Effects of propiogenic ingredients on serum concentration of insulin and progesterone in non-lactating cows

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ABSTRACT

Three experiments evaluated serum insulin and progesterone (**P4**) concentrations in grazing Gir $\times$ Holstein cows supplemented with monensin (**MON**) or propylene glycol (**PPG**; 2.5 mL/kg of live weight$^{0.75}$ per drench). Cows were non-lactating, ovariectomized, and received an intravaginal drug-releasing device containing 1.9 g of P4 to estimate treatment effects on hepatic P4 degradation. In Exp. 1, 15 cows received, in a crossover design containing 2 periods of 21 d, 0.1 kg/d of corn in addition to 2 g/d of kaolin (**CON**) or 0.2 g/d of MON. Blood samples were collected on d 13 and 20 of each period. Cows receiving CON had greater ($P < 0.05$) serum insulin concentrations compared with MON prior to and 6 h after feeding. However, MON cows had greater ($P = 0.01$) serum P4 concentrations compared with CON 18 h after feeding. In experiment 2, 15 cows received, in a replicated crossover design containing 2 periods of 24 h, a single drench of PPG or water (**WT**). Cows receiving PPG had greater ($P < 0.01$) serum insulin concentrations compared with WT from 0.5 to 3 h after drench. However, PPG cows had reduced ($P < 0.05$) serum P4 concentrations compared with WT at 1 and 2 h after drench. In experiment 3, 13 cows received, in a replicated 3×3 Latin square design containing 3 periods of 24 h, 3 PPG drenches administered 1 h apart (**PPG3x**), 3 WT drenches administered 1 h apart, or 1 PPG drench+2 WT drenches administered 1 h apart (**PPG1x**). Serum insulin concentrations increased proportionally to PPG dosage (treatment $\times$ hour; $P < 0.01$). However, mean serum P4 concentration was greater ($P < 0.01$) in WT cows compared with PPG1x and PPG3x, but similar ($P = 0.25$) between PPG1x and PPG3x cows. In conclusion, feeding propiogenic ingredients to grazing cows failed to substantially increase serum P4 concentrations.

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1. Introduction

Nutrition, more specifically energy intake, is one of the environmental factors that most influences reproductive function in beef and dairy females (Butler, 2005; Maas, 1987). The beneficial effects of energy intake on cattle reproduction are regulated, at least partially, by circulating hormones and metabolites such as glucose and insulin (Wettemann et al., 2003). As an example, insulin modulates circulating concentrations of progesterone (**P4**), a hormone required for establishment and maintenance of pregnancy in cattle (Spencer and Bazer, 2002). More specifically, insulin stimulates ovarian steroid synthesis (Spicer and Echternkamp, 1995), and also modulates hepatic expression of enzymes associated with catabolism of P4, such as CYP2C and CYP3A (Lemley et al., 2008; Murray, 1991).

Our research group recently reported that non-lactating cows in adequate nutritional status receiving i.v. glucose infusion to increase circulating insulin concentrations had

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greater plasma P4 concentrations compared to cohorts receiving saline solution, and this outcome was attributed to reduced hepatic P4 degradation given that cows were ovariectomized and supplemented with exogenous P4 (Vieira et al., 2010). Accordingly, Lemley et al. (2010) reported that feeding diets to stimulate insulin secretion reduced CYP2C and CYP3A activity in lactating dairy cows in adequate energy balance. Therefore, we hypothesized that feeding diets enriched with propiogenic ingredients, such as propylene glycol (**PPG**) and monensin (Nielsen and Ingvartsen, 2004; Van Maanen et al., 1978), are nutritional alternatives to increase circulating concentrations of insulin and P4 in bovine females. Hence, 3 experiments were conducted to evaluate serum concentrations of insulin and P4 in non-lactating ovariectomized cows receiving exogenous P4, as a model to estimate hepatic P4 degradation (Moriel et al., 2008; Lopes et al., 2009; Vieira et al., 2010), and offered diets formulated to meet or exceed nutritional maintenance requirements enriched with monensin (Exp. 1) or PPG (Exp. 2 and 3).

2. Materials and methods.

All 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).

2.1. Experiment 1

2.1.1. Animals and treatments

Fifteen non-lactating, non-pregnant, and ovariectomized Gir × Holstein cows [mean ± SE; live weight (**LW**)=619 ± 22 kg and body condition score (**BCS**)= 3.7 ± 0.1] were ranked by LW and BCS (Wildman et al., 1982), and randomly assigned to receive, in a crossover design containing 2 periods of 21 d each, 0.1 kg/d of ground corn in addition to (1) 2 g/d of kaolin (**CON**; rumen-inert indigestible substance) or (2) 0.2 g/d of Na monensin (**MON**; 2 g/d of Rumensin® 100, Elanco Saúde Animal, São Paulo, Brazil). The initial 5 d of each period served as adaptation, when CON cows received 0.1 kg/d of ground corn in addition to 1 g/d of kaolin, whereas MON cows received 0.1 kg/d of ground corn in addition to 0.1 g/ d of Na monensin. Cows were maintained in a *Brachiaria brizantha* pasture and received treatments individually through self-locking head gates every morning (0800 h). Upon feeding, cows readily consumed their respective treatments. The Na monensin dose utilized herein is the recommended dose for cattle receiving forage-based diets by the manufacturer (Elanco Saúde Animal). Nutritional content of corn and pasture were estimated to be 88% and 53% total digestible nutrients, 8.2% and 7.1% CP, and 8.7% and 76.4% NDF from samples collected prior to the experiment and analyzed by a bromatology laboratory (São Paulo State University, Botucatu, Brazil). Cows also 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.

2.1.2. Progesterone implants, blood sampling and analysis

During the adaptation period (d 0–5 of each period), all cows were inserted with a previously used (second use) intravaginal P4 releasing device (**CIDR**, originally containing 1.9 g of P4; Pfizer Animal Health, São Paulo, Brazil) to initially expose and adapt cows to exogenous P4. Cows received a new CIDR on d 5, which remained in the cows until the end of each respective period (d 21).

Cow LW was recorded prior to the beginning of the first period and at the last day of the second period to calculate cow average daily gain (**ADG**) during the experiment. Blood samples were collected on d 13 and 20 of each period, prior to (0 h) and 6, 12, 18, and 24 h relative to treatment feeding, for determination of serum glucose, insulin, and P4 concentrations. Samples were collected on d 13 and 20 to ensure that cows were completely adapted to treatments. Cows were feed-deprived for 4 h prior to the first blood collection (0 h), and remained without access to pastures between blood collections within d 13 and 20, to prevent any confounding effects between feed intake and treatments on circulating P4 concentrations (Vasconcelos et al., 2003). Immediately after the last blood collection of d 13 and 20, cows received their treatments and returned to pasture. 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 $3000 \times g$ at 4 °C for 30 min for serum collection. Harvested serum was stored frozen at -20 °C until further processing. Glucose was determined using a quantitative colorimetric kit based on glucose oxidase, phenol, and 4-aminoantipyrine (Katal® Biotecnológica Ind. Com. Ltda.; Belo Horizonte, Brazil). Concentrations of P4 and insulin were determined using Coat-A-Count kits (DPC Diagnostic Products Inc., Los Angeles, CA) solid phase ^{125}I RIA that were previously validated for bovine samples (Moriel et al., 2008). All samples were analyzed within 1 assay for each hormone. The intra-assay CV was 6.8% for P4, and 3.9% for insulin. The minimum detectable concentrations were 0.1 ng/mL of P4 and 0.05 μIU/mL of insulin.

2.2. Experiment 2

2.2.1. Animals and treatments

Fifteen non-lactating, non-pregnant, and ovariectomized Gir × Holstein cows (LW=622 ± 21 kg; BCS= 3.6 ± 0.1) were ranked by LW and BCS (Wildman et al., 1982), and randomly assigned to 2 groups (group 1, n=7; group 2, n=8). Throughout the experiment (d 0–22), cows were maintained in a *B. brizantha* pasture and received (as-fed basis) 2 kg/d of a supplement (50% corn and 50% soybean meal) through self-locking head gates every morning, in addition to ad libitum access to water and the mineral and vitamin mix described in Exp. 1. Nutritional content of supplement and pasture were estimated to be, respectively, 84% and 53% total digestible nutrients, 26.1% and 6.5% CP, and 10.5% and 78.2% NDF from

samples collected prior to the experiment and analyzed by a bromatology laboratory (São Paulo State University). On d 13, groups were randomly assigned to receive in a crossover design containing 2 periods of 24 h (d 14 and 15) each (1) 1 oral drench with PPG (Propilac®, M. Cassab Com. Ind. LTDA, São Paulo, SP, Brazil) at 2.5 mL/kg of $LW^{0.75}$ (311 ± 5 mL of PPG per cow), or (2) 1 oral drench with water at 2.5 mL/kg of $LW^{0.75}$ (311 ± 5 mL of water per cow; **WT**). The PPG treatment was based on Christensen et al. (1997) and the reported increase in circulating insulin concentrations upon a PPG drench. Treatments were applied once, at 0600 h. The same cross-over design was replicated on d 21 and 22 of the experiment.

2.2.2. Progesterone implants, blood sampling and analysis

All cows were inserted with a previously used (second use) CIDR prior to the beginning of the experimental period (d −5 to 0) to initially expose and adapt cows to exogenous P4. On d 0, cows received a new CIDR, which was replaced every 7 d to ensure that CIDR was inserted for at least 6 d prior to treatment administration. Cow LW was recorded on d 0 and 23 to calculate cow ADG during the experiment. Blood samples were collected prior to (−0.5 and 0 h) and 0.5, 1, 2, 3, 4, 5, 6, and 7 h relative to treatment application for determination of serum insulin and P4 concentrations. Cows were feed-deprived for 12 h prior to treatment application, and remained without access to pastures and supplements between blood collections, to prevent confounding effects between feed intake and treatments on circulating P4 concentrations (Vasconcelos et al., 2003). Immediately after the last blood collection, cows received supplement and returned to pasture. The feed deprivation period in Exp. 2 was greater than the feed deprivation period of Exp. 1 due to the greater concentrate intake in the present experiment (Vasconcelos et al., 2003). Blood samples were collected, stored, processed for serum extraction, and analyzed for insulin and P4 concentrations as in Exp. 1. Blood samples were not analyzed for serum glucose concentrations due to extensive sample degradation following the insulin assay. The intra-assay CV was 5.2% for P4, and 5.5% for insulin.

2.3. Experiment 3

2.3.1. Animals and treatments

Thirteen non-lactating, non-pregnant, and ovariecto-mized Gir × Holstein cows (LW=655 ± 22 kg; BCS=3.5 ± 0.1) were ranked by LW and BCS (Wildman et al., 1982), and randomly assigned to 3 groups (group 1, n=5; group 2, n=4; group 3, n=4). Throughout the experiment (d 0–23), cows were maintained in the same pasture and received the same supplementation program described in experiment 2. On d 13, groups were randomly assigned to receive, in a 3 × 3 Latin square design containing 3 periods of 24 h each (d 14, 15, and 16) (1) 3 oral drenches with PPG (**PPG3x**; Propilac®; M. Cassab Com. Ind. LTDA) at 2.5 mL/kg of $LW^{0.75}$, administered 1 h apart (h 0, 1, and 2 within each period; 323 ± 14 mL of PPG per cow/drench), (2) PPG drench (Propilac®,

M. Cassab Com. Ind. LTDA; 2.5 mL/kg of $LW^{0.75}$) at h 0 with the addition of 2 oral drenches with water (h 1 and 2) at 2.5 mL/kg of $LW^{0.75}$ each (**PPG1x**; 323 ± 14 mL of PPG or water per cow/drench), and (3) 3 oral drenches with water (**WT**) at 2.5 mL/kg of $LW^{0.75}$ (h 0, 1, and 2 within each period; 323 ± 14 mL of water per cow/drench). Treatments were applied at 0600, 0700, and 0800 h. The same Latin square design was replicated on d 21, 22, and 23 of the experiment.

2.3.2. Progesterone implants, blood sampling and analysis

Cows received P4 implants as described in Exp. 2. Cow LW was recorded on d 0 and 24 to calculate cow ADG during the experiment. Blood samples were collected every 30 min from −0.5 to 6 h relative to the first treatment application (0 h). Blood samples collected at 1 and 2 h were obtained immediately prior to each treatment application. As in Exp. 2, cows were feed-deprived for 12 h prior to treatment application and remained without access to pastures and supplements between blood collections. Blood samples were collected, stored, processed for serum extraction, and analyzed for glucose, insulin, and P4 concentrations as in Exp. 1. The intra-assay CV was 2.7% for P4, and 7.5% for insulin.

2.4. 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 in Exp. 1 contained the effects of treatment, day, collection hour, and all resultant interactions. Period was included as independent variable. Data were analyzed using cow as random variable. The specified term for the repeated statement was collection hour, subject was cow(treatment × period × day), and the covariance structure utilized was first-order auto-regressive, which provided the best fit for these analyses according to the Akaike information criterion. The model statement used in Exp. 2 and Exp. 3 contained the effects of treatment, collection hour, replication, and all resultant interactions. Period (replication) was included as an independent variable. Data obtained prior to treatment application (−0.5 and 0 h) were averaged and used as covariates, whereas cow was used as a random variable. The specified term for the repeated statement was collection hour, subject was cow(treatment × period × replication), and the covariance structure utilized was first-order auto-regressive, which provided the best fit for these analyses according to the Akaike information criterion. Results from Exp. 1 are reported as least squares means, whereas results from Exp. 2 and 3 are reported as covariately adjusted least squares means. Means were separated using LSD or PDIFF. Significance was set at $P \leq 0.05$, and tendencies were declared if $P > 0.05$ and ≤ 0.10. Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected.

3. Results

3.1. Experiment 1

During the experimental period, mean cow ADG ($\pm$ SE) was 0.20 ± 0.07 kg. No treatment effects were detected ($P = 0.88$) on serum glucose concentrations (61.0 vs. 60.9 mg/dL for CON and MON cows, respectively; SEM = 1.0).

A treatment × hour interaction was detected ($P = 0.05$) for insulin (Fig. 1) because CON cows had greater ($P < 0.05$) serum insulin concentrations compared with MON cows at 0 and 6 h relative to feeding. In addition, serum insulin concentrations significantly decreased from 0 to 24 h relative to treatment feeding in CON cows (hour effect; $P < 0.01$), but did not change for MON cows (hour effect; $P = 0.13$). A treatment × hour interaction was also detected ($P = 0.02$) for P4 (Fig. 2) because MON cows had greater ($P = 0.01$) serum P4 concentrations compared with CON cows 18 h relative to treatment feeding.

3.2. Experiment 2

During the experimental period, mean cow ADG ($\pm$ SE) was 0.57 ± 0.13 kg. A treatment × hour interaction was
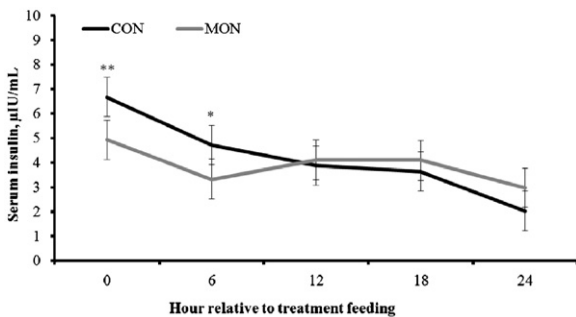
Fig. 1. Serum insulin concentrations ($\pm$ SEM) of forage-fed cows receiving 0.1 kg/d of ground corn in addition to (1) 2 g/d of kaolin (CON; rumen-inert indigestible substance) or (2) 0.2 g/d of Na monensin (MON). Treatments were offered after the first blood collection (0 h). A treatment × time interaction was detected ($P = 0.05$). Treatment comparison within time: [**]$P = 0.01$, [*]$P = 0.05$.
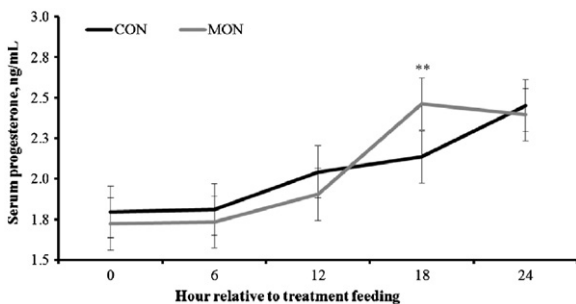
Fig. 2. Serum progesterone concentrations ($\pm$ SEM) of forage-fed cows receiving 0.1 kg/d of ground corn in addition to (1) 2 g/d of kaolin (CON; rumen-inert indigestible substance) or (2) 0.2 g/d of Na monensin (MON). Treatments were offered after the first blood collection (0 h). A treatment × time interaction was detected ($P = 0.02$). Treatment comparison within time: [**]$P = 0.01$.

detected ($P < 0.01$) for serum insulin (Fig. 3). Insulin concentrations prior to treatment application were significant covariates ($P < 0.01$), but did not differ ($P = 0.15$) between treatments (4.9 vs. 5.6 µIU/mL for WT and PPG, respectively; SEM = 0.97). Following treatment application, PPG cows had greater ($P < 0.01$) serum insulin concentrations compared with WT cows from 0.5 to 3 h (Fig. 3).

A treatment × hour interaction was also detected ($P = 0.02$) for serum P4 (Fig. 4). Concentrations of P4 prior to treatment application were significant covariates ($P < 0.01$), but did not differ ($P = 0.19$) between treatments (2.3 vs. 2.1 ng/mL for WT and PPG, respectively; SEM = 0.20). Following treatment application, PPG cows had reduced ($P < 0.05$) serum P4 concentrations compared with WT cows at 1 and 2 h (Fig. 4).

3.3. Experiment 3

During the experimental period, mean cow ADG ($\pm$ SE) was 0.50 ± 0.09 kg. A treatment × hour interaction was detected ($P = 0.05$) for serum glucose (Fig. 5). Glucose concentrations prior to treatment application were significant covariates ($P < 0.01$), but did not differ ($P = 0.47$) among treatments (83.4, 86.3, and 86.6 mg/dL for WT, PPG1x, and PPG3x, respectively; SEM = 2.9). Following treatment application, PPG1x cows had greater ($P = 0.05$) serum glucose concentrations compared to WT cows at 0.5 h. Cows receiving PPG3x had greater ($P < 0.05$) serum glucose concentrations compared to WT cows at 0.5, 1, 2.5, and 3 h relative to treatment application. Serum glucose concentrations were also greater ($P = 0.05$) for PPG3x compared with PPG1x cows at 3 h relative to treatment application.

A treatment × hour interaction was detected ($P < 0.01$) for serum insulin (Fig. 6). Insulin concentrations prior to treatment application were significant covariates ($P < 0.01$), but did not differ ($P = 0.71$) among treatments (5.7, 6.2, and 6.5 ng/mL for WT, PPG1x, and PPG3x, respectively; SEM = 1.12). Following the first treatment application (0 h), PPG1x cows had greater ($P < 0.05$) serum insulin concentrations compared to WT cows from 0.5 to 2.5 h. Cows receiving PPG3x had greater ($P < 0.05$) serum insulin concentrations compared to WT cows during the entire collection period, and had greater ($P < 0.05$) serum insulin concentrations compared to PPG1x cows at 1 h and from 2 to 6 h relative to treatment application (Fig. 6).
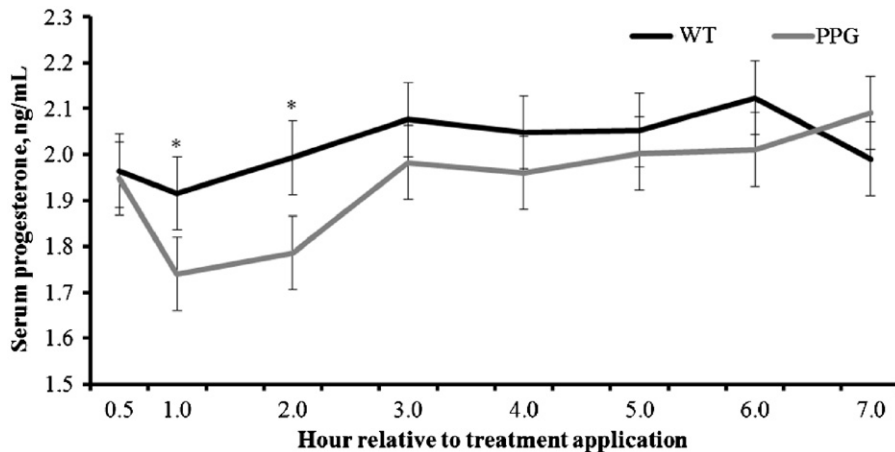
A treatment effect was detected ($P < 0.01$) for serum P4. Concentrations of P4 prior to treatment application were significant covariates ($P < 0.01$), but did not differ ($P = 0.49$) between treatments (0.98, 1.07, and 0.92 ng/mL for WT, PPG1x, and PPG3x, respectively; SEM = 0.10). Mean serum P4 concentrations were greater ($P < 0.01$) in WT cows compared with PPG1x and PPG3x cows, but similar ($P = 0.25$) between PPG1x and PPG3x cows during the sampling period (0.98, 0.88, and 0.85 ng/mL, correspondingly; SEM = 0.04).

4. Discussion

Recent research studies demonstrated that insulin may increase circulating concentrations of P4 in bovine
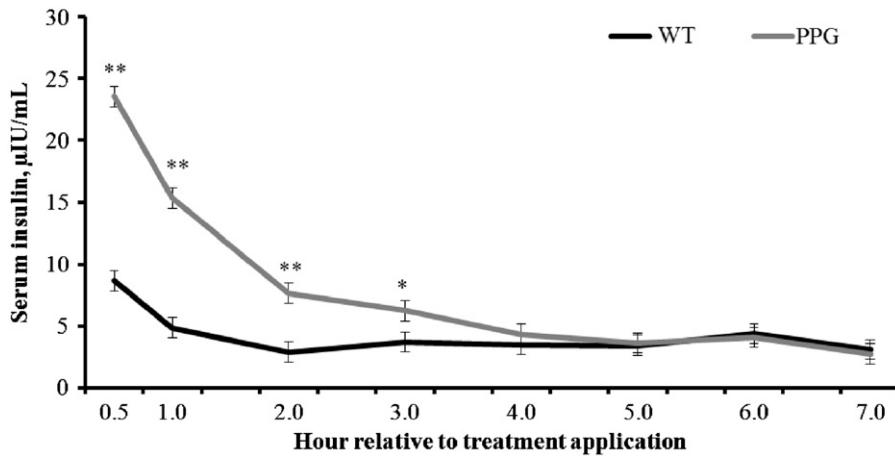
Fig. 3. Serum insulin concentrations ($\pm$ SEM) of forage-fed cows receiving (1) 1 oral drench with propylene glycol at 2.5 mL/kg of LW $^{0.75}$ (PPG), or (2) 1 oral drench with water at 2.5 mL/kg of LW$^{0.75}$ (WT). Values obtained from samples collected prior to treatment application (-0.5 and 0 h) were significant covariates ($P < 0.01$), although they did not differ ($P = 0.15$) between treatments (5.6 vs. 4.9 µIU/mL for PPG and WT, respectively; SEM $= 0.97$). Therefore, results reported are covariately adjusted least squares means. A treatment × time interaction was detected ($P < 0.01$). Treatment comparison within time: $^{**}P < 0.01$, $^{*}P = 0.02$.
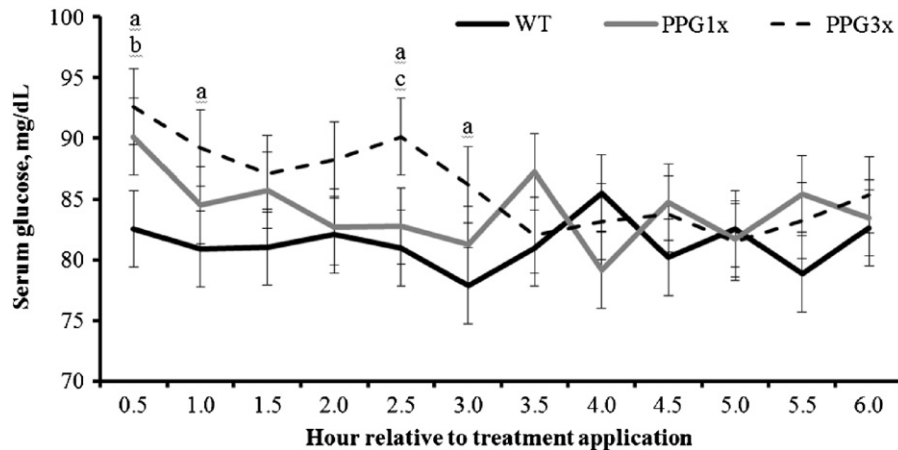

Fig. 4. Serum progesterone concentrations ($\pm$ SEM) of forage-fed cows receiving (1) 1 oral drench with propylene glycol at 2.5 mL/kg of LW$^{0.75}$ (PPG), or (2) 1 oral drench with water at 2.5 mL/kg of LW$^{0.75}$ (WT). Values obtained from samples collected prior to treatment application (-0.5 and 0 h) were significant covariates ($P < 0.01$), although they did not differ ($P = 0.19$) between treatments (2.3 vs. 2.1 µIU/mL for PPG and WT, respectively; SEM $= 0.20$). Therefore, results reported are covariately adjusted least squares means. A treatment × time interaction was detected ($P < 0.01$). Treatment comparison within time: $^{*}P < 0.05$.

females by reducing expression of hepatic enzymes associated with catabolism of P4, such as CYP2C and CYP3A (Butler et al., 2003; Lemley et al., 2008). Moreover, our research group reported that acute but transient increases in serum insulin following an intravenous glucose infusion increased serum P4 concentrations (Vieira et al., 2010), and promptly reduced mRNA expression of CYP2C and CYP3A (Vieira et al., 2013). Accordingly, Lemley et al. (2010) reported that propiogenic diets increased circulating insulin and reduced CYP2C and CYP3A activity compared with acetogenic diets, but did not substantially impact circulating P4 concentrations in lactating dairy cows in adequate energy balance. However, cows evaluated by Lemley et al. (2010) received diets immediately and during sample collection, whereas dietary carbohydrate profile and feed intake may override potential

benefits of insulin on circulating P4 catabolism (Moriel et al., 2008; Vasconcelos et al., 2003). Therefore, this series of experiments was conducted using ovariectomized cows receiving exogenous P4, with no access to feed prior to and during sample collection, to further evaluate if diets enriched with propiogenic ingredients increase circulating P4 concentrations by reducing basal hepatic P4 catabolism in bovine females in adequate nutritional status.

4.1. Experiment 1

According to the experimental design, cows were in adequate nutritional status based on the positive ADG detected during the experiment. No treatment effects were detected for serum glucose concentrations, although other

Fig. 5. Serum glucose concentrations ($\pm$ SEM) of forage-fed cows receiving (1) 3 oral drenches with propylene glycol (PPG3x) at 2.5 mL/kg of LW$^{0.75}$ (h 0, 1, and 2 within each period), (2) 1 oral drench with propylene glycol (h 0) and 2 oral drenches with water (h 1, and 2) at 2.5 mL/kg of LW$^{0.75}$ each (PPG1x), and (3) 3 oral drenches with water (WT) at 2.5 mL/kg of LW$^{0.75}$ (h 0, 1, and 2 within each period). Values obtained from samples collected prior to treatment application (-0.5 and 0 h) were significant covariates ($P < 0.01$), although they did not differ ($P = 0.47$) among treatments (86.6, 86.3, and 83.4 mg/dL for PPG3x, PPG1x, and WT, respectively; SEM=2.9). Therefore, results reported are covariately adjusted least squares means. A treatment $\times$ time interaction was detected ($P = 0.05$). Within hour, letters indicate the following treatment comparison ($P < 0.05$): a=WT vs. PPG3x, b=WT vs. PPG1x, and c=PPG3x vs. PPG1x.
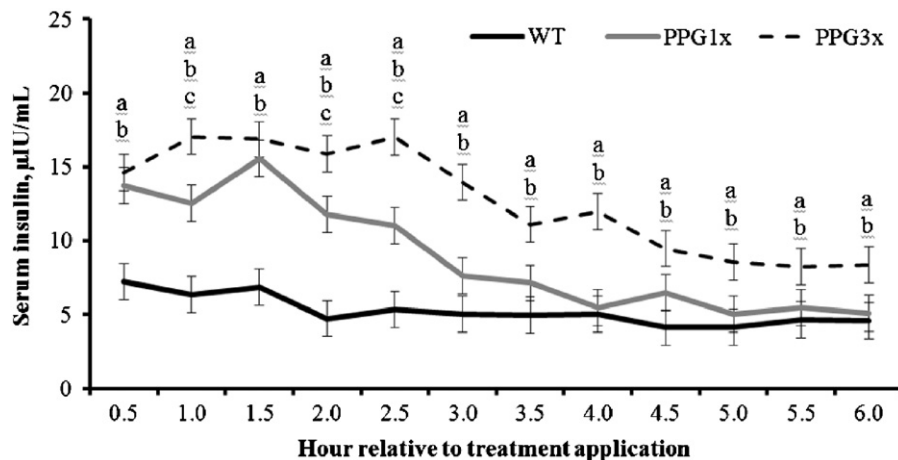
Fig. 6. Serum insulin concentrations ($\pm$ SEM) of forage-fed cows receiving (1) 3 oral drenches with propylene glycol (PPG3x) at 2.5 mL/kg of LW$^{0.75}$ (h 0, 1, and 2 within each period), (2) 1 oral drench with propylene glycol (h 0) and 2 oral drenches with water (h 1, and 2) at 2.5 mL/kg of LW$^{0.75}$ each (PPG1x), and (3) 3 oral drenches with water (WT) at 2.5 mL/kg of LW$^{0.75}$ (h 0, 1, and 2 within each period). Values obtained from samples collected prior to treatment application (-0.5 and 0 h) were significant covariates ($P < 0.01$), although they did not differ ($P = 0.71$) among treatments (6.5, 6.2, and 5.7 μIU/mL for PPG3x, PPG1x, and WT, respectively; SEM=1.12). Therefore, results reported are covariately adjusted least squares means. A treatment $\times$ time interaction was detected ($P < 0.01$). Within hour, letters indicate the following treatment comparison ($P < 0.05$): a=WT vs. PPG3x, b=WT vs. PPG1x, and c=PPG3x vs. PPG1x.

authors reported that monensin increases circulating glucose in feedlot and forage-fed cattle (Potter et al., 1976; Van Maanen et al., 1978). However, supporting our findings, Lalman et al. (1993) reported that grazing heifers supplemented with 200 mg/d of monensin had similar serum glucose concentrations compared to non-supplemented cohorts.

Serum insulin concentrations were greater in CON cows compared with MON cows at 0 and 6 h relative to feeding, contradicting the lack of treatment effects for serum glucose given that circulating insulin and glucose are, in general, positively associated (Nelson and Cox, 2005; Vieira et al., 2010). Nevertheless, Lalman et al. (1993) also reported that monensin supplementation reduced serum insulin concentrations in grazing beef heifers compared to non-supplemented cohorts. However, serum insulin concentrations decreased for CON cows during the sampling period but did not change in MON cows, suggesting that monensin supplementation prevented, or at least alleviated, the decrease in serum insulin induced by feed deprivation.

Serum P4 concentrations were greater for MON cows compared with CON cohorts at 18 h relative to treatment feeding. Based on our initial hypothesis, the transient

treatment effect detected on serum P4 concentrations could be attributed to the lack of variation in serum insulin of MON cows during the sampling period. However, serum insulin concentrations were either greater for CON cows, or similar between CON and MON cows during the study. These outcomes suggest that the increase in serum P4 concentration in MON cows 18 h relative to feeding should be associated with alternative, and unknown, physiological processes.

4.2. Experiment 2

As in Exp. 1, cows were in adequate nutritional status based on the positive ADG detected during the experiment. Similar to previous research evaluating PPG administration to dairy cows (Christensen et al., 1997; Grummer et al., 1994; Studer, 1993), cows receiving PPG herein had an immediate increase in serum insulin concentrations, which returned to levels similar to WT cows after 3 h relative to treatment administration. However, differing from our main hypothesis, PPG cows had reduced serum P4 concentrations compared to WT cows at 1 and 2 h relative to treatment infusion. This outcome may be attributed to increased hepatic blood flow (Kristensen and Raun, 2007) and consequent circulating P4 degradation (Sangsritavong et al., 2002) caused by PPG administration, albeit cows did not receive diets prior to and during treatment administration and sampling. More specifically, Kristensen and Raun (2007) reported that lactating dairy cows ruminally-infused with PPG at 5.0 mL/kg of $LW^{0.75}$ had increased hepatic blood flow compared with non-infused cohorts, whereas Sangsritavong et al. (2002) reported that hepatic blood flow is positively associated with hepatic P4 catabolism. Supporting this rationale, Moriel et al. (2008) suggested that increased hepatic blood flow overrides any potential inhibitory effect of insulin on hepatic P4 catabolism. Alternatively, Lemley et al. (2008) reported that hepatic expression of CYP2C and CYP3A decreased linearly according to the dose of insulin infusion. Therefore, we speculated that increasing the rate of PPG application to further increase serum insulin concentrations would be an alternative increase, or at least prevent the decrease observed herein, in serum P4 concentrations.

4.3. Experiment 3

Based on results from Exp. 2, the present experiment included the PPG3x treatment in which cows received a total of 7.5 mL/kg of $LW^{0.75}$ of PPG fractioned into 3 drenches to further increase and maintain serum insulin concentrations elevated for a longer period of time compared to PPG1x. In addition, PPG3x cows did not receive the entire PPG dosage in 1 administration to prevent additional increases in hepatic blood flow and consequent P4 catabolism (Vasconcelos et al., 2003). As in Exp. 1 and 2, cows were in adequate nutritional status based on the positive ADG detected during the experiment. Similar to the results reported by Grummer et al. (1994), increasing the PPG dose proportionally increased serum concentrations of glucose and insulin in the present experiment. In

fact, PPG3x had greater insulin concentrations compared with WT cows throughout the sampling period, as well as greater insulin concentrations compared with PPG1x cows during the majority of the sampling period. Similar to Exp. 2, serum insulin concentrations in PPG1x were greater than WT cows during the initial 2.5 h of the sampling period.

However, differing from our main hypothesis and supporting the results from Exp. 2, cows receiving PPG1x and PPG3x had a similar decrease in mean serum P4 concentrations compared with WT cows during the sampling period. This outcome may again be attributed to increased hepatic blood flow (Kristensen and Raun, 2007) and consequent circulating P4 degradation (Sangsritavong et al., 2002) caused by PPG administration. Moreover, PPG administration herein and in Exp. 2 did not increase serum insulin concentrations to levels previously reported to alleviate hepatic P4 catabolism (Vieira et al., 2010; Vieira et al., 2013), which may also have contributed to the treatment effects detected for serum P4 concentrations. Nevertheless, the similar serum P4 concentrations between PPG1x and PPG3x cows suggest that fractioning the high PPG dosage (7.5 mL/kg of $LW^{0.75}$) into 3 drenches effectively increased serum insulin and glucose concentrations without enhancing hepatic blood flow and consequent hepatic P4 catabolism.

5. Conclusion

Results from this series of experiments collectively indicate that feeding propiogenic ingredients to non-lactating forage-fed cows as a nutritional strategy to increase circulating insulin concentrations failed to substantially increase serum P4 concentrations. More specifically, monensin supplementation to grazing cows did not substantially increase basal insulin and P4 concentrations. Contrary to our hypothesis, PPG administration increased circulating insulin but reduced serum P4 concentration, likely by enhancing hepatic blood flow and consequent hepatic P4 catabolism. Nevertheless, additional research is warranted to develop management alternatives that exploit the ability of insulin to alleviate hepatic P4 catabolism, promoting increased circulating P4 concentrations and enhanced reproductive function of bovine females.

Conflict of Interest Statement

There is no conflict of interest in this manuscript.

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