Concentrations of Progesterone and Insulin in Serum of Nonlactating Dairy Cows in Response to Carbohydrate Source and Processing

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ABSTRACT

Two experiments were conducted to investigate the effects of carbohydrate source and processing on serum progesterone (P4) and insulin concentrations of nonlactating dairy cows. In experiment 1, 12 ovariectomized grazing Gir × Holstein cows were stratified by body weight and body condition score, and randomly assigned to receive a supplement containing either finely ground corn or citrus pulp in a Latin square crossover design. Diets were fed individually, twice daily at a rate of 10.9 kg of dry matter per cow. Cows received a controlled intravaginal P4-releasing insert before the beginning of the study, and inserts were replaced every 7 d. During the first experimental period, cows were adapted to treatments from d 0 to 13 and blood was collected on d 14, whereas during the second experimental period cows were adapted to treatments from d 0 to 6 and blood samples were collected on d 7. In both periods, blood samples were collected immediately before and at 1, 2, 3, 4, 5, and 6 h after the first supplement feeding of the collection day. In experiment 2, the cows utilized in experiment 1 were randomly assigned to receive a supplement based on finely ground corn, coarsely ground corn, or high-moisture corn in a Latin square crossover design. Cows were fed and received the controlled intravaginal P4-releasing insert as in experiment 1. Within each of the 3 experimental periods, cows were adapted to diets from d 0 to 6, and blood samples were collected on d 7 as in experiment 1. Time effects were detected in experiments 1 and 2 because insulin concentrations increased by 1 h (4.6 ± 0.90 vs. 7.4 ± 0.91 μ IU/mL for 0 and 1 h, respectively) and P4 concentrations decreased by 3 h (1.8 ± 0.12 vs. 1.2 ± 0.11 ng/mL for 0 and 3 h, respectively) after supplements were offered. In experiment 2, insulin concentrations were greater in cows fed high-moisture corn compared with those fed coarsely or finely ground corn (8.8 ± 1.05 , 5.7 ± 1.05 , and 6.1 ± 1.05 μ IU/mL, respectively).

Data combined from both experiments indicated that cows with median insulin ≥ 4.5 μ IU/mL before supplement feeding had greater P4 concentrations at 1 h, but lesser P4 concentrations at 5 h compared with cows with insulin < 4.5 μ IU/mL. Carbohydrate processing, but not carbohydrate source, affected serum insulin of nonlactating dairy cows.

Key words: carbohydrate, dairy cow, insulin, progesterone

INTRODUCTION

Corn and citrus pulp are important feedstuffs used as energy sources in dairy cattle diets. These feeds differ substantially in their composition, particularly their carbohydrate fractions (NRC, 2001). Pectin is the main carbohydrate of citrus pulp (Arthington et al., 2002), whereas starch predominates in corn (Rooney and Pflugfelder, 1986). Feeding diets rich in citrus pulp or pectin increased the proportion of acetic acid in the rumen fluid, whereas corn- or starch-based diets result in increased propionate concentration in the rumen of cattle (Hentges et al., 1966). In addition, ruminal propionate synthesis can be enhanced by corn processing methods that further expose the starch granules to ruminal microbes and digestive enzymes (Huntington, 1997), such as grinding, steam-flaking, and high-moisture processing. Propionate and acetate synthesized in the rumen are removed by the liver through the portal blood. The majority of propionate is taken up by hepatic cells and serves as a major substrate for gluconeogenesis. In contrast, acetate is mainly oxidized throughout body tissues to generate energy or to synthesize fat (Bergman, 1990). It is hypothesized that these differences in the metabolic fate of propionate and acetate could influence the circulating concentrations of insulin and progesterone (P4), as the synthesis and release of insulin by the pancreas is stimulated by the concentrations of blood glucose (Nussey and Whitehead, 2001).

An increased rate of blood flow to the liver increased the metabolism of P4 by hepatic cells (Sangsritavong et al., 2002), resulting in decreased concentrations of P4 after feeding (Sangsritavong et al., 2002; Vasconcelos et

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al., 2003). This is of physiological significance because lesser concentrations of P4 can result in increased frequency of both LH pulses and estradiol-17 β secretion (Fortune and Vincent, 1983; Kinder et al., 1996), extend the dominance of follicles (Smith and Stevenson, 1995), and potentially decrease conception rates and embryonic survival in cattle (McNeill et al., 2006). Insulin has a central role in influencing follicular dynamics, including acting directly on steroidogenesis (Butler et al., 2004). Cows ovulated earlier in response to dietary-induced elevated concentrations of insulin (Gong et al., 2002). Conversely, elevated concentrations of insulin had a negative impact on both the quality and development of oocytes and embryos (Fouladi-Nashta et al., 2005). Insulin altered the hepatic clearance of P4 by modulating the expression of the catabolic enzymes P450 2C and P450 3A (Murray, 1991; Lemley et al., 2008). Thus, it is plausible to postulate that feeding diets based on different carbohydrate sources and processing levels may have significant effects on reproductive functions of dairy cows by altering circulating concentrations of both P4 and insulin.

Hence, the objective was to investigate the effect of feeding different carbohydrate sources or sources subjected to different processing methods on circulating P4 and insulin concentrations in nonlactating dairy cows. The aim of experiment 1 was to evaluate the effect of adding supplements based on finely ground corn (FC) or citrus pulp (CT) on serum insulin and P4 concentrations. The objective of experiment 2 was to determine the impact of feeding FC, coarsely ground (GC), or high-moisture (HM) corn on serum insulin and P4 concentrations.

MATERIALS AND METHODS

Both experiments were 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

Experiment 1. Twelve nonlactating, nonpregnant, and ovariectomized Gir $\times$ Holstein cows (BW = 423 $\pm$ 53 kg; BCS = 2.5 $\pm$ 0.18) were stratified by BW and BCS (Wildman et al., 1982), and blocked by these variables into 2 squares of 6 cows each. Squares were randomly assigned to receive a supplement based on FC or CT in a Latin square 2 $\times$ 2 crossover design. Dietary treatments were formulated to be isocaloric and

isonitrogenous. The first period of feeding lasted 14 d (d 0 to 14), whereas the second period lasted 7 d (d 22 to 28). Before the beginning of the experiment (d –13 to 0) and between periods (d 15 to 21), cows received up to 8.4 kg/d of a transition supplement consisting of 37.5% of CT, 31.9% of soybean meal, 28.8% of FC, 0.7% of mineral mix, 0.7% of calcium carbonate, and 0.4% of urea on DM basis. Each cow received an intra-vaginal P4-releasing insert (CIDR) containing 1.9 g of P4 (Pfizer Animal Health, São Paulo, Brazil) at 1800 h on d –7, 7, and 21 of the experimental period so that at the last day of each experimental period (d 14 and 28) the CIDR in all cows had been in place for 7 d.

Experiment 2. The same cows utilized in experiment 1 were stratified by BW and BCS (Wildman et al., 1982), and blocked by these variables into 3 squares of 4 cows each. Squares were randomly assigned to receive FC (as in experiment 1), or supplements containing GC or HM in a Latin square 3 $\times$ 3 crossover design containing 3 periods of 7 d each. Dietary treatments were formulated to be isonitrogenous and to have the same amount of corn (DM basis). Cows received up to 8.4 kg/d of the same transition supplement offered in experiment 1 before the beginning of this experiment (d –7 to 0). Treatments were switched at the end of each period, on d 7 and 14. Each cow received a CIDR at 1800 h on d 0, 7, and 14 of the experimental period so that at the last day of each experimental period (d 7, 14, and 21), the CIDR in all cows had been in place for 7 d. Periods of 7 d (6 d for adaptation and 1 d for blood collection) were chosen for this experiment because all supplement treatments were based on corn, although corn processing methods differed.

Diets

Cows were maintained in *Brachiaria brizantha* pastures (50% total digestible nutrients, 4.4% CP, 39% ADF, 70% NDF; DM basis) during both experiments, and received supplements twice daily at 0600 and 1800 h, at a daily rate of 10.9 kg of DM/cow. Composition and nutritional profile of supplements are described in Table 1. The pastures utilized in this experiment were not fertilized before or during the experimental periods. 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 were offered for ad libitum consumption throughout the experiment.

Blood Sampling and Analysis

Blood samples were obtained on the last day of each period (d 14 and 28 for experiment 1 and d 7, 14, and

Table 1. Ingredient composition, nutrient profile, and intake rate of supplements fed to cows during experiment 1 and 2

Item	Treatment ^{1,2}			
	CT	FC	GC	HM
Ingredients, % of DM				
Citrus pulp	52.8	22.3	22.3	22.3
Coarsely ground corn	—	—	43.5	—
Finely ground corn	15.5	43.5	—	—
High-moisture corn	—	—	—	43.5
Soybean meal	31.3	33.0	33.0	33.0
Urea	0.4	0.4	0.4	0.4
Limestone	—	0.8	0.8	0.8
Nutrient profile, ³ % of DM				
DM	89.4	88.8	88.8	80.0
Total digestible nutrients	73.6	73.5	73.5	74.5
CP	17.0	17.2	17.1	17.1
Starch	14.5	28.8	28.8	27.2
Ca	0.5	0.5	0.5	0.5
P	0.3	0.3	0.3	0.3
Daily supplement intake, kg/cow				
DM	10.9	10.9	10.9	10.9
Total digestible nutrients	8.0	8.0	8.0	8.1
CP	1.8	1.9	1.9	1.9
Starch	1.6	3.1	3.1	3.0
Ca	0.06	0.06	0.06	0.06
P	0.03	0.03	0.03	0.03

¹CT = supplement based on citrus pulp; FC = supplement based on finely ground corn; GC = supplement based on coarsely ground corn; HM = supplement based on high-moisture corn.

²During experiment 1, cows were offered CT and FC according to treatment allocation. During experiment 2, cows were offered FC, GC, and HM according to treatment allocation.

³Estimated using NRC (2001).

21 for experiment 2), immediately before and at 1, 2, 3, 4, 5, and 6 h after the morning supplement feeding, for determination of P4 and insulin concentrations. Blood was collected from either the coccygeal vein or artery 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 .

Concentrations of P4 and insulin were determined using Coat-A-Count kits (DPC Diagnostic Products Inc., Los Angeles, CA) solid-phase ^{125}I RIA. All samples were analyzed within 1 assay for each hormone. The intraassay CV was 7.8% for insulin and 1.7% for P4.

Statistical Analysis

For both experiments, data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). The model statement contained the effects of treatment, square, time, and the resultant interactions. Data were analyzed using cow(square) and period as random variables. Insulin and P4 values obtained during both experiment were tested for normality with the Shapiro-Wilk test from the UNIVARIATE procedure of SAS, and results indicated that all data were dis-

tributed normally ($W \geq 0.90$). For comparison of P4 concentrations in cows with insulin above or below the median before feeding, data from both experiments were analyzed with the UNIVARIATE procedure of SAS for median determination and the MIXED procedure of SAS to determine insulin effects on P4 concentrations. The model statement for this analysis contained the effects of insulin (above or below median), time, and the interaction, whereas cow(period by experiment), period(experiment), and experiment were the random variables. Satterthwaite approximation was used to determine the denominator degrees of freedom for all tests of fixed effects. Results are reported as least squares means. Means were separated using LSD in experiment 1 and PDIFF in experiment 2. Significance was set at $P \leq 0.05$, and tendencies were declared if $P > 0.05$ and ≤ 0.10 .

RESULTS AND DISCUSSION

Experiment 1

No treatment effects were detected on P4 (1.10 vs. 1.07 ng/mL for FC and CT, respectively; SEM = 0.20) and insulin concentrations (5.5 vs. 5.6 $\mu\text{IU/mL}$ for FC and CT, respectively; SEM = 1.8). A time effect was de-

Figure 1. Serum concentrations of progesterone (ng/mL) and insulin (μ IU/mL), pooled across treatments, of nonlactating cows offered either finely ground corn or citrus pulp based supplements in experiment 1 ($n = 12$ experimental units/treatment). Data were pooled because no treatment effects were detected for both hormones ($P > 0.05$). Supplements were offered after blood was sampled at 0 h. A time effect was detected ($P < 0.01$). Time comparisons within hormones; lowercase letters correspond to progesterone (SEM = 0.15), and uppercase letters correspond to insulin (SEM = 1.9). Hours not bearing a common letter differ ($P < 0.05$).

tected ($P < 0.01$) for concentrations of both hormones. Concentration of insulin peaked 1 to 2 h after feeding, whereas those of P4 decreased and reached minimum values between 3 and 4 h after feeding (Figure 1). The time effects detected for P4 concentrations in both treatments were similar to previous reports (Sangsri-tavong et al., 2002; Vasconcelos et al., 2003; Cooke et al., 2007). The hypothesis tested by this experiment was that cows offered supplements based on corn would experience reduced concentrations of P4 and hastened increases in insulin after feeding compared with cows consuming CT-based supplements. The rationale was that feeding corn would increase blood flow to the liver and enhance glucose synthesis resulting from greater ruminal propionate production. Nevertheless, no treatment effects were detected to support our assumptions. Lack of treatment effects for P4 and insulin can be explained, at least in part, by similar hepatic blood flow between treatments (Reynolds and Huntington, 1988; Royes et al., 2001), similar acetate:propionate ratio, or to treatment differences in forage intake, although this response was not evaluated in the present experiment.

Experiment 2

The hypothesis tested in this experiment was that cows offered supplements based on HM would experience reduced concentrations of P4, but increased concentrations of insulin after feeding compared with cows consuming FC or GC because of increased rumen starch

Figure 2. Serum concentrations of progesterone (ng/mL) and insulin (μ IU/mL), pooled across treatments, of nonlactating cows offered supplements based on finely ground corn, coarsely ground corn, or high-moisture corn in experiment 2 ($n = 12$ experimental units/treatment). Supplements were offered after blood was sampled at 0 h. A time effect was detected ($P < 0.01$). Time comparisons within hormones; lowercase letters correspond to progesterone (SEM = 0.30), and uppercase letters correspond to insulin (SEM = 1.2). Hours not bearing a common letter differ ($P < 0.05$).

digestibility of HM (Huntington, 1997), which would hasten ruminal propionate production and consequently increase hepatic blood flow and glucose synthesis. One could argue that decreased ruminal degradability of FC and GC compared with HM could result in greater flow of starch to the small intestine of FC and GC cows, and consequently increase absorption and circulating concentrations of glucose in those animals. Still, our assumptions were supported by the results, and cows fed HM had greater ($P < 0.01$) insulin concentrations than those fed FC and GC (8.83, 6.15, and 5.72 μ IU/mL, respectively; SEM = 1.05). A time effect was detected ($P < 0.01$; SEM = 1.2) because insulin concentrations peaked for all treatments 2 h after feeding (Figure 2).

Regarding treatment effects on P4 concentrations, our assumptions were marginally supported by the results. Cows fed HM tended ($P = 0.09$) to have decreased mean P4 concentrations compared with GC cows (1.72 vs. 1.93 ng/mL, respectively; SEM = 0.20), but mean P4 concentration did not differ from that of FC cows (1.72 vs. 1.85 ng/mL, respectively; SEM = 0.20). As in experiment 1, a time effect was detected ($P < 0.01$) because P4 concentrations for all treatments decreased and reached minimum values 3 to 4 h after feeding (Figure 2). Although processing of corn tended to alter concentrations of P4 in serum when HM and GC were compared, additional treatment effects were not observed. It is possible that further treatment differences in P4 were not detected because of the greater insulin concentrations in the HM cows, which could

Figure 3. Serum progesterone concentrations (ng/mL) in nonlactating cows allotted to groups in which serum insulin concentrations (μ IU/mL) were either greater ($n = 31$) or less ($n = 28$) than the median concentration (4.5μ IU/mL) before supplement feeding (0 h). A group by time interaction was detected ($P < 0.05$; SEM = 0.23). Cows with insulin $\geq 4.5 \mu$ IU/mL had greater concentrations of progesterone with 1 h and a delayed, but more severe, decrease in progesterone concentrations with 5 h compared with cows with insulin concentrations $< 4.5 \mu$ IU/mL. Group comparison within time; * $P < 0.05$.

have attenuated, at least to some degree, the hepatic rate of P4 clearance.

When results from both experiments were combined and analyzed independently of treatments, cows having insulin equal or greater than the median concentration (4.5μ IU/mL) immediately before feeding had greater ($P < 0.05$) P4 concentrations at 1 h, but decreased P4 concentrations at 5 h after feeding, compared with cows with insulin $< 4.5 \mu$ IU/mL (Figure 3). These data indicate that cows having initially greater insulin concentrations experienced a delayed, but more severe, decrease in P4 concentrations because of hepatic clear-

Figure 4. Serum concentrations of progesterone (ng/mL) and insulin (μ IU/mL), pooled across treatments and experiment 1 and 2, of nonlactating cows offered supplements based on citrus pulp, finely ground corn, coarsely ground corn, or high-moisture corn. A total of 60 periods of 6 h were evaluated (12 cows; 5 periods/cow). Supplements were offered after blood was sampled at 0 h. A time effect was detected for both hormones ($P < 0.01$). Time comparisons within hormones; lowercase letters correspond to progesterone (SEM = 0.15), and uppercase letters correspond to insulin (SEM = 1.9). Hours not bearing a common letter differ ($P < 0.05$).

ance in response to feed intake compared with cows having initially lesser insulin concentrations. The delayed P4 decrease could be attributed to the inhibitory effects of greater insulin concentrations on hepatic P4 metabolism (Smith et al., 2006; Lemley et al., 2008). On the other hand, the greater reduction in P4 concentrations detected in these cows, 5 h after feeding, can be attributed to increased rumen fermentation, propionate production, and subsequent glucose synthesis which likely cause the initial elevated concentrations of insulin concentrations but also enhanced hepatic blood flow after feeding. The increased metabolism of P4 likely overrides a potential inhibitory effect of insulin on P4 catabolism (Figure 4), suggesting that P4 concentration is a negative mirror of VFA production, although further research is required to address this issue.

CONCLUSIONS

Nonlactating cows consuming supplements based on finely ground corn or citrus pulp had similar concentrations of P4 and insulin for 6 h postfeeding. On the other hand, nonlactating cows consuming supplements based on high-moisture corn had increased insulin concentrations compared with cows offered supplements containing finely or coarsely ground corn. In addition, results from this study suggest that greater insulin concentrations may delay hepatic clearance of P4 in response to feed intake. The important results of the increased concentrations of insulin and the decreased concentrations of P4 after feeding may provide evidence that peripheral concentrations of insulin can affect the clearance of P4, although further research is required to address this matter.

High-genetic-merit cows typically have elevated feed intake and increased blood flow to the liver (Sangsritavong et al., 2002; Vasconcelos et al., 2003), but reduced concentrations of insulin (Gutierrez et al., 2006), resulting in an increased clearance of P4. These factors influence negatively circulating P4 concentrations in lactating dairy cows (Sangsritavong et al., 2002; Smith et al., 2006). Therefore, a better understating of how nutritional management can affect insulin synthesis and hence the rate of hepatic P4 metabolism would be an important tool to improve the reproductive efficiency of dairy herds.

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