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Peripartum Injection of Vitamins (E and B_{12}) and Trace Minerals (Selenium and Iron) in Holstein Dairy Cows: Effect on Milk Production and Composition, Body Condition Score and Serum Metabolites

Research Article

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ABSTRACT

The aim of this study was to determine the effects of injection of vitamin E and selenium, vitamin B_{12} and iron or their combination during the transition period on milk production and composition, body condition score (BCS) changes and serum metabolites of dairy cows. A total of 40 Holstein dairy cows (659±57.9 kg of body weight (BW)) were divided into four groups based on parity, BW and BCS and randomly assigned to experimental treatments. Experimental treatments were T1: injection of NaCl % 0.9 as control treatment (C), T2: injection of 3000 IU of vitamin E and 30 mg of selenium (ESe), T3: injection of 700 µg of vitamin B_{12} and 254 mg of iron (B_{12}Fe) and T4: injection of 3000 IU of vitamin E and 30 mg of selenium plus 700 µg of vitamin B_{12} and 254 mg of iron (ESe+B_{12}Fe). Injection of ESe, B_{12}Fe or ESe + B_{12}Fe had no effect on BCS and its changes (P>0.05). Milk production, 4% fat-corrected milk (FCM), energy-corrected milk (ECM) and milk fat, protein and lactose content and yield did not influence by injection of ESe, B_{12}Fe or ESe + B_{12}Fe (P>0.05). Experimental treatments had no effect on serum β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), glucose, total protein, triglycerides, total cholesterol and HDL-cholesterol concentrations (P>0.05). It was concluded that injection of ESe, B_{12}Fe or their combination during transition period had no effect on milk production and composition, BCS changes, and serum metabolites concentrations of Holstein dairy cows.

KEY WORDS dairy cow, mineral, performance, transition period, vitamin.

INTRODUCTION

The transition period from 3 weeks before to 3 weeks after calving is the most critical period of life for dairy cows that accompanied by a stressful experience. During the transition period, dairy cows undergo dramatic alteration in their metabolism to supply the nutrients for milk production (Goff *et al*. 2002), and also dealing with decreasing dry matter intake (DMI) and negative energy balance (NEB) can lead to mobilization of large amounts of body fat, a concomitant increase in plasma non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) concentration, accumulation of lipids in the liver (Roche *et al*. 2009), lipid peroxidation and reactive oxygen species, and oxidative stress that reduced immune resistance (Sordillo and Aitken, 2009). These alterations altogether contribute to dairy cows increased susceptibility to develop metabolic disorders and infectious diseases such as ketosis, retained placenta, metritis, mastitis, abomasal displacement, and reduced immune resistance (Furll *et al*. 2010; Sordillo and Aitken, 2009;

Bicalho *et al*. 2014). Most of the metabolic disorders occur postpartum (Drackley, 1999), decreasing the milk production, impairing the reproductive performance, increasing the risk of culling and morbidity (Huzzey *et al*. 2007; Pereira *et al*. 2013). Several strategies have focused on maximizing DMI and energy in the transition period to reduce the effects of NEB, including supplementation with glycerol (DeFrain *et al*. 2004), nicotinic acid (Pires *et al*. 2007), cis-linoleic acid (Mosley *et al*. 2007), fat (Moallem *et al*. 2007), methionine (Preynat *et al*. 2009), choline (Chung *et al*. 2009), carnitine (Carlson *et al*. 2007) and monensin (Duffield *et al*. 2008; Pereira *et al*. 2013). Overall, current treatments had limited success, and more research is needed for an adjunct therapeutic agent that is effective in the treatment or prevention of fat mobilization syndrome in transition dairy cows. One potential alternative is the use of butaphosphan and cyanocobalamin (vitamin B_{12}) injections after calving, which have shown positive effects (Furll *et al*. 2010; Rollin *et al*. 2010; Pereira *et al*. 2013). Transition period is a stressful time that lead to decreases in trace mineral retention ability (Xin *et al*. 1993) and plasma concentrations of Ca and Zn (Goff *et al*. 2002), suggesting that other mineral concentrations could also be affected (Machado *et al*. 2013; Machado *et al*. 2014). Some studies suggested that long-term supplementation of trace mineral and vitamins above the predicted requirements may positively influence dairy cattle productivity, particularly during physiological stress (Kincaid and Socha, 2004; Rensis and Scaramuzzi, 2003; Khorsandi *et al*. 2016). Trace minerals and vitamins are essential as antioxidants for improvement of immune system in dairy cows (Sordillo, 2005). For instance, subclinical mineral deficiency of Se has been associated with immunosuppression (Sordillo and Aitken, 2009) and reproductive failure (Spears and Weiss, 2008), subclinical deficiency of Zn was associated with impaired growth (Enjalbert *et al*. 2006), and subclinical deficiency of Ca was associated with decreased milk production (Oetzel, 2013; Bicalho *et al*. 2014). Cows with subclinical hypocalcemia have reduced blood neutrophil counts as well as impaired neutrophil function and increased incidence of metritis compared with normocalcemic cows (Martinez *et al*. 2012; Bicalho *et al*. 2014). Moreover, an association between a greater degree of negative energy balance (characterized by elevated prepartum NEFA concentration and postpartum BHB concentration) and decreased immune response in cows that developed uterine disease compared with healthy cows has been reported (Hammon *et al*. 2006; Galvao *et al*. 2010; Bicalho *et al*. 2014). Therefore, an adequate supply of vitamins and minerals is important for ensuring an optimal transition from pregnancy to lactation. Iron as an antioxidant and energetic mineral can protect immune cells against reactive oxygen species (ROS) and improvement of NEB for transition dairy cows (Tomlinson *et al*. 2008).

In dairy cows, plasma iron (Fe) concentration is decreased during the acute phase response to immunological challenges (Kushner, 1982; Andrieu, 2008). Some study reported that plasma concentration of Fe in dairy cows decrease during late gestation (Furugouri *et al*. 1982; Weiss *et al*. 2010). Although Fe is a required nutrient for adult dairy cattle, essentially no research has been conducted evaluating its requirement in the last 50 years (Weiss *et al*. 2010).

A major source of dietary Fe for dairy cows is forage. Forages, because of soil contamination, often contain more than 200 mg/kg of Fe, which presumably should be adequate to meet or exceed a cow's requirement for Fe (Underwood and Suttle, 1999). Soil Fe, however, can have very low bioavailability (Hansen and Spears, 2009). Probably because of fetal growth, increased maternal blood volume, and low DMI (relative to that in lactating cows), Fe status may be reduced in late gestation dairy cows. Furthermore, an association between low hemoglobin concentrations and low milk yields has been shown in dairy goats (Atroshi *et al*. 1986; Weiss *et al*. 2010). Therefore, improved Fe status in late gestation may result in increased milk production in early lactation.

Providing additional vitamin B_{12} (B_{12}) could enhance the efficiency of energy production from propionate by a more active tricarboxylic acid (TCA) cycle (Rollin *et al*. 2010). Reduction in plasma concentration of B_{12} was frequently observed in early lactating cows due to increased demands for milk secretion, insufficient synthesis by ruminal bacteria and decreasing DMI (Girard *et al*. 2005; Weiss and Ferreira, 2006; Akins *et al*. 2013; Duplessis *et al*. 2014). For example, Kincaid *et al*. (2003) reported that serum vitamin B_{12} concentrations of 2.4, 2.0, and 1.2 ng/mL at −21, 7, and 120 d relative to parturition, respectively, and the decrease from 21 d prepartum to 7 DIM was greater for primiparous cows. Also, Kincaid and Socha (2007) showed a significant decrease in serum vitamin B_{12} concentration from 5.7 ng/mL at 55 d prepartum to 2.3, 2.0, and 1.9 ng/mL at −20, 7, and 120 d relative to parturition, respectively. Hence, B_{12} supply is considered a limiting factor for milk production in early lactation (Girard and Matte, 2005; Furll *et al*. 2010).

Vitamin E (E) is one of the best natural antioxidants that protect immune cells against free radical in dairy cows (Smith *et al*. 1997; Weiss and Spears, 2006; Spears and Weiss, 2008). Concentration of vitamin E is very low between one week before to 2 weeks after calving (Spears and Weiss, 2008). High producing dairy cows have moderate hepatic lipidosis after dry period and accumulate fat in the liver, which peaks at about two weeks postpartum. The ruminant's liver is incapable of rapidly increase lipoprotein

secretion and also E is transported in plasma mainly by very low density lipoprotein (Dutta-Roy *et al*. 1994) consequently, the risk of low plasma levels of E is more pronounced during the peripartum period (Baldi *et al*. 2000).

It is known that selenium (Se) improves immune responses, health and performance of dairy cows (Cebra *et al*. 2003). The concentration of serum Se decreased during the last two months of gestation which indicates the importance of administration of selenium during the late gestation in dairy cows (Abdelrahman and Kincaid, 1995; Moeini *et al*. 2009). Diets for ruminant animals are almost exclusively of plant origin and the Se content within plants can be extremely variable (Juniper *et al*. 2009; Wang *et al*. 2009). Also, the available Se concentration in soil is low in many regions of the world including most parts of Iran. Soils in many of the areas of Iran are selenium deficient and feedstuffs grown on these soils will not provide adequate dietary Se (Kojouri, 2002; Moeini *et al*. 2009). Consequently, selenium in diets can be deficient. Selenium deficiency has been reported to be involved in the pathogenesis of postnatal maladjustment syndrome (Guyot *et al*. 2004), fertility problems (Corah and Ives, 1991) and udder health (Smith *et al*. 1997) in dairy herds. Therefore, Se supplementation may be required to improve the performance and health of dairy cow. Furthermore, Se supplementation may enhance the nutritional quality of the milk product (Wang *et al*. 2009).

The above mentioned studies provided some useful information regarding the potential benefits of minerals and vitamins might have for transition cows, but it also created questions regarding the form of use, time and duration of injection or consumption and also possible biological mechanisms that led to the observed effects. On the other hand, quantifying the requirements of dairy cattle for trace minerals precisely is a particularly difficult task. Trace minerals are needed in minute amounts, but variation in feed composition and DMI can be high, making precise and accurate measurements of intake of trace minerals difficult (Bicalho *et al.* 2014). Although, NRC (2001) meet requirements of minerals and vitamins by diet for transition dairy cow, but limitations such as feed intake reduction, negative interactions between minerals and vitamins in diet, stress and incidence of metabolic and infectious diseases may decrease the amount of nutrient received and their bioavailability. On the other hand, inclusion of minerals in the diet does not ensure intake or absorption (Roche *et al*. 2009; Machado *et al*. 2013). Antagonists in drinking water (e. g. iron) can also have a negative effect on trace mineral absorption from the digestive tract (Spears, 2003). Therefore, administration of minerals and vitamins by injection could potentially provide an alternative way of feeding trace minerals and vitamins during the transition period.

Feeding supplemental organic Fe to late gestation and early lactation dairy cows had no effect on milk production and composition (Weiss *et al*. 2010). Previous studies (Moeini *et al*. 2009; Wang *et al*. 2009; Bayril *et al*. 2015) showed that vitamin E and Se injection or dietary during the transition period increased milk production of dairy cows, whereas, in studies of Liu *et al*. (2008) and Santos *et al*. (2016), had no effect. Furthermore, milk production increased by B_{12} injection or dietary during the transition period (Preynat *et al*. 2009; Pereira *et al*. 2013), whereas, in other studies (Girard and Matte, 2005; Furll *et al*. 2010; Akins *et al*. 2013; Weerathilake *et al*. 2018) had no effect. Also, Duplessis *et al*. (2014) reported lower milk fat and higher milk protein content in response to B_{12} injection during the transition period. These contrasting results could be due to differences in the composition of the diet and/or to the dose of vitamin and mineral and lactation period of dairy cow.

It could be that using a combination of trace minerals and vitamin is more beneficial than administrating alone. To the best of our knowledge, there is no research regarding the combination effect of injectable vitamin E, Se and B_{12}, Fe during the transition period on milk production and composition. We hypothesized that injection of vitamin E and Se (Ese) and vitamin B_{12} and Fe (B_{12}Fe) together during the transition period may improve metabolic indices, performance and health of dairy cows. Therefore, the objective of this study was to evaluate the effects of multiple injections of ESe, B_{12}Fe or their combination during transition period on milk production and composition, body condition score (BCS) and serum metabolites concentrations in Holstein dairy cows.

MATERIALS AND METHODS

Animals, treatment and management

This study was conducted in a commercial dairy farm located in Kermanshah province, west of Iran, (Bazoye Keshavarz Sarmast Co.). A total of 40 Holstein dairy cows (659±57.9 kg of body weight (BW)) at day 21 before expected calving were divided into 4 groups based on parity, BW and BCS and randomly allocated to experimental treatments. Diets were formulated using the dairy NRC (2001) software to meet the nutrient requirements of dairy cows (NRC, 2001).

All cows were housed in free-stall barn, fed the same total mixed ration (TMR) twice daily at 08:00 h and 16:00 h and had free access to drinking water throughout the experiment. Cows fed with pre-fresh diet for the last 3 weeks of gestation, fresh diet for the first 3 weeks of lactation and lactation diet from day 21 after calving to 90th days of lactation (Table 1).

Table 1 Ingredients and chemical composition of diet fed to pre-fresh, fresh and lactating dairy cows[1]

Ingredient (g/kg DM)	Pre-fresh	Fresh	Lactation
Alfalfa hay	117.6	139.0	108.6
Corn silage	627.5	541.7	540.0
Barley straw	19.6	8.3	6.4
Ground barley grain	19.0	31.1	34.5
Ground corn grain	112.8	143.4	158.9
Wheat bran	24.6	9.3	10.3
Rapeseed meal	19.0	21.7	24.1
Soybean meal	28.2	68.4	76.0
Meat meal	0.0	9.3	10.3
Calcium salts of palm fatty acids	0.0	9.3	10.3
Calcium carbonate	4.7	3.1	3.4
Salt	0.0	2.1	2.4
Sodium bicarbonate	4.7	6.2	6.9
Mineral and vitamin premix[2]	22.3	7.1	7.9
Chemical composition (g/kg DM)			
Crude protein	146.2	176.8	178.1
Ether extract	31.0	41.5	54.7
Ash	98.0	85.2	76.0
Neutral detergent fiber	382.7	325.0	320.2
Acid detergent fiber	240.2	214.2	213.5
Non fibrous carbohydrate[3]	342.1	371.5	371.0
Net energy for lactation (Mcal/kg DM)[4]	1.59	1.68	1.70
Calcium	12.5	8.5	8.9
Phosphorous	3.6	4.4	4.5
Magnesium	3.6	3.3	3.2
Potassium	10.0	11.2	11.5
Selenium	0.039	0.042	0.042
Iron	0.185	0.155	0.159
Zinc	0.59	0.88	0.87
Copper	0.015	0.018	0.019
Manganese	0.045	0.056	0.058

[1] Pre-fresh: cows in the last 3 weeks of gestation; Fresh: cows in the first 3 weeks of lactation and Lactation: cows after 3 weeks of lactation.
[2] Contained (per kg): Calcium: 140 g; Phosphorous: 20 g; Magnesium: 35 g; Organic Cr: 40 mg; S: 40 g; Mn: 1200 mg; Zn: 1000 mg; Cu: 800 mg; Co: 8 mg; I: 10 mg; Fe: 400 mg; Se: 15 mg; Niacin (B_3): 20000 mg; vitamin A: 350000 IU; vitamin D: 60000 IU; vitamin E: 4000 IU and Anionic salts for pre-fresh diet: 650 g.
[3] Non fibrous carbohydrate (NFC)= 1000 – (neutral detergent fiber+crude protein+ether extract+ash).
[4] Calculated according to NRC (2001).

Experimental treatments consisted of T1: injection of NaCl % 0.9 as control treatment (C), T2: injection of 3000 IU of vitamin E and 30 mg of selenium (ESe), T3: injection of 700 µg of vitamin B_{12} and 254 mg of iron (B_{12}Fe) and T4: injection of 3000 IU of vitamin E and 30 mg of selenium plus 700 µg of vitamin B_{12} and 254 mg of iron (ESe+B_{12}Fe). Mineral and vitamin solutions were injected at days 21 and 7 before and day 7 after calving. Vitamin E and Se solution (Vitesel, Nasr Co. Iran) and vitamin B_{12} and Fe solution (Cyanoferin, Nasr Co. Iran) were injected subcutaneously and intramuscularly, respectively.

Samples of TMR were collected weekly and analyzed for dry matter (DM; method 934.01-AOAC, 2007), ash (method 942.05-AOAC, 2007), crude protein (CP; method 976.05-AOAC, 2007), ether extract (EE; method 973.18-AOAC, 2007), neutral detergent fiber (NDFom) and acid-detergent fiber (ADFom) according to Van Soest *et al.* (1991).

Potassium (K), magnesium (Mg), copper (Cu), iron (Fe), zinc (Zn), selenium (Se) and manganese (Mn) were analyzed by atomic absorption by using an air-acetylene flame and calcium (Ca) by using a nitroxide acetylene flame (Atomic absorption, Analytikjena, nov AA 400P, Germany).

Phosphorus (P) was analyzed using an AutoAnalyzer (model BT 1500, Biotecnica Instrument Co, Rome, Italy) method by Pars Azmun kits (Pars Azmun Laboratory, Tehran, Iran).

Blood sampling and analysis

Blood samples were collected from the coccygeal vein before the morning feeding as well as solution injection at days 21 and 7 before expected calving, immediately after calving and days 7, 14 and 21 after calving using evacuated tubes without heparin anticoagulant. Serum was separated by centrifuging samples at 3000 × g for 15 min at 4 ˚C and stored at -20 ˚C until analysis. Concentrations of glucose, total protein, triglyceride, HDL-cholesterol, total cholesterol (Pars Azmun Co. Iran), β-hydroxybutyrate **(**BHB) and non-esterified fatty acids (NEFA; Randox kit, UK**)** were measured by an auto analyzer (Biotecnica Instruments, BT1500, Italy).

BCS, milk production and composition

All cows were scored for body condition at day 21 before expected calving, at calving day and day 30 after calving by a single investigator using a 5-point scale (Edmonson *et al.* 1989). The cows were milked three times daily at 08:30, 16:00 and 24:00 h. Milk production was recorded and milk samples were taken from all cows on the three consecutive milking at days 30, 60 and 90 of lactation. Fat corrected milk (4% FCM) is defined as milk with 4% fat (NRC, 2001).

Milk samples were analyzed for fat, protein and lactose content (MilkoScan; FUNKE GERBER, LactoStar, Germany). Milk protein, fat and lactose production were calculated by multiplying milk production from the respective day by protein, fat and lactose contents of the milk for each cow. Energy corrected milk (ECM) was calculated as (0.327×milk yield (kg/d)) + (12.95×fat yield (kg/d)) + (7.2×protein yield (kg/d)) according to Tyrrell and Reid (1965).

Statistical analyses

All data were statistically analyzed as a 2 × 2 factorial arrangement based on randomized block design using PROC MIXED of SAS with the following model:

$$Y_{ijkl} = \mu + ESe_i + B_{12}Fe_j + (ESe \times B_{12}Fe)_{ij} + P_k + A_l\,(P_k) + e_{ijkl}$$

Where:

Y_{ijkl}: dependent variable.

μ: overall mean.

ESe_i: effect of vitamin E and Se injection.

$B_{12}Fe_j$: effect of vitamin B_{12} and Fe injection.

$(ESe \times B_{12}Fe)_{ij}$: interaction effect of ESe and $B_{12}Fe$ injection.

P_k: effect of block (parity).

$A_l\,(P_k)$: random effect of cow within the block.

e_{ijkl}: random effect of residual error.

Significant differences among treatments were tested using LSMEANS with the PDIFF option with significance declared at $P \leq 0.05$, and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Effect of experimental treatments on BCS and its changes during the transition period are presented in Table 2. Injection of ESe, $B_{12}Fe$ or their combination (interaction of treatments) had no effect on BCS at calving day, day 30 after calving and BCS changes before and after calving (P>0.05).

Effect of ESe, $B_{12}Fe$ or their combination on milk production and composition are shown in Table 3. Milk production did not influence by experimental treatment at days 30, 60 and 90 of lactation (P>0.05). The interaction of treatments were not significant for milk production (P>0.05), however, tended to increase numerically compared to control group at days 30, 60 and 90 of lactation. Injection of ESe or its interaction with $B_{12}Fe$ had no effect on milk fat percentage during the experiment (P>0.05), whereas milk fat content of lactation tended to be lower (P=0.09) in cows received $B_{12}Fe$ solution compared with those received no $B_{12}Fe$ solution at day 30. The interaction effects of the investigated factors not significant for milk fat percentage (P>0.05), however, tended to decrease numerically compared to control group at days 30, 60 and 90 of lactation. Milk protein content at days 30 and 60 of lactation was similar for cows received ESe, $B_{12}Fe$ or their combination compared with the C group (P>0.05), whereas protein precent tended to be lower in milk produce by cows received $B_{12}Fe$ solution compared with those without $B_{12}Fe$ injection (P=0.06). Milk lactose content tended to be lower at days 30 (P=0.08) and 60 (P=0.07) of lactation in cows of the C treatment than other groups. Whereas, injection of ESe or $B_{12}Fe$ alone compared with no injection had no effect on milk lactose content at days 30, 60 and 90 of lactation (P>0.05).

The interaction of treatments were not significant for milk lactose percentage and milk lactose production (P>0.05), however, tended to increase numerically compared to control group at days 30, 60 and 90 of lactation.

Effect of experimental treatments on serum metabolites concentrations are presented in Table 4. Serum BHB concentration was similar among experimental treatments at day 7 before expected calving, calving day and days 7, 14 and 21 after calving (P>0.05). Injection of $B_{12}Fe$ during the transition period tended to decrease numerically serum BHB concentration at calving day and day 21 after calving compared with those without $B_{12}Fe$ injection (P=0.06).

Table 2 Effect of experimental treatments[1] on body condition score (BCS) and its changing

Item	-ESe		+ESe		SEM	P-value		
	-B_{12}Fe	+B_{12}Fe	-B_{12}Fe	+B_{12}Fe		B_{12}Fe *vs*. NI	Ese *vs*. NI	B_{12}Fe × ESe
				Body condition score				
Day 21 pre-partum	3.60	3.56	3.61	3.61	0.04	0.59	0.49	0.59
Calving day	3.28	3.34	3.31	3.31	0.03	0.34	0.88	0.34
Day 30 postpartum	3.07	2.95	2.97	3.02	0.06	0.59	0.84	0.17
Body condition score change								
Day 21 pre-partum-calving day	-0.32	-0.22	-0.30	-0.30	0.03	0.29	0.86	0.29
Calving day-day 30 postpartum	-0.21	-0.39	-0.34	-0.29	0.07	0.53	0.62	0.17

ESe: no injection of ESe; +ESe: injection of ESe; -B_{12}Fe: no injection of B_{12}Fe; +B_{12}Fe: injection of B_{12}Fe; B_{12}Fe *vs*. NI: comparison between injection of B_{12}Fe *vs*. no injection; Ese *vs*. NI: comparison between injection of ESe *vs*. no injection and B_{12}Fe × ESe: interaction effect.
SEM: standard error of the means.

Table 3 Effect of experimental treatments[1] on milk production and composition

Item	-ESe		+ESe		SEM	P-value		
	-B_{12}Fe	+B_{12}Fe	-B_{12}Fe	+B_{12}Fe		B_{12}Fe *vs*. NI	Ese *vs*. NI	B_{12}Fe × ESe
Day 30 of lactation								
Milk production (kg/d)	33.58	38.20	36.04	37.42	2.59	0.26	0.74	0.53
4% FCM[1] (kg/d)	30.40	30.71	31.56	29.69	2.02	0.71	0.97	0.60
ECM[2] (kg/d)	33.48	34.38	34.95	33.13	2.29	0.84	0.96	0.56
Fat (%)	3.16	2.82	3.06	2.83	0.15	0.09	0.77	0.72
Fat (kg/d)	1.09	1.12	1.20	1.09	0.10	0.69	0.72	0.50
Protein (%)	3.14	3.15	3.13	3.07	0.04	0.59	0.37	0.45
Protein (kg/d)	1.09	1.19	1.18	1.13	0.09	0.77	0.88	0.39
Lactose (%)	4.40	4.61	4.68	4.52	0.10	0.83	0.38	0.08
Lactose (kg/d)	1.55	1.75	1.73	1.66	0.15	0.71	0.77	0.38
Day 60 of lactation								
Milk production (kg/d)	37.73	39.34	37.63	38.80	2.87	0.64	0.91	0.94
4% FCM (kg/d)	33.11	33.08	32.97	32.40	2.43	0.90	0.86	0.91
ECM (kg/d)	36.37	36.80	36.54	35.94	2.62	0.97	0.89	0.85
Fat (%)	3.16	3.00	3.19	2.92	0.19	0.24	0.89	0.76
Fat (kg/d)	1.19	1.07	1.19	1.13	0.11	0.45	0.79	0.80
Protein (%)	3.09	3.17	3.21	3.12	0.04	0.91	0.47	0.09
Protein (kg/d)	1.17	1.21	1.24	1.20	0.09	0.77	0.99	0.68
Lactose (%)	4.43	4.66	4.73	4.58	0.10	0.71	0.30	0.07
Lactose (kg/d)	1.70	1.83	1.81	1.77	0.14	0.78	0.86	0.56
Day 90 of lactation								
Milk production (kg/d)	33.93	36.16	34.01	35.10	2.81	0.56	0.86	0.84
4% FCM (kg/d)	28.86	29.43	28.92	29.06	1.95	0.86	0.94	0.91
ECM (kg/d)	31.99	32.85	32.35	32.00	2.20	0.91	0.91	0.78
Fat (%)	3.06	2.79	2.32	2.86	0.26	0.17	0.53	0.73
Fat (kg/d)	1.01	0.99	1.02	1.00	0.07	0.77	0.95	0.99
Protein (%)	3.14	3.11	3.28	2.98	0.08	0.06	0.93	0.10
Protein (kg/d)	1.06	1.12	1.11	1.04	0.09	0.95	0.83	0.51
Lactose (%)	4.52	4.47	4.66	4.55	0.08	0.34	0.19	0.72
Lactose (kg/d)	1.54	1.62	1.58	1.60	0.13	0.72	0.92	0.82

[1] 4% FCM (fat corrected milk)= (0.4×milk production (kg/d)) + (15.0×(fat yield (kg/d)).
[2] ECM (energy corrected milk)= (0.327×milk production (kg/d)) + (12.95×fat yield (kg/d)) + (7.2×(protein yield (kg/d)).
ESe: no injection of ESe; +ESe: injection of ESe; -B_{12}Fe: no injection of B_{12}Fe; +B_{12}Fe: injection of B_{12}Fe; B_{12}Fe *vs*. NI: comparison between injection of B_{12}Fe *vs*. no injection; Ese *vs*. NI: comparison between injection of ESe *vs*. no injection and B_{12}Fe × ESe: interaction effect.
SEM: standard error of the means.

Likewise, serum BHB concentration at day 21 after calving decreased in cows received ESe injection than those without ESe injection (P=0.05). The treatment interaction effects were not significant for serum BHB concentration (P>0.05), however, tended to decrease numerically at day 7 before expected calving, calving day, days 14 and 21 after calving.

Serum concentration of NEFA at day 7 before expected calving, calving day and days 14 and 21 after calving did not influence by experimental treatments (P>0.05). However, serum NEFA concentration tended to be higher in cows in the C group compared with other groups (P=0.07).

Table 4 Effect of experimental treatments on serum metabolites concentration

Item	-ESe		+ESe		SEM	P-value		
	-B_{12}Fe	+B_{12}Fe	-B_{12}Fe	+B_{12}Fe		B_{12}Fe *vs.* NI	Ese *vs.* NI	B_{12}Fe × ESe
Beta-hydroxybutyrate (µM/L)								
Day 7 before calving	487.33	490.33	484.00	476.67	5.08	0.11	0.67	0.32
Calving day	699.33	685.17	692.67	675.33	7.94	0.06	0.31	0.84
Day 7 after calving	656.50	643.50	642.50	643.67	7.21	0.16	0.12	0.72
Day 14 after calving	541.67	529.17	532.50	526.00	7.14	0.22	0.36	0.73
Day 21 after calving	461.83	453.00	452.50	441.00	5.12	0.06	0.05	0.79
Non-esterified fatty acids (µM/l)								
Day 7 before calving	104.67	101.17	98.83	95.83	4.01	0.42	0.17	0.95
Calving day	458.00	438.30	448.17	428.83	9.49	0.06	0.32	0.98
Day 7 after calving	369.00	343.67	350.83	349.50	6.31	0.04	0.34	0.07
Day 14 after calving	314.83	307.00	304.83	297.17	5.57	0.01	0.09	0.17
Day 21 after calving	238.50	228.83	223.67	219.33	4.76	0.06	0.06	0.97
Glucose (mg/dL)								
Day 7 before calving	77.33	87.16	79.16	84.83	3.72	0.06	0.94	0.58
Calving day	117.01	114.28	105.26	126.45	11.89	0.44	0.98	0.33
Day 7 after calving	84.16	93.50	83.66	97.66	8.17	0.16	0.82	0.77
Day 14 after calving	61.16	62.16	56.83	70.00	3.64	0.06	0.63	0.11
Day 21 after calving	66.66	72.50	70.33	68.83	4.55	0.63	0.99	0.43
Total protein (g/dL)								
Day 7 before calving	7.16	7.07	6.83	7.01	0.29	0.88	0.51	0.64
Calving day	8.05	7.16	7.62	8.06	0.40	0.60	0.57	0.12
Day 7 after calving	6.76	6.40	6.83	6.23	0.32	0.17	0.88	0.73
Day 14 after calving	7.18	6.77	7.10	7.13	3.51	0.88	0.35	0.60
Day 21 after calving	7.55	7.41	7.54	8.11	0.32	0.52	0.29	0.28
Triglyceride (mg/dL)								
Day 7 before calving	49.33	50.66	47.83	45.50	3.51	0.88	0.35	0.60
Calving day	40.33	49.33	42.66	40.66	4.52	0.44	0.49	0.23
Day 7 after calving	40.00	39.66	41.83	42.16	3.46	0.99	0.53	0.92
Day 14 after calving	33.00	36.33	34.00	36.00	1.77	0.12	0.78	0.78
Day 21 after calving	33.00	28.16	36.16	32.50	2.45	0.09	0.14	0.81
Total cholesterol (mg/dL)								
Day 7 before calving	149.17	159.00	146.00	134.00	8.69	0.90	0.12	0.22
Calving day	137.67	158.00	134.83	141.83	7.04	0.06	0.19	0.35
Day 7 after calving	146.67	174.50	151.33	162.67	17.37	0.27	0.83	0.64
Day 14 after calving	144.67	173.00	152.33	164.00	10.46	0.07	0.94	0.43
Day 21 after calving	192.17	208.83	214.67	197.17	14.85	0.71	0.97	0.26
HDL-cholesterol (mg/dL)								
Day 7 before calving	83.83	86.16	88.06	84.50	5.71	0.90	0.81	0.60
Calving day	82.83	87.50	78.16	88.66	4.07	0.07	0.67	0.48
Day 7 after calving	75.83	95.00	86.33	89.83	6.27	0.67	0.08	0.22
Day 14 after calving	72.16	89.00	86.16	76.66	7.78	0.64	0.91	0.10
Day 21 after calving	77.33	70.00	85.83	86.50	5.13	0.94	0.10	0.94

ESe: no injection of ESe; +ESe: injection of ESe; -B_{12}Fe: no injection of B_{12}Fe; +B_{12}Fe: injection of B_{12}Fe; B_{12}Fe *vs.* NI: comparison between injection of B_{12}Fe *vs.* no injection; Ese *vs.* NI: comparison between injection of ESe *vs.* no injection and B_{12}Fe × ESe: interaction effect.
SEM: standard error of the means.

Serum NEFA concentration at days 7 and 14 after calving was lower significantly (P<0.05) and tended to be lower at calving day (P=0.06) and day 21 after calving (P=0.06) in cows received B_{12}Fe injection compared with those without B_{12}Fe injection. Furthermore, serum NEFA concentration at days 14 (P=0.09) and 21 (P=0.06) after calving tended to be lower in cows received ESe injection than those without ESe injection. The interaction of treatments were not significant for serum NEFA concentration (P>0.05), however, tended to decrease numerically at day 7 before expected calving, calving day, days 14 and 21 after calving. Serum glucose concentration at day 7 before expected calving, calving day and days 7, 14 and 21 after calving was similar among experimental treatments (P>0.05). Injection of ESe solution compared with no injection had no effect on serum glucose concentration during the transition period (P>0.05). However, serum glucose concentration tended to be numerically higher when cows received B_{12}Fe injection compared with those without B_{12}Fe injection at day 7 before expected calving and day 14

after calving (P=0.06). The treatment interaction effects were not significant for serum glucose concentration (P>0.05), however, tended to increase numerically at calving day, days 14 and 21 after calving. Serum total protein concentration did not influence by injection of ESe, $B_{12}Fe$ or their combination in transition dairy cows (P>0.05). Experimental treatments had no effect on serum triglyceride (TG) concentration during transition period (P>0.05).

However, serum TG concentration at day 21 after calving tended to be lower numerically in cows injected with $B_{12}Fe$ solution compared with those without $B_{12}Fe$ injection (P=0.09). The interaction of treatments were not significant for serum TG concentration (P>0.05). Serum concentrations of total cholesterol and HDL-cholesterol at day 7 before expected calving, calving day and days 7, 14 and 21 after calving were similar among experimental treatments as well as in cows received ESe injection compared with those without ESe injection (P>0.05). Whereas, serum total cholesterol concentration at calving day (P=0.06) and day 14 after calving (P=0.07) and HDL-cholesterol concentration at calving day (P=0.07) tended to be higher numerically in cows injected with $B_{12}Fe$ than those without injection . The interaction of treatments were not significant for serum total cholesterol and HDL-cholesterol (P>0.05).

The lack of effect of ESe, $B_{12}Fe$ or their combination on BCS and its changes during the transition period in the present study was consistence with previous studies, who observed similar BCS when dairy cows received injection of antioxidant minerals solution (Se, Mn, Cu and Zn) and vitamin E (Daugherty *et al*. 2002), vitamin B_{12} (Akins *et al*. 2013; Weerathilake *et al*. 2018) or vitamin B_{12} and P (Pereira *et al*. 2013) during the transition period. Similar to the results of the present study, Graulet *et al*. (2007) and Preynat *et al*. (2009) observed no treatment effect on pre- and post-calving BW and BCS for cows receiving a combination of folic acid and vitamin B_{12} supplement. Duplessis *et al*. (2017) reported no benefit to injections of vitamin B_{12} on body condition over the transition period (Duplessis *et al*. 20017). However, in the study of Duplessis *et al*. (2014), the folic acid and vitamin B_{12} supplement significantly decreased BW losses from 7 until 55 DIM and tended to diminish BCS losses. These differences among experiments could be partially explained by the number of animals involved in each study (a total of 805 dairy cows in study of Duplessis *et al*. (2014), versus 24 dairy cows in study of Graulet *et al*. (2007) and Preynat *et al*. (2009) and 40 dairy cows in this study).

Body condition was defined as the ratio of body fat to nonfat components in the body of a live animal. The BCS of a dairy cow is an assessment of body fat mobilization and energy balance, so is an important factor in dairy cattle management. Roche *et al*. (2013) indicated that if the BCS of dairy cows maintained at 3-3.25 during transition period, the risk of metabolic disorders occurrence is minimized (Roche *et al*. 2013). High BCS (>4) in early lactation decrease DMI and increase blood BHB in dairy cows (Hayirli *et al*. 2002), therefore BCS is considered as index of energy balance in animal (Duplessis *et al*. 2014). Concentrations of BHB (at day 7 before expected calving, calving day and days 7, 14 and 21 after calving) and NEFA (at day 7 before expected calving, calving day and day 21 after calving) plasma (Table 4) and milk fat content (Table 3) were not affected by vitamin and mineral supplementations. However, the numerical decrease in concentration of BHB (at day 7 before expected calving, calving day and days 7, 14 and 21 after calving) and NEFA (at day 7 before expected calving, calving day and day 21 after calving) plasma in cows that received vitamin and mineral supplementations and significant decrease in concentration of NEFA (at days 7 and 14 after calving) plasma in cows that received $B_{12}Fe$ were consistent with the numerical decrease in BCS in cows that received vitamin and mineral supplementations. These results suggest that there was possibly less mobilization of body fat reserves for cows in the vitamin and mineral groups and therefore, the BCS response was consistent among cows.

Similar to the results of the present study, injection of a solution contained Se, Mn, Cu and Zn, as antioxidant minerals (Machado *et al*. 2013; Ganda *et al*. 2016) and vitamin B_{12} (Girard and Matte, 2005; Furll *et al*. 2010; Akins *et al*. 2013; Weerathilake *et al*. 2018) and dietary vitamin E supplementation (Santos *et al*. 2016), vitamin E and Se (Bourne *et al*. 2008; Liu *et al*. 2008; Anwar *et al*. 2014) or Fe (Weiss *et al*. 2010) during the transition period had no significant effect on milk production in dairy cows. However, injection of vitamin E and Se (Moeini *et al*. 2009; Bayril *et al*. 2015), vitamin B_{12} (Preynat *et al*. 2009; Pereira *et al*. 2013), dietary E or Se supplementation (Wang *et al*. 2009; Bayril *et al*. 2015; Schafers *et al*. 2017; Vasil' *et al*. 2017) in transition period increased milk production of dairy cows. In various studies, the effect of mineral and vitamin supplements on cow's milk production and composition can be varied depending on the form of use, time and duration of injection or consumption of supplements in dairy cows. For example, in the study of Furll *et al*. (2010), supplemented lactating animals with a 2 doses of B_{12} at 1 and 2 weeks before calving, whereas the work of Pereira *et al*. (2013) aimed to evaluate the effect of a 4 doses of B_{12} every 5 days from calving to 20 days in milk and in the study of Preynat *et al*. (2009), injected B_{12} as weekly from 3 weeks before to 16 weeks after calving. A meta-analysis by Moghimi-Kandelousi *et al*. (2020) indicated that positive effects of vitamin E supplementation on milk production in some studies (Bayril *et al*. 2015; Schafers *et al*.

2017; Vasil' *et al*. 2017) can be explained by the fact that prepartum vitamin E supplementation may increase DMI through increased glucose availability and levels, leading to increasing nutrient availability for milk synthesis. Moeini *et al*. (2009) and Bayril *et al*. (2015) suggested that the well-known protective role of glutathione peroxidase and vitamin E on membrane integrity and decrease oxidative stress might represent at least one of the mechanisms through which Se and vitamin E enhanced milk production.

Serum glucose concentration (Table 4) and milk lactose content (Table 3) were not significantly different among treatments, which may explain the similar milk production by dairy cows in the current study.

Numerical increase in milk lactose percentage and production in cows injected with B_{12}Fe compared to the control group can be related to the increase in blood glucose concentration (Table 4) in these cows. Because vitamin B_{12} by interfering with the metabolism of propionate and gluconeogenesis and thus the synthesis of glucose can be used for the synthesis of lactose in the mammary glands and thus the synthesis of milk production (Furll *et al*. 2010; Costa *et al*. 2019). Lactose is the main carbohydrate in cow' milk, it is responsible for the osmotic equilibrium between blood and alveolar lumen in the mammary gland and consequently milk volume (Costa *et al*. 2019).

Roche *et al*. (2009) reported a negative relationship between calving BCS and milk production. Therefore, no effect of experimental treatments on milk production of dairy cows in the present study may be partly attributed to similar BCS (Table 2) in these animals.

Similar 4% FCM and ECM in the present study can be explained by no difference in milk production and composition (Table 3).

In the present study, we found no difference in milk fat, protein and lactose content at days 30, 60 and 90 of lactation (Table 3) that was in agreement with previous studies who reported no effect of injection of antioxidant minerals (Machado *et al*. 2013) and dietary Se supplementation (Falkowska *et al*. 2000), vitamin E (Santos *et al*. 2016), vitamin E and Se (Liu *et al*. 2008) or vitamin B_{12} (Weerathilake *et al*. 2018) on milk fat, protein and lactose content in dairy cows. Milk fat, protein and lactose are mainly synthesized by epithelial cells of mammary glands from blood metabolites (Jenkins and McGuire, 2006). One explanation for the similar milk production and milk fat, protein and lactose content at days 30, 60 and 90 of lactation between treatments groups in the current trial could be the timing of injection of mineral and vitamin supplements and milk sample collection. Supplemental minerals and vitamins could have been cleared through homeostatic processes or have been stored in body reserves. Given the circumstances of the present study, it was not possible to perform liver biopsies on d 30 to assess the effect of mineral and vitamin supplements on other body reserves. In addition, the lack of differences in milk yields and composition between vitamin and mineral supplementations and control groups indicated that the dose and interval injections was not beneficial to improve milk production.

Spears (2003) found that Se and vitamin E supplementation had positive effects on energy status in postpartum dairy cows. This might be related to the effect of Se and vitamin E on health and the immune system in animals (Baldi, 2005; Pilarczyk *et al*. 2012). In the present study, cows that received ESe were numerically higher milk production compared with control cows that probably related to improvement of immune status.

In early lactation, milk production requires more energy than what can be provided by DMI. This results in a negative energy balance leading to a mobilization of body fat reserves and increased circulating NEFAs to meet requirements for milk production and maintenance. Fat from body reserves can be taken up by the mammary gland and secreted in milk (Remppis *et al*. 2011; Duplessis *et al*. 2014) and can increase thereafter milk fat concentration. In other words, cows with an excessive negative energy balance in early lactation generally produce a lower milk production and higher milk fat concentration. Vitamin B_{12} is involved as a coenzyme for the entry of propionate into the Krebs cycle for providing energy and subsequently being used for gluconeogenesis (Furll *et al*. 2010). In the present study, the concentration of NEFA serum in cows injected with B_{12}Fe compared to no injection was significantly lower at days 7 and 14 after calving and numerically lower at day 21 after calving (Table 4), that may be the reason for the tendency to reduce milk fat in cows injected with B_{12}Fe. On the other, tendency to decreasing milk fat concentration in cows injected with B_{12}Fe can be related to the numerical increase in milk production because the percentage of milk fat and milk production are inversely related, due to the secretion of fat into the milk and the loss of energy from fat that can be used to synthesize milk. By tendency to decreasing milk fat concentration as compared with control cows, it could probably be hypothesized that the vitamin supplement changed energy partitioning in early lactation.

The second role of vitamin B_{12} is its requirement as a co-factor for the synthesis of methionine via the transfer of a methyl group from 5-methyl-tetrahydrofolate to homocysteine (McDowell, 2000; Bernabucci *et al*. 2010). Methionine is generally regarded as one of the first limiting amino acids in milk protein synthesis (NRC, 2001) and plays a key role in the synthesis of S-adenosylmethionine as a methyl donor (McDowell, 2000; Bernabucci *et al*. 2010), and consequently has a major impact on milk production, and therefore can be a reason for the tendency to increasing

milk production in cows injected with B_{12}Fe in present study.

In addition to B_{12}, injection of Fe in present study, probably result in increased milk production numerically. Atroshi *et al*. (1986) reported that an association between low hemoglobin concentrations and low milk yields has been shown in dairy goats (Atroshi *et al*. 1986). Weiss *et al*. (2010) indicated that if supplemental Fe had increased milk production, the most likely mode of action would be increased oxygen carrying capacity of the blood or improved health (e.g., less mastitis) via reduced oxidative stress because of enhanced antioxidant status and improved immune function. Published data with lactating dairy cows is lacking but anemic dairy calves grow slower than healthy calves, and supplemental dietary Fe can increase growth rate when given to Fe-deficient calves (Thomas *et al*. 1954). However, in the study of Weiss *et al*. (2010), adding 30 mg/kg of supplemental Fe from a Fe-amino acid complex to diets fed to dry cows, transition cows, and early lactation cows, which greatly exceeded NRC recommendations for Fe (approximately 20 mg/kg of diet DM), did not affect measures of Fe status and did not affect milk production and milk composition and concluded that control cows appeared adequate in Fe and cows generally did not respond to additional Fe. Also, in present study, injection of 3 doses of Fe supplement (each dose contain 254 mg Fe) at intervals of 2 weeks, had no significant effect on the performance of dairy cows and it seems that cows generally did not respond to additional Fe supplement.

In the present study, interaction of treatments (B_{12}Fe×ESe) compared to control had numerically higher milk production at days 30, 60 and 90 of lactation that can be related to effects of vitamin E, Se and Fe on improvement of immune status (Baldi, 2005; Weiss *et al*. 2010; Pilarczyk *et al*. 2012) and effect of vitamin B_{12} on gluconeogenesis and providing energy (Furll *et al*. 2010) and synthesis of methionine as one of the first limiting amino acids in milk protein synthesis (NRC, 2001) for dairy cows. Also, vitamin E may increase DMI through increased glucose availability and levels, leading to increasing nutrient availability for milk synthesis.

The interaction of treatments (B_{12}Fe×ESe) compared to control had numerically lower milk fat percentage at days 30, 60 and 90 of lactation that may be related to effect of vitamin B_{12} on decrease in mobilization of adipose tissue and serum NEFA concentration (Table 4).

The interaction of treatments (B_{12}Fe×ESe) compared to control had numerically higher milk lactose percentage and production at days 30, 60 and 90 of lactation that may be related to effect of vitamin B_{12} on propionate metabolism and gluconeogenesis (Furll *et al*. 2010).

Effect of vitamin E, Se and vitamin B_{12} on blood concentration of NEFA and BHB in transition dairy cows was controversial in previous studies. For example, injection of 1000 IU of vitamin E before expected calving decreased serum NEFA concentration at days 21, 14 and 7 (Pontes *et al*. 2015) and injection of antioxidants mineral solution contained Se, Mn, Cu and Zn (Machado *et al*. 2014) decreased serum BHB concentration at d 230 and 260 of gestation and d 35 after calving in transition dairy cows. In contrast, injection of antioxidants mineral solution (Se, Mn, Cu and Zn) at days 230 and 260 of gestation (Bicalho *et al*. 2014), 10 mg of vitamin B_{12} from day 21 before calving to 8 weeks after calving (Graulet *et al*. 2007), 5 mg of vitamin B_{12} from 8 weeks before to 8 weeks after calving (Weerathilake *et al*. 2018) and injection of B_{12} from 3 weeks before to 16 weeks after calving (Preynat *et al*. 2009) had no effect on serum NEFA and BHB concentrations in dairy cows. It was no research to investigate the effect of Fe on serum NEFA and BHB concentration in transition dairy cows. Increase in serum NEFA concentration leads to liver triacylglycerol accumulation and is detrimental to milk production and reproductive performance (Pereira *et al*. 2013). In the current study, reduction of serum NEFA and BHB concentrations in cows received B_{12}Fe compared to no injection may be attributed to the essential role of B_{12} in propionate metabolism through the gluconeogenesis pathway for providing energy (Girard and Matte, 2005). A relative deficiency of B_{12} would be expected to decrease glucose availability and affect energy balance (Preynat *et al*. 2009). Methylmalonyl-CoA mutase is a B_{12}-dependent enzyme that transforms methylmalonyl-CoA into succinyl-CoA to enter the TCA cycle (McDowell, 2000; Akins *et al*. 2013). It can be assumed that a deficiency of B_{12} could affect the efficiency of energy production from propionate and NEFA and decreases glucose availability (Furl *et al*. 2010). By increasing propionate metabolism and Krebs cycle activity, the process of gluconeogenesis is activated and glucose production is increased, which in turn affects insulin secretion.

Insulin stops lipolysis and possibly reduces fatty acid metabolism through reduction in both hepatic ketogenesis and hepatic lipidosis and therefore reduction in blood NEFA and BHB (Rollin *et al*. 2010). On the other hand, B_{12} is also essential for methionine synthesis by participating in the methionine synthase enzyme which provides the methyl group for compounds such as choline and carnitine, and these two compounds are involved with fat metabolism and transport, thus reduce fat accumulation in the liver (Preynat *et al*. 2009; Akins *et al*. 2013) and may be effective in reducing the incidence of metabolic disorders such as ketosis and fatty liver in transition dairy cows.

Machado *et al.* (2014) reported that injection of trace minerals (selenium, copper, zinc, and manganese) in lactating dairy cows by reducing the oxidative stress may decrease serum BHB concentration than control cows (Machado *et al.* 2014). Bernabucci *et al.* (2010) also reported that oxidative stress alters the metabolism of carbohydrates, proteins and lipids, and damages tissues including adipose tissues, and possibly resulting in the breakdown of adipose tissue and releasing of fatty acids (Bernabucci *et al.* 2010). As regards, Se, Fe and vitamin E have antioxidant properties, probably in the present study, one of the reasons for the decrease in serum NEFA in cows receiving vitamins and minerals compared to control group was related to the improvement of antioxidant capacity. Also, Moghimi-Kandelousi *et al.* (2020) indicated that vitamin E supplementation may increase DMI through increased glucose availability and levels, leading to increase nutrient availability for energy supply and thus reduce the breakdown of lipid tissue (Chandra *et al.* 2013). In the present study, the interaction of treatments (B_{12}Fe×ESe) were not significant for serum NEFA and BHB concentrations, however, tended to decrease numerically at day 7 before expected calving, calving day, days 14 and 21 after calving that can be related to synergistic effects of B_{12}Fe and ESe on decrease fat accumulation in the liver by increase glucose availability, providing energy and Fat transport from the liver through synthesis of choline and carnitine, reduce oxidative stress and maybe increase DMI. Roberts *et al.* (2012) reported that precalving NEFA ≥0.4 mmol/L and NEFA ≥0.8 mmol/L in weeks +1 and +2 relative to calving were each associated with an increased risk of culling within the first 60 DIM. Similarly, BHB ≥0.7 mmol/L in week −1, BHB ≥1.2 mmol/L in week +1, and BHB ≥1.6 mmol/L in week +2 were each associated with an increased risk of culling within the first 60 DIM (Roberts *et al.* 2012). In the present study, plasma concentration of NEFA and BHB in all experimental groups were in the normal range for transition dairy cows.

In the present study, we found a tendency to increase serum glucose concentration at day 7 before and day 14 after calving when cows received B_{12}Fe compared to no injection. Similarly, injection of B_{12} (5 µg/kg of BW) at days 14 and 7 before expected calving increased blood glucose concentration of dairy cows at day 1 after calving (Furll *et al.* 2010). However, injection of 5 mg vitamin B_{12} from 8 weeks before to 8 weeks after calving (Weerathilake *et al.* 2018) and 10 mg from day 60 before expected calving to day 150 after calving (Akins *et al.* 2013), dietary supplemental B_{12} (0.5 g/d) from 3 weeks before to 8 weeks after calving (Graulet *et al.* 2007) and injection of B_{12} from 3 weeks before to 16 weeks after calving (Preynat *et al.* 2009) had no effect on blood glucose concentration in dairy cows. Vitamin B_{12} converts propionate to glucose by interfering in the TCA cycle and ultimately generating energy (Girard and Matte, 2005; Kreipe *et al.* 2011), which could be a reason for the tendency to increase in serum glucose concentration in the present study. In the present study, the interaction of treatments (B_{12}Fe×ESe) were not significant for serum glucose concentrations, however, tended to increase numerically at calving day, days 14 and 21 after calving that can be related to effects of B_{12} on propionate metabolism and gluconeogenesis and production of glucose and effect of ESe and Fe on decrease oxidative stress and through this increase DMI and thus increase glucose availability. There was no research about the effect of Fe on blood glucose, total protein, triglyceride, total cholesterol and HDL-cholesterol concentration in transition dairy cows. The lack of effect of ESe on serum metabolites concentration in the current study was consistence to previous studies. For example, addition of 375 IU vitamin E to the diet of dairy cows had no effect on serum glucose, triglyceride and HDL-cholesterol concentration, but decreased total cholesterol (Santos *et al.* 2016). Likewise, supplemental Se (0.5 mg/kg of DM) had no effect on blood glucose and total cholesterol concentrations of dairy cows (Calamari *et al.* 2011). Furthermore, feeding dairy cows a diet supplemented with minerals (Cu, Se, Co, I, Mn, Zn) and vitamin (A) from day 21 before expected calving to day 60 after calving had no effect on blood triglyceride concentration (Khorsandi *et al.* 2016). However, in the present study, we found a tendency to decrease serum total cholesterol concentration at day 7 before expected calving and calving day and serum triglyceride concentration at day 7 before expected calving, calving day and 7 after calving when cows received ESe compared to no injection. Dhingra and Bansal (2006) reported that in rats, Se supplementation has been reported to decrease the 3-OH-methyl-glutaryl CoA reductase expression (Dhingra and Bansal, 2006) and leading to decreased serum total cholesterol levels (Yang *et al.* 2010). The interaction of treatments were not significant for serum TG, total and HDL-cholesterol concentrations.

CONCLUSION

Administration of three injections of ESe, B_{12}Fe or their combination at days 21 and 7 before expected calving and day 7 after calving have no effect on BSC changes around calving, milk production and composition and serum metabolites concentrations in dairy cows. However, their injection during transition period may improve the health status and reduce the incidence of metabolic disorders in dairy cows.

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