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Physiologic Responses to Feeding Rumen-Protected Glucose to Lactating Dairy Cows

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Abstract

Lactating Holstein cows were enrolled in a study beginning before first insemination. Cows were supplemented with a rumen-protected glucose (RPG) product to test the hypothesis that circulating progesterone concentrations could be increased by increasing blood glucose, which causes an increase in insulin, subsequently decreasing progesterone clearance by liver enzymes. Supplementation occurred at 0, 2.2, 4.4, or 8.8 lb per head per day to test a dose response. Treatment began 3 days before ovulation and continued until day 12 of the estrous cycle. Rumen-protected glucose did not impact serum concentration of glucose before or after feeding, but the change in insulin concentration (post-feeding – pre-feeding) was greater for the control cows compared with cows that received the three doses of RPG. Crude protein (CP) intake and milk urea nitrogen (MUN) increased linearly with treatment, but dry matter intake (DMI) and milk yield were unaffected by treatment. Concentrations of progesterone were unaffected by treatment, and pregnancy risk at first insemination was reduced by treatment. Rumen-protected glucose failed to increase serum insulin or progesterone concentrations.

Keywords

glucose, insulin, progesterone

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Summary

Lactating Holstein cows were enrolled in a study beginning before first insemination. Cows were supplemented with a rumen-protected glucose (RPG) product to test the hypothesis that circulating progesterone concentrations could be increased by increasing blood glucose, which causes an increase in insulin, subsequently decreasing progesterone clearance by liver enzymes. Supplementation occurred at 0, 2.2, 4.4, or 8.8 lb per head per day to test a dose response. Treatment began 3 days before ovulation and continued until day 12 of the estrous cycle. Rumen-protected glucose did not impact serum concentration of glucose before or after feeding, but the change in insulin concentration (post-feeding – pre-feeding) was greater for the control cows compared with cows that received the three doses of RPG. Crude protein (CP) intake and milk urea nitrogen (MUN) increased linearly with treatment, but dry matter intake (DMI) and milk yield were unaffected by treatment. Concentrations of progesterone were unaffected by treatment, and pregnancy risk at first insemination was reduced by treatment. Rumen-protected glucose failed to increase serum insulin or progesterone concentrations.

Introduction

Progesterone is essential for maintenance of pregnancy and inhibits estrus expression in dairy cows. Peripheral concentrations of progesterone are affected by both milk yield and the rate of metabolism in the liver. Selection for increased milk yield during several decades has resulted in greater milk yield and the necessity to feed cows less roughage and higher-energy diets to support milk synthesis. High-energy diets, in turn, chronically increase liver blood flow, resulting in increased clearance of progesterone. Clearance of progesterone reduces peripheral concentrations of progesterone available to reproductive tissues, and the rate at which this occurs is a function of liver blood flow and activity of liver enzymes, particularly cytochrome P450 2C and cytochrome P450 3A. Therefore, decreasing liver enzymatic activity may increase peripheral concentrations of progesterone.

Previous research has shown that feeding a high-starch diet causes an increase in insulin production, resulting in decreased activity of liver cytochrome P450 enzymes. Insulin, a metabolic mediator between nutrition and reproduction, is secreted in response

to increased concentrations of circulating glucose and functions to maintain stable concentrations of blood glucose. Previous research also indicated that insulin can effectively decrease progesterone clearance in vivo. Glucose is a key nutrient required during lactation for milk synthesis and maintenance of other body tissues, including those involved in various reproductive processes. Because most dietary carbohydrate is converted into volatile fatty acids in the rumen, a cow must synthesize glucose by gluconeogenesis in liver. The extensive demand for glucose by the mammary gland to synthesize milk may decrease the amount of glucose readily available to other body tissues, including those tissues involved in reproductive processes.

Rumen-protected glucose should facilitate more glucose being delivered to the small intestine for absorption as opposed to relying solely on its synthesis in the liver. Because circulating glucose induces secretion of insulin, a resulting increase in insulin may decrease the activity of liver enzymes involved in clearance of progesterone, and thus increase peripheral concentrations of progesterone. Increased concentrations of progesterone at estrus or during the 7 to 10 days preceding a timed insemination are associated with improved pregnancy risk in lactating dairy cows.

Therefore, the objective of the current study was to determine the effect of supplementing an RPG product on blood serum concentrations of glucose, insulin, and progesterone. We hypothesized that supplementing RPG would increase concentrations of glucose and insulin, resulting in decreased activity of liver enzymes (cytochromes P450 2C and P450 3A), thus increasing circulating concentrations of progesterone.

Materials and Methods

Sixty-one Holstein cows were enrolled in a study before first insemination at Kansas State University. Cows calved in a maternity barn on a straw-bedded pack and were subsequently housed in a sand-bedded free-stall facility. Cows were then housed in a tie-stall barn from 58 ± 3 to 72 ± 3 DIM and fed individually during the experiment. Treatments included a daily supplement of 0 (control, n = 13), 2.2 (n = 15), 4.4 (n = 15), or 8.8 lb/day (n = 16) of an RPG product (Grain States Soya, West Point, NE). Ground corn was top-dressed with the treatment supplement, so each cow received a total supplement (RPG ± ground corn) of 8.8 lb per day.

Cows were subjected to an ovulation-synchronization program to synchronize ovulation (day 0; Figure 1). Briefly, cows received injections of $PGF_{2\alpha}$ at 48 ± 3 DIM, an injection of GnRH at 51 ± 3 DIM, and $PGF_{2\alpha}$ at 58 and 59 ± 3 DIM. An injection of GnRH was administered 56 hours after $PGF_{2\alpha}$ (d -0.5) to induce ovulation of the dominant follicle. The products used were 100 μg GnRH (Factrel) and 25 mg $PGF_{2\alpha}$ (dinoprost tromethamine) from Zoetis Inc. (Kalamazoo, MI). Cows were eligible to continue in the experiment if ovulation was detected by the appearance of a new CL by day 2 and elevated progesterone (≥ 1 ng/mL) by day 4. Cows were reintroduced in the herd at 72 ± 3 DIM (day 12) and estrous cycles were resynchronized in cows for insemination (GnRH on 72 ± 3, $PGF_{2\alpha}$ on 79 ± 3 and 80 ± 3, GnRH on 81 ± 3, and timed AI on 82 ± 3 DIM).

Feed intake was recorded daily. Cows were milked thrice daily. Milk samples collected once 3 days before initiation of treatment and again on day 11 of treatment were analyzed for concentrations of fat, true protein, lactose, and somatic cells. Energy-corrected milk was calculated according to Dairy Records Management Systems as (0.327 × milk yield) + (12.95 × fat yield) + (7.65 × protein yield). Body condition score and body weight were recorded at enrollment (58 ± 3 DIM).

Blood samples were collected via coccygeal puncture on day 0, 2, and 4 to analyze concentrations of glucose and insulin. Samples were collected 1 hour before and 8 hours after the morning feeding to determine pre-feeding and post-feeding concentrations of glucose and insulin. Progesterone was measured in blood serum samples collected on day 2 and daily from day 4 through 12.

Results and Discussion

Feed Intake, Milk Production, and Milk Composition

Dry matter intake, milk yield, and milk composition are summarized in Table 2. As anticipated, starch intake decreased ($P < 0.01$) linearly with increasing dose of RPG. In contrast, intake of CP and ethanol-soluble carbohydrates (sugars) increased ($P < 0.01$) linearly with increasing dose of RPG. Neither milk nor energy-corrected milk yield were impacted by RPG dose. Dose of RPG had no effect on yields of milk fat, lactose, or SCC. In contrast, milk protein concentration tended ($P = 0.10$) to differ among treatment doses. Milk urea nitrogen concentration increased linearly ($P < 0.01$) with increasing dose of RPG.

Metabolic Analytes

The difference in post-feeding and pre-feeding concentrations of serum glucose and insulin are shown in Figure 2. The change in concentration of insulin from pre-feeding to post-feeding was greater ($P < 0.01$) for control cows compared with cows supplemented with any dose of RPG (Figure 2A). Changes in pre-feeding to post-feeding concentrations of glucose did not differ among treatments (Figure 2B).

Reproductive Traits

Concentrations of progesterone increased from day 2 to 12 of the estrous cycle but were unaffected by treatment (Figure 3). This experiment was not designed to offer sufficient power to detect differences in pregnancy risk; however, pregnancy risk (69.2%) was greater ($P < 0.01$) for control cows compared with all RPG-treated cows, with 2.2 lb/day (14.2%), 4.4 lb/day (42.9%), and 8.8 lb/day (25%) all resulting in numerically decreased pregnancy risk. Volume of the corpus luteum on day 8 of the estrous cycle averaged 9.5 ± 1.5, 10.3 ± 1.5, 9.4 ± 1.5, and 12.0 ± 1.4 cm^3, respectively, but did not differ among treatments.

Fertility continues to be a leading economic concern for the dairy industry. Measures to increase pregnancy rates and reduce early pregnancy loss would improve the efficiency of reproduction. In the current study, we focused on reducing clearance of progesterone from the peripheral circulation by supplementing RPG and found that the insulin response was diminished with RPG diets relative to the control. Supplementation with RPG caused a linear increase in CP intake and MUN concentration with increasing

dose but had no impact on milk yield or DMI. Increasing MUN is associated with decreased fertility. Therefore, we conclude that RPG failed to alter insulin concentration as hypothesized and did not affect progesterone concentration.

Table 1. Ingredient and nutritional composition of the basal diet[1]

Ingredients	% Dry matter
Corn silage	22.5
Triticale silage	15.0
Alfalfa hay[2]	3.1
Alfalfa hay[3]	3.1
Corn gluten feed[4]	22.8
Whole cottonseed	4.0
Corn grain, finely ground	13.4
Concentrate mix[5]	16.1

	Basal diet	Ground corn	RPG[6]
Nutrient, % of dry matter (DM) (unless otherwise specified)			
DM, % as-fed	47.8	86.3	82.9
Crude protein (CP)	18.2	9.8	43.2
Soluble protein, % CP	18.2	9.8	43.2
Acid detergent fiber	23.9	3.9	4.9
Neutral detergent fiber	36.5	9.8	20.2
Starch	12.3	69.7	1.1
Ethanol-soluble carbohydrates (simple sugars)	8.1	5.5	38.6
NE_L, Mcal/lb	0.75	0.94	0.86

[1]Nutrient composition values presented are results of near infrared analysis of the basal diet.
[2]Lower quality alfalfa with 22.1% CP.
[3]Higher quality alfalfa with 23.9% CP.
[4]Sweet Bran (Cargill Inc., Blair, NE).
[5]Concentrate premix consisted of 59.9% expeller soybean meal (SoyBest; Grain States Soya, West Point, NE), 12.0% limestone, 10.5% sodium bicarbonate, 7.48% Ca salts of long-chain fatty acids (Megalac R; Arm & Hammer Animal Nutrition, Princeton, NJ), 2.40% magnesium oxide, 2.14% of a 1.50% stock salt, 1.50% trace mineral salt, 1.50% potassium chloride, 1.50% vitamin E (20 kIU/g), 0.94% Biotin 100 (ADM Alliance Nutrition, Quincy, IL), 0.25% selenium premix (0.06%), 0.23% 4-Plex (Zinpro Corp., Eden Prairie, MN), 0.15% vitamin A premix (30 kIU/g), 0.12% Zinpro 120 (Zinpro Corp.), 0.06% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 0.04% vitamin D premix (30,000 IU/g), 0.01% ethylenediamine dihydriodide premix (3.65% I).
[6]Rumen-protected glucose product.

Table 2. Dry matter intake, milk yield, and milk composition in cows supplemented with varying doses of rumen-protected glucose (RPG)[1]

	Treatment,[2] lb RPG/day					*P*-value		
Item	0	2.2	4.4	8.8	SEM	RPG[3]	Linear[4]	Quadratic[4]
Dry matter intake, lb/day	53.5	54.6	52.8	56.1	1.63	0.62	0.42	0.51
Starch intake, lb/day	12.0	10.8	9.4	7.3	0.24	< 0.01	< 0.01	0.06
Crude protein intake, lb/day	11.4	12.3	12.8	15.0	0.37	< 0.01	< 0.01	0.09
Sugar intake[5] lb/day	3.9	4.7	5.3	6.9	0.13	< 0.01	< 0.01	< 0.01
Milk,[6] lb/day	109.8	111.3	107.6	111.1	2.8	0.96	0.88	0.57
Energy-corrected milk, lb/day	105.3	108.9	107.6	107.8	2.4	0.29	0.60	0.46
Fat, %	3.8	3.9	4.0	4.0	0.1	0.20	0.36	0.72
Fat, lb/day	4.0	4.6	4.2	4.6	0.2	0.29	0.28	0.65
Lactose, %	5.0	5.0	5.0	5.0	0.1	0.81	0.78	0.49
Lactose, lb/day	5.5	5.9	5.3	5.5	0.2	0.48	0.76	0.87
Milk urea nitrogen, mg/dL	15.3	16.7	17.4	20.1	0.6	< 0.01	< 0.01	0.73
Protein, %	2.7	2.6	2.7	2.6	0.1	0.10	0.15	0.74
Protein, lb/day	2.9	3.1	2.9	2.9	0.2	0.79	0.67	0.95
Somatic cell linear score	3.5	3.1	3.4	3.2	0.2	0.94	0.83	0.73

[1]Milk components and energy-correct milk (ECM) were measured in milk samples collected on day 8 of the supplemental period. Dietary nutrient components were assessed from feed samples collected weekly and composited every 2 weeks.
[2]Lactating dairy cows were supplemented with either 0 (control), 2.2, 4.4, or 8.8 lb of a rumen-protected glucose product in replacement of finely ground corn grain.
[3]*A priori* contrasts of the 0 lb (control) were compared with the combined 3 treatment means.
[4]*A priori* orthogonal contrasts for unevenly spaced treatment doses to determine linear and quadratic effects of dose.
[5]Free ethanol-soluble carbohydrates.
[6]Mean milk production from day 0 through 12. Treatment did not impact milk production but there was an effect of day ($P < 0.01$).

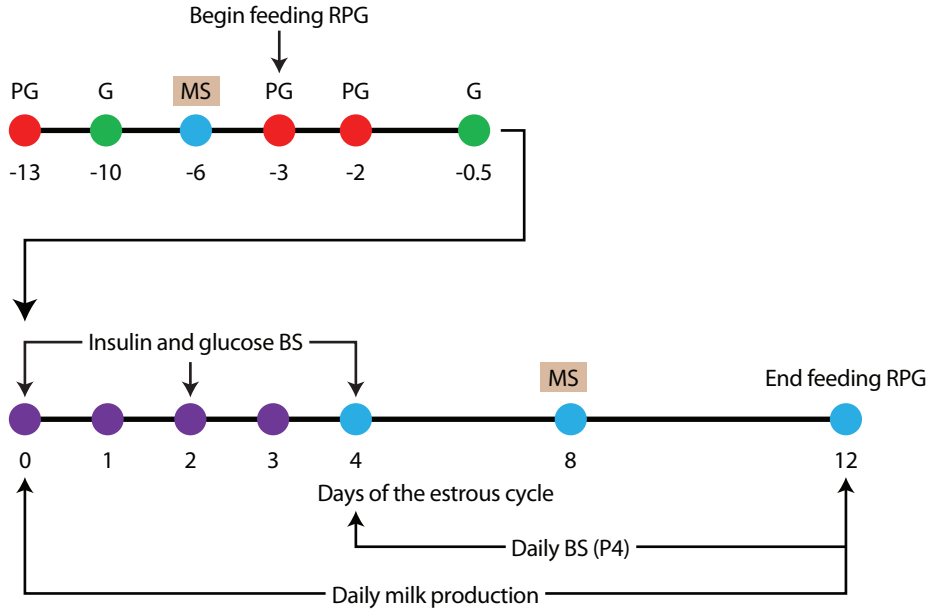

Figure 1. Illustration of the ovulation-synchronization scheme, blood collection (BS) and milk sampling (MS) schedule for supplementation of rumen-protected glucose at varying doses. Ovulation was synchronized with an injection of $PGF_{2\alpha}$ (PG) on day -13, followed by an injection of GnRH (G1) on day -10. Injections of PG were given 24 hours apart on day -3 and -2 to induce complete luteal regression. An injection GnRH (G) was given on day -0.5 to cause ovulation and begin a new estrous cycle (25 mg PG; 100 µg of GnRH). Blood samples were collected on days 0, 2, and 4 before feeding and again 8 hours after feeding to measure changes in insulin and glucose from before to after feeding. Progesterone (P4) was measured on day 2, and then daily from day 4 through 12. Corpus luteum volume was measured on day 8. Milk samples were collected on day -3 and 8 to determine milk components. Daily milk production was recorded from day 0 through 12.

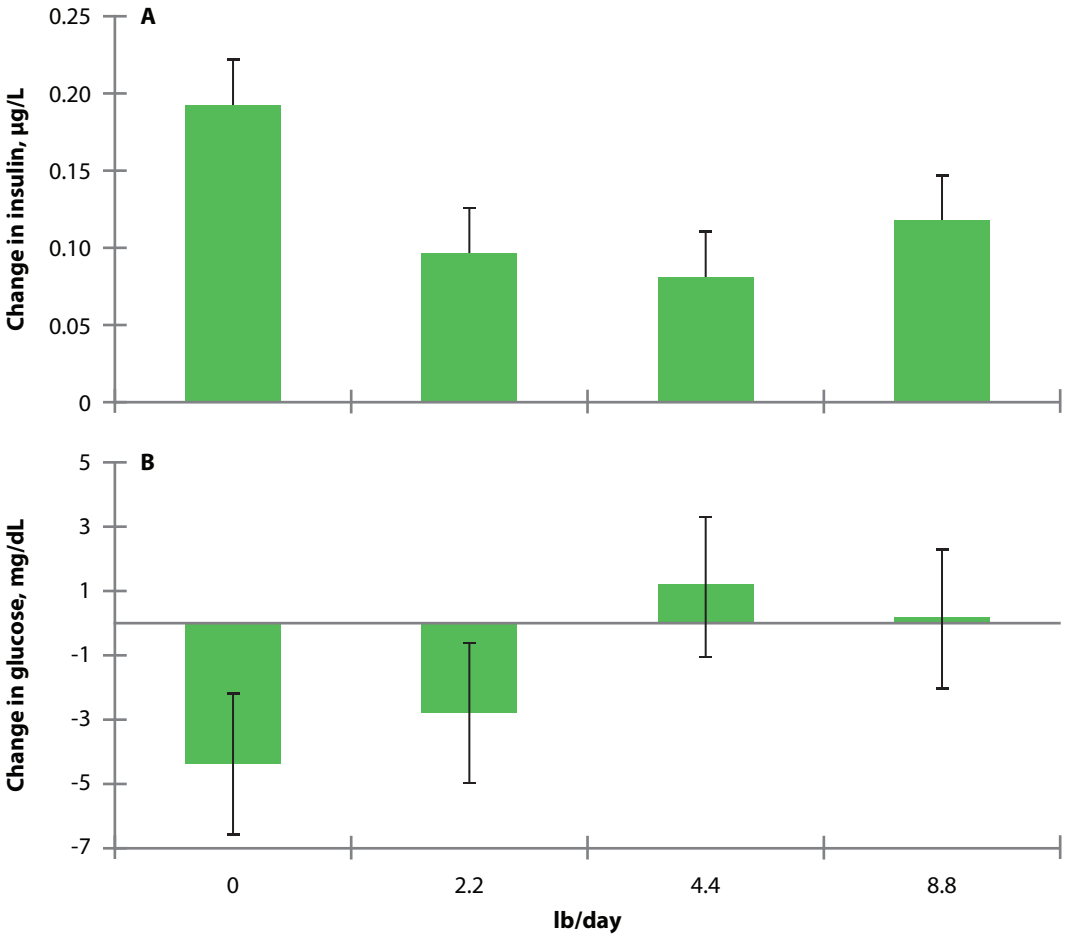

Figure 2. Composite change (post-feeding "minus" pre-feeding) in concentrations of insulin (A) and glucose (B) measured in blood samples collected on days 0, 2, and 4 pre-feeding and 8 hours later (post-feeding). Control cow receiving 8.8 lb of ground corn (0 lb RPG) had greater ($P < 0.01$) change in concentrations of insulin than the cows receiving either of the 3 doses of RPG. No differences ($P = 0.26$) in treatments were detected for the change in glucose concentration from before to after feeding.

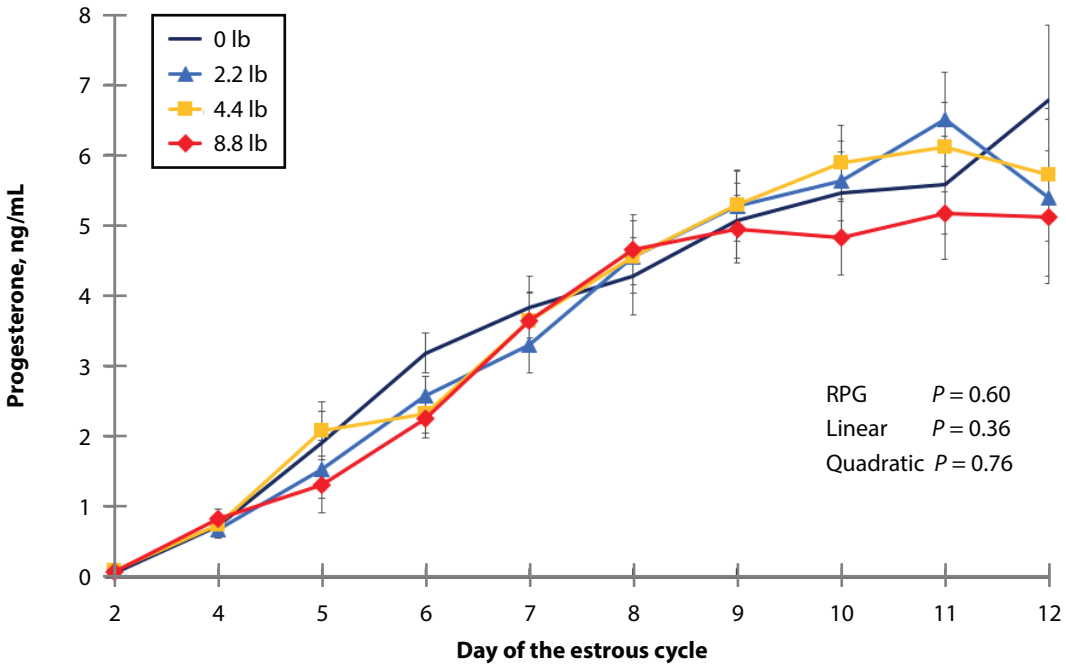

Figure 3. Daily progesterone concentrations in cows supplemented with rumen-protected glucose (RPG). Progesterone increased ($P < 0.01$) from day 2 through 12 of the estrous cycle but was not affected by dose of rumen-protected glucose treatment.