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Blood Iron Status in Dairy Cows during Early Lactation - Relationship with Hematological, Biochemical, Endocrine and Inflammatory Response

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ABSTRACT

***Background*:** Iron (Fe) is microelement, essential to ensure some metabolic physiological process. Although Fe is a stable element in the body that is tightly regulated, metabolic stress, lipolysis, ketogenesis, endocrine change, insulin resistance and inflammation in early lactation can influence Fe status in blood. The objectives of this experiment were to determine the Fe status of cows in early lactation and determine whether measures of Fe status were in relation with hematological, metabolic, endocrine and inflammatory response in cows during early lactation.

***Materials, Methods and Results*:** The experiment included 30 healthy cows in the 1st 6 weeks of lactation. Laboratory analysis includes determination of Fe status (blood Fe, unsaturated iron-binding capacity (UIBC), total iron-binding capacity (TIBC), transferrin saturation percentage (TS%) and metabolic response of cows (complete blood count, non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), cholesterol, triglycerides, glucose, total bilirubin (TBIL), aspartate amino-transferase (AST), gamma-glutamyl transferase (GGT), cortisol, T3, T4, insulin, RQUICKIBHB, TNF-α, and haptoglobin). Blood samples were collected by puncture of coccygeal vein. Fe increased from the 1st to the 6th week of lactation, with significantly lower concentrations in the 1st 2 weeks of the experiment ($P < 0.01$). TIBC and UIBC were lower in 1st 2 or 3 weeks of experiment ($P < 0.01$), while the TS% was unchanged. Hematological response included a significant lower MCV and HCT ($P < 0.01$) and tendency to decrease HGB and RBC ($P < 0.1$) in 1st 3 weeks of lactation compared to 2nd 3 weeks. WBC and PLT was unchanged, but NLR was higher in 1st 3 weeks of lactation ($P < 0.05$). Metabolic response was characterized by higher concentration of NEFA, BHB, TBIL, AST, GGT ($P < 0.01$) and NEFA:CHOL ratio ($P < 0.05$), and lower concentration of GLU, TGC, CHOL ($P < 0.01$) and ALB ($P < 0.05$) in 1st 2 or 3 weeks of lactation compared to period from 4 to 6 week. Endocrine response included lower concentration of insulin ($P < 0.01$), T3 and T4 ($P < 0.1$), and higher concentration of cortisol and RQUICKIBHB index of insulin resistance ($P < 0.01$) in 1st 2 or 3 weeks of experiment compared to period from 4 to 6 weeks. Inflammatory response included higher concentration of TNF-α and haptoglobin ($P < 0.01$) in 1st 2 or 3 weeks of experiment compared to period from 4 to 6 weeks. Fe concentration showed significant positive correlation with TIBC, UIBC, HGB, MCV, GLU, T3, T4 and INS and negative correlation with NLR, NEFA, BHB and RQUICKIBHB. TIBC and UIBC showed negative correlation with TBIL, AST, GGT, and NEFA:CHOL ratio and positive with ALB and TGC. Fe showed negative correlation with TNF-α, while TIBC and UIBC showed negative correlation with haptoglobin. Metabolic and inflammatory parameters had higher magnitude of change when Fe was extrapolated on deficit level (9 µmol/L), compared to TIBC, UIBC, TS% and red blood cell parameters as functional indicators of Fe status.

***Discussion*:** Fe, TIBC and UIBC showed significant change in 1st 6 weeks of lactation. Decrease in Fe in cows could be related to general homeorhetic adaptation to the onset of lactation, since Fe correlates with indicators of lipolysis, ketogenesis, and endocrine parameters. The lower TIBC and UIBC observed in cows in 1st 3 weeks when Fe was lower is an unexpected finding, since Fe decrease usually increases TIBC and UIBC. TIBC correlates with hepatocytes indices of the functional status and lipid infiltration, so it is possible that TIBC decreases because of changes in lipid metabolism and acute response in the liver. Blood Fe, TIBC and UIBC can be useful indicators for assessing metabolic stress in early lactating cows.

Keywords: cow, iron, early lactation, metabolic stress.

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INTRODUCTION

Iron (Fe) is essential to ensure the physiological process of health, animal's growth and productivity [43], and participates in a wide variety of metabolic processes, including oxygen transport, deoxyribonucleic acid synthesis, electron transport, helps to convert blood sugar to energy and boosts the immune system in animals [44]. Iron deficiency is rare in herbivores (except in young individuals), due to its good presence in plants, soil and water [37].

Although it can be concluded that Fe is a stable element, there are several reasons why it is necessary to examine Fe status in cows in the periparturient period. Cows in late gestation and early lactation have lower hemoglobin concentration [13], and hemoglobin correlates well with body Fe depots in cows [27]. Fe availability and concentration decrease in blood of cows during early lactation [32]. Metabolic changes in early lactation include lipolysis, insulin resistance, inflammation and fatty liver [7] which may be significantly related to lower Fe concentration, as demonstrated by the example of metabolic syndrome [16], which has a similar pathophysiology to peripartal metabolic stress. Inflammation in early lactation changes liver response and decrease transferrin value [1,24] with change of unsaturated iron-binding capacity (UIBC), total iron-binding capacity (TIBC), transferrin saturation percentage (TS%) as indicators of iron status.

The objectives of this experiment were to determine the Fe status of cows in early lactation and determine whether measures of Fe status were in relation with hematological, metabolic, endocrine and inflammatory response in cows during early lactation.

MATERIALS AND METHODS

Animals and blood sampling

A total of 30 clinically healthy Holstein Friesian cows were included in experiment. Cows were in 2nd and 3rd lactation, with optimal body condition score (BCS) ranging from 3 to 3.5. The cows were fed a total mixed ration, as described in our previous study [22]. Chemical composition of the rations was according to the recommendations of the National Research Council [29]. The content of Fe in the concentrate mixture for cows was a minimum of 40 mg/kg for early lactation, 20 mg/kg for stable lactation and 80 mg/kg in supplementary mixtures, according to Serbian national Rulebook on the quality of animal feed ("Sl. glasnik RS", br. 4/2010).

Blood was sampled weekly from the coccigeal vein for 6 consecutive weeks. All blood samples were taken in the morning between 6 and 8 am. For the purposes of laboratory analyses, blood was sampled into serum separating and EDTA evacuated tubes[1]. Blood samples were transported to laboratory in cool boxes on ice. Laboratory processing and analysis of samples were performed on the same day. Complete blood count was done after blood with EDTA was mixed on roller and wormed to room temperature. Blood serum was obtained after clotting for 3 h at room temperature and centrifugation (1500 *g*, 10 min), sera were immediately analyzed. All analyses were performed at the Laboratory of pathophysiology, Department of Veterinary Medicine University of Novi Sad.

Laboratory analysis

Iron and hematology status were determined by the following parameters: iron in blood serum (Fe), unsaturated iron-binding capacity (UIBC), total iron-binding capacity (TIBC), transferrin saturation percentage (TS%), red blood cells count (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW) and neutrophil to lymphocyte ratio (NLR). Metabolic stress was assessed by measuring blood serum parameters: non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHB), cholesterol, triglycerides, glucose, total bilirubin (TBIL), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), cortisol, T3, T4, insulin, RQUICKI-BHB index of insulin resistance and NEFA to cholesterol ratio. Hematological analysis were conducted by an automatic hematology counter Mek-6550[2]. Biochemical analysis were run on an automated biochemical analyzer Chemray analyzer[3] using standard kits[4]. An automatic immunoassay analyzer AIA-360 was used for analysys of insulin, cortisol, T3 and T4[5]. The concentrations of TNF-α and haptoglobin were determined by standard kits[6] the ELISA reader and washer[4]. RQUICKI-BHB was obtained by calculation formula [31] and TS% was calculated by online platform (https://www.omnicalculator.com/health/transferrin).

Statistical analysis

Influence of week was determined using ANOVA analysis and the post-hock Tukey´s test. The

correlation between Fe and iron binding capacity (TIBC and UIBC) with blood parameters was determined using the Pearson correlation coefficient. At the end, a "what-if" analysis and a sensitivity analysis were performed to determine how the examined blood parameters would change if the value of Fe was below the minimum reference value (an example was taken for Fe = 9 μmol/L). The obtained results were presented graphically. The SPSS statistical software[7] was used for analysis. Statistical significance was set at $P < 0.01$ (strong significance), $P < 0.05$ (moderate significance) or $P < 0.1$ (trend).

RESULTS

The influence of the week was statistically significant, and the greatest differences were observed in the period of the 1st 3 weeks, compared to the last 3 weeks of the experiment. Our experimental model showed changes in Fe during the study, making it suitable for studying Fe-related changes in cows. Fe increased from the 1st to the 6th week of lactation, with significantly lower concentrations in the 1st 2 weeks of the experiment ($P < 0.01$). Indicators of Fe transport in blood changed, so that the value of TIBC and UIBC were lower in 1st 2 or 3 weeks of experiment ($P < 0.01$), while the value of TS% was unchanged. Hematological response included a significant lower MCV and HCT ($P < 0.01$) and tendency to decrease HGB and RBC ($P < 0.1$) in the 1st 3 weeks of lactation compared to the 2nd 3 weeks. WBC and PLT was unchanged, but NLR was higher in 1st 3 weeks of lactation ($P < 0.05$). Results are presented in Table 1.

Metabolic response during early lactation was characterized by higher concentration of NEFA, BHB, TBIL, AST, GGT ($P < 0.01$) and NEFA:CHOL ratio ($P < 0.05$), and lower concentration of GLU, TGC, CHOL ($P < 0.01$) and ALB ($P < 0.05$) in the 1st 2 or 3 weeks of lactation compared to period from 4 to 6 week. Endocrine response included lower concentration of insulin ($P < 0.01$) T3 and T4 ($P < 0.1$), and higher concentration of cortisol and RQUICKIBHB index of insulin resistance ($P < 0.01$) in the 1st 2 or 3 weeks of experiment compared to period from 4 to 6 week. Inflammatory response included higher concentration of TNF-α and haptoglobin ($P < 0.01$) in the 1st 2 or 3 weeks of experiment compared to period from 4 to 6 week. Results are presented in Table 2.

Fe showed significant correlation with hematological parameters and parameters of general adaptation, while TIBC and UIBC showed stronger correlation with liver functionality parameters. Fe concentration showed significant positive correlation with TIBC, UIBC, HGB, MCV, GLU, T3, T4 and INS and negative correlation with NLR, NEFA, BHB and RQUICKIBHB. TIBC and UIBC showed negative correlation with TBIL, AST, GGT, and NEFA:CHOL ratio and positive with ALB and TGC. Fe showed negative correlation with TNF-α, while TIBC and UIBC showed negative correlation with haptoglobin. The results are presented in Figure 1. Other red blood cells indicators of Fe status (HGB, MCV, RBC) and TS% did not show significant correlation with metabolic, endocrine or inflammatory parameters (results not shown).

	Fe	TIBC	UIBC
Fe	1.00	0.75	0.62
TIBC	0.75	1.00	0.69
UIBC	0.62	0.69	1.00
TS%	0.12	0.06	0.07
RBC	0.32	0.09	0.12
HGB	0.31	0.12	0.11
MCV	0.33	0.10	0.08
HCT	0.11	0.07	0.09
MCH	0.13	0.05	0.06
MCHC	0.10	0.09	0.10
WBC	0.12	0.09	0.11
NLR	-0.61	0.11	0.09
PLT	0.05	0.02	0.04
NEFA	-0.49	0.11	0.10
BHB	-0.47	0.12	0.10
GLU	0.52	0.09	0.05
TBIL	0.13	-0.52	-0.48
AST	0.12	-0.45	-0.45
GGT	0.09	-0.48	-0.49
TGC	0.08	0.29	0.31
CHOL	0.09	0.08	0.07
NEFA:CHOL	0.11	-0.39	-0.36
TPROT	0.11	0.15	0.16
ALB	0.15	0.30	0.31
T3	0.31	0.07	0.06
T4	0.29	0.09	0.04
INSULIN	0.42	0.10	0.05
RQUICKIBHB	-0.36	0.13	0.08
CORT	-0.27	0.11	0.09
TNF-α	-0.43	-0.09	-0.13
HAPTO	-0.13	-0.45	-0.41

Figure 1. Correlation of Fe, TIBC and UIBC with blood parameters in cows. Abbreviations: Iron blood serum (Fe); Unsaturated iron-binding capacity (UIBC); Total iron-binding capacity (TIBC); Transferrin saturation percentage (TS%); Red blood cells count (RBC); Reticulocyte count (RET); Hemoglobin (HGB); Mean corpuscular volume (MCV); Hematocrit (HCT); Mean corpuscular hemoglobin (MCH); Mean corpuscular hemoglobin concentration (MCHC); Red blood cell distribution width (RDW); White blood cells (WBC); Neutrophil to lymphocyte ratio (NLR); Platelet (PLT).]

Using the linear relationship and regression allows us to investigate what would happen to the intensity of the deviation of the blood parameters when Fe = 9 µmol/L (extrapolated for clinical Fe deficiency). The length of the bars indicates the magnitude of the impact of Fe deficiency on the blood parameters, and the position of the bars indicates the range of the blood parameters. The blood parameters, from the most sensitive to the least sensitive, respond to Fe deficiency as follows: a) positive deviation: NEFA, TNF-α, NLR, NEFA:CHOL, BHB, GGT, CORT, RQUICKIBHB, AST, TBIL, WBC; b) negative deviation: INS, TGC, TIBC, UIBC, GLU, T4, CHOL, MCV, T3, ALB, TS%, PLT, HCT, HGB, RBC, TPROT, MCH. The results are presented in Figure 2.

Table 1. Iron (Fe) and iron-binding capacity concentration, TS% and hematological parameters in dairy cows during early lactation.

Blood parameters*	Week after calving						*P*
	1	2	3	4	5	6	
Fe µmol/L	17.9 ± 7.7^{a}	18.6 ± 8.12^{a}	22.9 ± 8.41^{b}	24.6 ± 7.83^{b}	25.6 ± 8.25^{b}	26.2 ± 8.91^{b}	< 0.01
TIBC µg/dL	279 ± 10.1^{a}	285 ± 11.1^{a}	303 ± 9.99^{b}	348 ± 10.4^{c}	375 ± 12.3^{d}	382 ± 11.6^{d}	< 0.01
UIBC µg/dL	142 ± 11.3^{a}	155 ± 11.4^{b}	164 ± 12.5^{c}	189 ± 13.1^{d}	192 ± 12.2^{d}	195 ± 10.9^{d}	< 0.01
TS %	33.7 ± 8.01^{a}	36.1 ± 8.76^{a}	39.2 ± 9.03^{a}	39.4 ± 8.78^{a}	38.1 ± 8.54^{a}	38.2 ± 8.14^{a}	NS
RBC × 10^{12}/L	4.86 ± 0.9^{a}	4.72 ± 0.95^{a}	4.75 ± 1.01^{a}	4.9 ± 0.9^{a}	4.99 ± 0.86^{a}	5.25 ± 0.8^{a}	NS
HGB g/L	69.2 ± 10.2^{a}	70.2 ± 10.5^{a}	70.3 ± 10.5^{a}	73.5 ± 10.8^{a}	74.7 ± 10.5^{a}	75.6 ± 10.6^{a}	NS
MCV fL	57.4 ± 1.11^{a}	57.5 ± 1.09^{b}	58.2 ± 1.1^{c}	59.05 ± 0.99^{d}	59.1 ± 0.99^{d}	59.2 ± 1.03^{d}	< 0.01
HCT L/L	27.9 ± 2.1^{a}	28 ± 2.5^{a}	25.5 ± 2.2^{b}	30 ± 2.3^{c}	30.2 ± 2.4^{c}	31.1 ± 2.25^{c}	< 0.01
MCH pg	14.2 ± 0.49^{a}	14.3 ± 0.52^{a}	14.05 ± 0.5^{a}	14.2 ± 0.49^{a}	14.35 ± 0.56^{a}	14.4 ± 0.51^{a}	NS
MCHC g/L	247.3 ± 4.5^{a}	248.1 ± 4.9^{a}	249.2 ± 4.6^{a}	242.5 ± 4.5^{a}	244.7 ± 4.4^{a}	243 ± 4.5^{a}	NS
WBC × 10^{9}/L	8.13 ± 3.5^{a}	7.92 ± 3.2^{a}	7.41 ± 3.1^{a}	8.15 ± 2.9^{a}	6.95 ± 2.7^{a}	7.83 ± 3.1^{a}	NS
NLR n/n	1.07 ± 0.12^{a}	0.95 ± 0.13^{a}	0.81 ± 0.15^{b}	0.75 ± 0.14^{b}	0.64 ± 0.13^{c}	0.58 ± 0.14^{c}	< 0.01
PLT × 10^{9}/L	411 ± 103^{a}	395 ± 120^{a}	450 ± 114^{a}	382 ± 103^{a}	391 ± 112^{a}	463 ± 107^{a}	NS

*Abbreviations: Iron blood serum (Fe); Unsaturated iron-binding capacity (UIBC); Total iron-binding capacity (TIBC); Transferrin saturation percentage (TS%); Red blood cells count (RBC); Reticulocyte count (RET); Hemoglobin (HGB); Mean corpuscular volume (MCV); Hematocrit (HCT); Mean corpuscular hemoglobin (MCH); Mean corpuscular hemoglobin concentration (MCHC); Red blood cell distribution width (RDW); White blood cells (WBC); Neutrophil to lymphocyte ratio (NLR); Platelet (PLT). ^{a,b,c,d}Different superscript mean statistic significant difference between group.

Table 2. Blood biochemical, endocrine and inflammatory parameters in dairy cows during early lactation.

Blood parameters*	Week after calving						*P*
	1	2	3	4	5	6	
NEFA mmol/L	0.79 ± 0.1^{a}	0.74 ± 0.07^{a}	0.66 ± 0.09^{b}	0.57 ± 0.08^{c}	0.51 ± 0.08^{d}	0.42 ± 0.09^{e}	< 0.01
BHB mmol/L	0.92 ± 0.08^{a}	1.02 ± 0.09^{b}	0.9 ± 0.1^{c}	0.81 ± 0.1^{c}	0.73 ± 0.09^{d}	0.66 ± 0.1^{e}	< 0.01
GLU mmol/L	2.91 ± 0.31^{a}	3.31 ± 0.35^{b}	3.58 ± 0.34^{c}	3.6 ± 0.35^{c}	3.68 ± 0.4^{d}	3.71 ± 0.38^{d}	< 0.01
TBIL µmol/L	9.92 ± 3.9^{a}	8.15 ± 4.1^{a}	7.4 ± 3.5^{a}	3.9 ± 2.8^{b}	4.1 ± 3.1^{b}	3.6 ± 2.9^{b}	< 0.01
AST IU/L	97.3 ± 10.8^{a}	92.6 ± 11.2^{a}	89.6 ± 10.2^{b}	85.1 ± 9.3^{b}	87.3 ± 9.8^{b}	84.6 ± 10.1^{b}	< 0.01
GGT IU/L	32.4 ± 6.1^{a}	33.1 ± 5.5^{a}	30.8 ± 6.4^{a}	25.9 ± 5.2^{b}	24.1 ± 6.0^{b}	23.9 ± 5.7^{b}	< 0.01
TGC mmol/L	0.12 ± 0.04^{a}	0.15 ± 0.04^{a}	0.21 ± 0.04^{b}	0.24 ± 0.05^{b}	0.25 ± 0.05^{c}	0.26 ± 0.06^{c}	< 0.01
CHOL mmol/L	2.31 ± 0.52^{a}	2.25 ± 0.54^{a}	2.51 ± 0.49^{a}	2.72 ± 0.57^{b}	2.85 ± 0.55^{b}	3.01 ± 0.55^{b}	< 0.01
NEFA:CHOL	0.34 ± 0.19^{a}	0.33 ± 0.18^{a}	0.26 ± 0.18^{a}	0.21 ± 0.17^{a}	0.18 ± 0.16^{b}	0.17 ± 0.15^{b}	< 0.05
TPROT g/L	59.8 ± 6.1^{a}	62.6 ± 6.3^{a}	63.4 ± 5.9^{a}	63.8 ± 5.9^{a}	64.5 ± 6.3^{a}	64.9 ± 6.5^{a}	NS
ALB g/L	29.5 ± 3.7^{a}	30.8 ± 3.5^{a}	32.5 ± 3.2^{a}	33.3 ± 2.9^{b}	34.5 ± 3.6^{b}	34.5 ± 3.6^{b}	< 0.05
T3 nmol/L	2.19 ± 0.51^{a}	2.25 ± 0.5^{a}	2.21 ± 0.49^{a}	2.36 ± 0.6^{a}	2.39 ± 0.52^{a}	2.56 ± 0.48^{a}	< 0.1
T4 nmol/L	43.7 ± 9.5^{a}	45.9 ± 10.2^{a}	47.1 ± 9.9^{a}	48.2 ± 10.1^{a}	50.1 ± 9.6^{a}	51.9 ± 9.4^{a}	< 0.1
INSULINmU/L	3.6 ± 1.02^{a}	4.1 ± 1.11^{a}	4.9 ± 1.07^{b}	5.2 ± 1.12^{b}	5.7 ± 1.14^{c}	5.9 ± 1.22^{c}	< 0.01
RQUICKIBHB	0.47 ± 0.05^{a}	0.46 ± 0.07^{a}	0.45 ± 0.04^{a}	0.43 ± 0.04^{b}	0.42 ± 0.05^{b}	0.41 ± 0.04^{b}	< 0.01
CORT nmol/L	95.6 ± 25.9^{a}	87.2 ± 30.2^{a}	65.2 ± 28.7^{b}	75.3 ± 31.4^{b}	51.9 ± 29.1^{c}	51.8 ± 33.5^{c}	< 0.01
TNF-α ng/mL	13.22 ± 2.1^{a}	12.51 ± 2.2^{a}	10.23 ± 1.99^{b}	9.52 ± 1.86^{b}	7.43 ± 1.95^{c}	7.21 ± 2.04^{c}	< 0.01
HAPTO g/L	0.75 ± 0.05^{a}	0.67 ± 0.05^{a}	0.52 ± 0.04^{b}	0.56 ± 0.04^{b}	0.41 ± 0.05^{c}	0.43 ± 0.05^{c}	< 0.01

*Abbreviations: Non-esterified fatty acids (NEFA); Beta-hydroxybutyrate (BHB); Glucose (GLU); Total bilirubin (TBIL); Aspartate aminotransferase (AST); Gamma-glutamyl transferase (GGT); Triglycerides (TGC); Cholesterol (CHOL); Total protein (TPROT); Albumin (ALB); Creatinine (CREAT); Triiodothyronine (T3); Thyroxine (T4); insulin (INS); Revised Quantitative Insulin Sensitivity Check Index-BHB (RQUICKI-BHB); cortisol (CORT); haptoglobin (HAPTO). ^{a,b,c,d}Different superscript mean statistic significant difference between group.

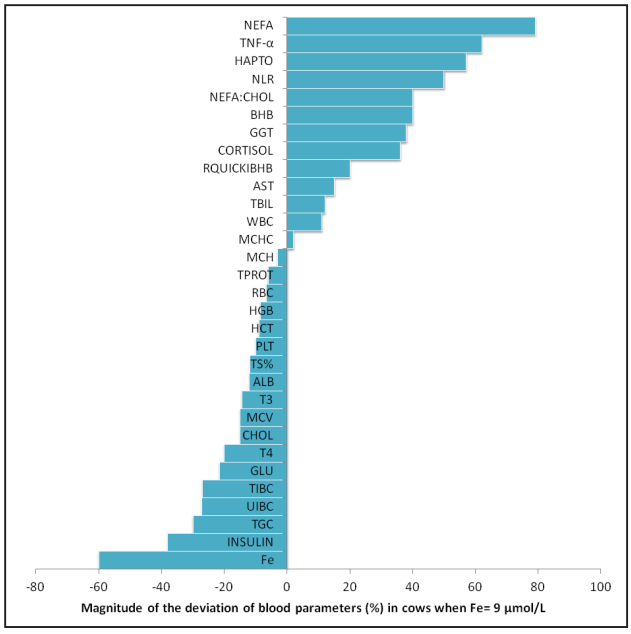

Figure 2. Sensitivity analysis for blood parameters in Fe deficient cows when Fe = 9 µmol/L. Abbreviations: Iron blood serum (Fe); Unsaturated iron-binding capacity (UIBC); Total iron-binding capacity (TIBC); Transferrin saturation percentage (TS%); Red blood cells count (RBC); Reticulocyte count (RET); Hemoglobin (HGB); Mean corpuscular volume (MCV); Hematocrit (HCT); Mean corpuscular hemoglobin (MCH); Mean corpuscular hemoglobin concentration (MCHC); Red blood cell distribution width (RDW); White blood cells (WBC); Neutrophil to lymphocyte ratio (NLR); Platelet (PLT).

DISCUSSION

In previous studies Fe concentrations in cows were 18-36 µmol/L [41], 20-30 µmol/L [3], and suggested reference values ranged from 10 to 29 µmol/L [33]. Our results agreed with previously reported Fe values in cattle. Serum Fe is mainly bound to transferrin, and in routine analysis we determined the capacity of transferrin to bind Fe through three indices: TIBC, UIBC and TS% [21]. The Fe was lower in cows in the 1st weeks after calving, as were TIBC, UIBC, HCT, and MCV. The decrease in Fe, TIBC and UIBC agrees with the results obtained previously [13]. Miltenburg *et al*. [25] also reported that serum Fe and Fe binding values decreased at calving in crossbred Friesian cattle. TIBC and UIBC value and change in our experiment agreed with those determined by other authors [32]. Most Fe in the body is bound to hemoglobin, and additionally the total number of erythrocytes and their volume (MCV) are routine indicators of the status of iron in the body [21]. Calving can have a significant effect on the value of the erythrocyte parameters, so that in early lactation there is a lower value of RBC and MCV compared to the other lactation periods [26,35]. Cows were under metabolic stress which was higher in the 1st 3 week of lactation compared to the 2nd 3 weeks and characterized by: negative energy balance (lower GLU and higher NEFA and BHB), fatty liver (lower TGC, CHOL and higher NEFA:CHOL, TBIL, AST, GGT), homeorhetic endocrine change (lower insulin and thyroid hormone and higher CORT and insulin resistance RQUICK-IBHB index) and inflammation (higher concentration of TNF-α and haptoglobin). Our results are in relation with previous studies which showed this type of change in blood parameters [4,9,18,28,30].

Fe correlates with NEFA and BHB. Fe deficiency can lead to impairment of mitochondrial respiration, resulting in a change of the fatty acid oxidation process, thus potentially contributing to fatty infiltration of liver [17]. Around conception time, Fe deficiency tends to increase plasma and intrahepatic TCG concentrations [8], and Fe deficiency may limit fatty acids uptake [14]. The decrease in Fe in early lactation cows could be a protective mechanism against uncontrolled and intense lipolysis and ketogenesis. In early lactation, acute stress is present, and low Fe concentration attenuates lipolysis stimulated by catecholamines [19]. Fe concentration in cows decreased during inflammation, which has been observed in very severe inflammation such as mastitis and endotoxemia, but also in much weaker ones such as dehorning [39,40]. Fe and NLR show a negative correlation in cows. NLR is increased in early lactation cows due to various inflammatory and stressful processes in the body [34]. TNF-α is a pro-inflammatory cytokine that promotes hypoferremia [12], which are in relation to the negative correlation between these 2 parameters in our experiment. TIBC and UIBC negatively correlated with haptoglobin, due to competitive reverse change between positive (haptoglobin) and negative (transferrin) acute phase proteins production in liver during inflammation [1]. It has been shown that insulin production can be maintained only when Fe concentration is adequate, so Fe deficit or overload will impair insulin secretion [11]. Some previous studies showed that mixed insulin resistance occurred in Fe deficient animals [36], which in our experiment obviously depended on the physiological status of the individual. Fe and stress mediator CORT was in negative correlation which is in agreement with previous finding described that stress decrease Fe concentration [33]. Decrease in Fe means decrease in T3 and T4 hormone. Relation

between Fe and thyroid function was found in pregnant women with iron deficiency anemia [42].

Lipids have been found to accumulate in the liver of Fe-deficient adults [2]. Altered TIBC was found in hepatic cholestasis during pregnancy, as well as in liver cirrhosis, where negative correlation of TIBC with TBIL was observed [5], which was also the case in our experiment. The diagnosis of Fe deficiency is very complex when functional status of the liver is altered because Fe deficiency leads to changes in the functioning of the liver, but also liver diseases lead to changes in the concentration of Fe and its functional parameters [15]. The decrease in TIBC found in cows is an unexpected finding, because in Fe deficiency values of UIBC and TIBC should increase. In cows in the periparturient period, changes in TIBC and UIBC were not following the dynamics of Fe status [6,23], which was also noted in this study. In review by Fertrin [10], some considerations were made about TIBC. TIBC measures the total amount of Fe that can be bound by transferrin in the blood, and transferrin is produced by the liver. Inflammation decreases the production of negative acute phase proteins by the liver and thus decreases transferrin concentration in the blood [1].

Our results showed that metabolic and inflammatory parameters had higher magnitude of change when Fe was extrapolated on deficit level (9 μmol/L), compared to TIBC, UIBC, TS% and red blood cell parameters as functional indicators of Fe status. Decrease in Fe did not result in dramatic changes in erythrocyte parameters. Some studies suggest that there is a difference between functional Fe deficiency and anemia due to Fe deficiency [20,38], and in cows Fe deficiency in early lactation is obviously functional.

CONCLUSIONS

Fe, TIBC and UIBC showed significant change in the 1st 6 weeks of lactation. Correlation analyzes showed that the decrease in Fe in cows could be related to general homeorhetic adaptation to the onset of lactation, since Fe correlates with indicators of lipolysis, ketogenesis, and endocrine parameters. The lower TIBC and UIBC observed in cows in the 1st 3 weeks when Fe was lower is an unexpected finding, since Fe decrease usually increases TIBC and UIBC. TIBC correlates with hepatocytes indices of the functional status and lipid infiltration, so it is possible that TIBC decreases because of changes in lipid metabolism in the liver. Blood Fe, TIBC and UIBC can be useful indicators for assessing metabolic stress in early lactating cows, and both parameters should be used together as they are related to different aspects of homeorhesis.

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