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Lipolytic response of New Zealand and overseas Holstein-Friesian dairy cows challenged with epinephrine

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ABSTRACT

Sixteen New Zealand Holstein-Friesians (NZ HF) and 15 overseas HF (OS HF) grazing pasture or fed a total mixed ration (TMR) were subjected to an epinephrine challenge during early lactation. A genotype x diet interaction trend (P=0.10) was observed; when grazing, OS HF displayed the greatest glycerol response, with the reverse occurring on TMR. No genotype or diet differences were observed for the glycerol or non-esterified fatty acid response. Dairy cows of both genotypes fed TMR produced a glucose response 31% greater than both genotypes grazing pasture. These preliminary results suggest that genotype x diet-induced differences in the rate of lipolysis may occur in Holstein-Friesians during early lactation. These differences may be a function of small differences in energy balance. Feeding TMR was associated with a greater gluconeogenic response to epinephrine, which probably resulted from greater hepatic reserves of glycogen or simply a greater energy demand by the mammary gland.

Keywords: Holstein-Friesian; genotype; diet; epinephrine; body reserves.

MATERIALS AND METHODS

Design

The epinephrine challenge was conducted in the third year (2000) of a multi-year trial, the first year of which was described in detail by Kolver et al. (2000). All procedures were approved by the AgResearch Ruakura Animal Ethics Committee. Cows were fed a TMR (25% maize silage, 21% grass silage, 2% hay, 10% whole cottonseed, 42% concentrate) ad libitum, or grazed pasture (Grass) at generous allowances (>45 kg DM/cow/day). TMR was mixed in a vertical mixer wagon and fed twice daily. Only third-lactation cows in each herd were used for the challenge. The four treatments in this 2 x 2 factorial experiment were NZ Grass (n=8); OS Grass (n=7); NZ TMR (n=8); and OS TMR (n=8). The challenge was administered in early lactation over two days. Half of each of the four experimental groups was challenged on each day. Cows in the Grass treatment were challenged between 0800 and 1030 h and cows in the TMR treatment were challenged between 1100 and 1330 h. Cows in both the Grass and TMR treatments had been withdrawn from their respective feeds prior to milking and approximately 2 h prior to the beginning of the challenge period (grazing cows were milked at 0615 h and TMR cows at 0845 h).

Epinephrine challenge

Cows were fitted with one indwelling jugular catheter prior to the day of the challenge. Following milking, cows were haltered in an outside yard. Epinephrine was dissolved in sterile physiological saline at a concentration of 1 mg/ml, and challenge syringes made up to 5 ml with saline. Epinephrine (1.4 µg/kg live weight) was administered via the jugular catheter and was followed by 5 ml of saline. The dose of epinephrine used has previously been shown to result in the maximal stimulation of lipolysis (Sechen et al., 1990). Blood samples (10 ml) were withdrawn at -30, -15, -10, -5, 0, 2, 5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes relative to challenge (time zero). Blood was collected from the catheter into vacutainer tubes containing 100 ul of 35% (w/v) sodium heparin as the anticoagulant.
and immediately placed on ice. Plasma was harvested by centrifugation (3000 g for 15 min) and stored at –20°C until assayed.

**Measurements**

Plasma samples were analysed for concentrations of glucose (hexokinase method; Roche, Germany), non-esterified fatty acids (NEFA) (colourimetric method; Wako, Japan), and glycerol (colourimetric method; Randox, UK). All assays were performed on the Hitachi 717 analyser (Roche) at 30°C by Alpha Scientific Ltd (Hamilton New Zealand).

Milk yield and composition were measured on a daily basis for five days prior to the challenge. Live weight and body condition score were assessed one day prior to the challenge. Energy balance calculations were based on the change in live weight within treatment.

**Statistical analysis**

Post-challenge metabolite concentrations were corrected for pre-challenge concentrations by subtraction of the animal’s mean concentration in the five pre-challenge samples from post-challenge samples. This correction allowed comparison of treatment effects on post-challenge concentrations free of the confounding effects of variation in pre-challenge values. Results are presented as the deviation of the post-challenge values from pre-challenge base line.

Metabolite responses to the challenge were calculated as the area under the response curve (Sechen et al., 1990). Peak plasma concentrations of NEFA, glycerol and glucose occurred 5-15 min after epinephrine administration and returned to base line by 45-60 min. To minimise the contribution of clearance and counter-regulatory effects, the response area was calculated from the time of challenge to 20 min post-challenge (Sechen et al., 1990). Response areas were corrected for differences in base line concentration. A general analysis of variance (Genstat, Version 3.2) was then applied to the corrected post-challenge values and to the response areas to test genotype, diet, and the genotype x diet effects. All means presented are least squares. Significant effects were declared at P<0.05 and trends at P<0.10.

**RESULTS**

The epinephrine challenge was administered to cows in early lactation (62 ± 6.5 days in milk; mean ± SD) producing high levels of milk and milksolids (Table 1). Third-lactation cows fed TMR produced more milk and milksolids than cows grazing pasture, and had a greater live weight and calculated DM intake than their grazing counterparts. Holstein-Friesians of OS genetics produced statistically similar milk and milksolids yields compared to NZ HF on both diets, were of greater live weight, and had a lower body condition score. A genotype x diet interaction was apparent for calculated DM intake and energy balance; when grazing pasture, OS HF had a lower DM intake and energy balance than NZ HF; however when fed TMR, OS HF had a higher DM intake and energy balance than NZ HF (Table 1).

For all treatments, concentrations of glycerol peaked at 5 min, and NEFA and glucose at 10 min post-challenge (Figure 1). An exception was the NZ Grass treatment, which had a peak glycerol concentration 2 min post-challenge. Figure 1 expresses the response as the change from base line following administration of epinephrine. Base line values (Mean of 5 time points ± SD) for NZ Grass, OS Grass, NZ TMR, and OS TMR were 32.1 ± 1.7, 22.2 ± 6.3, 24.8 ± 2.7, and 21.3 ± 3.5 mmol/l glycerol; 0.38 ± 0.01, 0.32 ± 0.03, 0.34 ± 0.02, 0.35 ± 0.04 mmol/l NEFA, and 3.8 ± 0.1, 3.7 ± 0.03, 3.6 ± 0.1, and 3.7 ± 0.07 mmol/l glucose, respectively.

The area under the curve, as a measure of tissue responsiveness to epinephrine, indicated no treatment differences in glycerol or NEFA response, but a trend for a genotype x diet interaction in the glycerol response was observed (Table 2). When grazing pasture, OS HF tended to have a greater glycerol response than NZ HF; however, when fed TMR, OS HF had a smaller glycerol response than NZ HF. No difference in glucose response was detected between NZ and OS HF; however, both genotypes fed TMR produced a glucose response 31% greater than both genotypes grazing pasture (31.3 vs. 23.9 mmol.min.l⁻¹, respectively).

**DISCUSSION**

The response of tissues to circulating hormones can be increased in two ways, namely increased receptor occupancy, or intracellular amplification of the signal. Epinephrine binds to both the β- and α2-adrenergic receptors on adipocytes. The enhanced plasma concentrations of NEFA after an epinephrine challenge represent the mobilisation of fatty acids, that is the net effect of β-induced enhanced lipolysis and fatty acid re-

**TABLE 1**: Milk production, live weight, and energy balance characteristics of New Zealand (NZ) and overseas (OS) Holstein-Friesians in early lactation grazing pasture (Grass) or fed total mixed ration (TMR).

<table>
<thead>
<tr>
<th>Genotype (G)</th>
<th>Diet (D)</th>
<th>NZ</th>
<th>OS</th>
<th>SED</th>
<th>Genotype (G)</th>
<th>Diet (D)</th>
<th>G x D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grass</td>
<td>TMR</td>
<td>Grass</td>
<td>TMR</td>
<td>SED</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days in milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk yield (kg/cow/d)</td>
<td>52</td>
<td>71</td>
<td>57</td>
<td>66</td>
<td>11</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Milksolids yield (kg/cow/d)</td>
<td>27.3</td>
<td>35.3</td>
<td>27.9</td>
<td>40.8</td>
<td>2.6</td>
<td>NS</td>
<td>0.001</td>
</tr>
<tr>
<td>Live weight (kg)</td>
<td>500</td>
<td>532</td>
<td>565</td>
<td>604</td>
<td>21</td>
<td>0.001</td>
<td>0.05</td>
</tr>
<tr>
<td>Body condition score</td>
<td>4.9</td>
<td>5.1</td>
<td>4.1</td>
<td>4.4</td>
<td>0.4</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>DM intake² (kg/cow/d)</td>
<td>20.5</td>
<td>21.0</td>
<td>18.3</td>
<td>28.8</td>
<td>1.6</td>
<td>NS</td>
<td>0.01</td>
</tr>
<tr>
<td>Energy balance² (MJME/d)</td>
<td>46.4</td>
<td>15.3</td>
<td>22.5</td>
<td>52.9</td>
<td>16.9</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

¹All cows were in their third lactation.
²Calculated from energy requirements.
TABLE 2: Plasma metabolite responses to an epinephrine challenge administered to New Zealand (NZ) and overseas (OS) Holstein-Friesians1 in early lactation grazing pasture (Grass) or fed total mixed ration (TMR).

<table>
<thead>
<tr>
<th>Genotype (G)</th>
<th>NZ</th>
<th>OS</th>
<th>P&lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet (D)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Grass</td>
<td>TMR</td>
<td>Grass</td>
</tr>
<tr>
<td>NEFA, mmol.min⁻¹</td>
<td>3.03</td>
<td>4.81</td>
<td>3.60</td>
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<tr>
<td>Glycerol, umol.min⁻¹</td>
<td>290</td>
<td>390</td>
<td>359</td>
</tr>
<tr>
<td>Glucose, mmol.min⁻¹</td>
<td>24.3</td>
<td>32.6</td>
<td>23.5</td>
</tr>
</tbody>
</table>

1All cows were in third lactation.
2Response area (0-20 min after dose of epinephrine) was calculated for each animal and has been corrected for base line concentrations.

The response in plasma glycerol concentrations to an epinephrine challenge reflects lipolysis, as adipose tissue has very little glycerol kinase activity. The difference between estimates of lipolysis and mobilisation represent re-esterification (Sechen et al., 1990; Strosberg, 1992).

We observed no significant treatment difference in NEFA response to epinephrine, but did record a trend for a genotype x diet interaction response in glycerol concentrations. Cows in the OS Grass and NZ TMR treatment groups displayed a greater glycerol response to epinephrine than cows in the NZ Grass and OS TMR groups. The greater glycerol response implies a greater rate of lipolysis occurring in the OS Grass and NZ TMR treatments. Several mechanisms may be responsible for this result. Jaster & Wegner (1981) found that the greater lipolytic response of lactating versus non-lactating cows to epinephrine was associated with an increased number of β-adrenergic receptors per adipocyte with no change in binding kinetics. They also suggested that additional differences existed in the intracellular amplification system. A possible post-receptor mechanism for an enhanced rate of lipolysis may be via an increase in activity of lipolytic enzymes, the most likely candidate being hormone-sensitive lipase (Sechen et al., 1990).

The increased lipolysis may be associated with the differences in energy status between treatments. Analysis of calculated DM intake and energy balance revealed a genotype x diet interaction: in the grazing group, OS HF had a lower energy balance and DM intake than NZ HF, however, in the TMR groups the reverse was true. Although all treatments were in positive energy balance, an approximately 31 MJME/d difference in energy balance existed between OS Grass and NZ TMR treatments, compared to NZ Grass and OS TMR. It may be argued that differences in positive energy balance are unlikely to influence rate of lipolysis, nevertheless, cows in less positive energy balance (OS Grass and NZ TMR) displayed higher rates of lipolysis than cows in greater positive energy balance (NZ Grass and OS TMR). It is of interest to note that our calculations estimate that by d 62 of lactation, cows grazing pasture and cows fed TMR were both in positive energy balance. Greater differences in response to epinephrine may have resulted when cows were in negative energy balance earlier in lactation. It is also of speculative interest to note that the greater rates of lipolysis occurred in the genotype x diet treatments (OS Grass and NZ TMR) which were most divergent from the dietary conditions within which the respective genotypes were selected. This may suggest the requirement for genetic adaptation to extremely different diets. It is difficult to explain the lower calculated DM intake of OS Grass compared to NZ Grass when the opposite was true when these genotypes were fed TMR. Perhaps the inability of OS Grass animals to achieve high DM intake on pasture is part of the reason for the lower body condition score observed at the time of the challenge and during previous years of this experiment.

The increased glycerol, but not NEFA, response to epinephrine in the OS Grass and NZ TMR treatments could suggest a diet x genotype-induced increase in fatty acid re-esterification in the adipocyte. However, an explanation for an increase in re-esterification at a time when lipolysis is...
high and energy balance is less positive than NZ Grass and OS TMR is not obvious. Numerically the NEFA response did increase in concert with the greater glycerol response in the NZ TMR (but not OS Grass) treatment, but statistical power may not have been sufficient to detect this increase.

Regardless of genotype, cows fed TMR responded to epinephrine by producing more glucose than cows grazing pasture. The greater apparent conversion of glycerol to glucose by cows fed TMR may reflect an increased demand for glucose by cows producing 2.8 kg milk solids/cow/d compared with cows producing 2.2 kg milk solids/cow/d from pasture. Alternatively, cows fed TMR may have had greater glycogen reserves than cows grazing pasture, with more glycogen being converted to glucose. Increased gluconeogenesis by cows fed TMR may be the reason why the energy balance of cows grazing pasture and cows fed TMR was similar (34 MJME/cow/d) at 62 d in milk. These results present some difficulty determining whether the response to the epinephrine challenge reflected mechanisms that were driving lactation, or were simply a reflection of the differences in energy demand between treatments.

CONCLUSION
A greater glucogenic response to epinephrine by cows fed TMR may have reflected greater hepatic reserves of glycogen or simply a greater energy demand by the mammary gland. These preliminary results also suggest a trend for a genotype x diet-induced difference in adipose tissue responsiveness to epinephrine in Holstein-Friesians during early lactation. These differences were observed for the glycerol, but not NEFA, response. The difference in lipolysis may be a function of small differences in energy balance. Repeated challenges during early, mid and late lactation are required to confirm these observations.

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REFERENCES