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Effects of bovine somatotropin injection on serum concentrations of progesterone in non-lactating dairy cows

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ABSTRACT

The objective of this experiment was to evaluate the effects of bovine somatotropin administration on serum concentrations of glucose, insulin, NEFA, IGF-I, and progesterone (**P4**) in ovariectomized non-lactating dairy cows receiving exogenous P4, as a model to estimate treatment effects on hepatic P4 degradation. Ten non-lactating, non-pregnant, and ovariectomized Gir × Holstein cows were assigned to the experiment (d −14 to 27). On d 0, cows were ranked by BW and BCS, and randomly assigned to one of two treatments: (1) bovine somatotropin (*BST*; $n=5$) or (2) saline control (**control**; $n=5$). Cows assigned to the BST treatment were administered s.c. injections containing 500 mg of sometribove zinc on d 0, 9, and 18 of the experiment, whereas control cows concurrently received a 10-mL s.c. injection of 0.9% saline. On d −2, cows were inserted with an intravaginal releasing device containing 1.9 g of P4, which remained in the cows until the end the experiment (d 27). Cow BW and BCS were assessed on d −14, 0, and 27. Blood samples were collected daily from d 0 to d 27, at 0 (immediately before), 1, and 2 h relative to concentrate feeding for determination of serum glucose, insulin, NEFA, P4, and IGF-I concentrations. Concentrations of glucose, NEFA, and insulin obtained prior to feeding (0 h) were used to determine pre-prandial revised quantitative insulin sensitivity check index (**RQUICKI**). No treatment effects were detected for BW ($P=0.72$) and BCS change ($P=0.79$) during the experiment. Beginning on d 2 of the experiment, BST cows had greater ($P\leq0.01$) serum IGF-I concentrations compared with control cohorts (treatment × day interaction; $P<0.01$). Cows receiving BST had greater ($P\leq0.05$) insulin concentrations compared with control cohorts from d 8 to d 11, d 16 and 17, as well as from d 19 to d 21 of the experiment (treatment × day interaction; $P<0.01$). Cows receiving BST had greater ($P\leq0.01$) mean glucose and NEFA concentrations, as well as reduced ($P<0.01$) mean RQUICKI during the experiment compared with control cohorts. No treatment effects, however, were detected ($P=0.73$) for serum P4 concentrations. In conclusion, results from this experiment indicate that hepatic P4 catabolism is not directly regulated by circulating IGF-I, whereas BST administration decreases insulin sensitivity in non-lactating dairy cows in adequate nutritional status.

1. Introduction

Nutrition substantially impacts productive and reproductive traits in dairy cattle (Butler, 2005). Therefore, nutritional strategies that promote milk production and benefit reproductive efficiency of dairy cows are warranted (Lucy, 2001). The development of such strategies is dependent on the recognition of physiological mechanisms that associate nutrition with reproductive function in dairy females. More specifically, nutrition has been shown to regulate cattle reproduction, at least partially, via circulating hormones and metabolites such as insulin and IGF-I (Wettemann and Bossis,

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2000). As an example, insulin modulates circulating concentrations of progesterone (**P4**; Lopes et al., 2009), a steroid required for establishment and maintenance of pregnancy (Spencer and Bazer, 2002), by stimulating luteal P4 synthesis (Spicer and Echternkamp, 1995) and alleviating hepatic P4 catabolism by CYP2C and CYP3A enzymes (Murray, 1991; Lemley et al., 2008).

Our research group reported that non-lactating dairy cows in adequate nutritional status receiving intravenous glucose infusion to increase endogenous insulin concentrations had greater plasma P4 concentrations compared with cohorts receiving saline, and this outcome was attributed to reduced hepatic P4 degradation given that cows were ovariectomized and supplemented with exogenous P4 (Vieira et al., 2010). Supporting this research approach and results, Vieira et al. (2013) evaluated similar cows receiving the same dosage of glucose infusion as Vieira et al. (2010), and reported reduced hepatic mRNA expression of CYP2C and CYP3A compared with saline-receiving cohorts. However, glucose supplementation may also increase circulating concentrations of other hormones associated with reproductive and hepatic function, including IGF-I (Jones and Clemmons, 1995). Therefore, we hypothesized that the insulin-stimulated decrease in hepatic P4 catabolism may also be associated with circulating IGF-I.

Administration of bovine somatotropin is an alternative to increase circulating concentrations of IGF-I in non-lactating dairy cattle, independently of baseline glucose and insulin concentrations (Bilby et al., 2004). Based on this rationale and our hypothesis, this experiment evaluated the effects of bovine somatotropin administration on serum concentrations of glucose, insulin, NEFA, IGF-I, and P4 in ovariectomized non-lactating dairy cows receiving exogenous P4, as a model to estimate treatment effects on hepatic P4 degradation (Moriel et al., 2008; Lopes et al., 2009; Vieira et al., 2010).

2. 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).

2.1. Animals and treatments

Ten non-lactating, non-pregnant, and ovariectomized Gir × Holstein cows (mean ± SE; BW = 640 ± 27 kg and BCS = 3.4 ± 0.2) were assigned to the experiment (d −14 to 27). On d 0, cows were ranked by BW and BCS (Wildman et al., 1982), and randomly assigned to one of two treatments: (1) bovine somatotropin (**BST**; $n=5$) or (2) saline control (**control**; $n=5$). Cows assigned to the BST treatment were administered s.c. injections containing 500 mg of sometribove zinc (Lactotropin®; Elanco Saúde Animal, São Paulo, Brazil) on d 0, 9, and 18 of the experiment, whereas control cows concurrently received a 10-mL s.c. injection of 0.9% saline.

Cows were maintained in a *Brachiaria brizantha* pasture from d −14 to d 27, and individually received (as-fed basis) 2 kg/cow daily of a concentrate from d −14 to −3, and 4 kg/cow daily of the same concentration from d −2 to 27, through self-locking head gates at 0800 h. The 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. 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. Nutritional content of concentrate and pasture were estimated to be 76 and 53% TDN, 22.9 and 7.1% CP, and 12.5 and 76.4% NDF from samples collected prior to the experiment and analyzed by a bromatology laboratory (São Paulo State University, Botucatu, Brazil).

2.2. Progesterone implants, sampling, and blood analysis

From d −14 to −2, cows were inserted with a previously used (third 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 −2, which remained in the cows until the end the experiment (d 27).

Cow BW and BCS were assessed on d −14, 0, and 27. Blood samples were collected daily from d 0 to d 27, at 0 (immediately before), 1, and 2 h relative to concentrate feeding for determination of serum glucose, insulin, NEFA, P4, and IGF-I concentrations. Blood samples were 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, maintained at 4 °C for 24 h to allow clotting, and centrifuged at 3000 × 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 (Katal Biotecnológica Ind. Com. Ltda.; Belo Horizonte, Brazil). Insulin and P4 concentrations were determined using Coat-A-Count kits (DPC Diagnostic Products Inc., Los Angeles, CA) solid phase ^{125}I RIA previously used for bovine samples (Moriel et al., 2008). Concentrations of NEFA were determined using an enzymatic colorimetric kit (Randox Brasil Ltda., São Paulo, Brazil). Concentrations of IGF-I were determined using an immunometric chemiluminescence immunoassay (Immulite 2000 IGF-I Assay, Erlangen, Germany) previously used for bovine samples (Falkenberg et al., 2008). The intra- and inter-assay CV were, respectively, 7.4 and 3.8% for glucose, 5.8 and 7.1% for NEFA, 9.2 and 9.7% for insulin, and 6.8 and 3.7% for P4. All samples were analyzed for IGF-I concentration within a single assay, and the intra-assay CV was 6.4%.

2.3. Revised quantitative insulin sensitivity check index

Concentrations of glucose, NEFA, and insulin obtained prior to feeding (0 h) were used to determine pre-prandial revised quantitative insulin sensitivity check index (**RQUICKI**). This methodology has been used to estimate insulin sensitivity in dairy cows (Holtenius and Holtenius, 2007; Gross et al., 2011; Grünberg et al., 2011), according to the equa-