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Compromised liver functions during the breeding period of clinically healthy Holstein cows

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Summary

Background: The sub-clinical carry-over effects of post-partum (PP) conditions and the problems independent of parturition may affect the cows' performance during the breeding period. **Aims:** It was hypothesized that some mid-lactation cows may have compromised liver functions and calculating liver activity index (LAI; -1.5 to +1.5) may be helpful in detecting such conditions. **Methods:** Plasma lipid and protein profiles, retinol and ceruloplasmin were measured in 37 Holstein cows on days 60, 90, and 120 PP. Liver activity index was calculated using the measures of cholesterol, albumin and retinol. The results were interpreted using some health and performance indices. **Results:** The mean LAI was 0.00 ± 0.61. Sixteen cows (45.7%) were LAI^- and 19 cows (54.3%) were LAI^+. In LAI^+ cows the concentrations of cholesterol and albumin were unchanged, but in LAI^- cows both of them increased through the study ($P<0.05$). Greater concentrations of low density lipoproteins (LDL) ($P<0.01$) and lower concentrations of γ- and total globulins ($P<0.05$; day 60) were detected in LAI^+ cows. Ceruloplasmin was not different between the LAI groups with the highest level on day 90. Cholesterol had the strongest and the most repeated correlations with LAI during the study. The correlation of albumin with LAI faded on day 120 and vitamin A had a relationship only on day 60 PP. Triglycerides (TGs), very low density lipoproteins (VLDL) (day 60) and LDL (days 60 and 90) had positive correlations with LAI. All globulin fractions showed negative correlations with LAI on day 60. Seventy percent of the cows without endometritis (day 30 PP) and 33% of the infected cows were LAI^+ during the breeding period. About 79% of the cows with body condition score (BCS) loss ≤0.75 (day 60) and 38% of the cows with BCS loss >0.75 were LAI^+ during the breeding period. **Conclusion:** Compromised liver functions may exist in dairy cows during the breeding period and may be detected by calculating LAI. The relationship of LAI during the progressed lactation with herd's performance needs to be investigated.

Key words: Breeding period, Dairy cow, Liver activity index

Introduction

In dairy cows, the debilitating early post-partum (PP) conditions are anticipated to be resolved before the beginning of the breeding period, typically around 60 days PP (Lucy *et al*., 2014) with good nutritional and management practices during the preceding stages including dry, transition and early lactation (Overton and Waldron, 2004; Drackley *et al*., 2005; Castro *et al*., 2012; Lucy, 2016). However, the carry-over effects of PP conditions may affect the cows' productive and reproductive performance during the breeding period (Ribeiro *et al*., 2013; Lucy *et al*., 2014; Mohebbi-Fani *et al*., 2016). Besides, some problems may potentially originate several weeks after parturition. Decreasing levels of glucose together with increasing levels of non-esterified fatty acids (NEFA) in clinically healthy cows during days 60 to 120 of lactation have been shown (Mohebbi-Fani *et al*., 2018). A continuous acute phase response (APR) characterized by elevated levels of haptoglobin and serum amyloid-A (positive acute phase proteins (PAPP)) has been reported during 21-150 days in milk (DIM) with a weak inverse correlation between haptoglobin level and milk yield (Mohebbi-Fani *et al*., 2016). Acute phase response may consume a part of available energy and protein for immune responses, resulting in impaired animal performance without leaving clinical signs. Potentially, reduced hepatic synthesis of proteins such as albumin and lipoproteins (negative acute phase proteins (NAPP)) are anticipated.

Such subclinical conditions in mid-lactation cows may be important at herd level. Cows' insemination is often done based on the primary methods of assessing reproductive health (e.g. presence or absence of corpus luteum on ovary, characteristics of uterus, etc.). These methods usually detect the extreme clinical cases (Lucy *et al*., 2014) but not the sub-clinical disorders or metabolic insufficiencies. When the simple and less expensive diagnostic tools fail to illuminate and/or localize the problem, blood analysis may be helpful (Bertoni and Trevisi, 2013). Interpretation of the results, however, may be difficult due to a number of limiting factors such as minor affection of main blood metabolites (Mohebbi *et al*., 2005a), lack of reliable reference values (Mohebbi-Fani *et al*., 2010; Bertoni and Trevisi, 2013) and lack of control groups. Instead, composite metabolic indices such as liver activity index (LAI) may aid in evaluating the metabolic status of the cows (Bertoni and Trevisi, 2013). Liver activity index which targets the NAPP, varies between -1.5 and +1.5 and is calculated (within the suspect herd) using the average blood levels of albumin, cholesterol (indirect measure of lipoproteins) and retinol (indirect measure of retinol binding protein) on days 7, 14, and 28 of lactation. A low (or negative) LAI represents severe inflammation at or around calving in each cow. Lower dry matter intake and lower energy efficiency in the first weeks of lactation have been associated with low LAI (Trevisi *et al.*, 2007, 2010). The index has been recommended as a retrospective or prospective tool for assessing the transition period management and defining the probable further problems.

It was hypothesized in the present study as followed:
1) Some high producing dairy cows may not be metabolically perfect during the breeding period, suffering from inflammatory conditions and compromised liver functions
2) Such conditions may not be detectable by analyzing the main blood metabolites
3) LAI may be helpful in detecting some insufficiencies that otherwise may be left unnoticed

To assess these hypotheses, plasma lipid profile (total cholesterol, triglycerides (TGs), high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL)), protein profile (total protein, albumin and globulin fractions), retinol and ceruloplasmin were measured in Holstein cows on days 60, 90, and 120 of lactation. Liver activity index was calculated based on the measures of cholesterol, albumin and retinol. Some peri- and post-partum problems and performance indices of the cows (dystocia, metritis within 10 days PP, endometritis on day 30 PP, body condition score (BCS) loss on days 60, 90, and 120 of lactation, services per conception, days open) were also used for interpretation of the results.

Materials and Methods

Animals and samplings

Thirty-seven high producing Holstein cows (20 first lactation and 17 adults with average standard 305-day milk production of 10,000 kg) were enrolled into the study during 60-120 DIM. The first lactation and adult cows were housed separately in sand bed free stalls with natural ventilation. They were offered *ad libitum* a total mixed ration containing (dry matter basis) alfalfa hay (17.30%), corn silage (25.90%), barley grain (16.33%), corn grain (16.10%), wheat bran (5.90%), soybean meal (11.23%), poultry meat and bone meal (4.74%), trace mineral and vitamin supplements (0.93%), calcium carbonate (0.31%), white salt (0.31%), sodium bicarbonate (0.94%), and monensin (360 and 400 mg for 1st calf heifers and adults, respectively). Cows were milked 2 times per day at 05:00 and 17:00 h. The cows had received all required post-calving examinations and medications to get ready for insemination after a voluntary waiting period of 60 days. The results of clinical examinations were recorded. All cows were clinically healthy at the sampling times. Monthly milk yield (during the entire lactation), the intervals between calving to the first oestrus and calving to the first insemination, service per conception ratio and days open of the cows were obtained from the records and the data base of the farm.

Blood samples were collected from coccygeal vessels into ethylenediaminetetraacetic acid (EDTA) containing vacuum tubes on days 60, 90 and 120 (±5 days) of lactation. The samples were taken between 09:00 and 10:30 h. Plasma was separated before 1300 the same day by centrifugation at 750 × g for 10 min and was stored in a few separate Eppendorf tubes at -20°C for further experiments.

Measurements

Lipid profile (total cholesterol, triglycerides, HDL, LDL, VLDL)

Plasma was analyzed for total cholesterol using a commercial kit (Ziest Chem Diagnostics, Tehran, Iran) based on the modified Abell-Kendall/Levey-Brodie (A-K) method. Plasma triglyceride (TG) was measured based on the enzymatic procedure using a commercial kit (Ziest Chem Diagnostics, Tehran, Iran). Lipoproteins including HDL-cholesterol and LDL-cholesterol were measured by quantitative enzymatic colorimetric method using kits supplied by Ziest Chem Diagnostics, Tehran, Iran. All reactions were measured using Digital UV/VIS spectrophotometer (CE 292, series 2, Cecil Instruments, Cambridge, England). VLDL-cholesterol was estimated as one-fifth of the concentration of TGs (Friedewald *et al.*, 1972).

Protein profile (total protein, albumin and globulin fractions)

Plasma total protein was measured by Biuret method (Commercial kit; Pars Azmoon, Tehran, Iran). Protein fractions (albumin, α-globulin, β-globulin, and γ-globulin) were determined by cellulose acetate electrophoresis (time: 15 min; voltage: 180v; apparatus: Elphor 5; Germany).

Retinol (vitamin A)

The concentration of plasma vitamin A was measured by the high-performance liquid chromatography (HPLC) method based on the protocol described by Johnson-Davis *et al.* (2009) by using ultraviolet detection. The HPLC system consisted of a solvent delivery pump (JASCO 980-PU, Tokyo, Japan), a reversed-phase column (Luna C18, 250 × 4.6 mm; Phenomenex, CA, USA) and a UV-Vis detector (Jasco, UV-975, Tokyo, Japan).

Ceruloplasmin

The concentration of ceruloplasmin was measured according to its para-phenylenediamine oxidase activity (pH=5.4 at 37°C) using the spectrophotometric method described by Sunderman and Nomoto (1970).

Calculation of liver activity index

The index was calculated as described by Bertoni and Trevisi (2013). Accordingly, LAI is calculated using the blood levels of albumin, total cholesterol, and retinol on days 7, 14, and 28 PP (transition phase and beginning of early lactation). In the present study the values of days 60, 90, and 120 of lactation (transition from early- to mid-lactation and beginning days of mid-lactation; namely the breeding period) were substituted for the values of days 7, 14, and 28. The means of population values of albumin, total cholesterol and retinol were subtracted from each cow's value and then were divided by the corresponding standard deviation. The LAI score for each cow was the mean of the 3 partial values obtained as above. The cows were grouped as positive and negative LAI groups (LAI$^+$ and LAI$^-$) for statistical analysis.

Statistical analysis

Blood parameters were analyzed as mean±SD at P<0.05 using the SPSS statistical software (version 16.0, SPSS, Inc., Chicago, IL, USA). The trends of changes during the study period were assessed in all cows (n=37) and separately in LAI$^+$ and LAI$^-$ cows using analysis of variance (ANOVA) for repeated measures. The trends of changes were also compared between parities (1 and ≥2) and standard milk productions below and above 10,000 kg. The separate data of days 60, 90, 120 and the pooled data of the whole study period were compared between LAI$^+$ and LAI$^-$ cows using independent sample t-test. The differences between various sampling days within LAI$^+$ and LAI$^-$ cows were studied using one way ANOVA and LSD post-hoc test. The correlations between the blood parameters and LAI were studied by Pearson's correlation test. Chi-square test was used to compare the numbers and the proportions of LAI$^+$ and LAI$^-$ cows regarding various conditions including dystocia, metritis within 10 days PP, endometritis on day 30 PP, BCS loss (<0.75 vs. ≥0.75) on days 60 and 90, services per conception (<3 vs. ≥3) and days open (<120 vs. ≥120).

Results

General changes of blood metabolites

The concentration of blood lipids and proteins are shown in Table 1. Cholesterol concentration was not different among different sampling days (P>0.05) although it showed an increasing trend (P=0.012) during the study. TG and VLDL levels showed decreasing patterns of changes (P<0.001) with significant drops (P<0.05) on day 90 PP. The trends of changes of LDL and HDL were not significant during the study, but LDL level was significantly lower on day 60 (P<0.05) compared to the succeeding sampling days (Table 1). Albumin did not change significantly but total protein (P<0.003), α-globulins (P<0.001), γ-globulins (P<0.001) and total globulins (P<0.001) decreased during the study with significant drops on day 120 PP. Vitamin A was almost unchanged and ceruloplasmin increased both linearly and quarterly (P<0.001) during the study with significantly higher concentrations on day 90 compared to days 60 and 120 PP (P<0.05). The concentrations and trends of changes of blood lipids and proteins were not different (P>0.05) between parities and different milk production levels.

Liver activity index and variations of blood metabolites

Considering some missing data (2 cows), LAI was calculated for 35 cows. The LAI ranged from -1.19 to +1.44 with a borderline mean of 0.00 ± 0.61 during days 60 to 120 PP. Sixteen cows (45.7%) were LAI$^-$ (-0.53 ± 0.36) and the remaining 19 cows (54.3%) were LAI$^+$ (+0.45 ± 0.35) (P<0.001). Parity and milk production level had no significant effects on LAI.

Cholesterol, albumin and vitamin A (used for LAI calculation) were significantly different between the two sets of cows (Table 2), being higher in LAI$^+$ group. The differences of cholesterol and albumin were observed on various sampling days and were also reflected in the pooled data of the whole study period. The differences of

Table 1: General trends of changes of the blood parameters in Holstein cows through days 60 to 120 of lactation (n=37)

Variables	Day 60	Day 90	Day 120	P-value for trends of changes	Reference values
Cholesterol (mmol/L)	4.95 ± 2.10	6.02 ± 3.74	6.12 ± 1.91	0.012	2.07-3.11
Triglyceride (mmol/L)	0.11 ± 0.02^a	0.07 ± 0.04^b	0.07 ± 0.04^b	0.001	
VLDL (mmol/L)	0.021 ± 0.004^a	0.015 ± 0.008^b	0.013 ± 0.007^b	0.001	
LDL (mmol/L)	2.23 ± 1.81^a	3.60 ± 3.79^b	3.32 ± 1.93^b	NS	
HDL (mmol/L)	3.09 ± 1.28	2.60 ± 1.21	2.75 ± 0.90	NS	
Total protein (g/L)	83.51 ± 1.55^a	85.42 ± 1.74^a	74.31 ± 1.35^b	0.003	67.4-76.6
Albumin (g/L)	36.12 ± 13.37	40.46 ± 11.06	38.27 ± 8.04	NS	30.3-34.8
Alpha globulin (g/L)	11.42 ± 3.65^a	12.42 ± 3.97^a	8.14 ± 2.86^b	0.001	7.5-8.8
Beta globulin (g/L)	9.76 ± 3.10	9.24 ± 3.18	8.75 ± 0.23	NS	8.0-11.2
Gamma globulin (g/L)	25.92 ± 8.85^a	22.90 ± 7.33^a	18.61 ± 7.37^b	0.001	16.9-22.3
Total globulin (g/L)	47.10 ± 13.67^a	44.56 ± 12.86^a	35.49 ± 11.10^b	0.001	30-34.8
Vitamin A (mg/dl)	25.13 ± 1.48	25.27 ± 1.50	25.39 ± 1.55	NS	
Ceruloplasmin (mg/L)	0.062 ± 0.017^a	0.127 ± 0.030^b	0.109 ± 0.028^c	0.001 (L-Q)	

VLDL: Very low density lipoproteins, LDL: Low density lipoproteins, HDL: High density lipoproteins, NS: Non-significant, L: Linear change, and Q: Quadratic change. $^{a, b, c}$ different letters in each row correspond to significant differences between sampling days at P<0.05

Vitamin A, however, were limited to day 60. In LAI^{+} cows the concentrations of cholesterol and albumin did not change significantly between various sampling days (Table 2), but in LAI^{-} cows both of them increased toward day 120 PP (P<0.05). The trends of changes of cholesterol tended (P<0.069) to be different between the LAI groups being more prominent in the LAI^{+} cows particularly on day 90. The trends of changes of albumin were significantly different between the LAI groups (P=0.044, Table 2). While the LAI^{+} cows had almost constant albumin concentrations, the LAI^{-} group showed increasing levels of albumin toward day 120 PP with a sharp rise on day 90. The trends of changes of vitamin A were not significant between the LAI groups.

The LAI^{+} cows had significantly greater concentrations of LDL (P<0.01) on days 60 and 90 PP and the entire period of the study. The LAI^{+} cows had lower concentrations of γ-globulins and total globulins (P<0.05) on day 60 PP (Table 2). The changes of blood globulins were almost similar between the two sets of cows, decreasing toward day 120 PP. Ceruloplasmin was not different between the LAI groups and its concentrations within groups were similarly highest on day 90.

Correlations of LAI with blood metabolites

Among the three analytes used for LAI calculation, cholesterol had the strongest and the most repeated correlations with LAI (Table 3). The correlations of albumin with LAI faded on day 120 and vitamin A had a relationship only on day 60 PP. Among the other lipid fractions TG and VLDL had positive correlations with LAI on day 60 PP. LDL had positive correlations with LAI on days 60 and 90 PP. All globulin types showed negative correlations with LAI on day 60 PP.

Table 2: Blood metabolites of cows with positive and negative liver activity index during days 60 to 120 of lactation

Variables	LAI	Day 60	Day 90	Day 120	Mean of the whole study period	P
Cholesterol (mmol/L)	Positive	5.95±2.31**	7.78±4.25**	6.35±1.87	6.69±1.80**	0.069
	Negative	3.74±1.09$^{a, **}$	3.93±1.77$^{a, **}$	5.76±1.99^{b}	4.47±0.97**	
Triglyceride (mmol/L)	Positive	0.11±0.02$^{a, \dagger}$	0.06±0.02^{b}	0.08±0.04$^{b, c}$	0.08±0.02	NS
	Negative	0.10±0.02$^{a, \dagger}$	0.09±0.06^{a}	0.06±0.04^{b}	0.08±0.02	
VLDL (mmol/L)	Positive	0.022±0.004$^{a, \dagger}$	0.012±0.004^{a}	0.016±0.008^{b}	0.016±0.004	NS
	Negative	0.020±0.004$^{a, \dagger}$	0.018±0.012^{a}	0.012±0.008^{b}	0.016±0.004	
LDL (mmol/L)	Positive	112.08±77.76$^{a, **}$	204.56±159.82$^{b, **}$	124.43±75.45$^{a, c}$	146.05±62.27**	0.035
	Negative	45.98±25.13$^{a, **}$	60.42±80.70$^{a, b, **}$	130.04±77.52^{c}	70.83±25.26**	
HDL (mmol/L)	Positive	116.30±43.68	98.32±44.41	106.82±35.21	107.15±24.39	NS
	Negative	111.92±48.53	96.18±35.28	103.03±37.21	103.71±17.59	
Total protein (g/L)	Positive	85.91±14.05	86.40±17.76	76.68±14.83	83.00±10.05	NS
	Negative	81.95±17.70	82.67±16.22	72.66±12.20	79.09±9.25	
Albumin (g/L)	Positive	42.85±9.37**	42.12±12.30	40.09±8.97**	41.69±5.24**	0.044
	Negative	29.03±13.50$^{a, **}$	37.41±9.15^{b}	36.84±6.60$^{b, c, **}$	34.42±4.43**	
Alpha globulin (g/L)	Positive	10.46±2.36^{a}	12.29±3.75^{a}	8.26±3.11^{b}	10.34±2.28	NS
	Negative	12.62±4.75^{a}	12.41±4.38^{a}	8.00±2.68^{b}	11.01±2.62	
Beta globulin (g/L)	Positive	9.01±1.94	9.58±3.20	8.78±2.21	9.13±1.65	NS
	Negative	10.74±3.97	8.73±3.22	8.76±2.50	9.41±2.50	
Gamma globulin (g/L)	Positive	23.11±5.78$^{a, *}$	21.91±7.25^{a}	18.66±4.69^{b}	21.23±4.34	0.023
	Negative	29.47±10.52^{a,*}	23.78±7.40^{a}	18.87±10.14^{b}	24.04±6.50	
Total globulin (g/L)	Positive	42.58±8.48$^{a, *}$	43.78±11.96$^{a, b}$	35.70±8.70^{c}	40.69±6.99	0.032
	Negative	52.83±16.65$^{a, *}$	44.92±13.94$^{a, b}$	35.63±14.09^{b}	44.46±9.79	
Vitamin A (mg/dl)	Positive	25.68±1.39*	25.21±1.54	25.51±1.69	25.47±0.88	NS
	Negative	24.54±1.38*	25.39±1.46	25.23±1.46	25.03±0.73	
Ceruloplasmin (mg/L)	Positive	0.059±0.017^{a}	0.124±0.027^{b}	0.110±0.026^{c}	0.098±0.013	NS
	Negative	0.064±0.017^{a}	0.128±0.033$^{b, c}$	0.107±0.32^{c}	0.100±0.022	

LAI: Liver activity index, P: For differences of trends between LAI groups, VLDL: Very low density lipoproteins, LDL: Low density lipoproteins, HDL: High density lipoproteins, and NS: Non-significant. * P<0.05, ** P<0.01, and † 0.05>P<0.1 in each column. $^{a, b, c}$ different letters in each row correspond to significant difference at P<0.05

Table 3: The observed correlations between plasma metabolites and LAI during days 60 to 120 of lactation

DIM	Day 60	Day 90	Day 120	The whole study period
Correlations with LAI				
Cholesterol	0.50 (0.002)	0.61 (0.001)	0.36 (0.04)	0.74 (0.001)
Albumin	0.49 (0.003)	0.37 (0.029)	-	0.65 (0.001)
Vitamin A	0.43 (0.009)	-	-	0.42 (0.013)
TG	0.34 (0.044)	-	-	-
VLDL	0.34 (0.044)	-	-	-
LDL	0.47 (0.011)	0.58 (0.001)	-	0.70 (0.001)
α-globulins	-0.47 (0.004)	-	-	-
β-globulins	-0.41 (0.015)	-	-	-
γ-globulins	-0.39 (0.019)	-	-	-
Total globulins	-0.48 (0.004)	-	-	-

LAI: Liver activity index, DIM: Days in milk, TG: triglyceride, VLDL: Very low density lipoproteins, and LDL: Low density lipoproteins

Table 4: Frequencies of negative and positive LAI during days 60 to 120 of lactation concerning some performance indices and peri-partum problems (the values in the parentheses correspond to percentage)

Periparturient problem/performance index	Category	Frequencies of LAI during days 60-120 PP		
		Positive	Negative	P-value
Dystocia	Normal (n=17)	10 (58.8)	7 (41.2)	0.366
	Help (n=17)	8 (47.1)	9 (52.3)	
Metritis (within 10 days PP)	Healthy (n=23)	14 (60.9)	9 (39.1)	0.166
	Sick (n=11)	4 (36.4)	7 (63.4)	
Endometritis (day 30 PP)	Healthy (n=20)	14 (70)	6 (30)	0.034
	Infected (n=15)	5 (33.3)	10 (66.7)	
BCS loss (d. 60)	≤0.75 (n=14)	11 (78.6)	3 (21.4)	0.021
	>0.75 (n=21)	8 (38.1)	13 (61.9)	
BCS loss (d. 90)	≤0.75 (n=15)	11 (73.3)	4 (26.7)	0.052
	>0.75 (n=20)	8 (40)	12 (60)	
Service per conception	≤3 (n=28)	16 (57.1)	12 (42.9)	0.398
	>3 (n=7)	3 (42.9)	4 (57.1)	
Days open	≤120 (n=15)	8 (53.3)	7 (46.7)	0.532
	>120 (n=19)	11 (57.9)	8 (42.1)	

LAI: Liver activity index, BCS: Body condition score, and PP: Post-partum

Liver activity index and peri- and post-partum conditions

As shown in Table 4, seventy percent of the cows without uterine infection (endometritis) on day 30 PP were LAI$^+$ later during the breeding period. Meanwhile, a significantly lower percentage (33%) of the infected cows were LAI$^+$ during the same period (P=0.034). While about 79% of the cows with BCS loss ≤0.75 were LAI$^+$, only 38% of the thin cows (BCS loss >0.75) were LAI$^+$ during the breeding period (P=0.021). Almost similar results were obtained with the level of BCS loss on day 90 PP (P=0.052). The cows with a BCS loss ≤0.75 on day 60 PP were LAI$^+$ (+0.25 ± 0.47) compared to the thin cows that were LAI$^-$ (-0.17 ± 0.41) (P=0.044; data not shown). Some numerically better values were detected for LAI$^+$ cows considering dystocia, metritis and service per conception ratio (Table 4).

Discussion

Blood lipids and proteins

The concentration of cholesterol showed an increasing trend through the study with no significant difference between the sampling days. Cholesterol levels were higher than the published reference values (2.07-3.11 mmol/L; Kaneko *et al.*, 2008). According to Bartley (1989), cows show hypercholesterolemia during lactation and the levels of LDL and HDL also increase in parallel. In the present study, LDL concentration rose on day 90 PP but HDL level showed no significant changes (Table 1). Indeed, the trends of changes of both LDL and HDL were not significant. Increased concentrations of total cholesterol and LDL and decreased levels of HDL have been observed in humans with hypothyroidism (Rizos *et al.*, 2011). A concurrent study to the present one (Mohebbi-Fani *et al.*, 2018) showed significant decrease in the levels of circulating thyroid hormones in the same cows from day 60 to 120 PP. Mohebbi-Fani *et al.* (2009) reported cholesterol, LDL and HDL levels comparable to those found in the present study without significant differences between early- and mid-lactation cows.

Although affection of the liver functions by decreased levels of thyroid hormones is likely, it is concluded from the results of the present study that hepatic lipidosis may also be a factor for affection of the liver activity during the breeding period. The concentrations of TG and VLDL were highest on day 60 PP (0.11 and 0.021 mmol/L, respectively) and decreased afterwards and remained almost constant on days 90 and 120 PP (Table 1). Some higher levels of TG (0.15 mmol/L) and VLDL (0.03 mmol/L) have been reported in mid-lactation cows (71-150 days PP; Mohebbi-Fani *et al.*, 2009) as well as cows within 26-45 days PP (Mohebbi-Fani *et al.*, 2005). It is concluded that the decrease in TG and VLDL could be due to compromised liver functions probably as a result of lipid accumulation in the liver (see below; the LAI section). A concurrent study (Mohebbi-Fani *et al.*, 2018) has shown elevated plasma levels of NEFA during the breeding period. This may be a potential cause of hepatic lipidosis.

Albumin concentrations were statistically unchanged during the present study and they were even higher than the published reference values (30.3-34.8 g/L; Kaneko *et al.*, 2008). Albumin has a large pool size (Herdt, 2000; Mohebbi-Fani *et al.*, 2005) with a long half-life about 16.5 days (Kaneko *et al.*, 2008) and may not change rapidly. While the concentrations of albumin were unchanged, the concentrations of α-, γ- and total globulins decreased after day 60 PP (Table 1). On day 120 PP the concentrations of α-, γ- and total globulins were significantly lower than their levels in the previous sampling days. With these changes in globulin fractions, an increase in the level of albumin could be anticipated due to some extrahepatic saving of amino acids, but such a change was not observed. Meanwhile, β-globulins were unchanged and ceruloplasmin (an α-globulin) showed a sharp quadratic rise on day 90 (Table 1). Decreased γ- and α-globulins may be signs of relief of the animals from infectious conditions during the breeding period. However, unchanged β-globulins and increased ceruloplasmin may be signs of mild stressful and inflammatory conditions (Cray *et al*., 2009), during which some acute phase proteins may be synthesized in the liver at the expense of albumin synthesis. This conclusion may also be explained by the borderline LAI (0.00 ± 0.61) detected in the present study as well as the decreasing levels of blood total protein (Table 1).

Liver activity index and its components

The mean LAI for the studied cows (n=35) was 0.00 ± 0.61 (-1.19 to +1.44) during days 60-120 PP. Sixteen cows (45.7%) were LAI^- (-0.53 ± 0.36) and the remaining (n=19; 54.3%) were LAI^+ (0.44 ± 0.35) (P<0.001). As anticipated, albumin, cholesterol and vitamin A (used for LAI calculation) were significantly different between LAI^+ and LAI^- cows (Table 2). While the mean (and apparently normal) concentrations of albumin were unchanged during the study, the trends of its changes were different between the LAI groups (P=0.044). The LAI^+ cows had almost constant albumin concentrations through the study. The LAI^- cows, however, showed increasing levels of albumin during the study period (day 60 to 120 PP; P<0.05). These cows had lower albumin levels on all sampling days with a subnormal level on day 60 PP (Table 2). Albumin had positive correlations with LAI on days 60 and 90 PP (Table 3). Accordingly, it is concluded that some cows during the breeding period may have deficient albumin synthesis in the liver.

In addition to albumin that is a NAPP, the other two components of LAI (cholesterol and retinol) are also indirect measures of NAPP (Bertoni and Trevisi, 2013). In the present study, the LAI^- cows had lower cholesterol levels on days 60 and 90 PP (P<0.001) and cholesterol had the strongest positive correlations with LAI repeated in all sampling days (Tables 2 and 3). It is concluded that the synthesis of lipoproteins in the liver may be compromised during the breeding period even if their concentrations appear to be normal. The LAI^- cows tended (P<0.1) to have lower concentrations of VLDL on day 60 (Table 2). This, together with the significant decrease of VLDL after day 60 (Table 1), could probably be due to the accumulation of lipids in the liver, compromising its functions. Elevated plasma levels of NEFA during days 60 to 120 PP have been reported (Mohebbi-Fani *et al*., 2018) and may be a potential cause of hepatic lipidosis. The lower level of LDL in LAI^- cows (P<0.001; Table 2) on days 60 and 90 PP and the entire period of the study may explain this conclusion. LDL is the product of VLDL catabolism (Bartley, 1989) and varies in parallel with the changes of TGs and VLDL. Lower blood levels of LDL have been reported in cows with hepatic lipidosis as a result of lower production of VLDL in the liver (Rayssiguier *et al*., 1988). The positive relations of TG, VLDL and LDL with LAI (Table 3) may also explain the possibility of the affection of the hepatic functions in the studied cows. The concentrations of retinol were different between LAI groups only on day 60 PP. Also its correlation with LAI values was detected only on day 60. It appears that the synthesis of this protein in the liver may be less affected than the synthesis of lipoproteins and albumin.

Peri- and post-partum conditions

The data of Table 4 generally reveal that the cows that had better conditions in various aspects of dairy farming were LAI^+ during the breeding period. The indices closer to the time of breeding (BCS loss on days 60 and 90 PP, endometritis on day 30) had significant influences on the frequencies of the LAI^+ cows. However, a cause and effect conclusion may not be made by the results of the present study. In fact, the inflammatory conditions (in general) and compromised liver functions during the breeding period may possibly be the continuation of the early PP problems or may happen independent of those problems.

Compromised liver function may exist in dairy cows during the breeding period and may affect their performance. Acute phase response due to stress, inflammation or infection may play a role in the occurrence of this condition, but may not be detected by measuring the main blood metabolites and acute phase proteins. Minor affection of the blood metabolites, lack of reliable reference values and lack of control groups are the major limiting factors. Liver activity index, calculated based on the concentrations of cholesterol, albumin and retinol during the breeding period (in 30-day intervals), may be a useful tool for detecting insufficiencies in the liver functions and practical evaluation of the herd management during the breeding period and the stages prior to it. The relationship of LAI during the progressed lactation with the herd performance indices needs to be investigated.

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Conflict of interest

We declare that we have no conflict of interest.

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