proposed that the cows in EL group (20-70 DIM) were consuming nutrients in amounts lower than their actual needs due to NEB and also due to the effects of heat stress. Cows in LL group (150-210 DIM), on the other hand, were proposed to receive nutrients in amounts closer to their actual needs. Thus, some differences in milk components and serum glucose, total protein, albumin and BUN of groups were expected. However, among all parameters determined in this study, only low levels of milk protein apparently revealed some abnormalities or undernutrition. Roughly, this could be the result of shortage in intake of energy, protein or both. However, milk fat and serum constituents did not help explain the exact reason(s), though they are usually considered as indices of energy and protein status.

Milk protein was low in all groups (from 2.71% in ML1 to 2.80% in LL, close to the lowest point of normal range) and did not

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**Fig. 1:** Mean ± SEM of daily milk production and percentages of milk fat, crude protein and true protein in heat stressed high producing cows, at various stages of lactation under similar management and nutritional conditions. EL: early lactation; ML: mid lactation; LL: late lactation; DIM: days in milk

and true protein, however, were low and almost similar among all groups with no significant difference (P>0.05)(Fig. 1). Serum glucose, total protein and albumin did not differ significantly among groups (P>0.05), but BUN was significantly lower in EL group (P<0.05) (Fig. 2). Some weak correlations were observed between blood
Table 1: Correlations between blood and milk constituents at various stages of lactation cycle

<table>
<thead>
<tr>
<th>Stage of lactation</th>
<th>Serum constituents</th>
<th>Milk volume</th>
<th>Milk crude protein</th>
<th>Milk true protein</th>
<th>Milk fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early lactation (DIM = 20-70, n = 32)</td>
<td>Serum glucose</td>
<td>-0.43*</td>
<td>0.35</td>
<td>0.35</td>
<td>0.38*</td>
</tr>
<tr>
<td></td>
<td>Serum total protein</td>
<td>-0.13</td>
<td>0.04</td>
<td>0.03</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Serum albumin</td>
<td>0.14</td>
<td>-0.19</td>
<td>-0.05</td>
<td>-0.04</td>
</tr>
<tr>
<td></td>
<td>BUN</td>
<td>0.14</td>
<td>0.04</td>
<td>0.14</td>
<td>-0.13</td>
</tr>
<tr>
<td>Midlactation 1 (DIM = 70-110, n = 28)</td>
<td>Serum glucose</td>
<td>-0.16</td>
<td>0.25</td>
<td>0.01</td>
<td>-0.49**</td>
</tr>
<tr>
<td></td>
<td>Serum total protein</td>
<td>0.02</td>
<td>0.07</td>
<td>0.10</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Serum albumin</td>
<td>-0.03</td>
<td>0.03</td>
<td>-0.13</td>
<td>-0.22</td>
</tr>
<tr>
<td></td>
<td>BUN</td>
<td>-0.11</td>
<td>-0.02</td>
<td>0.02</td>
<td>0.20</td>
</tr>
<tr>
<td>Midlactation 2 (DIM = 110-150, n = 30)</td>
<td>Serum glucose</td>
<td>0.02</td>
<td>0.36*</td>
<td>0.23</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td>Serum total protein</td>
<td>-0.07</td>
<td>0.47**</td>
<td>0.44*</td>
<td>0.61**</td>
</tr>
<tr>
<td></td>
<td>Serum albumin</td>
<td>0.07</td>
<td>0.07</td>
<td>0.13</td>
<td>-0.43*</td>
</tr>
<tr>
<td></td>
<td>BUN</td>
<td>0.25</td>
<td>-0.09</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Late lactation (DIM = 150-210, n = 27)</td>
<td>Serum glucose</td>
<td>-0.10</td>
<td>0.30</td>
<td>-0.26</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Serum total protein</td>
<td>-0.36</td>
<td>0.48*</td>
<td>0.59**</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Serum albumin</td>
<td>0.32</td>
<td>-0.65**</td>
<td>-0.45*</td>
<td>-0.03</td>
</tr>
<tr>
<td></td>
<td>BUN</td>
<td>-0.05</td>
<td>-0.16</td>
<td>-0.04</td>
<td>0.33</td>
</tr>
<tr>
<td>All stages (DIM = 20-210, n = 116)</td>
<td>Serum glucose</td>
<td>-0.13</td>
<td>0.29**</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Serum total protein</td>
<td>-0.15</td>
<td>0.27**</td>
<td>0.27**</td>
<td>0.34**</td>
</tr>
<tr>
<td></td>
<td>Serum albumin</td>
<td>0.04</td>
<td>-0.10</td>
<td>-0.06</td>
<td>-0.16*</td>
</tr>
<tr>
<td></td>
<td>BUN</td>
<td>-0.06</td>
<td>-0.03</td>
<td>0.03</td>
<td>0.12</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

follow the normal pattern of milk protein curve. A normally fed group of Holstein cows can be expected to have a milk protein percent of 2.7 to 3.0% by week 5 to 6 of lactation which then increases to as high as 3.6 to 3.8% by the end of lactation (Robinson, 2000). Energy intake is the primary nutritional factor that affects milk protein percentage and low milk protein levels observed in this study, may indicate low intakes of energy in all groups from early to late lactation. The level of dietary protein also affects milk protein (Bachman, 1992).

Energy shortage may be reflected by low fat tests particularly during early lactation (Hutjens, 2002). In the present study, however, despite expected energy shortage and lower intake of forages, fat tests were normal and increased toward the end of lactation. For high producing Holsteins, a fat test between 3.0 to 3.3% from 50 to 150 DIM is not a concern. After that, milk fat should be normal for the breed (3.66% for Holsteins; Hutjens, 2002). In the present study, milk fat was well above 3.3%, approaching 3.66% in the LL (3.65 versus 3.66%) (Fig. 1). Milk fat can easily be kept within acceptable limits by providing sufficient dietary fiber (Bachman, 1992) from forages and some concentrates such as beet pulp, wheat bran and whole cottonseed which were present in the diet used in this study.

Serum glucose was also within its normal range (1.9- 3.8 mmol/l, Kaneko, 1989) similarly in all groups (Fig. 2), showing no significant difference. In fact, glucose is an insensitive index of energy status since it is under strong hormonal
control (Herdt, 2000).

The concentrations of serum albumin (Fig. 2) appeared to be slightly subnormal in all groups (normal range: 30.3-35.5 g/l; Kaneko, 1989). However, these levels would not indicate protein deficiency, mainly due to similar results in all groups regardless of expected differences in energy and protein intakes. Serum albumin concentration is influenced by protein status (Herdt, 2000) and inadequate intake, digestion and absorption of protein results in hypoalbuminemia (Russel et al., 1997). Energy shortage may also result in hypoalbuminemia since it causes less synthesis of microbial protein in the rumen (Blowey, 1990). Since albumin has a large pool size (Herdt, 2000), and a long half-life of 16.5 days (Russel et al., 1997) it takes a long time before hypoalbuminemia develops. The duration of expected nutritional inadequacy in this study was nearly two months. Despite these facts, albumin concentrations were almost the same at various stages of lactation irrespective of negative or positive energy balance and level of production. There are opposite reports on changes of serum albumin during a lactation cycle. Little (1974) reported a negative correlation between levels of serum albumin and milk production, but Hewett (1974) found no significant difference in serum albumin concentrations at various stages of a lactation cycle. Despite the pattern of changes in serum albumin during lactation, if the levels obtained in this study were to be considered as hypoalbuminemia, then some hypoproteinemia and lowered BUN would also be expected. None of these were observed in this study.

Hypoproteinemia in mature cattle is usually the result of hypoalbuminemia or panhypoproteinemia (Russel et al., 1997). Panhypoproteinemia with dietary protein deficiencies is often preceded by hypoalbuminemia (Morris and Johnston, 2002). However, the level of serum total protein (Fig. 2) was similarly normal in all groups (normal range: 67.4-74.6 g/l; Kaneko, 1989). Deficient protein intake can result in lowered BUN concentration (Moore, 1997b). However, physiologic range of BUN is large, from 5 to more than 20 mg/dl (0.83- 3.32 mmol/l) in clinically normal animals (Moore, 1997b; Herdt, 2000). Lower BUN concentration in EL group (Fig. 2) could be transient due to lower feed intake during early lactation. Such a condition can be an early warning that low protein status may develop if protein intake is not increased (Manston, et al., 1975). However, in protein deficiency, hypoalbuminemia also accompanies lowered BUN (Payne, 1989). In the present study, if the concentrations of both BUN and albumin were lowered in EL group, low protein intake could be concluded. A general protein deficiency identically in all groups can not also be concluded because of similar results of albumin, total protein and BUN in cows, which had a wide range of days in milk and different physiologic conditions.

Weak correlations between serum total protein and milk components became apparent after day 150 (Table 1). A valid physiologic explanation cannot be brought for these correlations and other weak correlations observed in separate groups. Constant levels of the parameters at various stages of lactation could be the reason of the absence of strong correlations.

Another contributing factor for low milk protein tests in this study may be the season of the year. Milk protein is lower in summer (Robinson, 2000). However, to rule out nutritional factors and to make a conclusion on direct effect of hot weather, more detailed experiments on serum and milk chemistry appear to be necessary.

In summary, low milk protein appeared to be the main problem in the herd studied with its specific management practices during summer. Energy and/or protein shortages were probably the most prominent reasons of this problem because of the effects of heat stress. The effects of shortages in energy and/or protein are firstly reflected on milk protein, milk fat and serum glucose, total protein, albumin and BUN may not be affected over a long period of time and measuring them may not be a useful tool in defining the problem before it gets bigger. Crude protein and true protein are affected similarly and as a rough index of low intakes of energy and/or protein, measuring only crude protein may suffice. To differentiate between probable reasons of
lowered milk protein, it appears that more detailed experiments on milk and serum are necessary.

Acknowledgements

This work was financially supported by the Office of Vice-Chancellor for Research Affair, Shiraz University. The authors would also like to thank Mr. Parviz Asghari for his cooperation and his reliance on scientific works. Review of the manuscript by Dr. H. Rajaian is highly appreciated.

References


Fig. 2: Mean ± SEM of serum glucose, total protein, albumin and BUN in heat stressed high producing cows, at various stages of lactation under similar management and nutritional conditions. EL: early lactation; ML: mid lactation; LL: late lactation; DIM: days in milk (*P<0.05)