Effects of Dietary Supplementation of Scutellaria baicalensis Extract During Early Lactation on Milk Production of Dairy Cattle

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Abstract
Multiparous Holstein cows (n = 122) were used in a randomized block design to determine the effect of short-term and long-term postpartum administration of *Scutellaria baicalensis* extract (SBE) on 305-day milk yield, 120-day milk component yield, and early lactation milk markers of inflammation and metabolic function. Treatments were (1) control, (2) short-term (5-day) administration of the SBE (SBE5), and (3) long-term (60-day) administration of the SBE (SBE60). Treatments were included in a treatment pellet that was identical to the control pellet in ingredient source and composition except for the extract, and both pellets were provided via an automated milking system. Milk samples were collected on day 1, 3, and once during days 5–12 of lactation, followed by weekly sampling for the remainder of the 120 days collection period. Milk samples collected in the first 2 weeks were used for biomarker analysis (haptoglobin and β-hydroxybutyrate [BHBA]), and all samples were used for composition analysis. Cows were scored for body condition every 2 weeks prepartum and postpartum. Milk production, programmed pellet allocation, and actual provision of both pelleted feeds were recorded daily. There was no difference in daily treatment pellet feeding between SBE5 and SBE60 for the first 5 days of lactation. Total pellet intake was greater for SBE60 than SBE5 and control cows during the treatment period (weeks 1–9), but not during the carryover period (weeks 10–36). No treatment effects were observed for body condition, milk haptoglobin, or milk BHBA. Whole-lactation milk yield was increased for SBE60 compared to control, but SBE5 did not differ from control. Milk lactose and fat yields were significantly greater and milk protein yield tended to be greater for SBE60 than control. Treatment SBE60 decreased somatic cell count (SCC) compared to control during weeks 3–5 and 8, whereas SBE5 did not affect SCC. Mastitis incidence was lesser for both SBE5 and SBE60 compared to control. Time to pregnancy did not differ, but retention in the herd tended to be greater for SBE60 than control. In conclusion, despite no detected treatment effects on BCS or milk biomarkers of inflammation and metabolic status, supplementation of postpartum dairy cows with *Scutellaria baicalensis* extract for 60 days was effective at decreasing mastitis incidence and increasing milk yield.

Keywords
polyphenol, dairy cow, transition, inflammation

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Effects of Dietary Supplementation of *Scutellaria baicalensis* Extract During Early Lactation on Milk Production of Dairy Cattle

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**Summary**

Multiparous Holstein cows (*n* = 122) were used in a randomized block design to determine the effect of short-term and long-term postpartum administration of *Scutellaria baicalensis* extract (SBE) on 305-day milk yield, 120-day milk component yield, and early lactation milk markers of inflammation and metabolic function. Treatments were (1) control, (2) short-term (5-day) administration of the SBE (SBE5), and (3) long-term (60-day) administration of the SBE (SBE60). Treatments were included in a treatment pellet that was identical to the control pellet in ingredient source and composition except for the extract, and both pellets were provided via an automated milking system. Milk samples were collected on day 1, 3, and once during days 5–12 of lactation, followed by weekly sampling for the remainder of the 120 days collection period. Milk samples collected in the first 2 weeks were used for biomarker analysis (haptoglobin and β-hydroxybutyrate [BHBA]), and all samples were used for composition analysis. Cows were scored for body condition every 2 weeks prepartum and postpartum. Milk production, programmed pellet allocation, and actual provision of both pelleted feeds were recorded daily. There was no difference in daily treatment pellet feeding between SBE5 and SBE60 for the first 5 days of lactation. Total pellet intake was greater for SBE60 than SBE5 and control cows during the treatment period (weeks 1–9), but not during the carryover period (weeks 10–36). No treatment effects were observed for body condition, milk haptoglobin, or milk BHBA. Whole-lactation milk yield was increased for SBE60 compared to control, but SBE5 did not differ from control. Milk lactose and fat yields were significantly greater and milk protein yield tended to be greater for SBE60 than control. Treatment SBE60 decreased somatic cell count (SCC) compared to control during weeks 3–5 and 8, whereas SBE5 did not affect SCC. Mastitis incidence was lesser for both SBE5 and SBE60 compared to control. Time to pregnancy did not differ, but retention in the herd tended to be greater for SBE60 than control. In conclusion, despite no detected treatment effects on BCS or milk biomarkers of inflammation and metabolic status, supplementation of postpartum dairy cows with *Scutellaria baicalensis* extract for 60 days was effective at decreasing mastitis incidence and increasing milk yield.

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Introduction

Inflammation during the transition period has been well established and is associated with reduced milk yield and reproductive performance. Previous research demonstrated that short-term postpartum administration (3 days) of the nonsteroidal anti-inflammatory drugs (NSAID) sodium salicylate and meloxicam increases whole-lactation milk and protein yields. The fact that use of NSAIDs during early lactation is considered off-label drug use, has encouraged investigation of plant extracts as a natural alternative. Extracts from the *Scutellaria baicalensis* plant, containing several flavonoids, have shown anti-inflammatory and antioxidant properties in cell culture experiments.

The objective of this study was to determine the effect of short-term (5-day) and long-term (60-day) administration of *Scutellaria baicalensis* extract (SBE) after calving on milk yield and milk markers of inflammation and metabolic function. Secondary outcomes examined were effects of SBE on milk components, somatic cell count, time to pregnancy, disease incidence, and retention in the herd.

Experimental Procedures

Multiparous Holstein cows (*n* = 122) on a commercial farm were used in a randomized block design to determine the effects of short-term (5-day) and long-term (60-day) postpartum administration of SBE on 305-day milk yield and early lactation milk markers of inflammation and metabolic function. Cows were blocked by parity (2 and 3+), calving date, and risk factors (high risk block: calving difficulty score ≥ 3 or twins), then randomly assigned within block to one of three treatments. Upon calving, cows were moved into a fresh pen where they had free access to an automatic milking system (AMS; Austronaut A3, Lely Ltd., Maassluis, The Netherlands), but were encouraged through the AMS if their voluntary attendance was less than 3 visits that day. Cows were managed per site standard operating procedures.

Cows were fed a partial mixed ration (PMR) twice daily and were provided with pelleted concentrate feed in the AMS. *S. baicalensis* extract (Groupe CCPA, Janze, France) was combined with the dairy’s standard robot feed formulation and pelleted. The control and treatment pelleted feeds were stored in two feed bins that independently supplied the milking robots. Treatments were (1) control (*n* = 39), (2) short-term (5-day) administration after calving of the SBE pellet (*n* = 42; SBE5), and (3) long-term (60-day) administration after calving of the SBE pellets (*n* = 40; SBE60). Treatments began within 24 hours after calving. All cows received the control pellet, with the amount based on stage of lactation and milk production. Treatment cows were allocated 1.8 kg of the treatment pellet (delivering 100 g test material/day) in place of an equal amount of control pellet across all milkings for either 5 or 60 days. Pellet allocation was based solely on days in milk (DIM) during the first 50 days of lactation, then from day 51 until 2 weeks prior to dry off, total pellet allocation was based on a feed table, which incorporated milk production. The feeding program distributed the target amount of treatment feed across the average number of daily milkings per cow. Due to the nature of AMS, voluntary deviations from a cow’s average number of milkings resulted in slight excesses or shortfalls in actual provision of pellet compared to the targeted allocation, and instances when not all the feed allocated for that particular
milking was dispensed were recorded as rest feed. Reported pellet intake is the difference between total pellet allowance and rest feed.

The PMR, control pellets, and treatment pellets were sampled every 2 weeks and composited by month for nutrient analysis by Dairy One Forage Laboratory (Ithaca, NY). Nutrient analyses are reported as averages across the study for the PMR in Table 1 and the pelleted feeds in Table 2.

Milk samples were collected on days 1, 3, and once during days 5–12 of lactation, followed by weekly sampling for the remainder of the 120-day collection period. Milk samples collected during the first 2 weeks of lactation were used for both biomarker analysis (haptoglobin and β-hydroxybutyrate [BHBA]) and component analysis; subsequent samples were used only for composition analysis. Milk composition was analyzed by MQT Lab Services (Kansas City, MO).

Cows were scored every 2 weeks for body condition score (BCS) on a 5-point scale (1 = extremely thin to 5 = extremely obese) from week -3 to week 17 relative to calving. Daily milk production, DIM, number of milkings per day, programmed feed daily allocated and feed provided for both pelleted feeds, and rumination data were recorded on an individual cow basis and collected using the management software, Time for Cows (T4C, Lely Ltd., Maassluis, The Netherlands). Culling data were reported in PC Dart by the farm staff.

Results and Discussion

Treatment Provision and Total Pellet Offered

Test material delivered for the first 5 DIM was not different between SBE5 and SBE60 ($P = 0.41$; 80.8 and 83.1 ± 0.34 g/day, respectively). Mean test material provision for SBE60 ranged between 92 and 98 g/d during weeks 1–9 of lactation. Pellet feeding records (T4C) confirmed that no treatment feed was allocated to control cows nor to SBE5 cows after day 5 of lactation. Total pellet offered over the first 63 DIM (Table 3) differed by treatment and week, and had a treatment × week interaction (all $P < 0.001$; Figure 1). Pellet offered was greater for SBE60 cows compared to control cows during week 1–9 ($P < 0.001$) and tended to be increased across week 1–36 ($P < 0.10$). Daily rumination time through 120 DIM was not different for control cows compared to either SBE5 or SBE60 over weeks 1–9 or 10–17 (all $P > 0.55$) and no treatment × week interaction was observed ($P = 0.39$; Table 3).

Milk Production and Composition

Milk yield did not differ between SBE5 and control, during the treatment period (weeks 1–9; $P = 0.35$) or the carryover period (weeks 10–43; $P = 0.73$). Milk yield tended to increase for SBE60 compared to control during weeks 1–9 ($P = 0.07$) and was significantly increased during week 10–43 ($P = 0.04$; Figure 2). Whole-lactation milk yields (305-day) were 24,795, 25,596, and 27,924 ± 1,026 lb for control, SBE5, and SBE60; significant differences were detected between SBE60 and control ($P = 0.03$), but not between SBE5 and control ($P = 0.60$). Milking frequency was not affected by either SBE5 ($P = 0.60$) or SBE60 ($P = 0.19$) during the first 63 DIM, but milking frequency was increased for SBE60 during the carryover period compared
to control \((P = 0.04)\) whereas no difference was detected between SBE5 and control \((P = 0.48)\). As expected, milking frequency differed by week \((P < 0.001)\), but no overall treatment × week interaction was observed \((P = 0.11)\). Despite the difference in milking frequency, milk yield per milking did not differ by treatment during the treatment or carryover periods \((all \ P > 0.65)\).

Milk composition data during the first 17 weeks of lactation are summarized in Table 4. There were no treatment effects on milk fat or protein concentration during the treatment or carryover periods \((all \ P > 0.15)\). Milk lactose concentration tended to be increased for SBE60 compared to control during the treatment period \((P = 0.06)\), but not the carryover period \((P = 0.25)\), and was not affected by SBE5. Milk fat yield was increased in SBE60 during both the treatment and carryover period compared to control \((both \ P = 0.04)\), whereas SBE5 was not different from control in either period \((both \ P > 0.50)\). Milk protein yield tended to be increased for SBE60 compared to control in the treatment period \((P = 0.09)\) and was statistically greater during the carryover period \((P = 0.01)\), but again did not differ between SBE5 and control \((P > 0.13)\). Milk lactose yield was increased for SBE60 but not SBE5 compared to control during the treatment period \((P = 0.03\ and 0.26, \ respectively)\). During the carryover period, milk lactose yield continued to be greater for SBE60 compared to control \((P = 0.02)\), and SBE5 tended to increase milk lactose yield compared to control \((P = 0.07)\).

Somatic cell count was decreased by SBE60 compared to control during the treatment period \((P = 0.02)\) with a tendency for a difference in week 3 and significant effects in weeks 4–6 and 8 (Figure 3). Treatment SBE5 did not affect SCC \((P = 0.37)\) during weeks 1–9, and neither SBE5 or SBE60 affected SCC during the carryover period \((P = 0.29\ and 0.13, \ respectively)\).

Overall there was no treatment effect on BCS \((P = 0.44)\) with means being 3.40, 3.30, and 3.31 ± 0.06 for control, SBE5, and SBE60. As anticipated, body condition score differed by week \((P < 0.001)\), but there was no treatment effect on prepartum or postpartum BCS \((treatment \times \ week: \ P = 0.57)\).

### Milk Markers of Inflammation and Metabolism

Neither milk haptoglobin nor milk BHBA showed significant treatment effects \((P = 0.97\ and 0.89, \ respectively; \ Table 5)\) or treatment × DIM effects \((P = 0.45\ and 0.47)\). Milk haptoglobin concentrations were greatest the day after calving (when inflammation is greatest) and subsequently declined for day 3 and day 5–12 milk samples \((P < 0.001)\). The BHBA concentration also had a DIM effect \((P < 0.0001)\), increasing from day 1 to day 5–12 samples.

### Time to Pregnancy, Disease Incidence, and Herd Retention

Survival analyses through 305 DIM were completed for time to pregnancy and removal from the herd. There was no treatment effect on time to pregnancy \((P = 0.34)\). At 365 days after treatment initiation, 13 of 40 control, 15 of 44 SBE5, and 6 of 38 SBE60 cows had left the herd, and after accounting for other risk factors, SBE60 tended to decrease the risk of removal from the herd by 64% compared to control \((P = 0.07; \ risk \ ratio \ for removal: 0.41, 95\% confidence interval: 0.11, 0.99)\). Treatment SBE5 did not affect retention in the herd. Incidence of several diseases are reported in Table 6. The
only disease incidence affected by treatment was mastitis, being lesser for both SBE5 and SBE60 compared to control ($P = 0.04$ and $0.05$, respectively). Treatment SBE60 tended to decrease the hazard of leaving the herd compared to control and SBE5 ($P = 0.07$).

**Conclusions**
Supplementation of dairy cows with *Scutellaria baicalensis* for 60 days increased whole-lactation milk yield compared to control cows. Milk fat, protein, and lactose yields increased through 120 DIM and SCC was decreased during the treatment period for the 60-d treatment compared to control cows. Milk production parameters were not different for short-term administration (5-day) compared to control cows. Other than milk SCC and reduced incidence of mastitis, there were no suggestions of impacts on health outcomes. Time to pregnancy was unaffected, but retention in the herd was increased. Overall, long-term administration of *S. baicalensis* effectively increased milk production, although the mechanism by which this was achieved is unknown.

**Table 1. Nutritional composition of the partial mixed ration (PMR)**

| Nutrient                   | % of dry matter (DM) | Standard deviation |
|----------------------------|----------------------|--------------------|
| DM, % as-fed               | 57.06                | 0.27               |
| Crude protein              | 18.71                | 0.37               |
| Acid detergent fiber       | 20.89                | 1.54               |
| Neutral detergent fiber    | 31.96                | 2.31               |
| Net energy for lactation, Mcal/kg | 1.65       | 0.04               |
Table 2. Ingredient and nutritional composition of the control and treatment pellet

| Item                                                      | Control pellet | Treatment pellet | Standard deviation |
|-----------------------------------------------------------|----------------|-------------------|--------------------|
| **Ingredient, % of dry matter (DM)**                      |                |                   |                    |
| Ground corn                                               | 42.47          | 42.44             |                    |
| Wheat middlings                                           | 27.76          | 27.23             |                    |
| Wheat flour                                               | 15.16          | 10.10             |                    |
| Soybean meal (47.5%)                                      | 10.92          | 10.92             |                    |
| Molasses                                                  | 3.16           | 3.16              |                    |
| Super bind¹                                               | 0.53           | 0.53              |                    |
| Test feed premix²                                          | ---            | 5.62              |                    |
| **Nutrient, analyzed, % of DM (unless otherwise specified)** |                |                   |                    |
| DM, % as-fed                                              | 87.60          | 87.44             | 0.81               |
| Crude protein                                             | 17.33          | 17.30             | 0.38               |
| Acid detergent fiber                                      | 6.77           | 5.30              | 0.70               |
| Neutral detergent fiber                                   | 15.33          | 14.52             | 0.94               |
| NE₄,³ Mcal/kg                                             | 1.94           | 1.94              | 0.02               |

¹Modified lignin sulfonate pellet binder (Bonaventure Chemicals, Inc., Weston, FL).
²Test feed premix included wheat flour, calcium carbonate, natural flavoring and Scutellaria baicalensis extract.
³Net energy for lactation.
Table 3. Treatment means for weekly total pellet offered, milk yield, and milking frequency for cows fed control or *S. baicalensis* extract for either 5 days (SBE5) or 60 days (SBE60) following calving

|                          | Control | SBE5  | SBE60 | SEM  | Con vs. SBE5 | Con vs. SBE60 |
|--------------------------|---------|-------|-------|------|--------------|---------------|
| **Total pellet offered, lb/d** |         |       |       |      |              |               |
| d 1–63                   | 11.6    | 11.7  | 12.5  | 0.31 | 0.77         | < 0.01        |
| d 64–301                 | 11.0    | 11.4  | 11.6  | 0.33 | 0.13         | 0.02          |
| **Milk yield, lb/d**     |         |       |       |      |              |               |
| d 1–63                   | 93.6    | 99.1  | 104.1 | 4.4  | 0.35         | 0.07          |
| d 64–301                 | 78.0    | 79.9  | 88.2  | 4.2  | 0.73         | 0.04          |
| **Milking frequency, d⁻¹**|         |       |       |      |              |               |
| d 1–63                   | 3.24    | 3.34  | 3.48  | 0.21 | 0.60         | 0.19          |
| d 64–301                 | 2.56    | 2.67  | 2.84  | 0.18 | 0.48         | 0.04          |
| **Milk per visit, lb**   |         |       |       |      |              |               |
| d 1–63                   | 30.6    | 31.2  | 31.1  | 1.65 | 0.70         | 0.75          |
| d 64–301                 | 31.0    | 30.6  | 31.0  | 1.39 | 0.92         | 0.99          |
Table 4. Rumination time through 120 DIM and milk composition for the first 17 weeks of lactation of control cows and cows supplemented with *S. baikalensis* extract (SBE) for either 5 days (SBE5) or 60 days (SBE60) following calving

|                        | Control | SBE5  | SBE60 | SEM  | Con vs. SBE5 | Con vs. SBE60 |
|------------------------|---------|-------|-------|------|--------------|---------------|
| Rumination, min/d      |         |       |       |      |              |               |
| d 1–63                 | 429.9   | 427.3 | 429.0 | 8.20 | 0.76         | 0.92          |
| d 64–120               | 410.3   | 405.8 | 409.9 | 7.30 | 0.58         | 0.95          |
| Milk fat, %            |         |       |       |      |              |               |
| d 1–63                 | 3.84    | 3.84  | 3.84  | 0.17 | 0.95         | 0.99          |
| d 64–120               | 3.24    | 3.08  | 3.29  | 0.18 | 0.28         | 0.77          |
| Milk protein, %        |         |       |       |      |              |               |
| d 1–63                 | 3.16    | 3.10  | 3.12  | 0.06 | 0.40         | 0.54          |
| d 64–120               | 2.97    | 2.89  | 2.97  | 0.05 | 0.15         | 0.99          |
| Milk lactose, %        |         |       |       |      |              |               |
| d 1–63                 | 4.87    | 4.89  | 4.95  | 0.04 | 0.54         | 0.06          |
| d 64–120               | 4.92    | 4.95  | 4.97  | 0.04 | 0.46         | 0.25          |
| Milk fat, lb/d         |         |       |       |      |              |               |
| d 1–63                 | 3.55    | 3.68  | 3.90  | 0.18 | 0.50         | 0.04          |
| d 64–120               | 2.98    | 3.04  | 3.33  | 0.18 | 0.73         | 0.04          |
| Milk protein, lb/d     |         |       |       |      |              |               |
| d 1–63                 | 2.95    | 3.09  | 3.22  | 0.13 | 0.42         | 0.09          |
| d 64–120               | 2.71    | 2.95  | 3.11  | 0.11 | 0.13         | 0.01          |
| Milk lactose, lb/d     |         |       |       |      |              |               |
| d 1–63                 | 4.63    | 4.92  | 5.20  | 0.22 | 0.26         | 0.03          |
| d 64–120               | 4.56    | 5.03  | 5.18  | 0.20 | 0.07         | 0.02          |
| SCC, log_{10} cells/mL |         |       |       |      |              |               |
| d 1–63                 | 2.19    | 2.07  | 1.86  | 0.13 | 0.37         | 0.02          |
| d 64–120               | 2.13    | 1.98  | 1.91  | 0.14 | 0.29         | 0.13          |

SCC = somatic cell count.
Table 5. Milk haptoglobin and BHBA on days 1, 3, and 5–12 of lactation for control cows and cows receiving *S. baicalensis* extract (SBE) for either 5 days (SBE5) or 60 days (SBE60) after calving

|                      | Control | SBE5  | SBE60 | SEM  | Trt   | DIM  | Trt × DIM |
|----------------------|---------|-------|-------|------|-------|------|-----------|
| Haptoglobin, µg/mL   |         |       |       |      |       |      |           |
| d 1                  | 4.98    | 3.54  | 5.47  | 1.04 | 0.97  | < 0.001 | 0.45     |
| d 3                  | 1.53    | 1.70  | 1.44  | 0.35 |       |       |           |
| d 5–12               | 0.59    | 0.69  | 0.50  | 0.13 |       |       |           |
| BHBA, µM             |         |       |       |      |       |      |           |
| d 1                  | 264.0   | 265.3 | 249.4 | 23.6 | 0.89  | < 0.001 | 0.47     |
| d 3                  | 639.7   | 609.7 | 632.2 | 22.6 |       |       |           |
| d 5–12               | 729.1   | 746.7 | 717.8 | 18.6 |       |       |           |

*P*-values

1Treatment: *P*-value for treatment effect; DIM: *P*-value for time (days in milk) effect.

Table 6. Disease incidence through 250 DIM for control cows and cows receiving *S. baicalensis* extract (SBE) for either 5 days (SBE5) or 60 days (SBE60) after calving

|                      | Control | SBE5  | SBE60 |
|----------------------|---------|-------|-------|
| At-risk              | 39      | 43    | 40    |
| Fever                | 3       | 1     | 1     |
| Milk fever           | 1       | 2     | 2     |
| Displaced abomasum   | 0       | 0     | 0     |
| Retained placenta    | 2       | 5     | 4     |
| Metritis             | 3       | 4     | 6     |
| Lame                 | 2       | 2     | 0     |
| Off feed             | 3       | 2     | 1     |
| Mastitis*            | 13      | 6*    | 6*    |
| Other                | 0       | 1     | 2     |

*Mastitis incidence tended to differ by treatment (*P* = 0.06).

*Control vs. SBE5: *P* = 0.04.

*Control vs. SBE60: *P* = 0.05.
Figure 1. Weekly total pellet offered (control + treatment) of control cows and cows supplemented with *S. baicalensis* during the first 5 day (SBE5) or 60 day (SBE60) of lactation.

Data were analyzed by treatment period (weeks 1–9) and carryover period (weeks 10–36). Total pellet offered was increased for SBE60 compared to control during the weeks 1–9 \( (P < 0.01) \) and from weeks 10–43 \( (P = 0.02) \). Total pellet offered was not different between SBE5 and control during either weeks 1–9 \( (P = 0.77) \) or weeks 10–43 \( (P = 0.13) \). A treatment × week interaction was detected \( (P < 0.001) \), and differences between SBE60 and control are indicated by * \( (P < 0.05) \) and † \( (P < 0.10) \).
Figure 2. Milk yield of control cows and cows supplemented with *S. baicalensis* extract (SBE) during the first 5 days (SBE5) or 60 days (SBE60) of lactation. Data were analyzed by treatment period (weeks 1–9) and carryover period (weeks 10–43). Milk yield tended to be increased for SBE60 compared to control from weeks 1–9 (*P* = 0.07) and was significantly increased from weeks 10–43 (*P* = 0.04). Milk yield was not different between SBE5 and control during weeks 1–9 (*P* = 0.35) or weeks 10–43 (*P* = 0.73). A treatment × week interaction was detected (*P* < 0.03), and differences between SBE60 and control are indicated by * (*P* < 0.05) and † (*P* < 0.10).
Figure 3. Somatic cell count (SCC) of control cows and cows supplemented with *S. baicalensis* extract (SBE) during the first 5 days (SBE5) or 60 days (SBE60) of lactation. Data were analyzed for the treatment period (weeks 1–9) and carryover period (weeks 10–17). Somatic cell count was not different between SBE5 and control during weeks 1–9 ($P = 0.37$) or weeks 10–17 ($P = 0.29$). Somatic cell count was decreased for SBE60 compared to control during weeks 1–9 ($P = 0.02$), but not during weeks 10–17 ($P = 0.13$). No treatment × week interaction was detected ($P = 0.16$). Differences between SBE60 and control are indicated by * ($P < 0.05$) and † ($P < 0.10$).