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Variations of some adipokines, pro-inflammatory cytokines, oxidative stress biomarkers, and energy characteristics during the transition period in dairy cows

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Abstract

Limited information exists about the relationship of adipose tissue with inflammation, oxidative stress, and energy metabolism during the transition period in dairy cows. The objective of this study was to assess the changes and relation of some adipokines, cytokines, oxidative biomarkers, and serum biochemical parameters related to energy balance (EB) in cows during the transition period. Thirty multiparous Holstein cows were selected based on estimated parturition date, and blood samples were collected from jugular vein on one-week prepartum and one and three weeks postpartum and used to measure the parameters. The serum levels of beta-hydroxybutyric acid (BHB), non-esterified fatty acid, cholesterol, high-density lipoprotein (HDL), aspartate aminotransferase, and total antioxidant capacity increased significantly, and glucose, urea, triglyceride (TG), and low-density lipoprotein (LDL) decreased significantly after parturition. The serum values of adiponectin, resistin, leptin, and cytokines including interleukin 6 (IL-6) and tumor necrosis factor (TNF)-α were not changed significantly during the experiment. The results of the Pearson correlation revealed a significant negative correlation between BHB with glucose, albumin, cholesterol, HDL, LDL, and a positive correlation with TG and malondialdehyde. Also, there was a significant direct correlation between insulin and leptin, adiponectin, resistin, IL-6 and TNF-α in the whole experiment period. These emphasize the difficulty of dairy cows to manage the energy requirements during the transition period. It can be stated that adipokines and cytokines may have an essential role in the metabolic status in this period, and control of their production and, or secretion could be helpful in EB during the transition period.

Introduction

A cow's transition period is an interval between three weeks pre- and three weeks post-calving. During the transition period, cows enter into mild negative energy balance (NEB)[1] that affects metabolism in different tissues such as the liver and adipose tissue.[2]

Adipose tissue acts as an energy depot and an endocrine organ by secreting protein hormones adipokines that have roles in inflammation and the acute-phase responses.[3] They have major roles in lipid metabolism and insulin resistance/sensitivity.[4] The NEB reduced leptin concentration near parturition, and higher metabolic efficiency are some of the adaptations that occur due to a decrease or absence of leptin in the early stages of lactation.[5] Recently, it has been reported that there is a negative association between adiponectin concentration and body condition score (BCS)[6] and non-esterified fatty acid (NEFA) during the dry period.[7] The levels of resistin are associated with the insulin resistance, and enhance one week after calving similarly to NEFA levels in dairy cattle. Also, there is a significant negative correlation between plasma resistin levels and energy balance (EB).[8]

The pro-inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF)-α are produced by adipocytes and resident immune cells in adipose tissue.[9] In cattle, IL-6 has an essential role in lipoprotein metabolism, fatty acid oxidation, urea cycle, oxidative stress, transcription regulation, and protein degradation through proteasomes.[10] The TNF-α regulate the inflammation, manages energy metabolism and also has endocrine activity.[11]

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A better understanding of the adaptation mechanisms to lactation and adipose tissue roles in the management of EB and inflammation in transition period is a tangible need.[12] Previous studies were done with a limited group of variables which resulted in the contradictory conclusions.

The objective of this study was: first, to elucidate the changes in the serum concentration of some adipokines, IL-6, TNF-α, oxidative stress biomarkers, and serum metabolites in the transition period, and second, to determine the relationships between measured variables before and after parturition for detecting of adipokines roles on EB and prepartum inflammatory conditions. In the present study, we measured important variables in different categories together for decreasing the effects of confounding factors and better interpretation of results.

Materials and Methods

Animals and diet. The study was performed in a commercial dairy farm near Neyshabur city, north-eastern Iran. The duration of the study was about three months, from December 2017 to February 2018. The herd consisted of 1000 lactating cows. Thirty multiparous Holstein cows were assigned based on their anticipated calving date. The mean of parity was 3.70 ± 1.64, and the cows had a BCS between 3.25 to 4.25 in the far-off period (3.61 ± 0.28). The body conditions of all cows were scored in far off period based on a 5-point scale and an increment of 0.25. All scorings were performed by a single evaluator. The mean milk record (kg) of sampled cows at one, two, and three months after parturition were 44.72, 55.17, and 56.38 kg, respectively. The mean of the highest record was 60.52 kg. Animals selected for the study were clinically examined for postpartum diseases such as mastitis, metritis, displaced abomasum, and laminitis. Those detected to be suffering from any one of these diseases during the study were excluded, and new ones were enrolled. The cows were restricted in free-stall housing with no access to pasture. Mean milk production in the previous lactation was 7,061 kg. The cows were milked three times daily, approximately at 6:00 AM, 2:00 PM, and 10:00 PM. All cows received a balanced diet in accordance with the nutritional requirements of the transition period. Diets were formulated according to recommendations by the NRC,[13] and the animals were fed a total mixed ration (TMR) twice a day (8:00 AM and 3:00 PM; Table 1). The cows had free access to drinking water. This study has been received ethical review committee approval (3/47408).

Sample collection. Blood samples were taken through the jugular vein with a disposable syringe three times: 7 days before the expecting calving date (–1 week), 7 days (+1 week), and 21 days (+3 weeks) after calving. All blood samplings were taken approximately two hours before milking at noon, shortly before feeding. All blood samples were transferred into 9.00 mL commercial evacuated tubes (Hebei Xinle Sci & Tech Co. Ltd., Shijiazhuang, China) and coagulated at room temperature. Clotted blood was centrifuged at 1,800 *g* for 15 min (Z 306; Hermle, Wehingen, Germany) for serum separation. The serum was frozen at – 70.00 ˚C until analysis.

Table 1. Ingredients and chemical composition of experimental diets in close up and fresh dairy cows.

Ingredients (% DM)	Close up	Fresh
Alfalfa hay	14.00	12.06
Corn silage	37.60	31.88
Barley (straw)	4.00	0.00
Barley (grain)	12.30	15.50
Corn dry (grain)	16.20	19.90
Cottonseed	1.40	2.42
Fish meal	3.00	5.00
Soybean meal	7.80	8.00
Soybean (whole roasted)	2.00	2.49
Calcium carbonate	0.00	0.50
Sodium bicarbonate	0.00	1.20
Salt	0.00	0.35
Minvit premix[1,2]	1.70	0.70
Chemical components		
Net energy for lactation (Mcal Kg^{-1})	1.53	1.61
Crude protein	15.70	17.10
Neutral detergent fiber	39.00	38.00
Non fiber carbohydrate	34.00	36.00
Ether extract	3.80	4.10
Calcium	0.79	0.80
Phosphorus	0.42	0.49

Close-up: critical time in the dry period is the last three weeks before calving; DM: dry matter.

[1] Anionic pre-fresh Minvit premix containing: 250,000 IU kg^{-1} vitamin A, 40,000 IU kg^{-1} vitamin D3, 4,000 IU kg^{-1} vitamin E, 40.00 mg kg^{-1} Biotin, 12.00 g kg^{-1} Niacin, 168 g kg^{-1} Ca, 65.00 g kg^{-1} Mg, 1,300 mg kg^{-1} Mn, 2,210 mg kg^{-1} Zn, 600 mg kg^{-1} Cu, 10.00 mg kg^{-1} Co, 8.00 mg kg^{-1} Se, 12.00 mg kg^{-1} I, 52.00 g kg^{-1} S, 120 g kg^{-1} Cl.

[2] Fresh cow Minvit premix containing: 550,000 IU kg^{-1} vitamin A, 120,000 IU kg^{-1} vitamin D3, 5,000 IU kg^{-1} vitamin E, 100 mg kg^{-1} Biotin, 100 mg kg^{-1} Niacin, 160 g kg^{-1} Ca, 30.00 g kg^{-1} P, 40.00 g kg^{-1} Mg, 16,000 mg kg^{-1} Mn, 22,500 mg kg^{-1} Zn, 5,750 mg kg^{-1} Cu, 200 mg kg^{-1} Co, 125 mg kg^{-1} Se, 200 mg kg^{-1} I.

Biochemical analysis. The serum concentrations of NEFA and beta-hydroxybutyric acid (BHB) were measured using commercial kits based on enzymatic reactions (Randox, Crumlin, UK) by an automated biochemistry analyzer (BT 1500; Biotecnica, Rome, Italy). The minimum detectable concentration of BHB and NEFA kits were 0.10 and 0.072 mmol L^{-1} respectively. The coefficient of variation for BHB and NEFA were < 3.50% and < 5.00%, respectively. Albumin, glucose, cholesterol, triglyceride (TG), urea, aspartate amino transferase (AST), high density lipoprotein (HDL) and low density lipoprotein (LDL) were measured with the automated biochemical analyzer using commercially available kits based on colorimetric reaction (Pars Azmoon, Tehran, Iran). The control serum (Randox) was used to evaluate measurement accuracy. The intra and inter assay coefficient of variation for the measured

variables were: albumin (< 1.79% and < 1.60%), glucose (< 1.49% and < 1.19%), urea (< 5.41% and < 5.79%), TG (< 1.82% and < 1.60%), cholesterol (< 1.62% and < 1.22%), AST (< 3.25% and < 4.40%), HDL (< 0.82% and < 1.80%) and LDL (<0.67% and <1.45%). The lower limit of detection for the measured variables were: albumin, glucose, urea, TG, cholesterol, HDL and LDL respectively 0.20, 5.00, 2.00, 5.00, 5.00, 1.00 and 2.00 (mg dL^{-1}) and for AST was 2.00 (UL^{-1}).

Measurement of adipokines and cytokines. In this study enzyme-linked immune sorbent assay (ELISA) based on the biotin double antibody sandwich technology was used to assay the bovine TNF-α, IL-6, adiponectin, leptin, resistin, and insulin (Shanghai Crystal Day Biotech CO., LTD, Shanghai, China). The intra and inter-assay coefficient of variation for insulin, adiponectin, resistin, leptin, IL-6, TNF- α were < 8.00% and < 10.00%. The lower limit of detection for these variables were: insulin (0.11 mIU L^{-1}), adiponectin (0.12 μg mL^{-1}), resistin (0.10 ng mL^{-1}), leptin (0.05 ng mL^{-1}), IL-6 (10.50 ng L^{-1}) and TNF-α (5.56 ng L^{-1}).

Evaluation of serum oxidative parameters. All chemicals were purchased from Sigma-Aldrich (St. Louis, USA). Malondialdehyde (MDA) was determined according to Lefevre *et al.*[14] Serum protein was precipitated by adding 2.50 mL of 200 g L^{-1} trichloroacetic acid to 0.50 mL of serum. After centrifugation at 1,000 *g* for 10 min, the supernatant was drawn off and the pellet was rinsed with 2.50 mL of acetic acid (100 mL L^{-1} in distilled water). The protein precipitate was resuspended in 2.50 mL of acetic acid, and 3.00 mL of thiobarbituric acid (2.00 g L^{-1} in 2.00 mol L^{-1} Na_2SO_4) was added. The reaction mixture was heated in a boiling water bath for 30 min, and then rapidly cooled in an ice bath to stop the reaction. The chromophore was extracted in 4.00 mL of n-butanol, and the organic supernatant was isolated by centrifugation for 10 min at 3,000 *g*. Absorbance measurements were performed on n-butanol extracts. Absorbance measurements were carried out at a wavelength of 532 nm (M330; Camspec, Cambridge, UK), with n-butanol as the blank in the reference channel. Total antioxidant capacity (TAC) was measured spectrophotometrically at 532 nm. A 100 microliter of the serum was incubated for 60 min at 37.00 ˚C with reaction mixture contained 0.50 mL phosphate buffer (100 mmol L^{-1}, pH 7.40), 0.50 mL Na-benzoate (10.00 mmol L^{-1}), 0.20 mL of Fe-EDTA 2.00 mmol L^{-1} (Sigma-Aldrich) and 0.20 mL of H_2O_2 (10.00 mmol L^{-1}) (Sigma-Aldrich). Then 1.00 mL acetic acid (20.00%) and 1.00 mL TBA (0.80% wt/vol in 50.00 mmol L^{-1} NaOH) were added and incubated for 10 min at 100 ˚C (in a boiling water bath). The absorbance was measured at 532 nm against deionised water.[15]

Statistical analysis. The data were analyzed by SPSS Software (version 22.00; IBM Corp., Armonk, USA). Based on the results of the Kolmogorov-Smirnov test, kurtosis, and skewness of data, we assumed normal distribution of measured variables. Repeated measures analysis of variance (ANOVA) was used to assess the effects of time for all cows. Pairwise comparisons were done by the Bonferroni method and adjusted for multiple comparisons. Serum profile was used as the dependent variable. The correlations between measured variables were determined for each sampling time by the Pearson method. Statistical significance was declared at $p \leq 0.05$, and data expressed as mean ± SE.

Results

The results from this study are shown in Tables 2 and 3. As these results revealed, the levels of BHB, NEFA, glucose, urea, cholesterol, TG, LDL, HDL, AST, and TAC in sera significantly changed during the experiment course ($p < 0.05$), while time did not have any effects on other measured parameters.

These results also demonstrated the maximum concentration of BHB on 1 week after calving ($p < 0.05$) and a decreasing trend till 3 weeks after parturition, but it did not return to the pre-partum level. The concentration of NEFA elevated after calving and remained high up to the end of this study ($p < 0.05$). Serum glucose decreased significantly 1 week after parturition ($p < 0.05$) and then increased significantly on +3 weeks ($p < 0.05$). Evaluation of insulin concentration showed that despite its reduction on +3 weeks, there were no significant changes throughout the study.

Serum albumin diminished on +1 week ($p < 0.05$) as expected, and this reduction was followed by a non-significant increase on +3 weeks. Urea concentration declined significantly ($p < 0.05$) at +1 week of parturition and persisted during the experiment.

Cholesterol concentration augmented after parturition ($p < 0.05$) and reached its maximum level at +3 weeks in comparison with previous sampling times. A significant reduction in serum TG was seen after parturition, on +1 week and +3 weeks ($p < 0.05$). The HDL concentration in serum elevated after calving, and the highest level of HDL was seen on +3 weeks ($p < 0.05$). Serum LDL decreased significantly on +1 week followed by an increase on +3 weeks ($p < 0.05$). We observed the significant induction of AST activity on +1 week, and its activity diminished on +3 weeks.

The results of this study revealed that serum IL-6 and TNF-α reduced on +3 weeks (Table 2), but we did not find any significant differences in terms of IL-6 and TNF-α levels during the experiment period. Similar to cytokines, hormones originated from adipose tissues, including leptin, adiponectin, and resistin altered during the study. Despite of the reduction in these adipokines levels on +3 week against –1 week and +1 week, we did not detect any significant difference in their concentrations between the sampling times (Table 2).

Our findings indicated that TAC value elevated after parturition ($p < 0.05$) with a significant difference on +3 week. Also, we observed that MDA concentration in sera did not change during the study. The transition period significantly impacted serum BHB, NEFA, glucose, urea, albumin, cholesterol, TG, LDL, HDL, and TAC value ($p < 0.05$). Our results did not reveal any significant effects on other parameters, including cytokines and adipokines.

The results of the Pearson correlation were listed in Table 3. As shown, on −1 week revealed significant positive correlations between glucose and albumin, LDL and HDL, as well as IL-6 and TNF-α ($p < 0.05$). On the same day, urea was significantly correlated with LDL and HDL, cholesterol directly correlated with LDL, HDL, and TAC, and insulin was positively correlated with leptin, adiponectin, resistin, IL-6, and TNF-α ($p < 0.05$). Furthermore, at this time, leptin was noted to have a significant direct correlation with adiponectin, resistin, IL-6, and TNF-α, adiponectin was positively correlated with resistin, IL-6, and TNF-α, and resistin was shown be correlated with IL-6 and TNF-α ($p < 0.05$).

On +3 weeks, BHB was found to have a significant negative correlation with glucose, albumin, cholesterol, LDL, and HDL, in addition to a positive correlation with TG and MDA ($p < 0.05$). The findings of this study showed a significant negative correlation between NEFA and glucose ($p < 0.05$). Also, on +3 weeks, albumin had a significant positive correlation with cholesterol, HDL, and LDL.

Furthermore, urea had direct correlations with LDL; and LDL with HDL. We observed that cholesterol was positively correlated with LDL, HDL, insulin, and leptin; HDL with insulin and leptin ($p < 0.05$). Also, insulin had a relationship with leptin, adiponectin, resistin, IL-6, and TNF-α. Besides, leptin was noted to directly be correlated with adiponectin, resistin, IL-6, and TNF-α. Adiponectin was correlated with resistin, IL-6, and TNF-α. Resistin was shown to be correlated with IL-6, TNF-α, and IL-6 with TNF-α ($p < 0.05$).

Pearson correlation analysis on +3 weeks revealed that BHB had positive and reverse correlations with MDA and TAC, respectively. At this time, NEFA was directly correlated with TG and urea with insulin, leptin, adiponectin, and resistin. Also, there were direct correlations between cholesterol and LDL, HDL and LDL with HDL. Furthermore, insulin was correlated with leptin, adiponectin, resistin, IL-6, and TNF-α. Our results also revealed that adiponectin had significant direct correlations with resistin, IL-6, and TNF-α and leptin with adiponectin, resistin, IL-6, and TNF-α. In addition, resistin was correlated with IL-6 and TNF-α. IL-6 and TNF-α were indicated to be positively correlated ($p < 0.05$). At this time, MDA had significant negative correlations with insulin and leptin.

Discussion

The high need for glucose in lactating mammary glands in ruminants causes a significant increase in its production in the mother's liver, fat tissue and other tissues.[16] During the early lactation period, the rapidly increasing demands of glucose, amino acids, and fatty acids for milk production cannot be sufficiently compensated by feed intake alone.[17] As a result, cows start to mobilize body fat and muscle tissue to cover the energy output via milk. This leads to the high mobilization of NEFA from adipose tissue to meet the requirements for maintenance and milk production.[18]

Table 2. Serum concentration (mean ± SEM) of some energy related metabolites, adipokines, and oxidative indicators during the transition period in 30 cows.

Parameters	−1 week	+1 week	+3 weeks	*p*-value
BHB (mmol L^{-1})	0.44 ± 0.03^{a}	0.90 ± 0.13^{b}	0.66 ± 0.07^{b}	0.002
NEFA (mmol L^{-1})	0.83 ± 0.06^{a}	1.27 ± 0.09^{b}	1.26 ± 0.07^{b}	0.001
Glucose (mg dL^{-1})	76.07 ± 0.79^{a}	62.27 ± 1.72^{b}	67.27 ± 1.71^{c}	0.001
Albumin (g dL^{-1})	3.93 ± 0.04^{a}	3.67 ± 0.06^{b}	4.04 ± 0.15^{ab}	0.035
Urea (mg dL^{-1})	28.27 ± 0.81^{a}	25.07 ± 1.10^{b}	23.53 ± 0.74^{b}	0.001
Cholesterol (mg dL^{-1})	92.63 ± 3.20^{a}	94.60 ± 3.70^{a}	149.73 ± 4.41^{b}	0.001
Triglyceride (mg dL^{-1})	14.83 ± 1.40^{a}	7.73 ± 0.81^{b}	7.63 ± 0.66^{b}	0.001
LDL (mg dL^{-1})	16.80 ± 0.86^{a}	12.47 ± 0.55^{b}	20.17 ± 0.77^{c}	0.001
HDL (mg dL^{-1})	73.23 ± 2.56^{a}	77.77 ± 2.64^{a}	132.37 ± 4.46^{b}	0.001
AST (U L^{-1})	55.43 ± 1.71^{a}	106.63 ± 11.07^{b}	76.83 ± 4.84^{b}	0.001
Insulin (mIU L^{-1})	3.67 ± 0.30	3.67 ± 0.31	3.32 ± 0.23	0.611
MDA (mmol L^{-1})	0.21 ± 0.03	0.22 ± 0.03	0.24 ± 0.03	0.742
TAC (mmol L^{-1})	0.54 ± 0.01^{a}	0.57 ± 0.02^{a}	0.64 ± 0.02^{b}	0.001
Leptin (ng mL^{-1})	1.27 ± 0.09	1.26 ± 0.08	1.15 ± 0.07	0.151
Adiponectin (µg mL^{-1})	3.82 ± 0.35	3.81 ± 0.29	3.45 ± 0.22	0.344
Resistin (ng mL)	1.73 ± 0.11	1.78 ± 0.12	1.62 ± 0.08	0.229
IL-6 (ng L^{-1})	114.34 ± 26.58	114.23 ± 22.53	98.93 ± 17.77	0.836
TNF-α (ng L^{-1})	86.46 ± 6.95	89.67 ± 6.73	76.96 ± 4.51	0.074

BHB: beta-hydroxybutyric acid, NEFA: non-esterified fatty acid, LDL: low-density lipoprotein, HDL: high-density lipoprotein, AST: aspartate amino transferase, MDA: Malondialdehyde, TAC: total antioxidant capacity, IL)-6 interleukin-6, and TNF: tumor necrosis factor.
^{ab} Different superscripts in each row denote significant differences ($p < 0.05$).

Table 3. Correlation coefficient between some adipokines,cytokines,oxidative biomarkers,and serum biochemical parameters in 30 dairy cows during pre- and post-partum periods.

Parameters	Time	BHB	NEFA	Glucose	Albumin	Urea	Cholesterol	Triglyceride	LDL	HDL	AST	Insulin	Leptin	Adiponectin	Resistin	IL-6	TNF	MDA	TAC
	−1 week	1	0.33	-0.14	0.05	-0.27	-0.14	0.06	-0.17	-0.04	0.04	-0.06	-0.03	0.03	-0.03	0.12	-0.03	0.17	-0.16
BHB	+1 week	1	0.30	-0.57**	-0.37*	-0.23	-0.39*	0.43*	-0.50**	-0.41*	0.15	-0.31	-0.16	-0.04	-0.16	0.24	-0.13	0.41*	-0.14
	+3 week	1	-0.01	-0.23	0.13	0.19	-0.11	-0.22	-0.18	-0.11	0.28	-0.18	-0.12	0.02	-0.10	0.10	-0.02	0.52	-0.39
	−1 week		1	0.10	0.11	-0.11	-0.03	0.14	0.02	-0.18	0.17	-0.04	0.07	0.03	-0.05	-0.15	0.01	0.15	0.13
NEFA	+1 week		1	-0.37*	0.11	0.13	0.04	-0.06	-0.01	0.17	0.21	-0.26	-0.19	-0.11	-0.21	-0.02	-0.29	0.09	0.06
	+3 week		1	-0.05	0.27	0.05	0.28	0.53**	0.06	0.24	0.33	0.19	0.04	0.01	0.10	0.02	0.16	0.08	0.03
	−1 week			1	0.51**	0.06	0.11	0.20	0.34	0.02	0.18	0.05	-0.16	0.14	0.10	0.22	0.03	-0.30	0.09
Glucose	+1 week			1	-0.06	-0.01	0.04	-0.33	0.22	0.11	0.19	0.12	0.05	-0.02	-0.04	-0.18	0.11	-0.20	-0.18
	+3 week			1	-0.16	0.07	-0.17	0.03	0.06	-0.15	-0.18	-0.10	-0.04	-0.19	-0.19	-0.16	-0.17	-0.22	0.30
	−1 week				1	0.14	0.13	0.20	0.14	0.22	-0.07	0.11	0.11	0.04	0.05	0.05	-0.09	0.14	0.19
Albumin	+1 week				1	0.05	0.40*	0.09	0.50**	0.53**	-0.17	0.24	0.35	0.21	0.30	-0.03	0.15	-0.19	0.31
	+3 week				1	0.10	0.26	-0.03	-0.12	0.23	0.78**	0.08	0.07	0.09	0.18	0.11	0.07	0.22	0.02
	−1 week					1	0.34	0.11	0.38*	0.36*	-0.27	0.24	0.26	0.07	0.15	-0.11	-0.05	0.08	-0.16
Urea	+1 week					1	0.18	-0.17	0.38*	0.23	0.31	-0.18	-0.19	-0.29	-0.16	-0.31	-0.20	-0.03	-0.13
	+3 week					1	0.03	0.04	-0.09	0.07	0.02	0.42*	0.36*	0.40*	0.39*	0.14	0.24	-0.16	0.15
	−1 week						1	0.13	0.65**	0.86**	0.07	-0.09	-0.05	-0.12	-0.05	-0.12	-0.16	0.05	0.41*
Cholesterol	+1 week						1	0.15	0.73**	0.79**	-0.14	0.37*	0.39*	0.31	0.32	0.30	0.26	-0.21	-0.06
	+3 week						1	0.02	0.62**	0.86**	0.10	0.15	0.17	0.11	0.20	0.26	0.18	0.06	0.17
	−1 week							1	0.06	0.15	0.18	0.29	0.23	0.17	0.28	0.30	0.08	-0.16	-0.23
Triglyceride	+1 week							1	0.05	-0.04	-0.08	-0.10	0.06	-0.03	-0.01	0.20	0.01	0.16	-0.02
	+3 week							1	0.05	0.12	-0.08	0.15	0.05	0.01	0.09	-0.23	0.17	-0.07	0.29
	−1 week								1	0.62**	0.06	-0.20	-0.15	-0.24	-0.15	-0.24	-0.28	0.11	0.23
LDL	+1 week								1	0.77**	-0.05	0.14	0.17	-0.05	0.14	-0.04	-0.07	-0.10	-0.12
	+3 week								1	0.75**	-0.06	0.05	-0.08	-0.20	-0.09	-0.02	-0.12	-0.02	0.33
	−1 week									1	-0.01	-0.03	0.03	-0.17	-0.04	-0.18	-0.18	0.07	0.33
HDL	+1 week									1	-0.21	0.43*	0.45*	0.32	0.35	0.12	0.17	-0.12	-0.06
	+3 week									1	0.17	0.21	0.16	0.08	0.19	0.20	0.13	-0.07	0.20
	−1 week										1	0.08	0.18	0.08	0.20	0.30	0.30	0.06	0.02
AST	+1 week										1	-0.31	-0.26	-0.25	-0.22	-0.08	-0.14	0.23	-0.05
	+3 week										1	-0.09	-0.16	-0.10	-0.05	0.01	-0.05	0.35	-0.04
	−1 week											1	0.77**	0.83**	0.86**	0.55**	0.76**	-0.33	-0.24
Insulin	+1 week											1	0.92**	0.80**	0.86**	0.54**	0.75**	-0.16	-0.01
	+3 week											1	0.82**	0.74**	0.86**	0.35	0.58**	-0.46	0.11
	−1 week												1	0.76**	0.83**	0.46*	0.76**	-0.01	-0.13
Leptin	+1 week												1	0.86**	0.87**	0.65**	0.77**	0.04	0.06
	+3 week												1	0.69**	0.79**	0.31	0.53**	-0.43	0.04
	−1 week													1	0.89**	0.74**	0.90**	-0.31	-0.10
Adiponectin	+1 week													1	0.82**	0.68**	0.87**	-0.02	0.10
	+3 week													1	0.88**	0.63**	0.74**	-0.27	0.04
	−1 week														1	0.70**	0.87**	-0.29	-0.14
Resistin	+1 week														1	0.71**	0.84**	-0.03	0.18
	+3 week														1	0.55**	0.73**	-0.28	0.17
	−1 week															1	0.72**	-0.15	-0.19
IL-6	+1 week															1	0.63**	0.21	0.01
	+3 week															1	0.44*	-0.03	-0.08
	−1 week																1	-0.16	-0.09
TNF	+1 week																1	0.02	0.08
	+3 week																1	-0.05	0.13
	−1 week																	1	0.02
MDA	+1 week																	1	-0.08
	+3 week																	1	0.01

BHB: beta-hydroxybutyric acid, NEFA: non-esterified fatty acid, LDL: low-density lipoprotein, HDL: high-density lipoprotein, AST: aspartate amino transferase, TAC: total antioxidant capacity, IL-6: interleukin-6, and TNF: tumor necrosis factor, MDA: malondialdehyde.

* and ** denote significant correlation at $p < 0.05$ and $p < 0.01$, respectively.

The level of NEFA indicates the degree of fat mobilization from the deposits and reflects dry matter intake. As the mobilization of NEFA to the liver exceeds its ability to completely oxidize the fatty acids to supply energy, BHB production increases.[19] In this study, higher NEFA values after calving indicate lipid mobilization as a metabolic mechanism for adaptation to the postpartum period. Similar to our findings, it was reported that elevated NEFA concentrations around the time of calving and early parturition, are inversely related to glucose concentration.[20] It is established that low insulin concentration and reduced insulin sensitivity of the tissues around parturition increase lipid mobilization and induce further rises in serum NEFA concentrations.[21] Also the induction of serum BHB levels during the first and second weeks after calving were associated with considerable milk losses.[22] High level of BHB and NEFA during the postpartum period, especially 1 week after parturition suggest that these cows probably suffer from NEB due to milk production. At post- parturition, plasma insulin levels are reduced compared to the prepartum level because of decreased pancreatic function, and insulin responsiveness to glucose.[23]

Lower insulin levels during the postpartum period versus the prepartum period may be due to a decreased responsiveness of pancreatic β-cells to a state of hyperglycemia, caused by factors that inhibit the release of insulin, such as the increase of NEFA.[24] It has been reported that redirecting blood glucose towards the mammary gland triggers fat mobilization through lipolysis.[25] Insulin and NEFA changes in the present study reflected similar alterations in metabolic adaptation to the postpartum phase and were consistent with the previous report.[25] In ruminants, volatile fatty acids from the gastrointestinal tract are the primary energy source rather than direct sources of glucose[26] so short-chain fatty acids at higher concentrations are potent stimulators of insulin secretion than glucose. Thus, insulin plays a slightly different role in ruminants versus non-ruminants. Elevating volatile fatty acid concentrations during lactation can interfere with glucose-induced insulin secretion[27] so insulin levels in blood decreases during transition period and in contrast adipocytes become insulin resistant.[28] Evaluation of the correlation between the measured parameters revealed that after parturition (+1 week), BHBA and NEFA levels were negatively correlated with blood glucose, respectively ($r = -0.574$ and -0.374, $p < 0.05$) as the lipomobilization triggered by low blood glucose levels.

The results showed that TG concentrations were significantly higher before calving compared to postparturition. Probably decreased catabolism of TG or its overproduction resulted in a higher serum TG concentration in prepartum cows.[29] After calving, the onset of lactation increased the uptake of TG by the mammary gland for the milk fat formation.[30] It has also been reported that, in the transition from non-lactating to lactation period, breaking down of the adipose TG to compensate NEB results in changes in the serum concentration of lipid profiles. Decreased serum TG concentration in the early lactation is a typical pattern of the lipomobilization syndrome in the transition period.[31]

In the present study, serum total cholesterol value increased slightly after parturition and reached its maximum level at 3 weeks postpartum. van Dorland *et al*. reported thar 2 weeks after parturition, cholesterol concentration increased and reached its highest concentration.[32] During the pregnancy, utilization of cholesterol by steroidogenic endocrine organs, including the ovaries and placenta for steroid hormone synthesis may have resulted in a decrease in total cholesterol concentration.[33] Additionally, in our study, a drop in LDL concentration was seen on +1 week followed by a significant increase on +3 week in cows. The changes found in cholesterol and LDL levels are consistent with the results of previous studies.[34] Elevated levels of plasma cholesterol may be due to increase demand for steroids synthesis in the ovary tissue and milk production in mammary glands, in this regard cholesterol is either supplied from the diet (exogenous) or synthesized de novo by many cells of the body (endogenous) LDL pathway.[29,34]

Evaluation of AST activity in cows during the transition period revealed a significant increase after parturition, especially on the first week postpartum so elevated AST activity with a high cholesterol level can be a sign of the development of hepatic steatosis.[35]

In the present study, the lowest concentration of albumin was detected on +1 week. It was suggested that serum albumin tend to decrease shortly after parturition. This reduction may be due to lower synthesis of albumin by the liver, a high rate of albumin catabolism, and an increase in the loss of albumin into milk.[29]

The BUN was lowest on 3 week after parturition. This finding is in agreement with the results by Seifi *et al.*[29] In contrast, Peterson and Waldern indicated that serum BUN was lowest in the dry period, increased after calving and remained high during the lactation, which was probably related to the increase in feed intake.[36]

In transition dairy cows, NEB occurs due to increasing energy demands and insufficient voluntary feed intake. In this regard, insulin sensitivity in peripheral tissues is reduced to supply sufficient energy toward the conceptus and the mammary gland. This process leads to adipose tissue mobilization.[37] It was documented that adipose tissue remodelling altered the secretion patterns of adipokines at the parturition time, and lipid metabolism was modulated by these bioactive compounds.[9]

In our study, adiponectin levels in cows reduced after parturition although this reduction was not significant. Singh *et al*.[38] reported that adiponectin levels in serum and fat tissue decreased around parturition, indicating that the

reduction in adiponectin level could be attributed to parturition-associated hormonal alternations or increased secretion of adiponectin into colostrum. Higher levels of glucose in cattle with high circulating adiponectin was reported.[39] We did not find any correlation between glucose levels and adiponectin despite the report of a positive correlation from a previous study.[40] In agreement to our findings, Ohtani *et al.* [41] reported a non-significant negative correlation between glucose and adiponectin. These contradictory results may be due to variation in diet, metabolic statuses of cows, and milk yield.

According to our results, leptin values non-significantly reduced during the third week after parturition. This finding was in agreement with other investigations that reported decreased secretion of leptin in AT during the first week after calving. Energy deficit around parturition is mainly responsible for the lower concentration of plasma leptin in early lactating dairy cows.[9] In accordance with our findings, it was documented that in dairy cows, plasma leptin concentrations were high before calving, proportionally to BCS; then decreased at calving and remained low even when energy status improved.[8] It was also reported that the CNS, via sympathetic innervation of AT, had an essential role in decreasing leptin production in early lactation. β-adrenergic signals are inhibitors of leptin expression in adipocytes, and ruminant adipose tissue is sensitive to their metabolic effects in early lactation. Reduction in leptin transcription and secretion during the transition period may promote a rapid return to regular dry matter intake.[42]

The significant correlation between the plasma concentration of leptin and insulin, could represent that EB regulated the levels of parameters like leptin, insulin, NEFA, and glucose. Also, these factors may have a role in mediating the effect of EB on leptin synthesis. The effect of insulin on the upregulation of leptin synthesis is dependent on the adequate uptake of glucose. This fact revealed that cellular energy availability be the initial factor in regulating leptin synthesis.[6]

In the transition period in dairy cows, during the first week after calving, resistin concentration peaks and then returns to prepartum levels after 6 weeks of lactation.[9,42] Similar to our results, Reverchon *et al.*[8] reported that plasma resistin concentrations increased 1 week after calving, similar to NEFA levels in dairy cows. The high levels of resistin in the plasma and adipose tissue observed immediately after calving may contribute to lipid mobilization during early lactation in dairy cows.[43] Although there was not any correlation between resistin with BHB and NEFA concentrations in the present study, Weber *et al*. reported a positive association between plasma resistin and plasma NEFA and a negative correlation with milk production.[44] The difference in milk production and level of NEB between reports may be the causes of these different results.

Several studies reported the association between inflammatory cytokines such as TNF-α and IL-6 with metabolic and infectious diseases during the peripartum period.[12] It was also mentioned that inflammatory response without any signs of infections or pathological changes might occur during the transition period.[10] According to the results, IL-6 concentration was higher during pregnancy (before parturition) and then decreased 3 weeks after calving. Similar to our findings, it was shown that IL-6 concentrations before parturition were higher than those after parturition.[10,45] It has been shown that pregnancy is an immunosuppressive condition, in contrast maternal lymphocytes were activated and secreted most cytokines.[45] It was suggested that during pregnancy, Th1 cell function was restrained and on the contrary, the Th2 cell function became dominant in mice, and humans.[45] IL-6 is known to be a cytokine produced by Th2 cells.[46]

Similar to our findings, it was shown that TNF-α concentrations before parturition were higher than those after calving.[45] In prepartum, the expression of TNF-α in the liver was upregulated directly by interleukin-8 and IL-1β that were secreted from the placenta.[47] According to our findings, there was a significant positive correlation between adipose tissue derived pro-inflammatory adipokines, resistin, TNF-α, and IL-6. As mentioned before, the presence of inflammation in the postpartum period has been stated in several species, including cattle.[48]

During the transition period, inequality in the redox balance results in oxidative stress. In this situation, cellular damage and, or dysfunction occur that has been proposed as the link between the metabolic and immune systems of the cows.[49]

Similarly, this study showed that the anti-oxidative/ pro-oxidative status was related to alterations in metabolic parameters during the transition period. As we observed, MDA concentration increased after calving in this study. During the NEB, delivery and oxidation of NEFA were increased in the mitochondria of hepatocytes to produce energy. Consequently, the generation of a large amount of reactive oxygen species is caused by the induced NEFA oxidation resulting in raised lipo-peroxidation processes.[31,48] Our result indicated that the antioxidative power of the body was increased as a compensatory mechanism for the protection of tissues against oxidative stress. Castillo *et al.* suggest that an efficient antioxidant system helped by the minerals and vitamins received by diet, can protect against oxidative stress after calving.[50]

Based on the results of this study, in dairy cows in the transition period, NEB induces changes in the production of some cytokines and adipokines that significantly correlate with insulin secretion, and affect the energy metabolism and modulation of lipolysis and induce inflammation. Thus, the management of NEB during transition period has a critical role in production of dairy cows.

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Conflicts of interest

The authors declare that they have no conflict of interest.

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