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Effects of body weight loss on serum progesterone concentrations of non-lactating dairy cows

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Abstract

The objective was to evaluate serum concentrations of nonesterified fatty acids (NEFA), cortisol, insulin, and progesterone (P4) of dairy cows maintaining or mobilizing body weight (BW). Eleven non-lactating, non-pregnant, and ovariectomized Gir × Holstein cows were stratified by BW and body condition score (BCS), and randomly assigned to: 1) BW loss (six cows; LOSS) and 2) BW maintenance (five cows; MAINT). Treatments were achieved through a grazing schedule using three pastures. From Days −7 to 1 of the study, all cows were maintained in Pasture A (12 kg of dry matter/cow daily). From Days 2 to 30, LOSS cows were maintained in Pasture B (less than 1.0 kg of dry matter/cow daily), whereas MAINT cows were maintained in Pasture C (12 kg of dry matter/cow daily). However, from Days 3 to 30 of the study, cows from both treatments were regrouped daily into Pasture A from 0600 to 1200 h to allow LOSS cows to consume, on average, 4.5 kg/d of forage dry matter. On Day −66 of the study, all cows received an intravaginal drug releasing device containing 1.9 g of P4 (replaced every 14 d and removed on Day 3). Cow BW and BCS were assessed on Day 0 and 30 and blood samples were collected daily from Days 0 to 30 at 0600 and 1200 h. Changes in BW and BCS were greater ($P \leq 0.05$) in LOSS cows compared to MAINT cows. Within samples collected at 0600 h, serum NEFA concentrations were often greater ($P < 0.05$) in LOSS cows compared to MAINT after Day 14. Serum P4 concentrations were greater ($P < 0.05$) on Days 21 and 22, and tended ($P < 0.10$) to be greater on Days 16, 23, and 24 of the study in LOSS cows compared to MAINT. In conclusion, BW loss was associated with increased circulating concentrations of P4 in non-lactating ovariectomized dairy cows; this was mainly attributed to fat mobilization and consequent release of P4 stored in adipose tissues.

Keywords: Adipose tissues; Weight loss; Body condition score; Dairy cows; Progesterone

1. Introduction

Negative energy balance is highly associated with reduced reproductive performance of postpartum lactating dairy cows [1–3]. In addition to the well-documented detrimental effects of inadequate nutritional status on estrus resumption, fertility, and pregnancy maintenance [4], mobilization of fat can also have direct consequences on reproductive function of cattle. More specifically, substantial quantities of progesterone (P4) can be stored in adipose tissues of cows, and released into the circulation if these tissues are mobilized [5]. In fact, fat tissues in mid-estrous dairy cows may contain up to 10 times more P4 compared with equivalent weight amounts of plasma [6].

Progesterone is an integral hormone in many reproductive processes including attainment of puberty, resumption of estrous cycles, and also establishment and

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maintenance of pregnancy [7–9]. Several researchers have reported that blood P4 concentrations in cattle before or after breeding have been positively associated with conception rates [10–12]. However, elevated P4 before first postpartum ovulation can impair GnRH secretion and thus prevent the LH peak, potentially leading to failed ovulation and formation of ovarian cysts [13–15]. Development of follicular cysts is a common reproductive disorder in the dairy industry [16], affecting up to 19% of dairy cows [17–19], and impairing their reproductive performance by increasing the service period from 22 to 64 d [20,21].

Throughout pregnancy, cows are exposed to elevated circulating P4 concentrations. A considerable portion of this P4 may be sequestered by adipose tissue [5] and released into the bloodstream as body fat is mobilized in response to negative energy balance early postpartum. The resulting circulating P4 could inhibit ovulation and lead to the formation of ovarian follicular cysts [15]. To our knowledge, no in vivo studies have investigated the effects of body fat mobilization on circulating P4 concentrations. A better understanding of this mechanism will provide important information toward the enhancement of reproductive efficiency in dairy cattle. Based on this rationale, we hypothesized that dairy cows experiencing body weight (BW) loss would have increased circulating P4 from adipose tissues compared to cohorts maintaining BW. To test this hypothesis, we compared serum concentrations of non-esterified fatty acids (NEFA), cortisol, insulin, and P4 of non-lactating, ovariectomized dairy cows maintaining or losing BW.

2. Materials and methods

This study was conducted at the São Paulo State University—Lageado Experimental Station, located in Botucatu, São Paulo, Brazil. The cows 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 [22].

2.1. Animals and diets

Eleven non-lactating, non-pregnant, and ovariectomized Gir × Holstein cows (BW = 653 ± 23.3 kg; body condition score [BCS] = 4.3 ± 0.07) were stratified by BW and BCS, and randomly assigned to one of two treatments on Day −7 of the study: 1) BW loss (6 cows; LOSS) and 2) BW maintenance (5 cows; MAINT). Cows were evaluated for BCS in a 1–5 scale with 0.25 increments [23]. Treatments were achieved through a grazing schedule using three pastures (A, B and C), and designed according to the Cornell Net Carbohydrate and Protein System model [24] to induce BW loss (−0.9 kg/d) in LOSS cows, and BW maintenance in MAINT cows. From Days −7 to 1 of the study, all cows were maintained in Pasture A, a 4.5-ha *Brachiaria brizantha* pasture with adequate forage quality (average of 53% total digestible nutrients, 7.1% crude protein, and 76.4% neutral-detergent fiber; dry matter [DM] basis) and availability (average of 12 kg of DM/cow daily). From Days 2 to 30 of the study, LOSS cows were maintained on Pasture B, a 0.5 ha *B. brizantha* pasture with minimal forage availability (less than 1.0 kg of DM/cow daily), whereas MAINT cows were maintained in Pasture C, a 6.9 ha *B. brizantha* pasture with similar forage quality and availability compared to Pasture A. However, from Days 3 to 30, cows from both treatments were re-grouped daily into Pasture A from 0600 to 1200 h to allow LOSS cows to consume, on average, 4.5 kg/d of forage DM.

Both groups 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 ad libitum throughout the study. From Days −7 to 30, forage mass from each pasture was evaluated once a week before and after grazing, based on techniques previously described [25] but using six 1 m^2 quadrats\ha. Forage intake was estimated by comparing forage mass before and after grazing, whereas forage samples were collected weekly and analyzed for nutritional content by a bromatology laboratory (São Paulo State University—Botucatu, SP, Brazil).

2.2. Progesterone implants

All cows received an intravaginal progesterone releasing device (CIDR, containing 1.9 g of P4; Pfizer Animal Health, Sao Paulo, SP, Brazil) on Day −66. The CIDRs were replaced at 14 d intervals to elevate circulating P4 concentrations and stimulate P4 uptake by adipose tissues. On Day 3, CIDRs were removed, and cows remained without an exogenous P4 source until the end of the study.

2.3. Sampling and blood analysis

Both BW and BCS were assessed on Days 0, 9, 16, 23, and 30 of the study to evaluate treatment effects on these variables. Values obtained on Days 0 and 30 were used to calculate BW and BCS change. Blood samples were collected daily, from Days 0 to 30, at 0600 and 1200 h, to determine serum concentrations of NEFA,

insulin, cortisol, and P4. This sampling schedule was adopted to determine the effects of continuous BW loss on the serum measurements evaluated herein (0600 h samples), and also if forage intake of LOSS cows from 0600 h to 1200 h would alleviate some of the physiological consequences of BW loss (1200 h samples). Samples were analyzed for NEFA to evaluate adipose tissue mobilization [26], insulin to evaluate cow nutritional status [27–29] cortisol to evaluate a potential adrenal contribution to circulating P4 [30], and P4 to test our main hypothesis.

Blood samples were collected via coccygeal vein or artery into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ, USA), placed immediately on ice, and centrifuged at 3000 × *g* for 30 min for serum collection. Within 2 h after collection, harvested serum was stored frozen at −20 °C and remained frozen until further processing. A quantitative colorimetric kit was used to determine concentrations of NEFA (NEFA 30T; Randox Brasil Ltda., São Paulo, SP, Brazil). Concentrations of cortisol, P4, and insulin were determined using Coat-A-Count kits (DPC Diagnostic Products Inc., Los Angeles, CA, USA) solid phase ^{125}I RIA that were previously validated for bovine samples [31,32]. The intra- and inter-assay coefficient of variations were, respectively, 9.2 and 6.9% for cortisol, 6.3 and 7.3% for P4, and 9.2 and 6.9% for insulin. The minimum detectable concentrations were 0.1 ng/mL of P4, 0.5 ng/mL for cortisol, and 0.002 ng/mL of insulin.

2.4. Statistical analyses

Data were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC, USA) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement used for BW and BCS change contained the effects of treatment. Data were analyzed using cow(treatment) as a random variable. The model statement used for serum measurements contained the effects of treatment, day, hour, and the resultant interactions. Data were analyzed using cow(treatment) as a random variable. The specified term for the repeated statement was day with cow(treatment) as subject, whereas the covariance structure utilized was compound symmetry, which provided the best fit for these analyses according to the Akaike information criterion. All results are reported as least squares means. Means were separated using least square differences. Results are reported according to treatment effects if no interactions were significant, or according to the highest-order interaction detected. Pearson correlation coefficients were calculated between individual mean serum concentrations of cortisol, P4, and NEFA from Days 16 to 24 of the study. The GLM procedure was used to determine effects of treatment and collection hour on correlation coefficients. No significant effects or interactions were detected; therefore correlation coefficients reported herein were determined across treatments and collection hours. For all analyses, significance was set at $P \leq 0.05$, and tendencies were declared if $P > 0.05$ and ≤ 0.10.

3. Results

As expected, there was a treatment effect ($P = 0.04$) for BW change. All LOSS cows lost BW during the study (−0.95 kg of BW/d; SE = 0.21) whereas BW was nearly unchanged for MAINT cows (−0.07 kg of BW/d; SE = 0.23). Similarly, a treatment effect was detected for BCS ($P = 0.05$). All LOSS cows lost BCS during the study (−0.30 of BCS change; SE = 0.088), whereas BCS was unchanged (0.00 of BCS change; SE = 0.097) for MAINT cows.

There was a treatment × day × hour interaction ($P = 0.02$) for serum NEFA concentrations, since a treatment × day interaction was detected ($P < 0.01$; Fig. 1) for samples collected at 0600 h, but not for samples collected at 1200 h ($P = 0.87$; data not shown). At 0600 h, serum NEFA concentrations were greater for LOSS cows compared with MAINT cows during the majority of the study (Fig. 1). For samples collected at 1200 h, NEFA concentrations were similar ($P = 0.97$) between LOSS and MAINT cows (0.286 vs. 0.284 mmol/L; SEM = 0.0211).

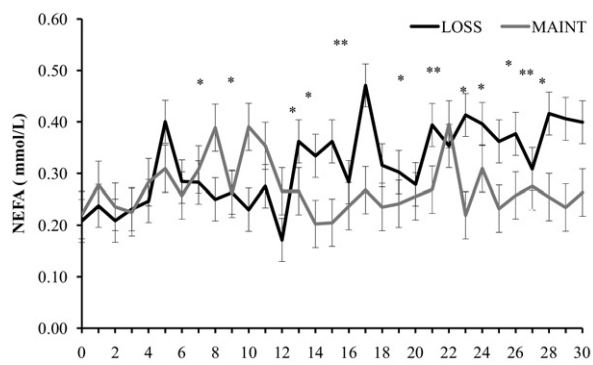

Fig. 1. Serum concentrations of NEFA in non-lactating ovariectomized dairy cows losing BW (LOSS) or maintaining BW (MAINT). Blood samples were collected daily at 0600 h. Treatment × day interaction ($P = 0.01$). Treatment comparison within time: **$P < 0.01$ and *$P < 0.05$.

A treatment × hour interaction was detected (P = 0.05) for serum insulin concentrations, mainly because an hour effect was detected for MAINT cows (P < 0.01), but not for LOSS cows (P = 0.25). Within MAINT cows, serum insulin concentrations were greater (P < 0.01) at 0600 h compared with 1200 h (0.231 vs. 0.179 ng/mL; SEM = 0.0615). Within LOSS cows, serum insulin concentrations were similar (P = 0.25) at 0600 h compared with 1200 h (0.145 vs. 0.130 ng/mL; SEM = 0.0562). No treatment effects were detected for serum insulin concentrations within sampling hours (P = 0.33 and 0.57 for 0600 and 1200 h, respectively).

There was a treatment × day interaction (P = 0.05) for serum P4 concentrations (Fig. 2). For both treatments, serum P4 concentrations decreased similarly and rapidly after CIDR removal (Day 3), and remained at negligible concentrations until Day 8. From Days 9 to 15, serum P4 concentrations increased within LOSS cows (day effect; P < 0.01), but remained consistently low within MAINT cows (day effect; P = 0.72), although daily means did not differ between treatments during this period (P > 0.14; Fig. 2). However, serum P4 concentrations were greater (P < 0.05) on Days 21 and 22, and tended (P < 0.10) to be greater on Days 16, 23, and 24 of the study in LOSS cows compared to MAINT (Fig. 2). Beginning on Day 25, P4 concentrations for LOSS cows decreased and reached negligible concentrations similar to those detected for MAINT cows.

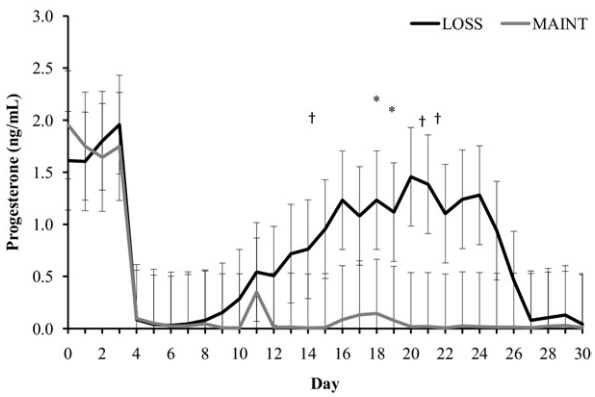

Fig. 2. Serum concentrations of progesterone in non-lactating ovariectomized dairy cows losing BW (LOSS) or maintaining BW (MAINT). All cows were inserted with an intravaginal progesterone releasing device containing 1.9 g of progesterone and replaced at 14-d intervals, from Days −66 to 3 of the study. Treatment × day interaction (P = 0.05). Treatment comparison within time: *P < 0.05, and †P < 0.10.

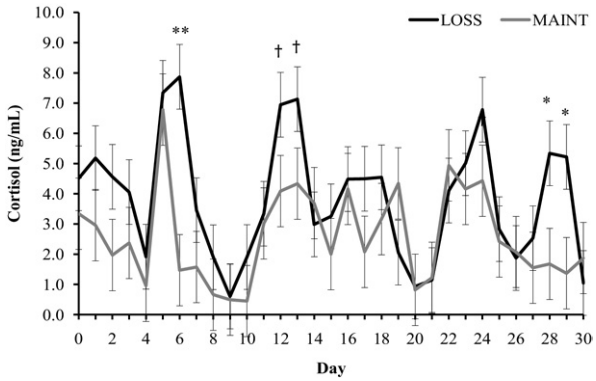

Fig. 3. Serum concentrations of cortisol in non-lactating ovariectomized dairy cows losing BW (LOSS) or maintaining BW (MAINT). Treatment × day interaction (P = 0.05). Treatment comparison within time: **P < 0.01, *P < 0.05, and †P < 0.10.

There was a treatment × day interaction (P = 0.05) for serum cortisol concentrations (Fig. 3), given that LOSS cows had greater (P < 0.05) cortisol concentrations compared with MAINT cows on Days 6, 28, and 29 of the study, whereas tendencies for the same outcome were detected on Days 12 and 13 of the study (Fig. 3).

Serum concentrations of NEFA and cortisol were positively correlated (P = 0.02; r = 0.66). There were no significant correlations among serum concentrations of P4 and NEFA (r = −0.26; P = 0.43), and P4 and cortisol (r = −0.45; P = 0.16), even when analyzed within LOSS cows only (P = 0.29 and 0.23, respectively).

4. Discussion

The treatment × day × hour interaction detected for NEFA in the present study was consistent with previous research indicating that BW loss increased circulating NEFA concentrations due to lipid mobilization, whereas re-alimentation promptly alleviated lipolysis and consequent release of NEFA into the blood [26,33]. Conversely, the reason for the lack of substantial treatment differences regarding insulin was unknown and was not expected, given that cattle mobilizing body tissues have been reported to have lower insulin concentrations compared with cattle maintaining BW and BCS [34]. In the present study, cows were offered forage-based diets, which do not stimulate insulin synthesis and secretion as much as grain-based diets [35–37], which may have contributed to the lack of treatment effects on serum insulin concentrations.

The treatment × day interaction detected for P4

supported our hypothesis, given that LOSS cows experienced elevated P4 concentrations during the study, but particularly after Day 16 when they also had greater serum NEFA concentrations compared with MAINT cows. However, serum concentrations of NEFA and P4 were not correlated from Days 16 to 24 when treatment effects on serum P4 and NEFA were concurrently detected, even when analyzed within LOSS cows only. Still, the mechanism by which P4 is released from adipose tissues and if NEFA is involved in this process is still unknown, and deserves further investigation [5]. The lack of treatment effects on serum P4 from Days 25 to 30 was attributed to depleted P4 reserves in adipose tissues of LOSS cows, given that cows from both treatments still had adequate BCS (4.06 vs. 4.33 BCS for LOSS and MAINT cows, respectively; $P = 0.07$, SEM = 0.095) and consequent fat reserves at the end of the study. However, P4 concentrations in fat tissues were not evaluated in the present study to support this assumption, which thus requires further investigation.

Nevertheless, it can also be speculated that BW loss elicited a stress reaction and stimulated synthesis of P4 by the adrenal gland, contributing to the increased serum P4 concentrations detected for LOSS cows in the present study. Indeed, BW loss stimulated by feed-restriction has been associated positively with adrenal synthesis of cortisol [38–40], whereas the adrenal gland is also capable of synthesizing significant amounts of P4 as an intermediate of corticosteroid synthesis when stimulated by stressors [30,41]. In the present study, serum concentrations of NEFA and cortisol were positively correlated, and LOSS cows had greater cortisol concentrations compared with MAINT cows on Days 6, 28, and 29 (Fig. 3). However, serum concentrations of P4 and cortisol were not correlated, even when analyzed within LOSS cows only, which suggested that adrenal contribution to circulating P4 was irrelevant in the present study [30,42,43]. Further, treatment differences on serum cortisol concentrations were not detected from Days 16 to 24 of the study, when LOSS cows had greater P4 concentrations compared to MAINT cows. Therefore, we concluded that treatment effects detected on serum P4 concentrations were mainly due to BW loss and consequent mobilization of P4 stored in fat tissues, given that cows were ovariectomized and did not receive any exogenous source of P4 after Day 3. Still, additional research is required to further investigate the mechanisms by which P4 is released from fat, because serum NEFA and P4 concentrations were not correlated in the present study.

In conclusion, BW loss increased circulating concentrations of NEFA and P4 in non-lactating ovariectomized dairy cows during the present study. At parturition, lactating cows typically experience a negative energy balance and can mobilize up to 1 kg of adipose tissue daily [44]. As a result, P4 stored in adipose tissues during gestation is released into the circulation [5], whereas serum P4 concentrations can be increased up to 1.5 ng/mL, as observed herein. This physiological mechanism may explain why BW loss could be associated with interval to first ovulation in dairy cattle [45], and the formation of ovarian cysts [13–15]. Further, spontaneous recovery of cystic ovaries is common in dairy cows [46], which can be attributed, at least in part, to reduced BW mobilization due to advance in lactation, and also depletion of P4 stored in adipose tissues. However, additional research should be conducted to better understand *in vivo* the mechanisms involving P4 sequestered by adipose tissues during gestation, BW loss during early lactation, P4 release by adipose tissues, and the consequent effects on reproductive function of lactating dairy cows.

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