Effects of close-up body condition score and selenium-vitamin E injection on lactation performance, blood metabolites, and oxidative status in high-producing dairy cows

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ABSTRACT

High-producing dairy cows with high pre-calving body condition score (BCS) are more susceptible to metabolic disorders and oxidative stress. The aim of present study was to evaluate the effects of close-up BCS and 3 times Se-vitamin E (SeE) injection on BCS change, blood metabolites, oxidative status, and milk yield in high-producing Holstein cows. A total of 136 multiparous cows were divided into 2 groups based on their BCS including high (HB = 4.00 ± 0.20) and moderate (MB = 3.25 ± 0.25) at 3 wk before expected calving time. Then, each group was divided into 2 subgroups: 3 rounds of SeE injection at 21 d before, and 0 and 21 d after calving (+SeE), and no SeE injection (–SeE). Four final experimental groups were HB+SeE, MB+SeE, HB–SeE, and MB–SeE (34 cows each). Results indicated that interaction effect of BCS and SeE affected serum glucose, and the MB+SeE group had the highest level. The HB cows lost more BCS compared with MB cows during the postcalving period. Moreover, serum insulin concentration increased after SeE injection. The HB cows had higher serum nonesterified fatty acids at 14 d after calving. The MB cows tended to have higher activity of blood glutathione peroxidase over the study period. Furthermore, the SeE-injected cows tended to have higher activity of blood glutathione peroxidase at 28 d after calving. Serum albumin level was increased by SeE injection. The HB cows had greater milk production than MB cows, and SeE-injected cows tended to have higher milk fat percentage and higher fat:protein ratio compared with nonsupplemented cows. It was concluded that SeE injection had beneficial effects on some blood metabolites, albumin as a blood antioxidative parameter, and

lactation performance in high-producing dairy cows, especially cows with moderate close-up BCS.

Key words: body condition score, antioxidant, blood metabolite, milk composition, transition cow

INTRODUCTION

One of the most practical methods for assessing energy balance in cows is monitoring BCS changes. Management of dairy cows' BCS can improve milk yield, herd health reproductive performance, and overall farm profitability (Bewley and Schutz, 2008). Dairy cows undergo tremendous endocrine and metabolic adaptations during the transition from pregnancy to lactation (Sundrum, 2015). Cows need to adjust their metabolism to increase in energy and nutrient requirements for milk production during the early phase of lactation (Jorritsma et al., 2003; Moyes et al., 2013; Sundrum, 2015). These changes occur exactly when DMI drops 10 to 30% and the cows face a physiological stage called negative energy balance (NEB; Esposito et al., 2014).

It is well established that energy partitioning between the mammary glands and peripheral tissues may increase plasma nonesterified fatty acid (NEFA), lipid peroxidation, and reactive oxygen species, and may reduce antioxidant capacity in high-producing dairy cows (Bernabucci et al., 2005; Castillo et al., 2005). Harrison et al. (1984) demonstrated that oxidative stress before parturition is probably the main contributing factor for metabolic and reproductive disorders in the milking period. During this period, balance between oxidants and antioxidants is necessary for improving the efficiency of dairy cows (Sordillo and Aitken, 2009). It is noted that cows with high BCS before calving loss more BCS after calving, which increases plasma reactive oxygen species, active thiobarbituric acid precursors, and thiol groups, and decreases plasma superoxide dismutase in periparturient dairy cows (Bernabucci et al., 2005). The antioxidant defense systems are essential for inhibiting oxidative damage in animals (Sordillo and Aitken,

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2009). Antioxidant supplements can decrease negative effects of BCS loss after calving, and supplementation with Se-vitamin E (**SeE**) can reduce mastitis, retained placenta, days to estrus, and abortion in dairy cows (Hoedemaker et al., 2009). It was reported that SeE supplementation in the pre-calving period can increase insulin sensitivity, immune responses, and antioxidant capacity after parturition (Abuelo et al., 2016; Mehdi and Dufrasne, 2016). Pilarczyk et al. (2012) reported that serum glutathione peroxidase (**GSH-Px**) had the highest activity in the first stage of lactation in dairy cows, which is reflective of biologically active Se level in the body. On the other hand, vitamin E is known to be a strong antioxidant agent, protecting cell membranes from the lipid peroxidation chain reaction (Baldi, 2005). Milad et al. (2001) reported that a single SeE injection increased whole blood GSH-Px activity in sheep.

We hypothesized that 3 rounds of SeE injection (10 mL/cow) before and after calving would have different effects on lactation performance, blood metabolites, and oxidative status around calving time in high-producing dairy cows with high and moderate close-up BCS. Thus, the objectives of this study were to investigate the relationship between close-up BCS and 3 rounds of SeE injection, as well as subsequent changes in BCS, blood metabolites, oxidative status, and milk yield in high-producing dairy cows.

MATERIALS AND METHODS

Animals, Housing, and Feeding

The experiment was conducted in a large commercial dairy herd located in the western district of Isfahan (Isfahan Emdad Keshte-Dame Co., Isfahan, Iran). The animal care advisory committee of the Isfahan University of Technology approved all experimental procedures (Iranian Council of Animal Care, 1995). Multiparous Holstein cows ($n = 136$, parity = 2.25 ± 1.2) were studied beginning 3 wk before the expected calving time. Cows were initially classified according to their BCS on 21 d before the expected time for calving as high BCS (**HB**; BCS = 4.00 ± 0.20 ; $n = 68$) or moderate BCS (**MB**; BCS = 3.50 ± 0.25 ; $n = 68$). Then, each group was divided into 2 subgroups, SeE injection (**+SeE**) and no SeE injection (**-SeE**). Finally, the 4 experimental groups with 34 cows each were (1) high BCS with SeE injection (**HB+SeE**), (2) high BCS without SeE injection (**HB-SeE**), (3) moderate BCS with SeE injection (**MB+SeE**), and (4) moderate BCS without SeE injection (**MB-SeE**). The experimental groups were balanced across expected calving date, parity (2.25 ± 1.2), and previous milk yield ($12,539 \pm 1,443$; 305

DIM). The SeE [10 mL of SeE, 0.5 mg of Se as sodium selenite, and 56 IU of vitamin E as DL- α -tocopherol acetate per mL, a commercial product; Eurovet Vitamin 50E+SE (Eurovet Animal Health BV, Bladel, the Netherlands), registration number: 12,092,219] was intramuscularly injected first 21 d before the expected calving date, at parturition, and 21 d after calving. The nonsupplemented groups were intramuscularly injected with 10 mL of isotonic saline (Abuelo et al., 2016). All cows were transferred to calving pens bedded with straw near the parturition time. After calving, the cows were housed in a freestall barn with open sides, milked 3 times daily (0500, 1300, and 2100 h), and allowed free access to water throughout the study. The TMR was distributed 3 times a day (after each milking time) and was available ad libitum (NRC, 2001; Table 1).

Measurement and Sampling

During the trial, the BCS of cows was recorded at 3 wk before expected calving (-21) and then at 0, 21, 42, 63, and 84 d postpartum using a 5-point scale with 0.25 intervals (Edmonson et al., 1989). Backfat thickness in the sacral region was measured using ultrasound (4.5–8.5 MHz frequency, Easi-Scan ultrasound scanner, BCF Technology Ltd., Livingston, UK), and then the data were converted to a BCS scale before analysis (Schröder and Staufenbiel, 2006). The backfat thickness measurements for all cows were performed by the same veterinarian and at a similar point in the sacral region. Blood samples were taken from the coccygeal vein approximately 4 h after morning feed distribution on d 0, 14, 28, and 42 postpartum. Blood samples were collected into evacuated tubes, placed on ice, and immediately transferred to the laboratory. Serum was separated by centrifugation ($3,000 \times g$ for 20 min at 4°C) and then stored at -80°C about 1 mo before analysis. Serum metabolites (glucose and albumin) were analyzed using an autoanalyzer (Abbott model Alcyon 300, Abbott Park, IL). Serum concentrations of NEFA and BHB were determined by an enzymatic colorimetric method using commercial kits (FA115 and RB1, 007, Randox Laboratories Ltd., Crumlin, UK). Intra- and interassay coefficients of variation were 2.3 and 5.1% for BHB, and 3.2 and 6.3% for NEFA, respectively. Erythrocyte GSH-Px activity as one of most important indices for body antioxidant capacity was determined at 340 nm based on the kinetic method, which measures the decrease in absorbance by the oxidized form of glutathione over time. This was measured using the GSH-Px kit (catalog no. RS 505, Randox Laboratories Ltd.) and an automatic analyzer (Alycon 300, dual voltage instrument). Insulin was assayed by ELISA (assay sensitivity of $0.5 \mu\text{IU/mL}$;

DiaPlus Instruments Inc., Chatsworth, CA). Intra- and interassay coefficient of variation values were 5.37 and 6.80%, respectively, for insulin.

Daily milk production of each cow was recorded monthly up to 180 DIM. Milk samples of each cow were collected in 50-mL Falcon tubes from 3 consecutive milkings and then composited and frozen at -20°C until analysis. The samples were analyzed for fat, protein, TS, and SCC using an automatic milk composition analyzer (MilkoScan134BN, Foss Electric, Hillerød, Denmark).

Statistical Analysis of Results

Data were analyzed using a completely randomized design with the factorial method and the MIXED procedure in SAS (SAS Institute Inc., Cary, NC). Close-up BCS level, previous milk yield, and parity included as fixed effects and cow as a random effect. The data with repeated measures over time (including BCS changes, glucose, insulin, NEFA, BHB, GSH-Px, albumin, and milk production and composition) were analyzed using the following model:

Table 1. Ingredients and nutrient composition of diets administered during the experimental period

Item	Diet ¹		
	Close-up	Fresh	Lactation
Ingredient (g/kg of DM)			
Alfalfa hay	141.4	178.2	121.3
Corn silage	364	202.7	219.1
Barley grain, ground	118	152.5	188.8
Corn grain, ground	118	152.3	142.4
Fish meal	21	26.6	11
Beet pulp	—	82.2	85.3
Soybean meal	42.1	63.6	90.3
Soy Plus ²	18.6	27	11.4
Wheat straw	35.3	—	6.8
Energy booster	—	5.9	15.1
Sodium bicarbonate	—	9.9	10.4
Canola meal	43.1	21.7	28.5
Cottonseed	24.4	27.7	21.7
Yeast	2.5	2	1.7
Magnesium oxide	2.4	2.9	1.7
Calcium carbonate	13.3	8	4.6
Toxin binder	2	1.5	1.1
Calcium phosphate	—	2.9	2.4
Anionic salt	16.1	—	—
Soybean whole extruded	21.8	28.1	23.4
Salt	—	3.2	3.4
Mineral and vitamin premix ³	16	1.1	9.6
Nutrient composition on DM basis			
DM (% as fed)	43	51	49
CP (%)	14.7	16.5	16.3
NDFom ⁴ (%)	34.8	30.3	29.6
ADFom ⁵ (%)	22.1	20.2	19.5
Ether extract (%)	3.5	4	4.7
Ash (%)	8	6.2	5.4
NFC ⁶ (%)	39	43	44
NE _L ⁷ (MJ/kg of DM)	6.38	6.88	7.1
Ca (%)	1.18	0.91	0.73
P (%)	0.38	0.46	0.46
Se (mg/kg)	1.01	0.28	0.66
Vitamin E (IU/kg)	260.43	34.48	161

¹Close-up: cows in the last 3 wk of gestation, fresh: cows in the first 3 wk of lactation, and lactation: cows after 3 wk of lactation.

²Mehr Bisotun Co., Isfahan, Iran.

³Mineral and vitamin premix: Co, 50 mg/kg; Cu, 2,000 mg/kg; I, 70 mg/kg; Fe, 800 mg/kg; Mn, 5,000 mg/kg; Se, 50 mg/kg; Zn, 5,000 mg/kg; vitamin A, 1,000,000 IU/kg; vitamin D₃, 360,000 IU/kg; vitamin E, 15,000 IU/kg.

⁴NDFom = neutral detergent fiber exclusive of residual ash.

⁵ADFom = acid detergent fiber exclusive of residual ash.

⁶NFC was calculated as $100 - (\text{NDF} + \text{CP} + \text{ether extract} + \text{ash})$.

⁷NE_L calculated according to the NRC (2001).

$$Y_{ijklm} = \mu + B_i + ES_j + B_i \times ES_j + P_k \\ + W_l + Animal + \beta_1 + e_{ijklm},$$

where Y_{ijklm} is the dependent variable; μ is the overall mean; B_i is the effect of BCS ($i = 1-5$); ES_j is the effect of SeE ($j = 0$ and 1); $B_i \times ES_j$ is the interaction of BCS and SeE, P_k is the effect of parity ($k = 2-5$); W_l is the effect of time; *Animal* is the random effect of cows nested within treatments; β_1 is the regression coefficient of previous milk yield (305 DIM); and e_{ijklm} is the random residual effect.

A model used for data which measured individually regardless of time (records of each time point):

$$Y_{ijkl} = \mu + B_i + ES_j + B_i \times ES_j + P_k \\ + Animal + \beta_1 + e_{ijkl},$$

where Y_{ijkl} is the dependent variable; μ is the overall mean; B_i is the effect of BCS ($i = 1-5$); ES_j is the effect of SeE ($j = 0$ and 1); $B_i \times ES_j$ is the interaction of BCS and SeE, P_k is the effect of parity ($k = 2-5$); *Animal* is the random effect of cows nested within treatments; β_1 is the regression coefficient of previous milk yield (305 DIM); and e_{ijkl} is the random residual effect.

Data were subjected to the method of normality test for residue. The treatment means were compared using a Tukey–Kramer test based on LSMEANS output from the SAS software. For SCC data to be normally distributed, it was log-transformed as SCS based on the following formula (Schutz, 1994):

$$SCS (\ln SCC) = [\ln (SCC/100,000)/0.693147] + 3.$$

The significance level was declared at $P \leq 0.05$, and a tendency toward significance was considered at $0.05 < P \leq 0.10$.

RESULTS

Change in BCS

The BCS value changes throughout the study (Figure 1) and at the different stages of the trial are shown in Table 2. At the start of the study, HB cows (HB+SeE and HB–SeE, $BCS = 4.03 \pm 0.04$) had higher ($P < 0.05$) BCS than the MB cows (MB+SeE and MB–SeE, $BCS = 3.52 \pm 0.04$), and the difference was maintained for the entire study. The BCS of all experimental groups decreased from d –21 to 42 (loss of BCS in HB cows was about twice of MB cows), then the BCS slightly increased up to d 84 (HB = +0.23 and MB = +0.22). Our results showed that the interaction of BCS and SeE

injection on BCS changes was not significant except for the period from 42 to 63 d postpartum, which tended to be significant ($P = 0.08$). The decrease in BCS was more pronounced ($P < 0.05$) in HB cows compared with MB cows (Figure 1). The BCS loss in HB cows was about 5 times more than in MB cows (–0.34 vs. –0.07) during the close-up period (–21 to 0 d). Finally, the HB cows lost approximately 1 point in score during a 9-wk period (–21 to 42 d), which was approximately 2 times more than MB cows. Our results showed that BCS changes in HB and MB cows were not affected by SeE injection. However, close-up BCS affected BCS change from –21 to 0, –21 to 42, and 21 to 42 d ($P < 0.05$). During these periods, HB cows lost more BCS than MB cows ($P < 0.05$).

Blood Metabolites

The effect of close-up BCS and SeE injection on serum metabolites over the entire study is presented in Table 3, and the serum metabolites changes are shown in Figure 2A–2E). Results indicated that the interaction between BCS and SeE injection affected serum glucose ($P < 0.05$) over the entire period of the study. The MB+SeE cows had higher ($P < 0.05$) serum glucose than MB–SeE cows (7.48 vs. 6.13 mg/mL) and the HB cows (HB+SeE and HB–SeE) had intermediate serum glucose concentration (6.14 and 7.16 mg/mL, respectively). This situation could be due to the interaction effect (SeE $\times$ BCS). Serum glucose concentration had more fluctuation in the HB–SeE cows compared with the others; in these cows glucose concentration markedly dropped during first 2 wk of lactation (from 8.56 to 5.82 mg/mL) then increased until d 28 (7.58 mg/mL), whereas it decreased again up to d 42 (6.22 mg/mL; Figure 2A), and at 14 d postpartum the cows with no SeE injection had the least ($P < 0.05$) serum glucose concentration. The interaction effect (BCS $\times$ SeE) on serum insulin tended to be significant ($P = 0.07$), and serum insulin was also affected by SeE injection ($P < 0.05$). The SeE-injected cows had higher ($P < 0.05$) serum insulin compared with nonsupplemented cows (10.75 vs. 8.04 μ IU/mL, respectively). The MB–SeE cows had the least serum insulin at calving day, and then at 14 d postpartum, their serum insulin increased to near the insulin levels of injected cows (Figure 2B). Serum insulin in HB–SeE cows increased sharply from d 28 to 42 postpartum and was greatest ($P < 0.05$) at d 42 (from 6.46 to 20.73 mIU/L, which was increased about 3.2-fold).

Serum NEFA concentrations were not affected by the interaction (BCS $\times$ SeE) nor SeE injection. The close-up BCS tended to have an effect on serum NEFA

Figure 1. Effects of close-up BCS and Se-vitamin E (SeE) supplementation on BCS changes (mean $\pm$ SEM) from 3 wk before expected calving to 12 wk postpartum in multiparous Holstein cows. HB+SeE = high BCS and Se-vitamin E injection; HB-SeE = high BCS and no Se-vitamin E injection; MB+SeE = moderate BCS and Se-vitamin E injection; MB-SeE = moderate BCS and no Se-vitamin E injection. *Significant differences are shown by asterisks ($P < 0.05$).

level after calving, and HB cows had greater NEFA ($P = 0.08$) than MB cows (0.57 vs. 0.48 mmol/L). However, in HB-SeE cows, serum NEFA level increased after parturition and the peak was at approximately 14 d postpartum (from 0.56 to 0.78 mmol/L, about 139%; Figure 2C). At this time point, HB-SeE cows had the highest ($P < 0.05$) NEFA concentration. The 2 experimental factors and their interaction (BCS $\times$ SeE) had no significant effects on serum BHB over the study period (Table 3). The MB+SeE cows had greatest ($P < 0.05$) serum BHB at 42 d postpartum (Figure 2D). The SeE injection and its interaction with BCS (BCS $\times$ SeE) had no effect on erythrocyte GSH-Px activity the entire study. However, the MB+SeE group had higher ($P < 0.05$) erythrocyte GSH-Px activity at 28 d postpartum (Figure 2E). Effect of BCS on erythrocyte GSH-Px activity tended to be significant ($P = 0.09$), and MB cows had higher GSH-Px than HB cows (49.97 vs. 44.39 U/g Hb). The BCS and its interaction with SeE (BCS $\times$ SeE) had no effect on serum albumin. The cows which received SeE (HB+SeE and MB+SeE) had higher ($P < 0.01$) serum albumin compared with non-supplemented cows (HB-SeE and MB-SeE). Highest serum albumin concentration was observed at 28 and 42 d postpartum in the HB+SeE and MB+SeE groups, respectively (Figure 2F).

Milk Production and Composition

Effect of close-up BCS and SeE injection on milk production and composition during a 180-d period is presented in Table 4. Neither milk production nor milk composition had been influenced by the interaction (BCS $\times$ SeE). Our results showed that BCS affected milk production, and HB cows (HB+SeE and HB-SeE) had greater ($P < 0.05$) milk production than those MB cows (MB+SeE and MB-SeE; 45.65 ± 0.96 kg/d for HB and 43.47 ± 0.96 kg/d for MB cows). Milk composition, except fat percentage and fat:protein ratio, was not affected by the treatments. The SeE injected groups (HB+SeE and MB+SeE) tended to have higher ($P = 0.09$) milk fat percentage (3.12 for +SeE and 2.98 for -SeE cows) and higher ($P < 0.05$) fat:protein ratio (1.08 for +SeE and 1.03 for -SeE cows) compared with nonsupplemented groups (HB-SeE and MB-SeE).

DISCUSSION

Change in BCS

The cows grouped as high close-up BCS (HB+SeE and HB-SeE) had greater changes in BCS compared with the moderate close-up BCS cows (MB+SeE and

Table 2. Effects of close-up BCS and Se-vitamin E (SeE) supplementation on BCS change (mean ± SEM) before and after calving in multiparous Holstein cows (d -21 to 84)

Days relative to calving	High BCS (HB) ¹				Moderate BCS (MB) ¹				BCS				SeE				P-value			
	+SeE		-SeE		+SeE		-SeE		SEM		High		Moderate		SEM		+		-	
Before calving (-21 to 0)	-0.29		-0.39		-0.06		-0.07		0.07		-0.34 ^a		-0.07 ^b		0.06		-0.18		-0.23	
After calving (0 to 84)																				
0 to 21	-0.26		-0.34		-0.32		-0.31		0.08		-0.3		-0.31		0.06		-0.18		-0.23	
21 to 42	-0.27		-0.25		-0.16		-0.1		0.07		-0.26 ^a		-0.13 ^b		0.05		-0.22		-0.18	
42 to 63	0.08		0.16		0.11		0		0.07		0.1		0.05		0.05		0.1		0.08	
63 to 84	0.14		0.08		0.1		0.18		0.06		0.11		0.14		0.05		0.12		0.13	
Two main periods ²																				
-21 to 42	-0.86		-0.98		-0.54		-0.49		0.08		-0.92 ^a		-0.51 ^b		0.06		-0.7		-0.74	
42 to 84	0.22		0.25		0.24		0.2		0.07		0.23		0.22		0.05		0.23		0.22	

^{a,b}Values within a row with different superscripts differ significantly ($P < 0.05$).
¹HB+SeE = high BCS and Se-vitamin E injection; HB-SeE = high BCS and no Se-vitamin E injection; MB+SeE = moderate BCS and Se-vitamin E injection; MB-SeE = moderate BCS and no Se-vitamin E injection.
²Consist of the periods of negative and positive BCS change (respectively d -21 to 42 and d 42 to 84 after calving).

Table 3. Effects of close-up BCS and Se-vitamin E (SeE) supplementation on serum metabolites concentration (mean ± SEM) in multiparous Holstein cows during the first 42 DIM (d 1 to 42)

Item	High BCS (HB) ¹				Moderate BCS (MB) ¹				BCS				SeE				P-value			
	+SeE		-SeE		+SeE		-SeE		SEM		High		Moderate		SEM		+		-	
Glucose (mg/mL)	6.15 ^{ab}		7.16 ^{ab}		7.48 ^a		6.13 ^b		0.37		6.65		6.81		0.26		6.82		6.65	
Insulin (μIU/mL)	9.18		8.66		12.31		7.41		1.18		8.92		9.86		0.89		10.75 ^a		8.04 ^b	
NEFA ² (mmol/L)	0.51		0.63		0.47		0.49		0.05		0.57		0.48		0.04		0.49		0.56	
BHB (mmol/L)	0.42		0.47		0.46		0.38		0.04		0.45		0.42		0.03		0.44		0.43	
GSH-Px ³ (U/g of Hb)	46.36		42.43		52.02		47.92		3.25		44.39		49.97		2.23		49.19		45.17	
Albumin (g/mL)	0.39		0.34		0.39		0.34		0.01		0.37		0.37		0.01		0.39 ^a		0.34 ^b	

^{a,b}Values within a row with different superscripts differ significantly ($P < 0.05$).
¹HB+SeE = high BCS and Se-vitamin E injection; HB-SeE = high BCS and no Se-vitamin E injection; MB+SeE = moderate BCS and Se-vitamin E injection; MB-SeE = moderate BCS and no Se-vitamin E injection.
²NEFA = nonesterified fatty acid.
³GSH-Px = glutathione peroxidase.

Figure 2. (A–F) Effects of close-up BCS and Se-vitamin E (SeE) supplementation on serum glucose, insulin, nonesterified fatty acid (NEFA), BHB, glutathione peroxidase (GSH-Px), and albumin (mean $\pm$ SEM) during the first 42 d postpartum in multiparous Holstein cows. HB+SeE = high BCS and Se-vitamin E injection; HB–SeE = high BCS and no Se-vitamin E injection; MB+SeE = moderate BCS and Se-vitamin E injection; MB–SeE = moderate BCS and no Se-vitamin E injection. *Significant differences are shown by asterisks ($P < 0.05$).

MB–SeE) during the study period. This result is in agreement with Pires et al. (2013) who compared cows with different pre-calving BCS. The HB cows lost approximately 1 point score during a 9-wk period from 3 wk before expected calving until 6 wk after parturition, which was approximately 2 times more than MB cows. It is believed that the recommended target BCS at calving is 3.25 (5-point scale) and that higher close-up BCS (≥ 3.5 , 5-point scale) could increase metabolic disorders (Roche et al., 2009) and oxidative stress (Bernabucci et al., 2005; Jamali Emam Gheise et al., 2017) in dairy cows.

Blood Metabolites

High-producing dairy cows experience manifest changes in their blood metabolites during the transition period. Therefore, monitoring plasma or serum metabolites related to energy balance and oxidative status is needed for better management of dairy cows (Celi and Gabai, 2015).

Our results showed that MB+SeE cows had the highest serum glucose concentration in the study. Furthermore, this experimental group had the highest glucose level ($P < 0.05$) at 14 d postpartum, which

Table 4. Effects of close-up BCS and Se-vitamin E (SeE) supplementation on milk production and milk composition (mean $\pm$ SEM) in multiparous Holstein cows during the first 180 DIM (d 0 to 180)

Item	High BCS (HB) ¹		Moderate BCS (MB) ¹		BCS			SeE		P-value					
	+SeE	-SeE	+SeE	-SeE	SEM	High	Moderate	SEM	+	-	SEM	BCS	SeE	BCS × SeE	Time
Milk production (kg/d)	46.08	45.22	42.40	44.54	1.24	45.65 ^a	43.47 ^b	0.96	44.24	44.88	0.97	0.04	0.56	0.17	<0.01
Fat (kg/d)	43.00	41.63	40.74	41.91	1.08	42.31	41.32	0.84	41.87	41.77	0.85	0.30	0.92	0.18	<0.01
ECM3.5 ² (kg/d)	42.98	41.94	40.79	42.13	1.06	42.46	41.46	0.84	41.88	42.04	0.85	0.29	0.87	0.20	<0.01
Fat (kg/d)	1.41	1.34	1.35	1.35	0.04	1.38	1.35	0.03	1.38	1.35	0.03	0.54	0.36	0.33	0.13
Protein (kg/d)	1.32	1.32	1.26	1.37	0.03	1.32	1.29	0.03	1.29	1.32	0.02	0.28	0.31	0.38	<0.01
Milk composition															
Fat (%)	3.09	2.98	3.15	2.99	0.08	3.03	3.07	0.06	3.12	2.98	0.07	0.61	0.09	0.76	<0.01
Protein (%)	2.88	2.96	2.94	2.91	0.04	2.92	2.93	0.03	2.91	2.93	0.03	0.79	0.55	0.13	<0.01
Fat:protein (ratio)	1.02	1.02	1.08	1.04	0.03	1.05	1.06	0.02	1.08 ^a	1.03 ^b	0.02	0.72	1.04	0.78	<0.01
Solids ⁴ (%)	5.96	5.94	6.10	5.91	0.11	5.95	6.00	0.09	6.03	5.93	0.09	0.61	0.29	0.40	<0.01
SCS ⁵	3.35	3.34	3.28	3.41	0.08	3.35	3.35	0.07	3.32	3.38	0.07	0.98	0.45	0.35	<0.01

^{a,b}Values within a row with different superscripts differ significantly ($P < 0.05$).

HB+SeE = high BCS and Se-vitamin E injection; MB+SeE = moderate BCS and Se-vitamin E injection; HB-SeE = high BCS and no Se-vitamin E injection; MB-SeE = moderate BCS and no Se-vitamin E injection.

$${}^2\text{FCM}3.5\% = (0.4324 \times \text{kg of milk}) + (16.216 \times \text{kg of milk fat}).$$
$${}^3\text{ECM}3.5\% = [(0.327 \times \text{kg of milk}) + (12.95 \times \text{kg of fat}) + (7.2 \times \text{kg of protein})].$$
⁴Solids = fat plus protein.
$$5\text{SCS (LnSCC)} = [\text{Ln (SCC/100,000)} / 0.693147] + 3.$$

could be related to less milk production, more DMI, or both. As mentioned above, the cows supplemented with SeE (HB+SeE and MB+SeE) had lower fluctuation in serum glucose at the different times of blood sampling. Spears (2011) found that SeE supplementation had positive effects on energy status in postpartum dairy cows. This might be related to the effect of SeE on health and the immune system in animals (Baldi, 2005; Pilarczyk et al., 2012). Jorritsma et al. (2003) demonstrated that severe NEB in cows led to fatty liver, which is characterized by lower blood glucose and insulin concentration, and also higher levels of NEFA and BHB in the early stage of lactation.

In the present study, serum insulin dramatically increased in HB-SeE cows from d 28 to 42 postpartum (Figure 2B), which might be due to insulin resistance in this group (Bernabucci et al., 2005). It has been clarified that cows supplemented with SeE before calving had higher postpartum insulin sensitivity (Mehdi and Dufrasne, 2016). Moreover, oxidative stress during NEB could activate nuclear factor kappa β pathway, which is responsible for stimulating insulin resistance (Ogihara et al., 2004; Shi et al., 2015). During the 28 d after parturition, the experimental groups injected with SeE had higher serum insulin concentration, compared with nonsupplemented cows (HB-SeE and MB-SeE) that had lower level of insulin. This status may be related to the positive effects of SeE on energy balance and glucose uptake by peripheral cells (Spears, 2011).

Concentrations of NEFA and BHB are commonly measured in dairy herds as indicators for fat mobilization after calving and energy status. It was reported that severe NEB leads to body tissue mobilization and affects some blood metabolites such as NEFA, BHB, glucose, insulin, and so on in postcalving dairy cows (Bewley and Schutz, 2008). Results of the present study showed that HB–SeE cows had the highest serum NEFA at 14 d postpartum (Figure 2C) and that values were near the critical threshold (0.8 mmol/L; Roberts et al., 2012; Pires et al., 2013). This could be related to lower serum glucose in this experimental group (Figure 2A). Moreover, higher serum NEFA in HB–SeE over the entire study, and especially 14 d after parturition, could be related to stronger BCS loss in these cows (Figure 1). It has been demonstrated that cows with more than 0.8 mmol/L of NEFA and 1.2 mmol/L of BHB at 1 and 2 wk after calving had a higher risk for metabolic disorders and culling risk (Roberts et al., 2012; Huzzey and Overton, 2013).

The GSH-Px was considered as an important antioxidant indicator of body Se status (Milad et al., 2001). Our results revealed that MB+SeE cows had highest erythrocyte GSH-Px activity at 28 d postpartum (Figure 2E). Furthermore, the activity of erythrocyte

GSH-Px in MB groups (MB+SeE and MB–SeE) was higher than in HB groups (HB+SeE and HB–SeE). Pilarczyk et al. (2012) demonstrated that dairy cows, during the first stage of lactation, should have highest serum Se concentration for greater serum and erythrocyte GSH-Px activity, and therefore can handle the risk of oxidative stress. Moreover, it is well known that albumin, as a primary source of serum thiol groups, has a significant effect on extracellular antioxidant capacity (Bernabucci et al., 2005; Celi and Gabai, 2015). Other antioxidant indices such as superoxide dismutase and thiobarbituric acid reactive substances should be considered in future studies.

Our results indicate that cows with moderate close-up BCS might have better oxidative status in the transition period (Dobbelaar et al., 2010). Results of the present study showed that serum albumin was increased by SeE injection. Mehdi and Dufrasne (2016) demonstrated that Se is bound to different serum metabolites (albumin, globulins, low-density lipoprotein, and very low-density lipoprotein) and that albumin is the main source of serum Se.

Milk Production and Composition

In accordance with previous reports (Berry et al., 2007; Jamali Emam Gheise et al., 2017), cows with high close-up BCS had greater potential for milk production. This condition could be related to the insulin resistance in fat cows and the homeorhesis mechanism for shifting blood glucose to the mammary glands. However, as mentioned earlier, this group of cows had higher serum NEFA, which increases the risk of reproductive and health problems. Our results showed that fat percentage and fat:protein ratio increased after SeE injection. Liu et al. (2008) reported that feeding SeE supplement increased milk fat percentage in Holstein dairy cows. However, the method of SeE supplementation was different and the mechanism is unknown.

CONCLUSIONS

Findings of the present study showed that close-up BCS, SeE injection, and their interaction (BCS $\times$ SeE) influenced some blood metabolites and lactation performance in high-producing Holstein cows. The BCS and SeE interaction affected serum glucose and insulin. The SeE injection had more positive effects on moderate close-up BCS cows. High close-up BCS cows clearly lost more BCS before and after calving, and these cows had higher serum NEFA, especially approximately 14 d postpartum. Cows with high close-up BCS produced more milk than moderate close-up BCS cows. However, this condition had a negative effect on their oxidative

status. Three rounds of SeE injection in the postpartum period of both MB and HB cows increased serum insulin and albumin, milk fat percentage, and milk fat:protein ratio. Finally, it was concluded that SeE injection has more positive effects on MB cows compared with HB cows. The effect of SeE injection in HB cows requires further research by individually DMI and NEB measurement, which we had limitations for that due to the herd experiment.

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