



# Effects of excessive energy intake and supplementation with chromium propionate on insulin resistance parameters, milk production, and reproductive outcomes of lactating dairy cows



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## ABSTRACT

This experiment compared insulin resistance parameters, milk production, and reproductive outcomes in: (1) lactating dairy cows consuming adequate or excessive energy, and (2) lactating dairy cows consuming excessive energy and receiving or not Cr-propionate supplementation. Seventeen primiparous and multiparous, non-pregnant, lactating Holstein cows (initial days in milk =  $76 \pm 2$ ) were assigned on d 0 to: (1) concentrate intake to meet their requirements of net energy for lactation ( $NE_L$ ) without Cr supplementation (MAN;  $n=5$ ), (2) concentrate intake to exceed their  $NE_L$  requirements without Cr supplementation (HIGH;  $n=6$ ), and (3) HIGH with 2.5 g/d of Cr propionate (HIGHCR;  $n=6$ ). Throughout the experiment (d 0 to 210), cows were offered corn silage for ad libitum consumption, and individually received a corn-based concentrate twice daily. Concentrate intake was formulated to allow diets to provide 100% of daily  $NE_L$  requirements of MAN, and 160% of daily  $NE_L$  requirements of HIGH and HIGHCR cows. Cow BW, BCS, and milk production were recorded weekly. Blood samples were collected weekly, prior to and at 2 and 4 h after the morning concentrate feeding. Six glucose tolerance tests (GTT) were performed, every 42 d, by infusing cows with 0.5 g of glucose/kg of BW. Follicle aspiration for in vitro embryo production was performed via transvaginal ovum pick-up 2 d after each GTT. Increase in BCS from d 0 to 210 was greater ( $P \leq 0.04$ ) in HIGH and HIGHCR vs. MAN. Milk production was similar ( $P=0.92$ ) between treatments. Within weekly samples, serum non-esterified fatty acids concentration were greater ( $P \leq 0.05$ ) for MAN vs. HIGH and HIGHCR. Serum insulin concentrations and insulin:glucose ratio were often greater ( $P \leq 0.05$ ) for HIGH, intermediate for HIGHCR, and lesser for MAN (treatment  $\times$  day interaction,  $P < 0.01$ ). During the GTT, serum insulin concentrations and insulin:glucose ratio were greater ( $P \leq 0.05$ ) for HIGH compared with HIGHCR and MAN from 10 to 60 min relative to the time of glucose infusion. Proportion of embryo produced per oocyte collected was greater ( $P \leq 0.02$ ) for MAN vs. HIGH and HIGHCR, and similar ( $P=0.59$ ) between HIGH and HIGHCR. In conclusion, lactating cows consuming excessive concentrate and  $NE_L$  experienced increased insulin resistance and reduced proportion of embryo produced per oocyte collected compared with cows consuming adequate amounts of energy, whereas Cr-propionate supplementation was effective in alleviating insulin resistance caused by excessive  $NE_L$  intake.

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## 1. Introduction

Inadequate nutrient intake is known to impair performance and welfare of dairy cattle (Van Saun and Sniffen, 1996). As an example, dairy cows often experience increased insulin resistance during the postpartum period, which is largely due to deficient

energy intake (Pires et al., 2007; Sinclair, 2010). Recently, our research group demonstrated that excessive energy intake also increases insulin resistance in non-lactating dairy cows (Leiva et al., 2014). This syndrome, characterized by persistent hyperinsulinemia, has been negatively associated with milk production, reproduction, and health parameters of dairy cattle (Adamiak et al., 2005; LeBlanc, 2010). However, the impacts of excessive energy intake on insulin resistance and production parameters in lactating dairy cattle are still unknown and warrant investigation, given that excessive energy intake is a common concern among late-lactating dairy cows (Van Saun and Sniffen, 1996).

Chromium is a critical component of the glucose tolerance

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factor and facilitates the action of insulin on body cells (Mertz, 1992) by enhancing auto-amplification of insulin signaling, maintaining the active conformation of insulin receptors, and thus promoting greater glucose uptake (Vincent, 2001). Accordingly, Cr supplementation has been shown to reduce insulin resistance in periparturient dairy cows under negative energy balance, particularly when an organic source of Cr is used (Subiyatno et al., 1996; Hayirli et al., 2001). Leiva et al. (2014) also reported that Cr-propionate supplementation prevented the increase in insulin resistance caused by excessive energy intake in non-lactating dairy cows. However, no research has yet evaluated this relationship in lactating dairy cows consuming excessive energy. Based on this information, we hypothesized that excessive energy intake increases insulin resistance in lactating dairy cows, and Cr-propionate supplementation is an alternative to alleviate this outcome. Hence, this experiment compared insulin resistance parameters, milk production, and reproductive outcomes in: (1) lactating dairy cows consuming adequate or excessive energy, and (2) lactating dairy cows consuming excessive energy and receiving or not Cr-propionate supplementation.

## 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 and approved by the São Paulo State University Animal Ethics Committee.

### 2.1. Animals and diets

Seventeen lactating, primiparous ( $n=8$ ) and multiparous ( $n=9$ ), non-pregnant Holstein cows (initial mean  $\pm$  SE; body weight [BW]= $537 \pm 22$  kg, body condition score [BCS]= $3.07 \pm 0.08$ , milk yield= $26.0 \pm 1.2$ , and days in milk= $76 \pm 2$ ) were assigned to the experiment (d – 15 to 210). On d 0, cows were ranked by parity, days in milk, milk yield, BW and BCS (Wildman et al., 1982), and assigned to one of three dietary treatments in a manner in which all treatment groups had equivalent parity distribution, and initial average days in milk, milk yield, BW and BCS: (1) concentrate intake to meet their requirements of net energy for lactation ( $NE_L$ ) without Cr supplementation (MAN;  $n=5$ ), (2) concentrate intake to exceed their  $NE_L$  requirements without Cr supplementation (HIGH;  $n=6$ ), and (3) HIGH with 2.5 g/daily of Cr-propionate (HIGHCR;  $n=6$ , with 10 mg of Cr/cow daily; KemTrace 0.4% Cr; Kemin Agrifoods South America, Indaiatuba, São Paulo, Brazil). All dietary treatments were formulated to exceed CP, mineral, and vitamin requirements (National Research Council (NRC) 2001), hence focusing treatment differences on  $NE_L$  intake.

Cows were maintained, according to parity, in two drylot pens with ad libitum access to corn silage, water, and a commercial mineral mix without the inclusion of Cr (22% Ca, 7.5% P, 6.5% Na, 1.0% K, 3.6% Mg, 2.0% S, 0.003% Co, 0.115% Cu, 0.004% I, 0.220% Mn, 0.003% Se, 0.400% Zn, 400,000 IU/kg of vitamin A, 100,000 IU/kg of vitamin D3, and 0.150% of vitamin E) from d – 15 to 210. Corn silage was provided in feed bunks that allowed 1.5 m of linear bunk space/cow and offered at daily rates to result in  $\geq 15\%$  (DM basis) of non-consumed silage, whereas the maximum daily provision of corn silage during the experiment was 13.9 and 11.6 kg of DM/cow for the multiparous and primiparous pens, respectively. Cows were milked twice daily in a side-by-side milking system (0600 and 1700 h), and individually received a concentrate through self-locking head gates immediately after milking. Concentrate composition was (as-fed basis) 40.4% of soybean meal, 56.6% of ground corn, and 3.0% of the aforementioned commercial

mineral mix. Twice monthly, 1 sample of the offered corn silage and 1 sample of the offered concentrate were collected. Samples of the same feedstuff were pooled into a single sample at the end of the experiment and analyzed for nutrient content via wet chemistry procedures by a bromatology laboratory (3rlab, Belo Horizonte, Brazil). Calculations of  $NE_L$  and  $NE_M$  used the equation proposed by the National Research Council (NRC) (2001). Concentration of DM was 40.5% in corn silage and 90.3% in the concentrate. Nutritive value (DM basis) was 5.81 MJ/kg of  $NE_L$ , 5.81 MJ/kg of  $NE_M$ , and 8.1% crude protein for corn silage, and 9.37 MJ/kg of  $NE_L$ , 9.37 MJ/kg of  $NE_M$ , and 28.5% crude protein for concentrate.

From d – 15 to 0, all cows received the aforementioned concentrate without Cr supplementation as an adaptation period, whereas concentrate intake was formulated to each individual cow so the diet (concentrate + corn silage) provided 100% of their  $NE_L$  requirements. From d 0 to 210, MAN cows continued to receive concentrate in amounts to allow their diets to provide 100% of their  $NE_L$  requirements, whereas HIGH and HIGHCR cows received concentrate in amounts to allow their diets to provide 160% of their  $NE_L$  requirements. Concentrate intake was adjusted weekly throughout the experimental period (d – 15 to 210) using the Spartan Dairy Ration Evaluator/Balancer (version 3.0; Michigan State University, East Lansing, MI, USA), according to treatment, parity, days in milk, milk yield, BW, and BCS, and corn silage intake estimated by the software. Chromium-propionate was offered in the amount recommended by the manufacturer (2.5 g/cow daily of KemTrace; Kemin Agrifoods South America) and similar to previous research from our group (Leiva et al., 2014), mixed with 97.5 g of finely ground corn and top-dressed daily into the morning concentrate feeding of each HIGHCR cow. Finely ground corn (97.5 g/cow) was also top-dressed into the morning concentrate feeding of HIGH and MAN cows, but without the addition of the Cr-propionate. Propionate intake was not adjusted among treatments because 2.5 g of additional propionate likely yields less variation on the variables assessed herein compared with the variation caused by sampling and measurement procedures. All cows completely consumed their concentrate allocation within 30 min after feeding.

### 2.2. Sampling

Cow BW, BCS, and milk production were recorded weekly during the experimental period (d – 15 to 210). These parameters were used to adjust concentrate intake of each cows on a weekly basis. Further, BCS was evaluated (Wildman et al., 1982) by the same two evaluators throughout the experiment, and evaluators were blinded to which treatment the assessed cow was assigned to.

Blood samples were collected weekly, prior to (0 h) and at 2 and 4 h after the morning concentrate feeding during the experiment for determination of serum glucose, insulin, and non-esterified fatty acids (NEFA; 0 h only) concentrations. Insulin to glucose ratio (I:G) was determined by dividing insulin and glucose concentrations within each sampling time (Bernhard et al., 2012). 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 (Grünberg et al., 2011), which is an approach to assess insulin resistance according to the equation proposed by Perseghin et al. (2001):  $RQUICKI = 1 / [\log(\text{glucose}) + \log(\text{insulin}) + \log(\text{NEFA})]$ .

Glucose tolerance tests (GTT) were performed on d – 2, 40, 82, 124, 166, and 208 of the experiment by intravenously infusing cows with 0.5 g of glucose/kg of BW. More specifically, cows were weighed the day before each GTT, and had access to water but

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