Early lactation milk production and energy partitioning in primiparous Holstein-Friesian cows with high or low milk protein concentrations in a seasonally-calving, pasture-based herd

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Abstract

A strong positive association between milk protein concentration (MP\%) and reproductive performance has been reported in many countries, but little has been done to elucidate and exploit the underlying mechanisms. The current research had the aim of comparing milk production, nutrient and energy partitioning, and physical characteristics between cows with high or low MP\% in early lactation. Milk yield and composition, blood plasma metabolite and hormone concentrations, and body condition score (BCS) were measured in a group of 85 primiparous Holstein-Friesian cows at DEDJTR, Ellinbank Centre Victoria, during the first 123 days of lactation. Results from cows within the quartiles with highest (Hi) and lowest (Lo) MP\% are presented. Hi MP\% cows had greater concentrations of milk fat, protein and lactose, but lower daily milk volume compared with Lo MP\% cows. There were no significant differences in daily yield of milk solids (milk fat plus protein) nor in daily net energy apportioned for milk production. Hi MP\% cows had greater blood plasma concentrations of glucose, insulin, insulin-like growth factor-1 (IGF-1) and leptin and maintained a greater BCS during early lactation. Overall, results were consistent with Hi MP\% cows partitioning more nutrients and energy to body condition at the expense of milk yield, except that this was not entirely supported by calculations of total energy output in milk. Further research is necessary to better understand causes of the positive association between MP\% and reproductive performance in dairy cattle.

Introduction

The declining fertility of dairy cattle in many countries over several decades has been associated with a focus on increasing per cow milk production and an increase in the proportion of Holstein-Friesian genes from sires of North American origin (Buckley, O'Sullivan, Metges, Evans & Dillon, 2003), but causal interrelationships between these variables are poorly understood. In pasture-based, low-input dairying systems of south-eastern Australia and elsewhere, seasonally-concentrated calving patterns are used to increase pasture utilisation by matching peak energy requirements with peak pasture growth rates (Auldist, O'Brien, Cole, Macmillan & Grainger, 2007). In such systems and others, low fertility results in substantial costs to dairy farmers, including high rates of cow wastage due to cows failing to conceive (Borman, 2004). The InCalf studies conducted mainly in Victorian herds in 1998 and 2009 showed that one of the factors having the greatest impact on the probability of conception within 6 weeks following the mating start date was the cow’s milk protein concentration (MP\%) (Morton, 2000, 2011). This positive association between MP\% and dairy cow fertility has also been shown in the United Kingdom, Ireland and the Netherlands (Buckley, O'Sullivan, Metges, Evans & Dillon, 2003; Xu & Burton, 1996), yet it is strongest in cows with moderate milk volumes, typical of those managed in pasture-based production systems of south-eastern Australia (Morton, 2000). If the factors underpinning this association could be understood and exploited, there could be substantial benefits for the Australian dairy industry, especially when viewed in the context of milk protein being more than twice as valuable as milk fat under the milk payment systems that predominate in many Australian processing companies.

Differences in the way cows partition energy in early lactation, when energy demands for lactation exceed energy intake, provide one possible explanation for the association. Post-partum negative energy balance (NEB) in dairy cows causes decreased MP\% due to a shortage of glucose for milk protein synthesis in the mammary gland (de Vries & Veerkamp, 2000). Negative energy balance, as indicated by a marked loss in body condition in early lactation when the energy requirements for milk production and maintenance exceed dietary energy intake, is associated with poor reproductive performance (Butler, 2003; Reist et al., 2003). However, Fahey, Morton & Macmillan (2003) demonstrated a positive association
between the reproductive performance of non-lactating heifers and their MP% in their first lactation, although it was not as strong as in multiparous cows. This nevertheless indicates that the biological determinants underpinning the association are not restricted to lactation-specific factors such as post-partum NEB.

The objective of this experiment was to compare milk production, body condition score (BCS) and blood plasma concentrations of selected metabolites and hormones between primiparous Holstein-Friesian cows with either high (Hi) or low (Lo) MP%, in order to gain an understanding of the mechanisms behind the association. The hypotheses tested were: (1) that, in comparison with Hi MP% cows, Lo MP% cows have blood plasma metabolite and hormone concentrations that indicate preferential partitioning of nutrients and energy towards milk synthesis in early lactation at the expense of body condition; and (2) that milk energy output in early lactation is greater for Lo compared with Hi MP% cows due to increased milk yield.

Material and Methods
Data was collected from 85 primiparous Holstein-Friesian cows from the research herd at DEDJTR, Ellinbank Victoria. Cows were managed as a single, seasonally-calving herd, with a mean calving date of August 1 2013. All cows experienced a common nutritional and management regimen. Their diet consisted of grazed perennial ryegrass pasture, an average of 3.7kg DM/day of grain (wheat and/or canola meal) fed in the dairy during milking, and pasture hay and silage fed in the paddock in summer and autumn. Cows were milked twice daily at c. 0600h and 1500h. All experimental procedures were approved by the DEDJTR Agricultural Research and Extension Animal Ethics Committee.

Milk volume was measured at each milking using a DeLaval Alpro milk metering system (DeLaval International, Tumba, Sweden), while a composite milk sample (p.m. plus a.m.) was taken using in-line milk meters once per week until November, and fortnightly thereafter. These samples were tested for concentrations of protein, milk fat (MF%) and milk lactose (ML%) using a near-infrared milk analyser (model 2000, Bentley Instruments, Chaska, MN, USA). Daily milk energy yield for each cow was estimated by calculating the daily yield of milk protein, fat, and lactose, and assigning these components net energy contents of 24.1 KJ/g, 38.3 KJ/g and 16.5 KJ/g, respectively (Sjaunja, Baevre, Junkkarinen, Pedersen & Setala, 1990).

Blood samples were collected weekly from calving until November, and thereafter at monthly intervals. Two 10ml blood samples were collected via coccygeal venepuncture into vacutainers containing powdered lithium heparin, and potassium EDTA (BD Vacutainer System, Belliver Industrial Estate, Plymouth, UK). Samples were gently inverted, stored on ice, and processed within 60 minutes of collection. Blood samples were centrifuged (Clements SG 400, Clements, Sydney, NSW, Australia) at 1800 g for 10 minutes at 4°C, the plasma was aspirated into two 5ml sample tubes, and then stored at -18°C prior to analysis. These blood samples were analysed for glucose, non-esterified fatty acids (NEFA), urea and β-hydroxybutyrate (BHBA) using commercially-available kits on an Olympus AU400 Clinical Chemical Analyser at the Animal Health Laboratories (Department of Agriculture and Food, Western Australia). Analyses for insulin, IGF-1, leptin and somatotropin were conducted using similar methodologies on a gamma counter (Packard Cobra-II, Auto Gamma) at the School of Animal Biology, University of Western Australia.

Cow BCS was assessed by trained technical staff at weekly intervals during early lactation using the 8-point scale of Earle (1976).

Statistical analyses
The cows were separated into quartiles based on their average MP% during early lactation (up to 123 days in milk for each cow), and only data from cows within the highest (Hi; 3.22 to 3.40%) and lowest (Lo; 2.87 to 3.00%) MP% quartiles were analysed, using GenStat 17 (2014).

Statistical analysis of the milk production and composition data, milk energy yield, cow BCS, and blood plasma metabolite and hormone concentrations was conducted by averaging the daily or weekly data over the early lactation period, and conducting univariable regression analyses between MP% and each variable.

Results
Mean daily milk volume was greater for Lo compared with Hi MP% cows during early lactation. Cows with Hi MP% also had greater MF% and ML% than Lo MP% cows, however the daily yield of milk solids (milk fat plus protein) did not differ significantly between quartiles (Table 1). Cows with Lo MP% had greater daily lactose yield compared with Hi MP% cows. There was also no significant difference in daily milk energy yield between the MP% quartiles (Table 1).

Pre-partum plasma metabolite and hormone concentrations were not significantly different between Hi and Lo MP% cows, indicating no difference in the nutrient status of these cows prior to their first lactation. Following parturition, cows with Hi MP% had greater plasma concentrations of glucose, insulin, IGF-1 and leptin (Table
1), and numerically lower average plasma concentrations of NEFA, urea, BHBA and somatotropin.

Pre-partum BCS did not differ significantly between Hi and Lo MP% cows (4.63 and 4.60 units, respectively). However, during early lactation, Hi MP% cows maintained a greater BCS in comparison to Lo MP% cows (Table 1).

Table 1. Selected variables for cows with high (Hi) or low (Lo) milk protein concentration (MP%) in early lactation (calving to 123 days in milk)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hi</th>
<th>Lo</th>
<th>SED</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk yield (L/day)</td>
<td>20.0</td>
<td>22.8</td>
<td>0.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MP%</td>
<td>3.27</td>
<td>2.94</td>
<td>0.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MF%&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.14</td>
<td>3.74</td>
<td>0.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ML%&lt;sup&gt;3&lt;/sup&gt;</td>
<td>5.19</td>
<td>5.13</td>
<td>0.03</td>
<td>0.027</td>
</tr>
<tr>
<td>Milk solids&lt;sup&gt;4&lt;/sup&gt; (kg/cow/day)</td>
<td>1.49</td>
<td>1.52</td>
<td>0.07</td>
<td>0.658</td>
</tr>
<tr>
<td>Milk energy yield (MJ/cow/day)</td>
<td>66.1</td>
<td>67.9</td>
<td>3.09</td>
<td>0.566</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>3.36</td>
<td>3.27</td>
<td>0.04</td>
<td>0.030</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>5.27</td>
<td>4.37</td>
<td>0.27</td>
<td>0.002</td>
</tr>
<tr>
<td>IGF-1&lt;sup&gt;6&lt;/sup&gt; (ng/ml)</td>
<td>16.20</td>
<td>11.39</td>
<td>1.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>0.58</td>
<td>0.52</td>
<td>0.02</td>
<td>0.006</td>
</tr>
<tr>
<td>BCS&lt;sup&gt;6&lt;/sup&gt; (units)</td>
<td>4.11</td>
<td>4.03</td>
<td>0.04</td>
<td>0.049</td>
</tr>
</tbody>
</table>

Values are the means for all cows in each quartile; SED = standard error of the difference; MF% = milk fat concentration; ML% = milk lactose concentration; ML solids = milk fat plus protein; IGF-1 = insulin-like growth factor-1; BCS = body condition score

Discussion

The positive association between MP% and dairy cattle fertility has been demonstrated in a number of studies and is well accepted (Buckley, O’Sullivan, Metges, Evans & Dillon, 2003; Pryce & Veerkamp, 2001). Despite this, the association is not helping to increase the profitability of dairy farming as the underlying mechanisms are not understood and so are not being exploited. This experiment has provided the first step in understanding the physiological and endocrinological differences between primiparous Holstein-Friesian cows with Hi and Lo MP% in a seasonally calving, pasture-based herd.

One possible explanation for the association is that there may be differences in the ways that Hi and Lo milk protein cows partition energy during early lactation. When milk yield reaches a peak some 6 weeks after parturition, energy demands of lactation are greater than the amount of energy that can be obtained from the diet and cows begin mobilising body tissue reserves in order to meet energy requirements (de Vries & Veerkamp, 2000). This state of NEB is known to reduce fertility, and has also been shown to result in reduced MP% (de Vries & Veerkamp, 2000) due to a higher proportion of available amino acids being used for gluconeogenesis and a reduction in their availability for milk protein synthesis (Auldist, Thomson, Mackle, Hill & Prosser, 2000). Hence, it is logical to expect that MP% and fertility may be related in this way.

In the current experiment, blood plasma concentrations of selected metabolites and hormones were consistent with Lo MP% cows partitioning more energy to milk production and less to body condition, and thus being in a more severe NEB than Hi MP% cows. For example, lower concentrations of glucose, insulin, IGF-1 and leptin have all been used previously as indicators of NEB (Delany et al., 2010). Thus our first hypothesis is accepted.

The suggestion that Lo MP% cows were in greater NEB is also consistent with these cows having higher milk volumes but lower BCS than Hi MP% cows in early lactation. This was demonstrated through lower plasma IGF-1 and leptin concentrations in Lo MP% cows, while the greater plasma insulin concentrations in Hi MP% cows would have promoted the uptake of glucose by peripheral tissues (Lucy, 2006), hence resulting in a greater BCS for these cows. Overall these observations indicate that Lo MP% cows may have greater difficulty conceiving during the first 6 weeks of the seasonally concentrated breeding program than Hi MP% cows (Butler, 2003), though it was not the objective of this experiment to assess this.

Despite the indications that Lo MP% cows were partitioning more energy towards milk production than Hi MP% cows, an examination of milk composition and milk energy content did not support this contention. Although milk volume was greater in Lo MP% cows, our results indicate that there were not large differences in daily yields of milk solids (fat plus protein, not including lactose) between Hi and Lo MP% cows. Similarly, when the daily milk energy yield was calculated using known energy values for the major milk components (this time including lactose), there was no significant difference in milk energy between Hi and Lo MP% cows. Our results suggest that differences in milk energy yield in early lactation are not the only cause of the differences in fertility between cows with divergent early lactation MP%, thus the second hypothesis is not supported.

The lack of difference in milk energy yield between the two MP% quartiles shows that, while some of the association between MP% and fertility may be related to differences in the way cows partition energy between milk and body tissue, there are other important factors that currently remain unclear. This is consistent with Fahey, Morton & Macmillan (2003) that the capacity of primiparous heifers to conceive when they were yet to commence lactation was significantly associated with their MP% in their subsequent lactation. It is also consistent with the observation of Morton (2000) that the association between MP% and dairy cow fertility persists.
at all stages of lactation, including mid and late lactation when cows have typically regained a positive energy status.

**Conclusion**

Overall this experiment has shown that Lo MP% cows have lower blood plasma concentrations of glucose, insulin, IGF-1 and leptin, reduced BCS in early lactation, and higher early lactation milk volume than Hi MP% cows. Collectively these observations suggest that Lo MP% cows proportion more energy to milk production at the expense of body tissue than Hi MP% cows. Nevertheless, estimates of the amount of milk energy produced by each quartile are not supportive of greater energy partitioning to milk synthesis by Lo MP% cows. We conclude that while differences in energy partitioning in early lactation may partially underpin the positive association between MP% and fertility in dairy cattle, other important factors are involved. Further research is required to fully elucidate these mechanisms so that the potential benefits of the association for the dairy industry can be captured.

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**References**


GenStat 17. (2014). VSN International Ltd. 5 The Waterhouse, Waterhouse Street, Hemel Hempstead, HP1 1ES, UK.


