



## 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  $-0.25 \pm 0.090$  for BCS, respectively; –22.4 and  $1.2 \pm 6.58$  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 (P<sub>4</sub>) and insulin (Sangsritavong et al., 2002; Butler, 2005).

Progesterone is a hormone required for adequate attainment of puberty, resumption of estrous cycles, and also 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 P<sub>4</sub> 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 devel-

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opment (Diskin et al., 2003; Webb et al., 2004), and ovarian steroid synthesis (Spicer and Echterkamp, 1995; Gutiérrez et al., 1997; Wettemann and Bossis, 2000). Accordingly, Gong et al. (2002) reported that dairy cows with elevated circulating insulin concentrations postcalving had shorter postpartum interval to ovulation compared with cohorts with reduced insulin concentrations.

In addition, insulin may affect reproduction in cattle by modulating hepatic expression of enzymes associated with catabolism of P4, such as cytochrome P450 2C and P450 3A (Murray, 1991; Lemley et al., 2008). Previous efforts from our research group established that dairy and beef cows with elevated insulin concentrations had greater mean P4 concentrations compared with cows with reduced insulin concentrations (Moriel et al., 2008; Lopes et al., 2009). However, these studies evaluated cows in moderate to positive energy balance. Insulin metabolism is highly dependent on nutritional status, and its synthesis, release, and metabolic effects differ in cattle in negative nutritional balance, such as periparturient dairy cows, compared with cattle in positive nutritional balance (Hove, 1978; Veerkamp et al., 2003).

To further test these mechanisms, we hypothesized that i.v. glucose infusion increases circulating concentrations of insulin and reduces hepatic clearance of P4 in dairy cows, but that these effects are dependent on cow nutritional status. Our objectives were to evaluate serum concentrations of NEFA, glucose, insulin, and P4 in nonlactating dairy cows in negative or positive nutritional balance, and administered or not with i.v. glucose infusion.

## MATERIALS AND METHODS

This experiment was conducted at the São Paulo State University, Lageado Experimental Station, located in Botucatu, São Paulo, Brazil. The animals utilized were cared for in accordance with the practices outlined in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999).

### 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 supplemental 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 basis) 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 Vendramini 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 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 procedures described by Curley et al. (2008). Treatments

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