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Original Article

The relationship between body condition score, thyroxin, and health condition and serum energy indices, insulin like growth factor-1, and lipids profile over the transition period in Holstein dairy cows

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Abstract

Background: Lipid mobilization increases significantly in cows around the time of calving; a correlation between excessive blood NEFA concentrations, oxidative stress, and impaired health status in transition dairy cattle was observed. **Aims:** The relationship between serum thyroxin (T4) values and energy indices and lipid profile in all cows, non-fat cows (NFCs), fat cows (FCs), healthy, and diseased animals were estimated in the present study. **Methods:** Blood samples were obtained from fifty multiparous cows on -14, +10, +20, and +30 days relative to parturition. They had similar diets and were kept under the same management conditions. **Results:** NEFA and BHBA values increased significantly on the 10th day of calving. Inversely, glucose, T4, triglyceride, LDL-C, and VLDL-C decreased significantly ten days after calving. There was a significant negative correlation between serum T4 and NEFA for all cows and FCs. Serum T4 and BHBA values had a significant negative correlation in NFCs, FCs, healthy, and diseased cows. In addition, serum T4 and fructosamine had a significant negative relationship in FCs and a significant positive correlation in diseased cows. Serum T4 values had a significant negative correlation with cholesterol, HDL-C, and a positive correlation with triglyceride and VLDL-C for all cows, NFCs, FCs, and healthy cows. **Conclusion:** The data emphasized the effects of negative energy balance during the transition period on serum lipids profile and thyroid function. In addition, the correlation between T4 and energy and lipids indices may indicate a possible effect of health and body condition status on thyroid responses.

Key words: Lipids profile, Negative energy balance, Thyroid hormone, Transition period

Introduction

All dairy cows experienced metabolic challenges during the transition period due to inadequate dry matter intake that cannot cover energy demands for body maintenance and milk production (Puppel and Kuczyńska, 2016). This state is characterized by a negative energy balance (NEB). If this continues, it will result in homeorhetic imbalances and reduction of liver and reproductive efficacy (Raphael and Sordillo, 2013).

In the NEB situation, low blood glucose concentrations and limited glycogen reserves of bovine hepatocytes lead to the support of body requirements by fat depots (Weaver *et al*., 2017). In response to homeorhetic changes, adipose tissues elevate triglyceride hydrolysis to non-esterified fatty acids (NEFA) and glycerol to supply energy requirements for various organs such as mammary glands, spleen, muscles, and liver. Hepatocytes can uptake large quantities of NEFA from blood associated with circulating NEFA levels and high hepatic blood flow (Herdt, 2000; Raphael and Sordillo, 2013). The liver has a critical role in regulating blood glucose concentrations and NEFA metabolism during the transition period. The rate of FFA release from adipocytes is affected by some hormones. However, the most potent ones appear to be the catecholamines, thyroid hormones, and the glucocorticoids. Through increasing the number of b1-adrenergic receptors on adipocytes, thyroid hormones become synergistic (not alone) with the catecholamines in promoting lipolysis (Engelking, 2010). The serum cholesterol amounts have a negative correlation with thyroid activity. The net effect of thyroid hormone on cholesterol metabolism is to increase the rate of its catabolism by the liver and excretion by bile, thereby lowering the serum cholesterol amount. In hypothyroidism, the net effect is a decrease in cholesterol catabolism and an increase in cholesterol (Kaneko, 2008).

Homeorhetic changes are adaptive responses that are critical to NEB improvement during the transition period (Hernández-Castellano *et al*., 2017a). Homeorhetic

adaptations manage the NEB state by limiting lipid mobilization to levels that can be metabolized entirely and optimally to provide energy demands (Hernández-Castellano *et al*., 2017b). However, excessive lipid mobilization causes the accumulation of triglyceride (TG) and ketone bodies in hepatocytes and blood, respectively. This intensifies NEB, and the risk of metabolic disorders such as the fatty liver and ketosis increases (Herdt, 2000).

Body condition score (BCS) is an index of energy reserves during the transition period (Roche *et al*., 2013). Genetic modification and high milk production results in cows losing more BCS after calving, and, thus, they are more susceptible to diseases (Roche *et al*., 2013). Based on previous clinical experiments, lipid mobilization increases significantly in over-conditioned cows around the time of calving, and a correlation between excessive blood NEFA concentrations, oxidative stress, and impaired health status in transition dairy cattles was observed (Bond *et al*., 2014).

The objectives of the present study were to determine the relationships between serum T4 value and energy indices and lipid profiles in fat cows (FCs) and non-fat cows (NFCs), healthy, and disease affected animals. In addition, it aimed to evaluate dynamic alterations of energy indices, hormones, and lipid profiles in dairy cows during the transition period.

Materials and Methods

Ethical statement

All research procedures (method, volume, and times of sampling) were approved by the Animal Care and Use Committee of the Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran based on EU standards for the protection of animals used for scientific purposes (registered under protocol No.: 18174/3). Blood samples were taken from the tail vein. The maximum blood volume was taken at every sampling time.

Cows, experimental design

This experiment was performed at a dairy herd of about 4000 lactating Holstein cows in Isfahan province, Iran. A total of 50 dry pregnant cows from 21 days before the expected calving date until 30 days after calving were used. All information regarding the previous history of all cows (such as reproduction, production, health status, parity, age within parity, calving expected date, and dry period BCS) had been documented and were available at the farm's Statistic and Data Center. The body condition of the selected cows was scored 21 days before the expected calving date and whole sampling times by one trained farm staff that had determined the dry BCS. The BCS was according to a scale method of 1 to 5 with increments of 0.25 (Ferguson *et al*., 1994). An extreme spectrum on BCS of selected animals due to suitable nutritional management in the farm was not seen (BCS minimum: 3; BCS maximum: 4.25). The subjects were categorized into two groups: non-fat cows (n=25) with BCS <3.75 (BCS minimum: 3; BCS maximum: 3.5; BCS means: 3.36), and fat cows (n=25) with BCS ≥3.75 (BCS minimum: 3.75; BCS maximum: 4.25; means: 3.87). Furthermore, the subjects entered second (n=18, NFC=9, and FC=9), third (n=12, NFC=5, and FC=7), fourth, and greater (n=20, NFC=11, and FC=9) lactation. They were housed in an enclosed barn with sand bedded free stall. In addition, the animals had free access to water and were fed twice a day throughout the experiment. The components and nutritional composition of the diets of dry and early lactation periods are presented in Table 1. After calving, cows were milked thrice daily at hours 8 a.m., 4 p.m., and 12 a.m.

Table 1: Ingredient and nutritional composition (% of total dry matter) during transition period

Items	Close up	Lactation
Component % of DM		
Alfalfa hay	18.3	20.8
Corn silage	31.4	20.2
Wheat straw	5.9	-
Barley grain	12.2	16.9
Corn grain	12.2	16.6
Soybean seed, extruded	12.3	14
Soybean meal	-	6.6
Fish meal	0.2	0.7
Calcium carbonate	1	0.8
Di-calcium phosphate	0.6	0.3
Vitamin supplement	2.5	0.5
Vitamin A (IU/kg of supplement)	250000	1300000
Vitamin D_3 (IU/kg of supplement)	40000	360000
Vitamin E (IU/kg of supplement)	2000	12000
Vitamin H_2 (mg/kg of supplement)	20	-
Minerals	2.5	0.8
Calcium chloride (g/kg)	150	-
Ammonium chloride (g/kg)	136	-
Magnesium oxide (g/kg)	40	-
Magnesium sulfate (g/kg)	30	-
Manganese sulfate (mg/kg)	800	10000
Zinc sulfate (mg/kg)	800	16000
Copper sulfate (mg/kg)	400	4000
Cobalt sulfate(mg/kg)	4	120
Selenium (mg/kg)	7	80
Calcium iodate (mg/kg)	12	150
Chromium-organic (mg/kg)	14	-
Energy and nutrients		
DM %	52.9	61.7
Crude protein	14.2	17.3
Net energy for lactation (Mcal/kg)	1.45	1.62
Neutral detergent fiber	42.1	35.8
Acid detergent fiber	20.9	17.3
Non-fiber carbohydrates	32.1	34.3
Ether extract	4.5	5.3
Salt	-	0.5

DM: Dry matter

Blood sampling

Blood sampling was performed at 14 ± 2 days before the expected calving date and 10, 20, and 30 days after calving. Blood specimens were withdrawn from the coccygeal vein into 9 ml commercial evacuated tubes (with clot activator) approximately 2 h after the morning milking time for serum preparation. The samples were transferred to the laboratory for serum separation. The blood samples in evacuated tubes were centrifuged at 1800 × g for 15 min, and the serum was separated

immediately. The serum samples were refrigerated at -20°C before analysis.

Biochemical analysis

The serum NEFA and BHBA were measured by commercial kits based on enzymatic reactions (Randox Laboratories Ltd., Ardmore, UK). The serum concentrations of glucose, cholesterol, triglyceride, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and fructosamine were measured using commercial kits (Parsazmoon, Tehran, Iran and BioSystems S. A, Barcelona, Spain for fructosamine). All variables were analyzed with a biochemical auto-analyzer (Biotecnica, BT 1500, Rome, Italy), according to the manufacturer's protocols. The control serum (Randox Laboratories Ltd., Ardmore, UK) was used to control measurement accuracy. The intra and inter-assay coefficient of variation for measured variables were: NEFA 4.81% and 4.32%, BHBA 3.78% and 5.25%, glucose 1.82% and 0.84%, fructosamine 2.7% and 4.3%, cholesterol 0.61% and 1.22%, triglyceride 1.82% and 1.04%, HDL-C 0.73% and 1.8%, and LDL-C 0.66% and 1.45%. VLDL-C was calculated using the formula:

VLDL-C= Ttiglyceride/5 (Friedwald *et al*., 1972)

ELISA assay

Serum bovine insulin-like growth factors 1 (IGF-1) and thyroxine (T4) concentrations were measured using commercially available bovine IGF-1 (Bioassay Technology Laboratory, Shanghai, China), T4 (Autobio Diagnostics Co., Ltd., Zhengzhou, China), and enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The intra- and inter-assay coefficient of variation, sensitivity, and specificity for these variables were: <8%, <10%, 0.53 ng/ml, 98% (IGF-1), 8.9%, 6.75%, 0.4 µg/dl, and 97.9% (T4), respectively.

Statistical analysis

The normality of variables was evaluated by PROC UNIVARIATE of SAS software, version 9.2 (SAS Institute Inc., Cary, NC). Variables with Shapiro-Wilk values P>0.05 were considered normal (glucose, IGF-1, T4, and fructosamine), whereas other variables were normalized using the natural logarithm.

The data of serum parameters were then analyzed using repeated-measures ANOVA (a mixed procedure in SAS, version 9.2). Serum parameters data were used as the dependent variables. "Fixed effect" was the time of sampling (-14, 10, 20, and 30 days relative to calving). BCS at the beginning of the dry period, health status, and parity were considered as covariates, and the cows were used as a random effect.

The model used for all serum metabolites included the effects of time of sampling (-14, 10, 20, and 30 days relative to calving), BCS category, parity group, and health situation. Parity was classified into three groups: cows with second parity, third parity, and fourth or more parity. The health status of cows was checked according to the recorded data and cows were divided into two groups: healthy cows (n=38) and disease affected cows (n=12). Nine disease affected cows were in NFC and three in FC categories. Recorded diseases included retained placenta, metritis, mastitis, milk fever, and displaced abomasum. Interactions between the time of sampling and the BCS were tested if the BCS effect was significant. In addition, the interaction between the BCS and significant covariates (parity and health situation) was tested and included in the final model if the effect of the covariates was significant.

To determine Pearson correlation coefficients between serum T4 and energy metabolites (NEFA, BHBA, glucose, and fructosamine) and serum lipids (cholesterol, triglyceride, LDL, and HDL) in BCS, parity, and health situation groups, CORR procedure of SAS was used.

Differences with $P \leq 0.05$ were considered as significant, and $0.05 < P \leq 0.10$ was considered as a tendency. Least square means (LSM) and standard errors (SE) were also presented.

Results

The BCS least-square means (LSMs) of the two NFC and FC groups on 5-times point (-21, -14, 10, 20, and 30 days relative to calving) are presented in Table 2. The BCS was significantly different between the two NFC and FC groups on -21, -14, +10, and +20 days of calving. In addition, the LSM and SE of daily milk production during the 120 days after parturition were 54.3 ± 1.8 kg/day in NFC and 55.1 ± 1.9 kg/day in FC groups without any significant difference between groups.

Table 2: Comparison of BCS least-square means between the two nonfat (NFC) and fat cows (FC) at different times

Days relative to calving	Group		SEM	P-values
	NFC	FC		
-21	3.36[b]	3.87[a]	0.05	<0.0001
-14	3.67[b]	3.99[a]	0.05	<0.0001
+10	3.37[b]	3.6[a]	0.05	0.0004
+20	3.18[b]	3.36[a]	0.05	0.0056
+30	2.99[a]	3.12[a]	0.05	0.07

NFC: Nonfat cows with BCS <3.75 at the beginning of the dry period; FC: Fat cows with BCS ≥3.75 at the beginning of the dry period; and SEM: Standard error of means. [a, b] Different superscripts presented a significant difference between the two NFC and FC groups

The results of serum concentrations of NEFA, BHBA, glucose, fructosamine, lipids profile, IGF-1, and T4 at four sampling times (-14, +10, +20, and +30 days relative to calving) are presented in Table 3. The sampling time and BCS had a significant effect on both NEFA and BHBA (P<0.05). The highest serum NEFA and BHBA were observed on the 10th day after parturition (Table 3). In addition, NEFA values were significantly greater (P<0.05) in FC than in NFC 30 days after calving (Fig. 1A). Serum BHBA of FC also had significantly higher (P<0.01) concentrations on day 20 of lactation (Fig. 1B).

Table 3: Least squares mean of serum energy metabolites and lipids profile of dairy cows during the transition period

Variables	Days relative to calving				SE[1]	P-values[1]
	LSM[1]					
	-14	+10	+20	+30		
NEFA (mmol/L)	0.62[b]	0.96[a]	0.82[a]	0.83[a]	0.1	<0.0001
BHBA (mmol/L)	0.54[c]	0.95[a]	0.68[b]	0.66[b]	0.14	0.0015
Glucose (mmol/L)	3.49[a]	3.19[b]	3.27[ab]	3.46[a]	0.13	0.03
Fructoseamin (µmol/L)	268.4[c]	267.9[c]	281.4[b]	293.2[a]	6.3	<0.0001
T4 (µg/dl)	7.46[a]	3.8[b]	3.67[b]	3.91[b]	0.3	<0.0001
IGF-1 (ng/ml)	304[a]	280.7[a]	257[a]	259.5[a]	35.8	0.3
Cholesterol (mmol/L)	2.2[d]	2.43[c]	3.92[b]	5.11[a]	0.2	<0.0001
Triacylglycerol (mmol/L)	0.27[a]	0.102[b]	0.12[b]	0.14[b]	0.03	<0.0001
HDL-C (mmol/L)	1.77[d]	2.1[c]	3.17[b]	4.15[a]	0.15	<0.0001
LDL-C (mmol/L)	0.53[a]	0.38[b]	0.55[a]	0.66[a]	0.03	<0.0001
VLDL-C (mmol/L)	0.12[a]	0.05[b]	0.056[b]	0.06[b]	0.01	<0.0001

[a, b, c, d] Different superscripts represent a significant difference between least squares means within a row (P<0.05). [1] LSM: Least square of means; SE: Standard error; and P-value indicate the significant level of fixed effect "days relative to calving"

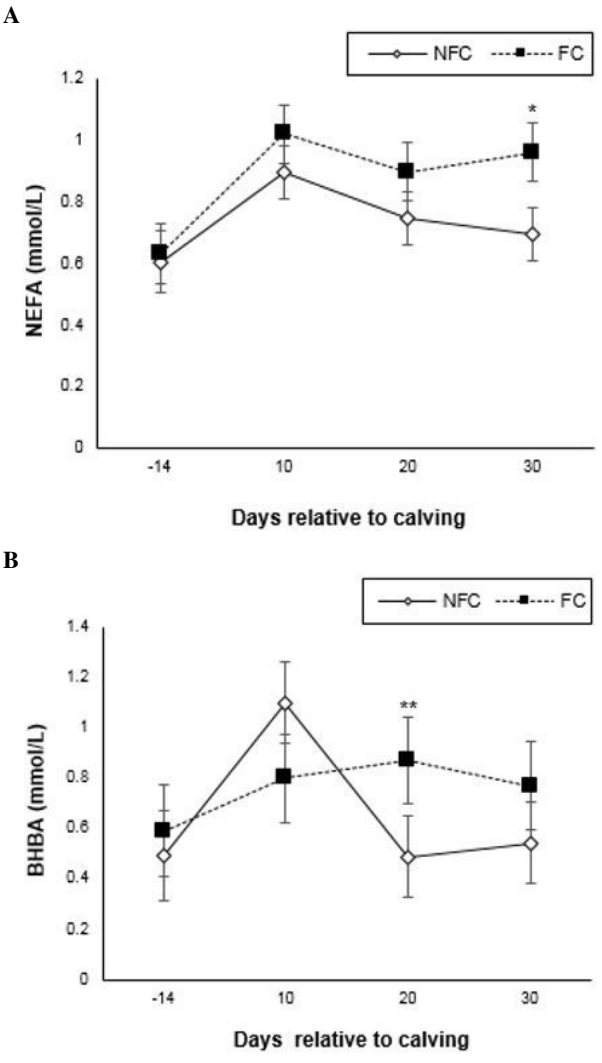

Fig. 1: Least square means and standard error of serum concentrations of NEFA (A) and BHBA (B) in animals enrolled in both non-fat cows (NFC, white diamond) and fat cows (FC, the black square) at transition period (-14, +10, +20, and +30 days relative to calving). * and ** indicates significant differences at P≤0.05 and P<0.01 between groups, respectively, within sampling time

Sampling time had a significant effect on serum glucose and fructosamine values (P<0.05). The serum glucose amount decreased significantly on day 10 compared to 14 days before calving and day 30 of lactation. The lowest fructosamine values were observed ten days after calving, whereas the highest values were detected 30 days after calving. Furthermore, disease affected cows had significantly lower serum fructosamine than the healthy ones (Fig. 2A).

The highest T4 values were observed 14 days before the expected calving date, while its values decreased significantly during the study (P<0.05). The sampling time did not affect IGF-1 values; moreover, healthy cows had lower serum T4 values than the disease affected cows (Fig. 2B).

There was a significant time effect on serum cholesterol, triglyceride, HDL-C, LDL-C, and VLDL-C concentrations (P<0.05). The average of both cholesterol and HDL-C was at its lowest values 14 days before the expected calving date, while it increased significantly after calving, and the highest values were detected 30 days after calving. The highest concentrations of triglyceride and VLDL-C were observed 14 days before calving, with a significant increase after calving. LDL-C concentration reduced significantly on day 10 of lactation in comparison with other sampling times (P<0.05).

Furthermore, the health situation and parity as covariates significantly affected cholesterol, HDL-C, and LDL-C concentrations (P<0.05). The interaction between BCS and health situation was also significant on serum cholesterol and HDL-C concentrations (P<0.01) (Fig. 3). Both cholesterol and HDL-C concentrations were significantly lower for fat disease affected cows than for non-fat disease affected cows and healthy cows. On the other hand, healthy cows had greater LDL-C concentrations than others (Fig. 2C). Moreover, the cows with second parity had significantly greater cholesterol, HDL-C, and LDL-C concentrations than the ones with fourth or more parities (Fig. 4).

The relationship between serum T4 and energy metabolites, and lipid profiles are represented in Table 4. There was a significant negative correlation between serum T4 and NEFA, cholesterol and HDL-C for the

total sampled cows. In addition, a significant positive correlation was observed between serum T4 values and triglyceride and VLDL-C. No significant correlation was observed between serum T4 values and BHBA, fructosamine, glucose, and LDL-C.

In addition, the correlation coefficient for serum T4 with energy metabolites and lipid profiles was calculated when the cows were classified into NFC and FC based on BCS. A significant negative correlation was found between serum T4 values with BHBA, cholesterol, and HDL-C in NFC group, whereas there was a significant negative correlation between serum T4 values and NEFA, BHBA, fructosamine, cholesterol, and HDL-C in FC group. In addition, a significant positive correlation was observed between serum T4 with triglyceride and VLDL-C in the FC group. There was no significant correlation between serum T4 and glucose and LDL-C concentrations in FC and NFC groups.

The serum T4 values had a significant negative correlation with serum values of BHBA, cholesterol, and HDL-C, and a significant positive correlation with serum triglyceride and VLDL-C in healthy cows. There was no

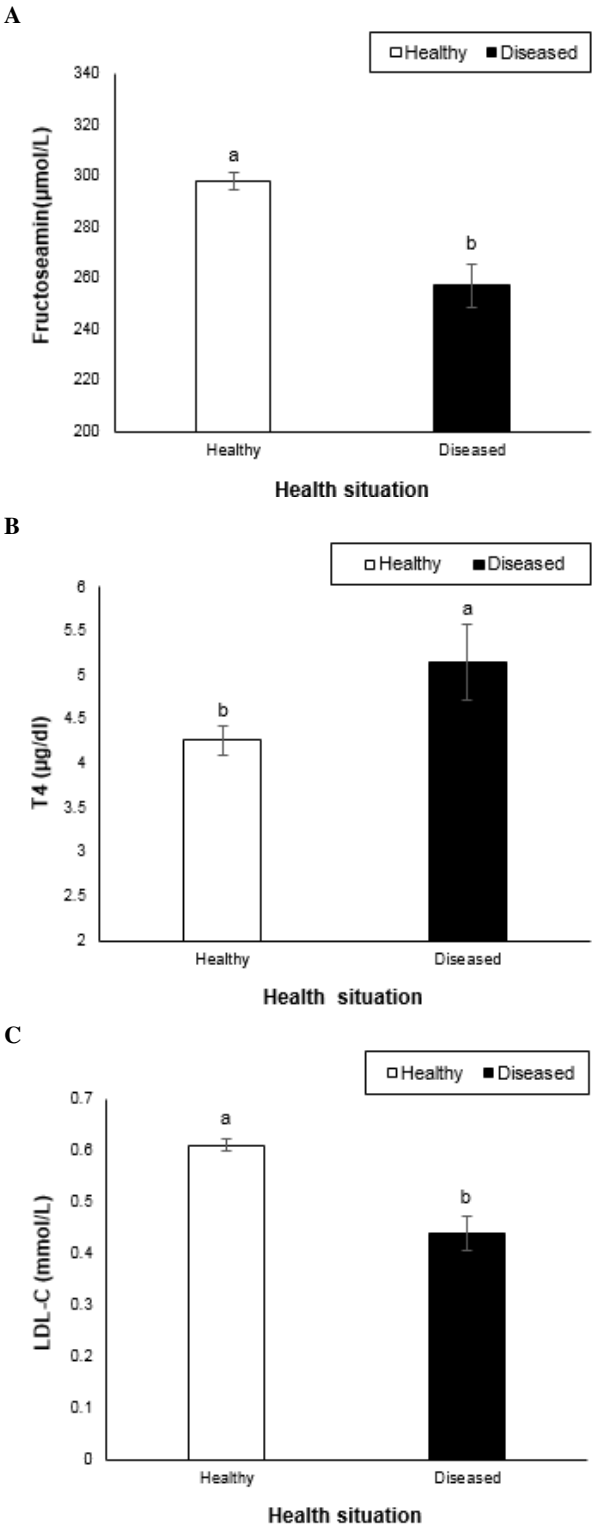

Fig. 2: Least square means and standard error of serum concentrations of fructosamine (fructosamine, A), thyroxin (T4, B), and LDL-C (C) in dairy cows categorized into healthy and diseased cows. Values with different superscripts [a, b] present significant differences (at P≤0.05) between the two health status by independent sample t-test

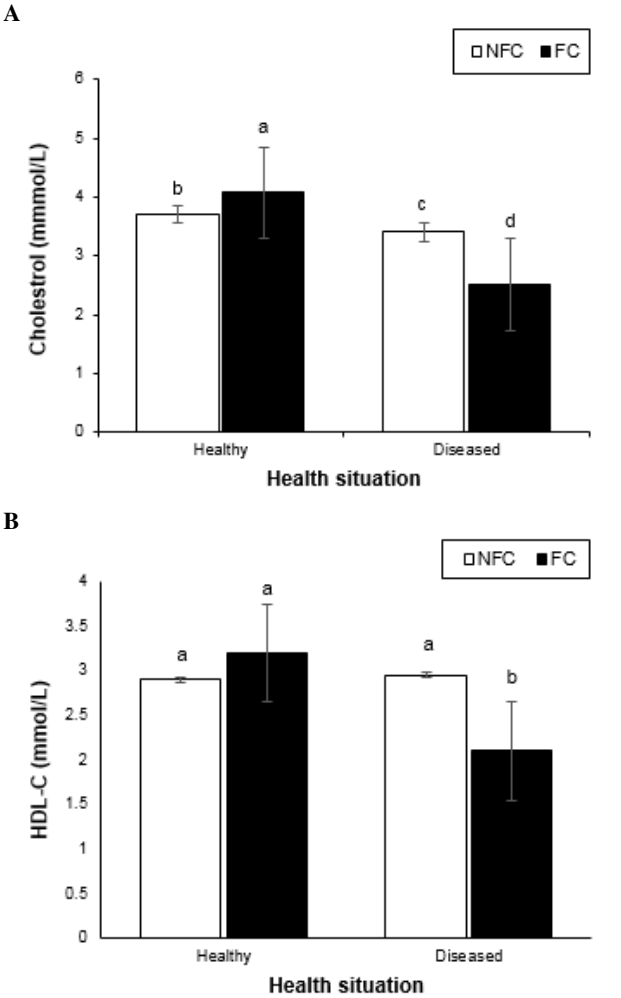

Fig. 3: Least square means and standard error of serum concentrations of cholesterol (A) and HDL-C (B) in healthy and diseased cows that enrolled in the two groups of nonfat cows (NFC, white column) and fat cows (FC, black column). Values with different superscripts [a, b, c, d] present significant differences (at P≤0.05) between NFC and FC groups by two-way ANOVA

Table 4: The correlation coefficient (r) between serum T4, energy metabolites, and lipid profiles in all cows, NFC, FC, healthy, and diseased cows

		NEFA	BHBA	Fructosamine	Glucose	Chol	TG	HDL-C	LDL-C	VLDL-C
T4	Total cows	-0.26**	-0.06	-0.003	0.07	-0.3**	0.27**	-0.3**	0.02	0.31**
	NFC	-0.2	-0.24*	-0.03	0.15	-0.32**	0.13	-0.35**	-0.042	0.13
	FC	-0.23*	-0.23*	-0.23*	0.003	-0.44**	0.34**	-0.41**	-0.01	0.34**
	Healthy	0.03	-0.25**	-0.09	0.07	-0.36**	0.35**	-0.35**	-0.05	0.35**
	Diseased	-0.39	-0.6**	0.58**	0.06	0.29	0.38	0.19	0.6**	0.38

Chol: Cholesterol, NFC: Nonfat cows, FC: Fat cows, NEFA: Non-esterified fatty acids, BHBA: Beta hydroxybutyric acid, TG: Triglyceride, HDL-C: High-density lipoprotein, LDL-C: Low-density lipoprotein, and VLDL-C: Very low-density lipoprotein. * P<0.05, and ** P<0.01

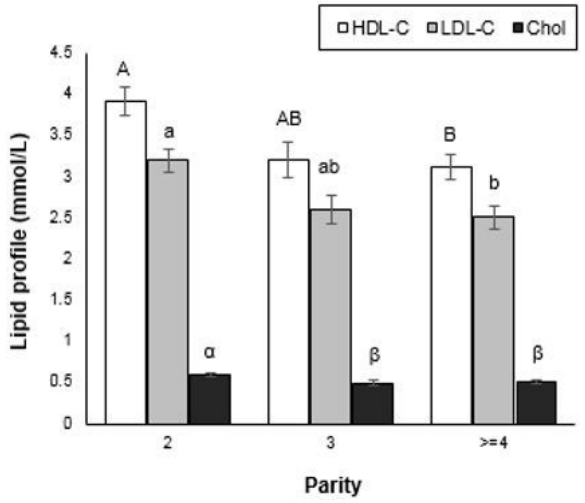

Fig. 4: Least square means and standard error of serum concentrations of lipids in dairy cows categorized into 2nd, 3rd, and 4th or more parities. Values with different superscripts present significant differences (at P≤0.05) between the three categories by one-way ANOVA

significant correlation between serum T4 and glucose concentrations in healthy and disease affected cows. On the other hand, a similar significant negative correlation was observed between serum T4 and BHBA. A significant positive correlation for disease affected cows was observed between serum T4, fructosamine, and LDL-C.

Discussion

The lowest serum NEFA and BHBA were observed g 14 days before the expected calving date. The inadequate dry matter intake during late pregnancy to early lactation does not cover energy demands for body maintenance and milk production (Puppel and Kuczynska, 2016). On the other hand, limited glycogen reserves of bovine hepatocytes support energy requirements by triglyceride hydrolysis and lipid mobilization (Herdt, 2000). NEFA values were significantly greater in FC than in NFC 30 days after calving. Furthermore, serum BHBA of FC had significantly higher concentrations on the 20th day of lactation. These results were predictable and are in accordance with those of previous studies (Al Ibrahim *et al*., 2010; Pires *et al*., 2013; Roche *et al*., 2013; Jamali Emam *et al*., 2017). They reported higher BHBA and NEFA values in the first week after parturition and then remained relatively constant in cows with high and medium BCS. Fat cows during the dry period had higher BHBA and NEFA values in comparison with NFC. They experience a more profound energy deficit than NFC during the transition period. After parturition, fat cows experience more lipolysis than NFC due to a greater decrease in dry mater intake and they transfer more fatty acids to liver for energy production and these events cause the accumulation of triglycerides in the liver and may lead to fatty liver syndrome. Sorondo and Cirio (2009) reported that glucose and fructosamine decreased five weeks after calving and this was associated with milking progress and increased BHBA concentrations. In our study, we have observed that disease affected cows had significantly lower serum fructosamine compared to healthy cows. This is in accordance with the previous study (Mostafavi *et al*., 2015). They indicated that cows suffering from liver lipidosis had lower serum fructosamine concentrations, and this might be attributed to insulin resistance and decreased gluconeogenesis (Mostafavi *et al*., 2015).

Thyroidal hormone changes are one of the adaptive responses that are critical to improving NEB around parturition (Piechotto *et al*., 2014). In the present study, serum T4 concentrations were significantly affected by times of sampling. The highest amounts of T4 were found 14 days before expected calving, and its values declined after calving. According to previous data, thyroid hormones decreased after parturition to keep energy balance and optimal milk yield (Fratric *et al*., 2013; Natalija *et al*., 2017; Paulikova *et al*., 2017). In addition, lower amounts of thyroxine after parturition increased the level of lipolysis and resulted in lipid accumulation in the liver and liver steatosis (Šamanc *et al*., 2010). Therefore, a significant negative correlation between serum T4 and NEFA in total animals has emphasized the above-mentioned studies. Serum T4 values also had a significant negative correlation with NEFA in FC and BHBA in NFC. Low T4 concentrations may expand lipid mobilization in FC. Furthermore, it has been shown that mitochondrial dysfunction and hypothyroidism are involved in lipid accumulation in the liver (Paulikova *et al*., 2017). Our findings suggested a close link between thyroid hormone, BCS, and NEB.

In the present study, healthy cows had significantly lower serum T4 values than disease affected cows (Fig. 2B). It was reported that dairy cows suffering from fatty liver and ketosis have lower serum T3 and T4 values (Djokovic *et al*., 2010; Samanc *et al*., 2010). According

to a significant negative correlation between serum T4 and BHBA in both healthy and diseased cows and a significant positive correlation between serum T4 and fructosamine in disease affected cows, it seems that higher T4 values in disease affected cows were a compensatory mechanism to increase available glucose and to minimize lipid mobilization for maintenance and milk yield (Romo *et al*., 1997).

The energy status during the transition period directly influences lipid profiles as potential landmarks of hepatocyte's function (Kessler *et al*., 2014). All cows in the present study, regardless of their BCS, showed gradual enhancement in the total serum cholesterol and HDL-C values during the study. The Liver adapts toward increased cholesterol biosynthesis during the lactation period. During late pregnancy, the decrease in total cholesterol and HDL-C are typically due to large requirements of the fetus and steroidogenic organs such as ovaries and placenta (Pysra and Opalka, 2000). Kessler *et al*. (2014) showed that hepatocytes could optimally provide critical proteins involved in cholesterol biosynthesis and lipoprotein components after calving. In contrast, Gross *et al*. (2015) and Kulka (*et al*. 2016) reported that downregulation of sterol regulatory element-binding factor 1 (SREBF-1) decreased cholesterol values after parturition. HDL-C is the main blood cholesterol carrier in cows that transfers cholesterol to steroidogenic organs (Hu *et al*., 2010). Its anti-inflammatory and antioxidant roles can improve liver responses to adaptation changes during the transition period (Turk *et al*., 2016). On the other hand, serum triglyceride, LDL-C, and VLDL-C had the lowest values on the 10th day of lactation. There are two reasons for the reduction of triglyceride and VLDL after calving. First, there is limited capacity of bovine hepatocytes for reconverting NEFA back to triglyceride and exporting triglyceride in the form of VLDL (Preynat *et al*., 2010; Chalmeh *et al*., 2015). Second, there is the stress condition around the time of calving, which triggers catecholamines secretion and increases liver lipidosis (Folnozic *et al*., 2015). In addition, decreased LDL concentrations are attributed to the limitation of VLDL synthesis and release from hepatocytes (Kulka *et al*., 2016). Moreover, it was found that the reduction of LDL-C values could be associated with cholesterol concentrations, since LDL-C is the primary lipoprotein for supplying milk cholesterol (Arfuso *et al*., 2016). In the present study, there was expanded lipid mobilization during the first ten days of lactation. The highest NEFA and BHBA are coordinated with the lowest serum glucose, fructosamine, triglyceride, VLDL-C, and LDL-C. These results confirm that mild liver lipidosis is associated with the development of the lactation period.

We have also observed a significant negative relationship between serum T4, cholesterol and HDL-C in all cows, NFC, and FC. It is well-known that bile acids were synthesized from cholesterol that was suppressed in hypothyroidism. Therefore, the amounts of serum cholesterol and HDL-C were increased in the hypothyroidism conditions (Bond *et al*., 2014). In addition, food-restricted ruminants lower their maintenance requirements, slowing down the basal metabolism rate by lowering circulating levels of thyroid hormones. The net effect of thyroid hormone on cholesterol metabolism is to increase the rate of its catabolism by the liver and excretion by bile, thereby lowering the serum cholesterol amount (Kaneko, 2008). This decrease in thyroid hormones may have beneficial effects on the cholesterol amounts because there is a higher need for lipoproteins to carry lipids to mammary gland. Decreased thyroid hormones has been noted in adaptations to NEB in accordance with other metabolic and hormonal changes. It appears that the tendency for reduction in glucose concentration in NEB initiates a cascade of changes which mediates the aforementioned adaptations through some changes in hormones and metabolites. Drop in thyroxin secretion and also less formation of T3 has been reported in NEB (Mohebbi Fani *et al*., 2009).

A significant positive correlation was observed between serum T4 values, triglyceride and VLDL-C in total cows and FC. Similarly, these correlations were seen in healthy cows. Mohebbi Fani *et al*. (2009) found that time affects the pattern of serum thyroid hormones correlations and lipids profile among dairy cows. They reported only a significant negative correlation between free T4 and triglyceride and VLDL-C in late pregnancy, while they observed no significant relationship between thyroid hormones and triglyceride and VLDL-C when samples of different stages of lactation were pooled (Mohebbi Fani *et al*., 2009). According to the results mentioned above, the subjects experienced mild liver lipidosis following lactation development. It is well-known that liver 5-deiodinase activity decreases in lactating cows suffering from liver lipidosis. Therefore, the liver accumulation of triglycerides may be accompanied by decreased serum thyroid hormones (Romo *et al*., 1997).

The total cholesterol, HDL-C, and LDL-C concentrations were significantly greater in healthy cows than in disease affected cows. We also observed that fat ill cows had the lowest values of total serum cholesterol and HDL-C concentrations. HDL-C contains the most values of serum cholesterol. Therefore, the changes of serum cholesterol and HDL-C accordingly occur in the same direction. It seems that inflammatory conditions might influence cholesterol packaging by hepatocytes and decrease the serum amounts of cholesterol and lipoproteins. This process may be more remarkable in fat cows due to the larger secretion of inflammatory mediators from adipose tissue (adipokines such as IL-6).

Previous investigations are in line with our findings, which have shown a positive relationship between the production of total cholesterol rate and health status after calving (Raphael and Sordillo, 2013; Turk et al., 2013; Newman *et al*., 2016). Furthermore, it was shown that cows with higher blood HDL-C values at the dry-off period have better fertility indices after calving (Crociati *et al*., 2017).

Moreover, cows with second parity had significantly

higher cholesterol, HDL-C, and LDL-C concentrations compared to fourth or more parities. In contrast, it was reported that plasma cholesterol and triglyceride concentrations were not affected by parity in late-pregnant heifers and dry Holstein cows (Brscic *et al*., 2015). It is likely that younger animals have higher metabolic rates and hepatocellular activities. Thus, younger cows can face metabolic stress more efficiently.

Our data emphasize the effects of negative energy balance during the transition period on lipid metabolism and thyroid function. In addition, the correlation between T4, energy, and lipid fractions may indicate a possible effect of health and body condition status on thyroid responses.

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Conflict of interest

The authors declare that they have no conflict of interest.

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