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Association of changes among body condition score during the transition period with
NEFA and BHBA concentrations, milk production, fertility, and health of Holstein cows


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ABSTRACT

Our objective was to evaluate the association between body condition score (BCS) change during the transition period with fertility, non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BHBA) concentrations, milk yield, and health problems of Holstein cows in a retrospective cohort study. Holstein cows (n = 232) were assessed for BCS (5 point scale; 0.25 point increments) and had blood collected at 21 and 7 d before, on the day of, and 7 and 21 d after calving. Blood samples were assayed for NEFA and BHBA concentrations. All cows received a timed artificial insemination (TAI) at 65 ± 3 days in milk (DIM) following a Presynch-Ovsynch protocol with a progesterone implant during the Ovsynch protocol. Cows were grouped based on BCS change after calving as to whether they: 1) lost (L), 2) maintained
(M), or 3) gained (G) BCS. Data were analyzed by logistic regression with GLIMMIX and ANOVA with repeated measures using the MIXED procedures of SAS. Both NEFA and BHBA concentrations after calving differed (P < 0.01) for cows that lost, maintained, or gained BCS from 21 d before to 21 d after calving (NEFA: 0.51 ± 0.01; 0.45 ± 0.01; 0.42 ± 0.01 mmol/L; BHBA: 0.73 ± 0.02; 0.70 ± 0.02; 0.68 ± 0.02 mmol/L; respectively; mean ± SEM). By design, BCS change after calving differed (P < 0.01) among groups and was -0.38; 0.00; and 0.35 for cows in groups L, M, and G, respectively. At 21 d before and 21 d after calving, BCS differed (P < 0.01) among groups and was \{before (2.97, 2.70, and 2.57) and after (2.54, 2.70, and 2.90)\} for cows in groups L, M, and G, respectively. Between evaluated days, higher circulating NEFA and BHBA concentrations were observed 7 d after calving. Change in BCS affected (P < 0.01) pregnancy/AI (P/AI), days to first ovulation, and percentage of cyclic cows at 50 DIM. At 32 d after TAI, P/AI differed (P < 0.01) for cows that lost [18% (11/84)], maintained [33% (26/80)], or gained [47% (32/68)] BCS. Cows that lost BCS during the transition period had more health events (P < 0.01), than cows that gained or maintained BCS. In conclusion, changes in BCS during the transition period affected NEFA and BHBA concentrations, fertility, and occurrence of health problems during the lactation.

**Keywords:** BCS, energy balance, diseases, fat mobilization, lactating cows, reproductive performance.
1 Introduction

The transition period, defined as the period from 3 wk before to 3 wk after calving, represents a challenge for dairy cows as milk production and dry matter intake (DMI) increase dramatically [1, 2]. Metabolic and hormonal changes occurring during the transition period in dairy cows entail numerous changes, with direct effects on health, productivity, and reproductive performance. During the transition period, dairy cows go through a period of negative energy balance (NEB), characterized by increased mobilization of energy reserves, specially fat and protein, to meet the demands of high milk production. High-producing dairy cows, on average, go through a period of NEB during the first weeks of lactation, when the energy demands for milk production exceed the energy intake obtained from the diet. Thus, during early lactation, fat reserves in some cows are mobilized into the bloodstream in the form of non-esterified fatty acids (NEFA) and contribute to overall energy requirements [3]. In the liver, some NEFA are oxidized or reesterified into triglycerides that are either exported as very low density lipoproteins or stored [4]. Measurement of NEFA and beta-hydroxybutyrate (BHBA) can be performed as indices of NEB or ketosis in transition animals [3] and excessive elevation of NEFA or BHBA can indicate metabolic problems [5]. Circulating NEFA concentrations and DMI usually have an inverse relationship [6]. Excess fat mobilization and NEB can attenuate function of the immune system [7], and are associated with negative effects on animal health and production [8, 9]. Maintaining health and productivity during the transition period is one of the greatest challenges dairy herds face.

Evaluation of body condition score (BCS) is a useful management tool to assess body fat stores of Holstein dairy cows [10-12]. Body condition score has received considerable attention
as a tool to aid in the management of nutritional programs in dairy herds [11, 13]. Roche et al. (2009) [13] noted that the BCS of cows at calving, the nadir BCS, and the post-partum BCS loss are associated with differences in milk production, reproduction, and health. Overconditioned cows with a BCS greater than 4.0 at calving had higher circulating concentrations of NEFA in early lactation until 7 wk postpartum compared with cows with moderate or low BCS [14]. Ketonemia, in turn, caused insulin resistance in dairy cows [15], consistent with studies linking high BCS to reduced peripheral insulin sensitivity in the lipomobilization state [16-18].

Multiple studies have reported a negative relationship of NEFA with reproduction. One experiment found that increased NEFA concentrations during the transition period were associated with decreased pregnancy rate at 70 d after the voluntary waiting period [3], whereas another found that high circulating NEFA was associated with a reduced 21-d pregnancy rate in herd level evaluations of 60 freestall herds [19]. In addition, a third study with 156 lactating dairy cows [20] reported that the probability of pregnancy at first timed AI (TAI) was decreased as serum NEFA concentrations on Day 3 postpartum increased. There are also other studies that have described a negative relationship between postpartum NEFA or loss of BCS and fertility [21-23].

Thus, our objectives were to evaluate the association between changes in BCS during the transition period, beginning at 21 d before expected calving, near the time of calving, and at 21 d after calving, and compare to the changes in circulating NEFA, circulating BHBA, milk production, pregnancy at first postpartum AI, and diseases. Our hypotheses were: (1) cows gaining BCS during entire evaluated period would have greater milk yield and lower NEFA and BHBA concentrations, with decreased incidence of diseases during lactation; (2) cows with greater circulating NEFA and BHBA in the transition period would have delayed first
postpartum ovulation and decreased fertility at first postpartum TAI; and (3) cows losing more
BCS during the transition period would have lower fertility;

2 Materials and Methods

2.1 Animals and management

This study was approved by the Bioethics Committee of School of Veterinary Medicine and
Animal Science – University of São Paulo, in accordance with the ethical principles of animal
experimentation.

The experiment was conducted on a commercial farm in São Paulo State, Brazil, from
June 2011 to March 2012. Multiparous lactating dairy cows (n = 232) were housed in freestall
barns bedded with mattresses and equipped with self-locking head gates at the feed line, in a
cross ventilation system. Cows had ad libitum access to food and water. Cows were milked three
times daily and were fed a total mixed ration.

The diets were formulated according to NRC (2001) [24] to meet the nutritional
requirements of each period (pre and postpartum). All cows received recombinant bovine
somatotropin every 14 d (500 mg/dose; Lactotropin; Elanco Co.) beginning at 80 ± 3 DIM until
dry-off. All cows were synchronized using a Presynch-Ovsynch protocol for first TAI with a
progesterone implant during the Ovsynch [25]. Protocol of synchronization started when cows
had 65 (± 2.01) days in milk. Prostaglandin F2α (cloprostenol; 25 mg; Sincrocio) and GnRH
(buserelin acetate; 100 µg; Sincroforte) were from OuroFino Animal Health Inc (Cravinhos,
Brazil).
Data for health status of the cows (clinical mastitis, metritis, ketosis, and pneumonia) were collected from the day of calving to 250 DIM. Cows were evaluated for metritis on Day 7 ± 4 and Day 14 ± 4 postpartum by palpation. Metritis was characterized by an enlarged uterus with fetid watery red-brown discharge [26]. Cows with metritis had rectal temperature measured and those with a temperature ≥ 39.5°C were classified with puerperal metritis.

At every milking all cows were examined for signs of clinical mastitis by the herd personnel immediately before milking. Clinical mastitis was characterized by the presence of abnormal milk or by signs of inflammation in one or more quarters. Respiratory problems were characterized by increased respiratory frequency associated with fever and presence of increased lung sounds at auscultation. Cows were considered to have elevated circulating NEFA if the concentration was ≥ 0.70 mM and subclinical ketosis if the BHBA concentration was ≥ 0.96 mM [3] in at least one of the two samples.

2.2 Assay of NEFA and BHBA

Blood samples were collected weekly throughout the experimental period (-28 to 84 d in relation to parturition) from the coccygeal vein or artery, prior to morning feeding. Blood samples were also collected within the first 24 h after parturition. The samples were collected in evacuated tubes of 10 mL for NEFA and BHBA. After collection, the samples were centrifuged at 2000 × g for 15 min, and the supernatant serum was frozen. The analyses of NEFA and BHBA were performed using commercial kits (Randox FA115 and Rambut RB100, Crumlin, UK, respectively) using the enzymatic colorimetric endpoint method. The reading was performed in a microplate reader (Asys Brand, Model UV-Plus Expert, Biochrom, Cambridge, UK).
2.3 Body condition score and milk production

Cows had their BCS evaluated by a single person using a 5-point scale with 0.25 increments: 1 = thin to 5 = fat [27], on Day -21 and -7 (before calving) and Day 7 and 21 (after calving). Milk weights were recorded at each milking and stored in the on farm computer software program (Dairy COMP 305; Valley Agriculture Software, Tulare, CA). Daily milk weights were extracted and used to calculate weekly average from 21 to 77 DIM. Weekly, milk samples were collected for fat concentration evaluation, using an infrared absorption system (MilkScan FT+, Foss Electric, Hillerød, Denmark). Milk production was corrected for 3.5% fat (FCM) according to the formula of Sklan et al. (1992) [28], where FCM = (0.432 + 0.1625 × milk fat content) × kg of milk. Cows were grouped based on BCS change between Day -21 and Day 21 in relation to calving as to whether they: 1) lost (L), 2) maintained (M), or 3) gained (G) BCS.

2.4 Ultrasound evaluation

Cows were evaluated by transrectal ultrasonography twice a week (Aloka SSD-500 with a 7.5-MHz linear-array transducer, Aloka, Tokyo, Japan), between 20 ± 4 and 50 ± 4 DIM, to assess presence of a CL and to determine time of postpartum cyclicity resumption. The size of the ovulatory follicle was determined by the largest follicle present in the ovary on day of AI that corresponded to an observed CL 7 d later. Pregnancy diagnosis was performed 32 d after TAI by using a portable scanner (Aloka SSD-500 with a 7.5-MHz linear-array transducer, Aloka, Tokyo, Japan) and pregnancy status was reconfirmed 70 d after TAI by palpation of the uterus. Cows
diagnosed pregnant at first pregnancy examination and subsequently diagnosed not pregnant at
the subsequent pregnancy examination were considered to have undergone pregnancy loss.

2.5 Statistical analyses

Milk yield, BCS and circulating NEFA and BHBA data were processed by using a mixed-model
procedure for repeated measurements (PROC MIXED, version 9.3; SAS Institute Inc., Cary, NC). The model included the fixed effects of experimental group (G, M, or L), random effect of
parity, fixed effect of week in lactation, and interaction of group by week. The AIC fit statistics
were used to choose the covariance structure.

To analyze health events, pregnancy and pregnancy loss rates, and ovulatory follicle,
PROC GLIMMIX (version 9.3; SAS Institute Inc., Cary, NC) was used and the model included
the fixed effect of experimental group and the random effect of parity. Differences between least
squares means were determined with the PDIFF option of SAS. Significance was declared at P ≤
0.05 unless otherwise indicated.

The intervals from calving to first ovulation and proportion of cows that had not ovulated
by 200 DIM were analyzed by the Cox proportional hazards ratio model using the PHREG
procedure of SAS. Adjusted hazard ratio (AHR) and 95% CI were calculated. The hazard in this
analysis determined the day of postpartum ovulation within the first 50 DIM. The model
included the fixed effects of experimental group (G, M, or L) and the random effect of parity.

3 Results
The percentages of cows that gained, maintained, or lost BCS from -21 to 21 DIM were 28%, 22%, and 50%, respectively. At Day -21, the cows in the L group had the greatest BCS (2.97), following by M (2.70), and the G group (2.57) had the lowest BCS (P < 0.01; Table 1). The L group had greater percentage of cows with BCS > 3 on Day -21 (P < 0.01; Figure 1) than the other groups. However, all cows had similar BCS on Days -7 (2.71) and Day 7 (2.71). At Day 21 postpartum, BCS was greater for cows that gained (2.90), intermediate for cows that maintained (2.70) and lower for cows that lost (2.54) BCS (P < 0.01; Table 1).

Milk yield was similar among experimental groups (Table 2 and Figure 2), and cows averaged 45.9 kg/d. However, week of lactation affected milk production (P < 0.01). Milk yield increased from the 3rd to 6th week and then was maintained until week 11. Experimental groups had no effect on fat corrected milk and cows averaged 45.2 kg/d. Similarly, FCM increased from the 3rd to 6th week and remained high until week 11 (P < 0.01).

The NEFA and BHBA concentrations for the three experimental groups are presented in Table 3. Prepartum NEFA did not differ (Figure 2) among groups, and there was no time effect or group by time interaction. Similarly, prepartum BHBA concentration did not differ among groups (Figure 2), time, or for the interaction group by time.

In contrast, postpartum NEFA concentrations differed (P = 0.01) among groups (Table 3). Cows that gained and maintained BCS had lower concentrations in comparison with cows that lost BCS. There was an effect of time (P < 0.01), and a tendency for a group by time interaction (P = 0.07). The postpartum BHBA concentration also differed among groups (P = 0.02). The group of cows that gained BCS had lower concentrations compared with cows that lost BCS, but there was no time or group by time interaction.
There was no difference among experimental groups for incidence of metritis or pneumonia (Table 4). Similarly, the incidence of ketosis did not differ among groups. Cows that gained BCS tended (P = 0.09) to have less mastitis than cows that lost BCS. Moreover, when we evaluated cows that had one or more health event, cows that gained and maintained BCS had fewer health events than cows that lost BCS (P < 0.01; Table 4).

The percentage of cows cycling at 50 DIM (Table 5) differed among experimental groups (P = 0.015), and was greatest for cows that gained BCS (100%; 69/69), intermediate for cows that maintained BCS (94.4%; 51/54), and least for cows that lost BCS (81.1%; 99/122). However, there was no effect of group on size of the ovulatory follicle. Nevertheless, days in milk at first postpartum ovulation (Figure 3) differed (P < 0.01) among groups, and was lower for cows that gained BCS, intermediate for cows that maintained BCS, and greater for cows that lost BCS.

The pregnancy diagnoses at 30 or 60 days after TAI both showed large differences for P/AI (P < 0.01) among groups (Table 5). At the 32 d pregnancy diagnosis, cows that gained BCS had greater (P < 0.01; Table 5) P/AI (53.0%; 35/66) than cows that maintained (26.9%; 14/52) or lost BCS (15.7%; 18/155). Similarly at the 60 d pregnancy diagnosis; cows that gained BCS had greater P/AI than cows that maintained or lost BCS. However, P/AI based on either pregnancy diagnosis did not differ between cows that maintained BCS compared to cows that lost BCS. Pregnancy loss from 30 to 60 days after TAI was not different among groups (Table 5).

4 Discussion
Various measures of energy balance, including loss of BCS, have been found to have effects on fertility in lactation dairy cows in many previous investigations. A novel contribution of this study is that it shows the effect of BCS in the prepartum period, and changes in BCS during the transition period on fat mobilization, fertility, milk yield, and health of lactating dairy cows. Some studies have reported that there is an inverse relationship between the shape of the lactation curve and the changes in BCS [13, 29, 30], since peak milk production occurs before the body begins to replenish body reserves. However, our study found a somewhat different pattern when the entire transition period was evaluated. Cows that had BCS loss, were heaviest at 21 d before expected calving and subsequently had the greatest BCS loss before calving (-0.26) and continued to lose BCS during the first 21 d after calving (-0.15). In contrast, the cows that gained BCS had the lowest BCS at 21 d before calving (2.57) and subsequently gained BCS prior to calving (+0.15) and continued to gain BCS after calving (+0.18). Previous studies have only evaluated changes in BCS after calving but reported that cows that gained or maintained BCS post-partum had lower BCS at calving compared to cows that lost BCS after calving [21, 31]. Stockdale (2001) [32] also reported a negative relationship between BCS at calving and changes in BCS during early lactation. Bewley and Schutz (2008) [33], suggested that the ideal BCS may be lower in cows with greater genetic merit for milk production, potentially producing a genetic trend for thinner dairy cows. Dechow et al. (2002) [34], evaluated correlations between BCS and loss of BCS based on 310,000 lactation records, and found that greater BCS at calving was phenotypically associated with greater loss of BCS in early lactation. Thus, our results showing that heavier cows have greater BCS loss is clearly consistent with previous data, although our study found the BCS loss was initiated in heavy cows even prior to calving.
In cows with greater genetic merit for milk production, lipolysis can be greater due to increased response to β-adrenergic stimulation, increased activity of hormone sensitive lipase (LIPE), and decreased lipogenesis compared with average genetic merit animals [35-38]. Because of faster lipolysis, it is generally recommended that modern high producing dairy cows have a moderate BCS (> 2.5 and ≤ 3.0) at the beginning of the transition period, which consequently will cause them to have a lower mobilization of body reserves and concentration of NEFA and BHBA. Although differences on subcutaneous and visceral adipose tissue [39] and relevance of this for fat mobilization, BCS, which is highly associated with subcutaneous adipose tissue, is an good predictor of mobilization potential. In addition, cows that gained BCS after calving were already on an upward trajectory prior to calving; whereas cows that would lose BCS after calving were already on a downward trajectory. Of special interest in our study, the BCS at 21 d before calving was more informative for BCS loss/gain than the BCS near calving, when all of the three groups were found to have a similar BCS.

Previous studies have reported that NEFA and BHBA concentrations during the early post-partum period were associated with BCS at calving and losses in BCS after calving. For example, high BCS at calving or a rapid loss in BCS after calving, or both, were associated with poor cow health [40, 41], and high postpartum circulating NEFA and BHBA concentrations [14, 42]. The degree of NEB, which can be identified by an increase in circulating concentration of NEFA and BHBA, and excessive NEB were both found to have detrimental effects on health, potentially due to a relationship between post-partum energy deficits and immunosuppression [8, 9]. On the other hand, the majority of studies have reported a negative association between BCS and DMI, consistent with variation between cows in appetite and DMI being a major driver of the variation in BCS changes during the early post-partum period [43-45]. The mechanisms are
not yet well defined, but are consistent with the proposed lipostatic theory [46]. Thus, increased
BCS at calving has been correlated with the BCS loss after calving and the magnitude of the
post-partum NEB [47]. Our results are consistent with extending this theory to include cows with
greater BCS during the 21 d prior to expected calving will experience, on average, a greater
magnitude of BCS loss during the 21 d before calving as well as the 21 d after calving. This
effect seemed to be independent of level of milk production and therefore we speculate that it
may be primarily driven by pre-partum and post-partum DMI.

Negative energy balance decreases dominant follicle growth and estradiol production,
possibly due to decreases in circulating insulin, IGF1, and LH pulse-frequency [48-50]. There a
number of endocrine and metabolic pathways that regulate the reproductive axis including
insulin and IGF1 [51, 52]. According to Wiltbank et al. (2002) [53], anovulation can result from
strong inhibitory actions of low amounts of follicular estradiol on GnRH secretion from the
hypothalamus resulting in lack of sufficient follicular growth and estradiol production to induce
a GnRH and LH surge and subsequent ovulation. The presence of this estradiol-mediated
negative feedback may be directly related to energy balance and this physiologic scenario may
underlie the delayed time to first ovulation in cows with greater BCS loss that was observed in
our study and in previous studies [54-56]. In addition, anovular cows have lower reproductive
efficiency in programs using detection of estrus or FTAI protocols [55, 56]. In the current study,
BCS loss during the transition period was associated with greater circulating concentrations of
NEFA and BHBA and reduced reproductive performance. Similar to our results, Carvalho et al.
(2014) [21] reported that about half of cows either gain (22.4%) or maintain (35.8%) BCS
during the transition period and that these cows have much better reproductive performance than
cows that lose BCS during the first 21 d post-partum. They reported P/AI at the 70 d pregnancy
diagnosis of 78.3% for cows that gained BCS, 36.0% for cows that maintained BCS, and 22.8% for cows that lost BCS. This is similar to the decrease of more than 50% in P/AI for cows that lost BCS compared to cows that gained BCS during the transition period. Loss of BCS early post-partum may have a negative effect on oocyte quality that may translate into a reduction in embryo quality by 7 d after breeding [21, 31].

In addition, cows that have greater loss of BCS during the transition period may have more health problems during early lactation. Diseases in the transition period have negative effects on reproduction, delaying resumption of cyclicity and reducing P/AI [57]. Cows in severe NEB may be more susceptible to infection [58] and, conversely, cows with health problems may have a reduced DMI and therefore a greater likelihood for NEB [59]. Individually, subclinical hypocalcemia, elevated NEFA concentration, metritis, and respiratory and digestive problems, reduced estrous cyclicity by d 49 postpartum [26], and have negative effects on early embryo quality, P/AI, and can increase pregnancy loss [57]. Elevated NEFA concentrations are associated with reduced P/AI [26] and NEFA and BHBA have both been associated with development of clinical diseases [3]. This is consistent with the interrelationships among excessive mobilization of body fat, post-partum health events, and periparturient morbidity that has been recently reviewed [60].

5 Conclusions

Body condition score at the beginning of the close-up period had effects on subsequent BCS loss both prior to and after calving. Furthermore, cows that gained BCS during the transition period, from 21 d prior to calving until 21 d after calving, had lower circulating NEFA and
BHBA concentration after calving and subsequently had fewer health problems after calving, earlier return to cyclicity, and greater fertility. These results are consistent with greater loss of BCS during the transition period being a key factor in prolonging the inhibition of ovarian activity after calving. The delay in first ovulation as well as negative effects of disease and NEB may underlie the negative association between BCS loss and fertility. Further, our results suggest that it may be useful to monitor and perhaps modify BCS during the 21 d prior to calving in order to optimize health and reproduction in high-producing dairy cows.

**Conflict of interest**

None of the authors of the above manuscript has declared any conflict of interest.

**Acknowledgments**

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**Table 1.** Body condition score (BCS; least squares means ± SEM) on Days -21, -7, 7, and 21, in relation to calving, for cows that lost, maintained, or gained BCS during the transition period.

<table>
<thead>
<tr>
<th>Item</th>
<th>Gained</th>
<th>Maintained</th>
<th>Lost</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>69</td>
<td>54</td>
<td>122</td>
<td></td>
</tr>
<tr>
<td>BCS at -21 DIM</td>
<td>2.57 ± 0.03c</td>
<td>2.70 ± 0.04b</td>
<td>2.97 ± 0.03a</td>
<td>&lt; 0.01</td>
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<tr>
<td>BCS at -7 DIM</td>
<td>2.72 ± 0.04</td>
<td>2.71 ± 0.06</td>
<td>2.71 ± 0.04</td>
<td>0.99</td>
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<tr>
<td>BCS at 7 DIM</td>
<td>2.72 ± 0.04</td>
<td>2.71 ± 0.06</td>
<td>2.69 ± 0.04</td>
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<tr>
<td>BCS at 21 DIM</td>
<td>2.90 ± 0.04a</td>
<td>2.70 ± 0.04b</td>
<td>2.54 ± 0.03c</td>
<td>&lt; 0.01</td>
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</tbody>
</table>

a-c Values within a row with different superscript letters differ at *P < 0.05.*

1 Cows had their BCS evaluated during the transition period (-21 to 21) using a 5-point scale with 0.25 increments.
Table 2. Effect of changes in body condition scores (BCS) during the transition period (-21 to 21 days in milk) on milk yield and fat corrected milk (least squares means ± SEM) for cows that lost, maintained, or gained BCS.

<table>
<thead>
<tr>
<th>Item</th>
<th>Change in BCS</th>
<th>P-values</th>
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<td>Gained</td>
<td>Maintained</td>
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<tr>
<td>N</td>
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<td>54</td>
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<tr>
<td>Milk yield, kg/d</td>
<td>46.5 ± 1.24</td>
<td>46.0 ± 1.38</td>
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<tr>
<td>Fat corrected milk, kg/d</td>
<td>45.7 ± 1.25</td>
<td>45.6 ± 1.41</td>
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a/c Values within a row with different superscript letters differ at P < 0.05.

1 Cows had their BCS evaluated during the transition period (-21 to 21) using a 5-point scale with 0.25 increments.
Table 3. Effect of changes in body condition score (BCS) during the transition period (-21 to 21) on circulating NEFA and BHBA (least squares means ± SEM) for cows that lost, maintained, or gained BCS.

<table>
<thead>
<tr>
<th>Change in BCS&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Prepartum, N</th>
<th>Lost</th>
<th>NEFA, mmol/L</th>
<th>BHBA, mmol/L</th>
<th>Postpartum, N</th>
<th>Lost</th>
<th>NEFA, mmol/L</th>
<th>BHBA, mmol/L</th>
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<tr>
<td></td>
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<td>54</td>
<td>0.39 ± 0.02</td>
<td>0.69 ± 0.02</td>
<td>69</td>
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<td>0.42 ± 0.02b</td>
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<td>0.38 ± 0.02</td>
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</tbody>
</table>

<sup>a</sup> Values within a row with different superscript letters differ at P < 0.05.

<sup>1</sup> Cows had their BCS evaluated during the transition period (-21 to 21) using a 5-point scale with 0.25 increments.
Table 4. Effect of changes in body condition score (BCS) during the transition period (-21 to 21) on incidence (%) of retained placenta, mastitis, ketosis and pneumonia for cows that lost, maintained, or gained BCS.

<table>
<thead>
<tr>
<th>Item</th>
<th>Change in BCS(^1)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gained</td>
<td>Maintained</td>
<td>Lost</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>66</td>
<td>52</td>
<td>116</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metritis</td>
<td>19.70 (13/66)</td>
<td>21.20 (11/52)</td>
<td>23.30 (27/116)</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Mastitis</td>
<td>16.70 (11/66)(^b)</td>
<td>17.30 (9/52)(^{a,b})</td>
<td>29.30 (34/116)(^a)</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Ketosis</td>
<td>15.20 (10/66)</td>
<td>19.20 (10/52)</td>
<td>26.70 (31/116)</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>9.10 (6/66)</td>
<td>11.50 (6/52)</td>
<td>14.70 (17/116)</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>&gt; 1 Health problem</td>
<td>39.4 (26/66)(^b)</td>
<td>46.2 (24/52)(^b)</td>
<td>62.9 (73/116)(^a)</td>
<td>0.007</td>
<td></td>
</tr>
</tbody>
</table>

\(^{ab}\) Values within a row with different superscript letters differ at P < 0.05.

\(^1\) Cows had their BCS evaluated during the transition period (-21 to 21) using a 5-point scale with 0.25 increments.
Table 5. Effect of changes in body condition score (BCS) during the transition period (-21 to 21) on ovulatory follicle diameter and first ovulation (least squares means ± SEM), pregnancies per IA (P/AI), pregnancy loss, days postpartum at first ovulation, and percentage of cyclic cows at 50 DIM for cows that lost, maintained, or gained BCS.

<table>
<thead>
<tr>
<th>Change in BCS</th>
<th>Gained</th>
<th>Maintained</th>
<th>Lost</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows, % (no./no.)</td>
<td>28 (69/245)</td>
<td>22 (54/245)</td>
<td>50 (122/245)</td>
<td></td>
</tr>
<tr>
<td>Ovulatory Folicle, mm</td>
<td>18.5 ± 0.5</td>
<td>19.0 ± 0.8</td>
<td>18.4 ± 0.4</td>
<td>0.76</td>
</tr>
<tr>
<td>P/AI 30 d, % (no./no.)</td>
<td>53.0 (35/66)a</td>
<td>26.9 (14/52)b</td>
<td>18.3 (21/115)b</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>P/AI 60 d, % (no./no.)</td>
<td>45.5 (30/66)a</td>
<td>25.0 (13/52)b</td>
<td>15.7 (18/155)b</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Pregnancy loss, % (no./no.)</td>
<td>14.3 (5/35)</td>
<td>7.1 (1/14)</td>
<td>14.3 (3/21)</td>
<td>0.79</td>
</tr>
<tr>
<td>First ovulation, d</td>
<td>33.9 ± 0.5c</td>
<td>37.9 ± 0.7b</td>
<td>47.1 ± 1.0a</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Cyclic cows at 50 DIM, %</td>
<td>100a (69/69)</td>
<td>94.4b (51/54)</td>
<td>81.1c (99/122)</td>
<td>0.015</td>
</tr>
</tbody>
</table>

a-c Values within a row with different superscript letters differ at P < 0.05.

1 Cows had their BCS evaluated during the transition period (-21 to 21) using a 5-point scale with 0.25 increments.
Figure 1. Distribution of cows that lost (◆; n = 122), maintained (■; n = 54) or gained (★; n = 69) BCS during the transition period (days -21 to +21 relative to parturition), according to BCS at Day -21 relative to parturition.

abc Different superscript letters differ at P < 0.05.
Figure 2. Milk yield (upper panel), serum NEFA (middle panel), and BHBA (lower panel) concentration (least squares means ± SEM) in relation to days in milk (DIM; P < 0.001), and body condition score (BCS) change during the transition period (P = 0.623) in cows that gained (a)
…; n = 69), maintained (…; n = 54), or lost (…; n = 122) BCS (days -21 to +21 relative to parturition).

abc Different superscript letters differ at P < 0.05.
Figure 3. Survival curve for days to postpartum first ovulation of dairy cows that gained (n = 69), maintained (n = 54) and lost (n = 122) BCS during the transition period (days -21 to +21 relative to parturition).
Associations among changes in body condition score during the transition period with NEFA and BHBA concentrations, milk production, fertility, and health of Holstein cows

Barletta et al.

**Highlights**

Changes in body condition score during peripartum affect cows health and fertility. During transition period, BCS change had no effect on dairy cows performance. Cows that lost BCS had higher incidence of diseases during lactation. Higher BCS loss during dry period increase NEFA and BHBA concentrations. Higher circulating NEFA and BHBA concentrations delay first postpartum ovulation.