Effects of intravenous glucose infusion and nutritional balance on serum concentrations of nonesterified fatty acids, glucose, insulin, and progesterone in nonlactating dairy cows

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ABSTRACT

The objective of this study was to evaluate serum concentrations of nonesterified fatty acids, glucose, insulin, and progesterone in nonlactating dairy cows according to nutritional balance and glucose infusion. Ten nonlactating, ovariectomized Gir × Holstein cows were stratified by body weight (BW) and body condition score (BCS) on d −28 of the study, and randomly assigned to 1) negative nutrient balance (NB) or 2) positive nutrient balance (PB). From d −28 to d 0, cows were allocated according to nutritional treatment (5 cows/treatment) into 2 low-quality pastures with reduced forage availability. However, PB cows individually received, on average, 3 kg/cow per day (as-fed) of a concentrate during the study. All cows had an intravaginal progesterone releasing device inserted on d −14, which remained in cows until the end of the study. Cow BW and BCS were assessed again on d 0. On d 0, cows within nutritional treatment were randomly assigned to receive, in a crossover design containing 2 periods of 24 h each, 1) intravenous glucose infusion (GLU; 0.5 g of glucose/kg of BW, as a 5% glucose solution administered, on average, at 32 mL/min over a 3-h period), or 2) intravenous saline infusion (SAL; 0.9% solution infused on average at 32 mL/min over a 3-h period). Prior to the beginning of each period, all cows were fasted for 12 h. Blood samples were collected, relative to the beginning of the infusion, at −12 and −11.5 h (beginning of fasting), and at −0.5, 0, 0.5, 1, 2, 3, 4, 5, and 6 h. Following the last blood collection of period 1, cows received (PB) or not (NB) concentrate and were returned to their respective pastures. Changes in BCS and BW were greater in NB cows compared with PB cows (−0.60 and −0.25 ± 0.090 for BCS, respectively; −22.4 and 1.2 ± 6.58 kg for BW, respectively). Cows receiving GLUC had greater glucose concentrations from 0.5 to 3 h relative to infusion compared with SAL cows. Insulin concentrations were greater in PB cows assigned to GLUC compared with SAL cohorts at 0.5 and 3 h following infusion, whereas NB cows assigned to GLUC had greater insulin concentrations compared with SAL cohorts at 0.5, 1, 2, and 3 h. Progesterone concentrations were greater in PB cows assigned to GLUC at 2, 3, and 4 h following infusion compared with SAL cohorts. In conclusion, the effects of glucose infusion on serum concentrations of insulin and progesterone in nonlactating dairy cows were dependent on cow nutritional status.

Key words: glucose infusion, insulin, nutritional status, progesterone

INTRODUCTION

During the last few decades in the United States, milk production per dairy cow increased whereas reproductive efficiency decreased (Lucy, 2001). This relationship can be associated with several factors, such as increased incidence of metabolic and reproductive diseases, intensified postpartum negative energy balance, and consequent increased postpartum body fat mobilization (Opsomer et al., 2000; Lucy, 2001). As an example, negative energy balance leads to reduced postpartum circulating concentrations of hormones such as progesterone (P4) and insulin (Sangsritavong et al., 2002; Butler, 2005).

Progesterone is a hormone required for adequate attainment of puberty, resumption of estrous cycles, and establishment and maintenance of pregnancy (Gonzalez-Padilla et al., 1975; Spencer and Bazer, 2002; Looper et al., 2003). Several researchers have reported that blood concentrations of P4 in cattle before or after breeding have been positively associated with conception rates (Fonseca et al., 1983; Folman et al., 1990; Demetrio et al., 2007). Insulin is considered a metabolic mediator between nutrition and reproduction of cattle, and modulates reproductive function by positively influencing LH synthesis and release by the pituitary (Monget and Martin, 1997), follicular deve-
Animals and Diets

Ten nonlactating, nonpregnant, and ovariectomized 
Gir × Holstein cows (BW = 587 ± 22.8 kg; BCS = 2.5 ± 0.07) were stratified by BW and BCS (Wildman et al., 1982) and randomly assigned to 1 of 2 nutritional 
treatments (5 cows/group) on d −28 of the experiment: 1) negative nutrient balance (NB) or 2) positive nutrient balance (PB). From d −28 to d 0, cows were 
allocated according to nutritional treatment into 2 
Brachiaria brizantha pastures with low forage quality 
(average of 53% total digestible nutrients, 7.1% CP, 
and 76.4% NDF; DM basis) and availability (average 
of 4.5 kg of DM/cow daily). Both groups received a 
complete commercial mineral and vitamin mix (7.7% 
Ca, 4.0% P, 3.0% Na, 0.20% K, 0.20% Mg, 2.0% S, 
0.002% Co, 0.03% Cu, 0.002% I, 0.02% Mn, 0.13% Zn, 
and 0.02% F) and water for ad libitum consumption 
throughout the experiment. However, PB cows received 
daily (as-fed basis), at 1200 h, 2 kg/cow of a supplementa-
tional concentrate from d −28 to d −14, and 4 kg/cow of 
the same concentrate from d −13 to d 0. Supplements 
were offered individually to cows through self-locking 
head gates.

The supplemental concentrate consisted of (DM ba-
sis) 62.5% of ground corn, 29% of soybean meal, 5% 
of mineral mix (18% Ca, 10.7% Na, 8% P, 1.2% S, 
0.5% Mg, 0.13% Cu, 0.007% Co, and 0.007% I), 2.5% 
of limestone, and 1% of urea. Nutritional content of 
concentrate was estimated to be (DM basis) 76% of 
total digestible nutrients, 22.4% of CP, and 12.5% of 
NDF. From d −28 to d 0, forage mass was evaluated 
weekly according to the techniques described by Ven-
dramini et al. (2008), whereas forage and concentrate 
samples were also collected weekly and analyzed for 
nutritional content by a bromatology laboratory (São 
Paulo State University, Botucatu, Brazil). Nutritional 
treatments were designed according to the Cornell Net 
Carbohydrate and Protein System model (Fox et al., 
2004) and formulated to induce BW loss (−0.9 kg/d) in 
NB cows and BW gain (0.2 kg/d) in PB cows.

Glucose Infusion and Sampling

On d 0, cows within nutritional treatment were 
randomly assigned to receive, in a crossover design 
containing 2 periods of 24 h each (d 1 and d 2), 1) i.v. 
glucose infusion (0.5 g/kg of BW; GLUC), or 2) i.v. 
saline infusion (SAL). Prior to the beginning of each 
period, all cows were fasted for 12 h, beginning at 1530 
h of the day before each period (d 0 and 1, respectively; 
approximately 5 h after PB cows completely consumed 
their supplements). Blood samples were collected, 
relative to the beginning of the infusion, at −12 and −11.5 
h (beginning of fasting), and at −0.5, 0, 0.5, 1, 2, 3, 4, 
5, and 6 h. Following the last blood collection of period 
1, cows received (PB) or not (NB) the supplementation 
and returned to their respective pastures. Cows were 
fasted before infusion to prevent any confounding ef-
effects between feed intake and infusion treatments on 
circulating concentrations of P4 (Vasconcelos et al., 
2003).

Immediately before infusions, all cows were fitted 
with indwelling jugular catheters according to the pro-
cedures described by Curley et al. (2008). Treatments
(GLUC or SAL) were administered via catheters over a period of 3 h (on average 32 mL/min); GLUC cows received a 5% (wt/vol) glucose solution (90:10 solution of physiological saline and Glicose 50%; Laboratório Prado S.A.; Curitiba, Brazil) according to their BW, whereas SAL cows received a comparable volume of physiological saline (0.9%). Catheters were removed after infusion was complete.

Cow BW and BCS were assessed at the beginning of the experiment (d −28) and before fasting on d 0 to determine nutritional treatment effects on BW and BCS change during the experiment.

**Progesterone Implants and Blood Analysis**

From d −28 to d −15, all cows were inserted with a previously used (third use) intravaginal progesterone releasing device (CIDR, originally containing 1.9 g of P4; Pfizer Animal Health, Sao Paulo, Brazil) to initially expose and adapt cows to exogenous P4. Cows received a new CIDR on d −14, which remained in the cows until the end of the experiment.

Blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL, Becton Dickinson, Franklin Lakes, NJ), placed immediately on ice, and centrifuged at 3,000 \( \times g \) for 30 min for serum collection. Harvested serum was stored frozen at −20°C until further processing. Quantitative colorimetric kits were used to determine concentrations of glucose (Katal Biotecnológica Ind. Com. Ltda., Belo Horizonte, Brazil) and NEFA (Randox Brasil Ltda., São Paulo, Brazil). Concentrations of P4 and insulin were determined using a Coat-A-Count kit (DPC Diagnostic Products Inc., Los Angeles, CA) solid-phase \(^{125}\text{I} \) RIA that was previously validated for bovine samples (Moriel et al., 2008). All samples were analyzed within one assay for each hormone. The intraassay CV was 5.8% for P4 and 6.9% for insulin. The minimum detectable concentrations were 0.1 ng/mL of P4 and 0.05 \( \mu \text{IU}/\text{mL} \) of insulin.

**Statistical Analysis**

Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement used for BW and BCS change contained the effects of nutritional treatment (NB and PB), infusion treatment (GLUC and SAL), collection hour, period as an independent variable, and all 2-way and 3-way interactions among nutritional treatment, infusion treatment, and collection hour. Data were analyzed using cow(nutritional treatment) as random variable. The specified term for the repeated statement was collection hour, subject was cow(nutritional treatment × period), and the covariance structure used was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. All results are reported as least squares means, and means were separated using LSD. Significance was set at \( P \leq 0.05 \), and tendencies were declared if \( P > 0.05 \) and \( \leq 0.10 \).

**RESULTS AND DISCUSSION**

During the experiment, BCS loss was greater \( (P = 0.02; \text{Table 1}) \) in NB cows compared with PB cows \( (−0.60 \text{ vs. } −0.25, \text{ respectively}; \text{SEM} = 0.090) \). Similarly, NB cows lost BW \( (\text{Table 1}) \) during the experiment, whereas BW of PB cows was almost unchanged \( (−22.4 \text{ vs. } 1.2 \text{ kg of BW change, respectively}; \text{SEM} = 6.58) \). These outcomes are in accordance with the differences in nutrient intake and consequent nutritional status between NB and PB cows.

However, concentrations of NEFA were similar \( (P = 0.65; \text{Table 1}) \) between PB and NB cows at the beginning of fasting \( (0.22 \text{ vs. } 0.21 \text{ mmol/L, respectively}; \text{SEM} = 0.021) \), despite differences in nutrient intake, BCS, and BW change. Also, no infusion effects were detected on serum NEFA, given that GLUC cows had similar \( (P = 0.17) \) NEFA concentrations compared with SAL cows \( (0.21 \text{ vs. } 0.23 \text{ mmol/L, respectively}; \text{SEM} = 0.014) \), independently of nutritional treatment.
(P = 0.80) or the interaction (nutritional treatment × infusion treatment; P = 0.13). These results were unexpected because circulating NEFA typically becomes elevated with inadequate nutrient intake and body fat mobilization in cattle (Grummer, 1995). In addition, previous research efforts demonstrated that circulating NEFA decreases in nonlactating or lactating dairy cows receiving glucose precursors, such as propylene glycol, particularly when cows are under a negative energy balance (Christensen et al., 1997; Butler et al., 2006).

At the beginning of fasting, glucose concentrations were greater (P = 0.05; Table 1) in NB cows compared with PB cows (72.0 vs. 67.3 mg/dL, respectively; SEM = 1.60), despite the differences in nutrient intake between nutritional treatments. Circulating glucose concentrations in cattle are highly dependent on hepatic gluconeogenesis from dietary ingredients following digestion (Young, 1977), particularly propionate originating from rumen fermentation (Reynolds et al., 1994; Huntington, 1997; Reynolds, 2005). However, cattle in inadequate nutritional status may use other gluconeogenic substrates, such as endogenous amino acids and glycerol from triacylglycerol, and also enhance gluconeogenesis to compensate for nutritional deficiencies and prevent significant decreases in circulating glucose, given that glucose is essential for many maintenance and productive functions of ruminants (Huntington, 1997).

An increase in experimental design, an infusion treatment × time interaction was detected (P < 0.01) for serum glucose independently of nutritional treatment (P = 0.77) or the resultant interaction (P = 0.18) because GLUC cows had greater glucose concentrations from 0.5 to 3 h relative to the beginning of infusion time compared with SAL cows (Figure 1).

At the beginning of fasting, serum insulin concentrations were greater (P = 0.04; Table 1) in PB cows compared with NB cows (27.12 vs. 17.86 μIU/mL, respectively; SEM = 2.865). These outcomes agree with differences in nutrient intake between PB and NB cows, given that circulating insulin concentrations have been positively associated with feed intake in cattle (Vizcarra et al., 1998; Bossis et al., 2000; Lapierre et al., 2000). Conversely, insulin is synthesized and secreted mainly in response to blood glucose concentrations (Nussey and Whitehead, 2001), which were greater in NB cows compared with PB cows. However, insulin is also secreted in response to other stimuli, such as gastrointestinal hormones and neural/paracrine mechanisms associated with feed intake (Nussey and Whitehead, 2001).

A nutritional treatment × infusion treatment × time interaction was detected (P < 0.01) for serum insulin concentrations because insulin responses to glucose infusion were reliant on nutritional status (Figure 2). Following infusion, PB cows assigned to GLUC had greater insulin concentrations compared with SAL cohorts at 0.5 and 3 h, indicating a biphasic increase in circulating insulin. On the other hand, NB cows assigned to GLUC had consistently greater insulin concentrations compared with SAL cohorts at 0.5, 1, 2, and 3 h following infusion, whereas a biphasic response was not detected. Hove (1978) also reported biphasic and single-phase insulin increases following glucose infusion in lactating dairy cows with adequate or inadequate feed intake, respectively, and suggested that

<table>
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<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>P-value</th>
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<tbody>
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<td>BCS change (1 to 5 scale)</td>
<td>NB -0.60</td>
<td>0.090</td>
<td>0.02</td>
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<td></td>
<td>PB -0.25</td>
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<tr>
<td>BW change, kg</td>
<td>NB -22.4</td>
<td>6.58</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>PB 1.2</td>
<td></td>
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<tr>
<td>NEFA, mmol/L</td>
<td>NB 0.21</td>
<td>0.021</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>PB 0.22</td>
<td></td>
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<tr>
<td>Glucose, mg/dL</td>
<td>NB 72.0</td>
<td>1.60</td>
<td>0.05</td>
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<tr>
<td></td>
<td>PB 67.3</td>
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<tr>
<td>Insulin, μIU/mL</td>
<td>NB 17.86</td>
<td>2.865</td>
<td>0.04</td>
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<tr>
<td></td>
<td>PB 27.1</td>
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<tr>
<td>Progesterone, ng/mL</td>
<td>NB 1.17</td>
<td>0.082</td>
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<td>PB 0.93</td>
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1Changes in BCS and BW were calculated from measures obtained on d −28 and d 0. Serum samples were collected at the beginning of the fasting period on d 1.

2NB cows were maintained in a Brachiaria brizantha pasture with low forage quality and availability; PB cows were maintained in a B. brizantha pasture with low forage quality and availability, but received daily 2 kg/cow of a supplemental concentrate from d −28 to d −14, and 4 kg/cow of the same concentrate from d −13 to d 1.

3According to Wildman et al. (1982).
these differences can be attributed to altered reserves of proinsulin in pancreatic β-cells due to nutritional status (Nelson and Cox, 2005). However, the results presented herein and by Hove (1978) are not sufficient to elucidate this mechanism in cattle; therefore, further research is required to address this subject.

At the beginning of fasting, serum P4 concentrations were greater \( (P = 0.05; \text{Table 1}) \) in NB cows compared with PB cows (1.17 vs. 0.93 ng/mL, respectively; SEM = 0.082). The cows used in the present study were ovariectomized; therefore, the main source for circulating P4 was the CIDR. These differences in P4 concentrations between NB and PB cows at the beginning of fasting can be attributed to many factors. First, PB cows had increased feed intake compared with NB cows; greater feed intake leads to increased hepatic blood flow and consequent hepatic P4 catabolism (Sangsrivatavong et al., 2002). Second, the adrenal gland is also capable of synthesizing significant amounts of P4 as an intermediate of corticoid synthesis when stimulated by stressors (O’Connor et al., 2000), and feed restriction can increase adrenal synthesis of cortisol (Murayama et al., 1986; Ward et al., 1992; Henricks et al., 1994) and perhaps adrenal P4 production (Cooke and Arthington, 2009). Third, P4 is stored into adipose tissues and can be released into the circulation when body fat is mobilized (Hamudikuwanda et al., 1996). Therefore, one can also speculate that P4 originating from the CIDR or adrenal glands was stored in fat tissues of NB cows, and because of significant BW and BCS loss (Table 1), it was released into the bloodstream and contributed to the increased serum P4 concentrations detected in NB cows compared with PB cows. Nevertheless, NEFA concentrations were similar between NB and PB cows at the beginning of fasting, and NEFA typically mirror the extent of body fat mobilization and correlate with P4 release from adipose tissues in cattle (Grummer, 1995; Hamudikuwanda et al., 1996).

A nutritional treatment × infusion treatment × time interaction was detected \( (P < 0.01; \text{Figure 3}) \) for serum P4 concentrations mainly because an infusion effect × time interaction was detected for PB cows \( (P < 0.01) \) but not for NB cows \( (P = 0.54) \). Further, PB cows assigned to receive GLUC had greater \( (P < 0.01) \) mean P4 concentrations from −0.5 h to 6 h relative to infusion compared with PB cows assigned to receive SAL (1.19 vs. 0.83 ng/mL, respectively; SEM = 0.119). These results, supporting our hypothesis, indicate that serum P4 concentrations were only altered by i.v. glucose infusion and subsequent increase in insulin concentrations within cows with adequate nutritional status. This increase can be attributed mainly to reduced hepatic catabolism of P4 in PB cows receiving GLUC compared with SAL cohorts, given that all cows were

Figure 1. Serum concentrations of glucose (mg/dL) in nonlactating cows infused intravenously with a 5% glucose solution (GLUC; 0.5 g of glucose/kg of BW, infused on average at 32 mL/min over a 3-h period) or with physiological saline (SAL; 0.9% solution infused on average at 32 mL/min over a 3-h period). Treatments were infused immediately following blood sampling at 0 h. An infusion treatment × time interaction was detected \( (P < 0.01) \). Treatment comparison within time: ** \( P < 0.01 \).
fasted to eliminate effects of feed intake on circulating P4 concentrations (Vasconcelos et al., 2003) and were ovariectomized and inserted with a CIDR at the same time and thus had equivalent sources of exogenous P4. Supporting the current findings, previous efforts from our research group indicated that ovariectomized cows in moderate to positive energy balance, inserted with CIDR, and with elevated insulin concentrations had greater mean P4 concentrations compared with cohorts with reduced insulin concentrations (Moriel et al., 2008; Lopes et al., 2009).

Other authors reported that lactating dairy cows drenched daily with propylene glycol (500 mL/cow daily), from d −10 to d 25 relative to parturition, had increased plasma concentrations of insulin during the study and reduced expression of hepatic P450 3A on d 25 compared with cohorts drenched with water (Butler et al., 2006; Lemley et al., 2008). Moreover, lactating dairy cows receiving continuous i.v. insulin infusion (1 μg/kg of BW per h) from d 10 to d 14 postpartum had increased plasma concentrations of insulin during the infusion period and reduced expression of hepatic P450 2A and P450 3A on d 14 compared with cohorts infused with saline (Butler et al., 2003; Lemley et al., 2008). Compared with the results reported herein, these cited studies (Butler et al., 2003, 2006; Lemley et al., 2008) reported inhibitory effects of insulin on hepatic expression of P4 catabolic enzymes in dairy cows under negative nutritional balance. However, these authors did not evaluate plasma P4 concentrations to directly assess the relationships among elevated plasma insulin, reduced P450 2A and P450 3A expression, and circulating P4. Further, in the present study, cows received glucose infusions only once during the experiment and the increase in insulin was acute but temporary (Figure 2), whereas in the cited studies insulin infusions were administered continuously during a 4-d period (Butler et al., 2003; Lemley et al., 2008) and daily propylene glycol drenches were administered for 35 d (Butler et al., 2006; Lemley et al., 2008) before collection of liver samples. Therefore, it can be theorized that dairy cows in negative nutritional balance require sustained increases in insulin concentrations to inhibit expression of hepatic P450 2A and P450 3A, and thus increase circulating P4 concentrations.

In conclusion, the effects of i.v. glucose infusion on circulating concentrations of insulin and P4 in nonlactating, ovariectomized dairy cows receiving exogenous P4 were dependent on cow nutritional balance. Cows assigned to adequate nutrient intake for 28 d before infusion and administered 0.5 g/kg of BW of glucose had a biphasic increase in serum insulin concentrations and increased serum P4 concentrations compared with cohorts infused with saline. On the other hand, cows

Figure 2. Serum concentrations of insulin (μIU/mL) in nonlactating cows in negative (NB) or positive (PB) nutrient balance, and infused intravenously with a 5% glucose solution (GLUC; 0.5 g of glucose/kg of BW, infused on average at 32 mL/min over a 3-h period) or physiological saline (SAL; 0.9% solution infused on average at 32 mL/min over a 3-h period). Treatments were infused immediately following blood sampling at 0 h. A nutritional treatment × infusion treatment × time interaction was detected (P < 0.01). Hours with letter designation indicates the following treatment differences (P < 0.05): a = GLUC-PB vs. SAL-PB; b = GLUC-NB vs. SAL-NB.
assigned to inadequate nutrient intake for 28 d before infusion and administered the same dose of glucose had a single-phase increase in serum insulin concentrations, but serum P4 concentrations were similar compared with cohorts infused with saline. Therefore, acute but temporary increases in circulating insulin only modulated P4 concentrations in cows with adequate nutritional status, whereas cows in negative nutritional balance, such as periparturient dairy cows, may require sustained increases in insulin concentrations to experience similar effects. These physiological mechanisms are important and require further investigation because circulating insulin and P4 are highly associated with reproductive function and success in dairy cattle. An example would be infusing PB and NB cows with exogenous glucose, with or without the addition of exogenous insulin, for longer periods of time. However, caution must be applied when extrapolating the results reported herein to lactating dairy cows. The physiological and metabolic aspects associated with parturition, resumption of reproductive function, and milk synthesis were not accounted for in the present experimental model given that nonlactating ovariectomized cows were used. Also, Gir × Holstein cows might not fully represent the physiological aspects of a high-producing dairy cow, given that milk yield capacity is considered moderate to low in Gir × Holstein cows (Madalena et al., 1979).

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