

ANALYSIS OF SELECTED HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF BLOOD OF LIMOUSIN CALVES IN THE EARLY NEONATAL PERIOD

Radosław Drozd, Anna Kirdzik, Dorota Jankowiak

West Pomeranian University of Technology, Szczecin, Poland

Abstract. The study involved a group of the Limousin breed calves to check changes in selected hematological and biochemical indices of blood as the number of red blood cells (RBC), hematocrit index (HCT), hemoglobin concentration (Hb) parameters, the total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC), concentrations of Fe and antioxidant status in the first ten days of neonatal period. In the analyzed period was found in blood of heifers increase of RBC value compared to the bulls group. The Hb and HCT indices were reduced systematically in time, regardless of gender. The values of TIBC increased steadily, while the degree of saturation of transferrin by iron (TS%) was reduced with decreasing of the plasma Fe concentration. It was also noticed downward trend in plasma antioxidant capacity value of studied calves. The observed changes in analyzed parameters of blood indicate on increased requirements of Limousin claves for iron supply in first days of life.

Key words: calves, iron, FRAP, antioxidant

INTRODUCTION

Birth and related with this change the intrauterine environment on completely different conditions is a huge challenge for newborn, requiring a series of anatomical and functional changes. In the breeding of cattle an important problem is

Corresponding author: Radosław Drozd, Department of Immunology, Microbiology and Physiological Chemistry, West Pomeranian University of Technology, Szczecin, al. Piastów 45, 70-311 Szczecin, Poland, e-mail: rdrozd@zut.edu.pl

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subclinical anemia [Mohri et al. 2010]. Its occurrence is associated with the rapid development and height daily body weight gains, especially in beef calves.

Delivered with a colostrum and milk the iron amount may not always be able to meet the demand of the rapidly developing the calves body. In adult ruminants iron is taken from the plant food that usually covers the whole of the animal body requirements for this microelement [Micek 2003]. However, in suckling ruminants with not fully developed digestive system, the recovery of this element from milk is significantly lower [Szymańska 2000, Kuleta 2005]. In addition, basic food of calves, milk is characterized by relatively low content of Fe. The concentration of Fe in cows milk varied based on the breed, age and lactation period. On the other hand, the regiments of calves body to iron also depends on the breed of cattle [Mohri et al. 2004, Tsioulcas et al. 2007, Mohri et al. 2010, Zarcula et al. 2010, Rocha et al. 2014]. Therefore, it is important to properly balanced diet of calves, depending on their age and breed [Kuleta 2005]. The consequence of the Fe deficiency are not only changes in hematological blood parameters but also changes in plasma antioxidant status [Nagababu et al. 2008]. The aim of this study was analysis iron status in plasma blood and changes in values of selected hematological, biochemical indicators and antioxidant status in the blood of Limousine breed calves in the early neonatal period.

MATERIAL AND METHODS

Animals and housing

The study was conducted on thirteen calves (seven heifers and six bulls) the Limousine breed in the first ten days of life. The study group consisted of animals born on three consecutive days. Calves with their mothers were kept at the uninsulated loose housing in cowshed with deep straw bedding. Cows had free access to food and water (automatic waterer). After the birth, the calves remain with their mothers for a period of 3 weeks. The research was conducted on the beginning the spring.

Blood hematological and biochemical parameters assay

Blood samples for analysis were collected from the external jugular vein, every day between 9:00 and 10:30 am. Blood for analysis were collected in heparinized tubes then samples were centrifuged and the resulting plasma was frozen and stored at -20° C until the execution of laboratory analysis.

In the whole blood samples was determined hematocrit (HCT), number of red blood cells (RBC), hemoglobin concentration (Hb). The assay was performed using a hematology analyzer Sysmex F800.

Iron concentration in plasma were determined using a diagnostic kit from Hydrex company.

Total iron binding capacity assay was performed with using diagnostic kit from BioMaxima company. The assays were conducted in accordance with the instructions provided by the manufacturers.

Unsaturated iron binding capacity (UIBC) was calculated from difference between assayed TIBC and Fe concentration.

The percent transferrin saturation (TS%) was calculated using the formula:

$$TS\% = \frac{Fe \ \mu mol \cdot l^{-1}}{TIBC \ \mu mol \cdot l^{-1}} \times 100\%$$

Ferric Reducing Ability of Plasma (FRAP) [Benzie and Strain 1996] method were used for plasma antioxidants potential assay. Briefly 10 ul plasma sample was transferred to 300 μ l preincubated to 37°C in microplate, FRAP reagent and mixed well. After 6 minutes absorbance was read at 593 nm (microplate reader TECAN m 200 pro). For assay calibration was used water solution of ferrous sulfate heptahydrate in range from 0.1 mM to 1 mM. Antioxidant status were expressed as micromole of Fe²⁺ equivalent.

Statistical calculations were performed using the package STATISTICA PL 9.0. Normality was tested using the Kolmogorov-Smirnov test. The homogeneity of variance was checked with using Levene's test. The data sets with no normality were transformed by Box-Cox transformation [Box and Cox 1964]. The evaluation of the significance of differences between group were made by repeated measures analysis of variance (rANOVA) and Tukey's post-hoc test. Differences were considered as significant at the levels of $P \le 0.05$, $P \le 0.01$. All data are expressed as an arithmetic mean \pm standard deviation (SD)

RESULTS

In the analyzed period of age calves number of red blood cells in heifers was in the range of from $7.46 \times 10^{12} \cdot l^{-1}$ to $9.24 \times 10^{12} \cdot l^{-1}$. The highest RBC value was recorded in the third day and the lowest in the fifth day of the heifers life (Table 1). In the group of bulls, the RBC values in the analyzed period were in range from $7.71 \times 10^{12} \cdot l^{-1}$ at the first day to the $6.57 \times 10^{12} \cdot l^{-1}$ at fourth day. In the whole analyzed period, value of the RBC was higher in heifers than in bulls (P ≤ 0.05).

Tabela 1. Po	równani	e wsk	aźników	hematolo	gicznych	RBC, HC	T, Hb ora	z stężenia	żelaza w	krwi bada	anych jałó	wek (♀) i bı	ıhajków (\Im)
Parameter	Sex						Day of life	 Dzień życ 	ia				Total
Parametr	Płeć		1	2	3	4	5	9	7	8	6	10	Ogółem
	С	x	9.21	8.72	9.24	7.52	7.46	8.33	7.68	8.39	8.59	8.23	8.34^{a}
)+	SD	1.97	3.07	1.02	1.21	1.20	2.09	1.63	1.59	1.90	1.37	0.64
1-1-2 Jol 2 Jol	K	X	7.72	7.31	6.61	6.57	6.73	6.95	7.97	6.70	6.88	7.21	$7.07^{\rm b}$
KDC, 10 '1	0	SD	1.02	0.66	0.55	0.25	0.49	0.79	2.07	0.72	0.86	0.96	0.48
	Total	X	8.51	7.84	8.12	7.23	7.22	7.48	7.28	7.59	8.05	7.69	I
	Ogółem	SD	1.25	1.48	1.65	1.22	1.16	1.26	1.16	1.38	2.47	1.30	I
	С	×	0.38	0.34	0.35	0.29	0.28	0.32	0.28	0.30	0.31	0.29	0.31
)+	SD	0.08	0.14	0.05	0.06	0.05	0.09	0.06	0.08	0.08	0.05	0.03
пСТ 1 .1-l	Б	×	0.30	0.27	0.25	0.25	0.25	0.26	0.30	0.25	0.25	0.26	0.26
пст, г.т	0	SD	0.04	0.01	0.02	0.01	0.02	0.04	0.07	0.02	0.02	0.04	0.02
	Total	X	0.34^{Aa}	0.30	0.31	0.28^{B}	0.27^{B}	0.29	0.29	0.27^{B}	0.29^{b}	0.28^{b}	Ι
	Ogółem	SD	0.07	0.06	0.07	0.05	0.06	0.06	0.06	0.06	0.11	0.06	I
	С	x	7.23	7.06	6.84	6.11	5.97	6.26	6.06	5.99	6.14	6.11	6.38
	+	SD	1.24	2.32	1.43	1.36	1.41	1.49	1.46	1.49	1.49	1.38	0.48
III. Louna dII	K	X	6.10	5.84	5.37	5.28	5.06	5.34	5.22	5.03	5.05	5.19	5.35
по, шши - т	0	SD	0.53	0.31	0.52	0.43	0.25	0.50	0.41	0.46	0.62	0.36	0.35
	Total	X	6.91^{Λ}	6.29^{AC}	6.20^{a}	5.80^{BC}	5.62^{BC}	5.81^{BC}	5.63^{BC}	5.52^{Bb}	5.84^{BC}	5.72^{BC}	Ι
	Ogółem	SD	1.07	1.01	1.19	1.10	1.22	1.10	1.19	1.42	2.00	1.19	I
	С	×	20.31	19.14	22.44	22.12	15.42	12.24	17.49	16.06	21.85	15.51	18.26
	+	SD	10.57	11.36	14.18	6.44	4.98	2.71	6.56	7.69	15.70	6.11	3.46
\mathbf{E}_{0} \mathbf{u}	Б	×	18.26	19.88	20.91	18.31	27.64	25.37	25.09	18.36	22.10	11.69	20.76
1.C, Juliot 1	C	SD	5.10	5.72	7.45	8.30	15.91	11.98	10.65	12.25	14.96	4.45	4.60
	Total	×	20.97	18.40	24.39^{A}	20.86	21.91	18.49	23.30^{A}	18.10	25.47^{a}	12.82^{Bb}	I
	Ogółem	SD	8.05	9.63	11.45	7.17	12.02	10.34	7.99	10.01	15.81	5.55	Ι
SD – standard c	eviation; 4	\ , B − d	lifferent sul	perscripts de	enote statistic	cally signific	cant differer	nces (P - val	ue ≤ 0.01);	a, b – differe	ent superscrif	ots denote statis	stically
significant diffe	rences (P -	- value	≤ 0.05).	;	•			4					i a a
SD – odchyleni	e standarde	owe; A,	B – różne	litery zazna	czają różnice	e statystyczn	lie istotne (I	2 ≤ 0,01); a,	b – różne li	tery zaznacz	ają różnice s	tatystycznie ist	othe ($P \le 0,05$).

Table 1. The comparison of the hematological parameters of whole blood RBC, HCT, Hb and Fe concentration in serum of studied calves – heifers (\Im) and calves – bulls (\Im)

Acta Sci. Pol.

46

The hematocrit indices in heifers was in the range of $0.27 \ l \cdot l^{-1}$ to $0.37 \ l \cdot l^{-1}$ while in bulls group was in range of $0.30 \ l \cdot l^{-1}$ to $0.25 \ l \cdot l^{-1}$. There was no gender related significant difference in the value of this parameter. However was indicated significant lowering of HCT in both group especially in the first five days of the animals life.

The hemoglobin concentration for bulls (6.10 mmol $\cdot l^{-1}$) and heifers (7.23 mmol $\cdot l^{-1}$) was the highest in the first day life. Intensive decreasing in Hb concentration was observed in both sexes in the first four days the newborns life. After this time, hemoglobin concentration in heifers and bulls remained on stable level up to the end of experiment. Hb content in the analyzed period was higher in heifers, compared to bulls, however without confirmed statistical significance.

The plasma iron content in both sexes fluctuated widely in the range of 12.23 μ mol $\cdot l^{-1}$ on the sixth day to 22.22 μ mol $\cdot l^{-1}$ in the third day in heifers and from 11.70 μ mol $\cdot l^{-1}$ in the tenth day of age to 27.64 in the fifth day in bull.

To the third day TIBC level in heifers were stable and varied within the range of 58.10 μ mol \cdot l⁻¹ and 64.22 μ mol \cdot l⁻¹ (Table 2). On the fourth day value of this indices increase to 75.85 μ mol \cdot l⁻¹ and continues to grow until the end of the analysis period, reaching in tenth day level 81.41 μ mol \cdot l⁻¹. A similar trend was observed in bulls but increase in the value of TIBC was recorded in the third day and the upward trend continued until the end of the studied period. TIBC values in the analyzed period were similar in both bulls and heifers.

The course of changes in the value of UIBC for both heifers and bulls was identical to the trend of changes in the value of TIBC. On the first day UIBC in heifers ranged average 37.80 μ mol \cdot l⁻¹, while for bulls 40.80 μ mol \cdot l⁻¹. In the tenth day, UIBC level significantly increased up to 65.90 μ mol \cdot l⁻¹ in heifers and up to 82.20 μ mol \cdot l⁻¹ for bulls. Between the sexes, there were no significant differences in the UIBC.

Similar to the Fe content, transferrin saturation degree in the reporting period was highly variable in both sexes analyzed. In heifers this parameter was in the range of 16,04% to 37,76% in bulls 11.9% to 47.0%. However level of the TS (%) in animals plasma systematically decreased over the experimental period. The value of antioxidant status was shaped in the range of 49.00 μ mol \cdot l⁻¹ in the sixth day to 74.00 μ mol \cdot l⁻¹ in the first day for heifers and bulls in the range of 45.0 μ mol \cdot l⁻¹ on the seventh day to 79.70 μ mol \cdot l⁻¹ on first day. In comparison to first day, significant decrease (P \leq 0.01) in plasma FRAP value was observed in the 4, 5, 7th and on the eighth day of newborns life. This difference was especially visible in heifers group.

47

Tabela 2. Po tra	równani nsferazy	ie waı / i war	tości całk tości FRAJ	owitej zdc P w krwi a	olności c malizowa	lo wiązani mych jałóv	a żelaza, vek (⊋) i ł	utajonej puhajków	zdolności (ح) (ح)	do wiązaı	nia żelaza,	stopnia v	vysycenia
Parametr	Sex						Day of life -	Dzień życia					Total
Parametr	Płeć	I	-	2	3	4	5	9	7	8	6	10	Ogółem
	C	×	61.09	72.49	72.47	85.01	82.73	91.67	99.64	90.04	89.89	95.25	84.03
)+	SD	15.36	31.74	28.07	27.73	37.82	29.23	31.50	32.17	44.32	26.74	11.99
	K	X	58.13	64.92	62.27	75.71	74.43	77.00	77.55	76.33	75.77	81.41	72.35
11BC, µmol · 1	o	SD	9.18	7.79	9.83	13.72	9.56	11.04	11.66	13.40	16.45	13.50	7.70
	Total	X	59.49	68.41	66.98	80.00	78.26	83.77	87.75	82.66	82.29	87.80	I
	Ogółen	1 SD	11.95	21.58	20.11	20.92	25.69	21.79	24.76	23.91	31.74	20.99	I
	C	×	58.13	64.92	62.27	75.71	74.43	77.00	77.55	76.33	75.77	81.41	60.99
)+	SD	9.18	7.79	9.83	13.72	9.56	11.04	11.66	13.40	16.45	13.50	12.57
	5	X	36.37	46.53	39.32	55.04	58.50	65.28	57.76	60.78	53.18	67.35	54.01
	0	SD	7.14	14.24	17.74	12.73	8.00	9.09	14.00	8.46	23.99	12.58	10.38
	Total	X	38.60^{Aa}	50.01^{A}	42.59^{A}	59.14^{b}	56.35^{a}	65.28^{BC}	63.98^{BC}	64.56^{BC}	56.82 ^a	74.98^{Bb}	Ι
	Ogółen	1 SD	12.54	22.95	26.63	19.71	23.29	16.83	23.70	18.61	31.54	19.91	I
	С	×	35.60	29.67	45.01	26.42	37.36	30.16	32.01	23.38	36.50	11.89	30.80
)+	SD	19.13	16.20	30.71	9.22	20.45	12.83	16.97	10.05	30.00	3.33	9.04
TC 0/	K	X	36.65	28.67	38.55	27.46	21.38	15.15	25.30	19.60	20.07	17.21	25.00
15, %0	O	SD	13.00	19.38	27.13	8.98	5.20	2.49	9.62	8.32	8.51	8.02	7.91
	Total	X	36.17^{Aa}	29.13^{a}	41.53^{B}	26.98^{B}	28.75 ^B	22.08	28.40^{B}	21.34	27.65^{a}	14.75 ^{Aa}	I
	Ogółen	1 SD	15.40	17.24	27.79	8.72	16.02	11.50	13.36	8.98	22.00	6.66	I
	С	x	84.04	67.95	64.62°	48.76	55.27	69.76	43.62	45.94	52.34	77.14	60.94
)+	SD	11.84	22.86	15.32	6.86	9.39	9.90	9.54	15.08	16.75	8.12	18.88
ED A D	5	×	79.70A	67.97	59.37	66.73	58.63	49.03	53.52	65.88	70.92b	62.47	63.42
L'INAT, JUIIU ' I	С	SD	18.21	11.82	15.36	14.54	17.91	12.58	26.49	31.24	16.84	19.17	21.23
	Total	X	82.04^{Aade}	67.96 ^{adc}	62.20	57.05 ^{bcf}	56.82 ^{bef}	60.19	48.19^{Bbe}	55.14 ^B	60.92	70.37 ^{dfc}	I
	Ogółen	1 SD	15.89	19.36	16.20	14.84	14.66	15.88	20.74	26.98	19.96	16.74	Ι
SD – standard d	eviation; ,	A, B – (lifferent supe	rscripts deno	te statistica	lly significan	t differences	(P − value ≤	0.01); a, b –	different sup	erscripts den	ote statistical	[y
significant diffe	rences (P	– value	≤ 0.05).										
SD – odchyleni	e standard	lowe; A	B – różne lit	tery zaznacza	ıją różnice :	statystycznie	istothe ($P \le 0$),01); a, b –	różne litery z	zaznaczają ro	óżnice statyst	ycznie istotne	$(P \le 0,05).$

The comparison of the total iron binding capacity, unsaturated iron binding capacity, transferase saturation and FRAP values in blood of the studied calves – heifers (\bigcirc) and calves – bulls (\heartsuit) Table 2.

R. Drozd et al.

DISCUSSION

The first days after birth is a period of intensive changes that reflected the values of hematological indices related to the adaptation of the body to the external environment. In the experimental calves regardless of gender, examined hematological indicators as Hb and HCT, were within the range of reference values [Winnicka 2008], while the content of red blood cells was higher than the upper range of those standards. The observed initial increase in the RBC may be the result of polycythemia, caused by hypoxia, which may take place in the last days of fetal life and affecting the induction of intensified erythropoiesis [Proytcheva 2009]. RBC similar values marked Muri et al. [2005] in the blood of calves Simmental breed, additionally noting the decreasing trend in the number of red blood cells over time. In the experimental calves especially in heifers also a marked downward trend in RBC, occurred in the studied period. This may be the result of increased plasma volume, due to the higher concentration of albumin. It can finally affect the plasma oncotic pressure and retain water in the blood vessels [Mohri et al. 2007]. The decrease in the RBC may also be the result of periodical lowering in the erythropoietin level, a hormone responsible for stimulation of the erythropoiesis in the bone marrow [Kuleta 2005]. With the change in the number of red blood cells in the analyzed calves was observed changes in the value HCT. Lowering the value of this index with the age of the calves was reported earlier for calves Holstein-Friesian breed [Rauprich et al. 2000] or in Polish Red-and-White cattle [Traczykowski 1997].

Reduction the value of HCT in addition to increased plasma volume is also related to the size of red blood cells [Adeli et al. 2013]. Erythrocytes of newborns are bigger than in adults. This affects the initial increase in the value of HCT, which with age of the animal is reduced due to decrease the size of these cells [Mohri et al. 2007]. With the decrease in the value of RBC and HCT undergoes also reduced hemoglobin content in both sexes analyzed calves. In the initial stage of life in Polish Black-and-White and Polish Red-and-White calves also was reported a decrease in Hb value [Albrycht et al. 1995, Traczykowski 1997]. However, in the examined calves, despite the decline in Hb, we can not talk about reduced its contents because it was located in the range of reference values for the adult cattle (4.96–8.69 mmol $\cdot l^{-1}$) [Winnicka 2008]. One of the relevant molecular components of hemoglobin are the iron ions. In the analyzed calves content of this element fluctuate strongly and often was at level lower than the reference values (21.50–35.80 μ mol · l⁻¹) [Winnicka 2008]. Reduced Fe content in plasma of calves in the first days of life in Holstein breed also found Muri et al. [2005]. In many earlier studies over the content of Fe in calves of dairy primiparous cows it found that 30% of the calves present serum deficient iron levels. It was explained by influence of reduced bearing transfer in fetal life and accumulation in the liver and spleen [Kume and Tanabe 1993]. The iron pool in the plasma is only a small part of this trace element in the body and the self determination of the content of this element is not a sufficient parameter to conclude that deficiency of this element. Furthermore the Fe concentration in plasma is subject to changes related to the times of the day [Tomaszewski 1997].

In the conditions of Fe deficiency increases the concentration of plasma proteins transporting it to allow enabling mobilization of the trace element out of the reticuloendothelial system, liver and spleen [Blum and Zuber 1975]. In the moment of the exhaustion of these resources is really noticeable reduction of Fe content in plasma and disruption of its transportation to the bone marrow [Naigamwalla et al. 2012]. In the experimental calves was observed an increase TIBC values, which reflects the increasing concentration of transferrin and rise in demand of calves body for iron. Mohri et al. 2004 also showed an increase in the TIBC in the first two weeks of calves life. With the increase in total iron binding capacity levels, has demonstrated changes in the value of the TS% signaling the increasing demand for iron, with declining resources of this element. However, the TS% values indicating the iron deficiency, which is $\leq 15\%$, was observed only on the ninth day of the calves life [Kaneko 1989].

Imbalance of iron homeostasis in the plasma can also affect the value of its antioxidant status. In humans, it was found reduction of the FRAP plasma in both women and men suffering from anemia caused by the iron deficiency [Madhikarmi and Murthy 2014]. However, RBC and Hb values that are above or within the reference range in the analyzed period, testify to the absence of symptoms of anemia in calves. Gaál et al. [2006] at the first days of life in blood Holstein-Friesian breed of calves reported an elevated plasma FRAP value, which was decreased in subsequent days. Therefore, they also showed an increased amount of oxidant in the plasma neonates compare to adults heifers. In the plasma of the analyzed calves, particularly in the first days was visible the downward trend in the antioxidant status value. In human newborn was established an occurrence significant unbalance between oxidant and antioxidant in their blood [Wiedemann et al. 2003]. After birth the neonates are under high environmental pressure by elevated oxygen availability, that can result in oxidative stress [Mutinati et al. 2014]. Moreover the rapid development of the newborn body is also strongly associated with the high rate of physiological changes and can also elicits the redox imbalance [Inanami et al. 1999 Lykkesfeldt and Svendsen 2007]. Iron ions are essential components of prosthetic groups present in the structure of the respiratory pathway enzymes and antioxidant enzymes as catalase. Insufficient amount of Fe may therefore affect the activity of enzymatic pathways, which are essential in defense responses to oxidative stress, finally potentiate the effects of oxidative stress in the body [Nagababu et al. 2008].

A decrease in antioxidant capacity in the analyzed calves is probably associated with the depletion of accumulated in fetal life antioxidants resources on which indirectly may affect the reduced availability of iron.

CONCLUSION

The period after the birth of the calves is a time in which they are particularly vulnerable to any disorder of metabolism associated with a deficiency of micronutrients and other nutrients. In the analyzed calves were observed typical for cattle decrease in serum iron concentration in the neonatal period. The reason you might be twofold from reduced its supply, along with mother's milk as well as the immaturity of metabolic pathways responsible for maintaining the homeostasis of micro-nutrient in the body of the animal.

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ANALIZA WYBRANYCH WSKAŹNIKÓW HEMATOLOGICZNYCH I BIOCHEMICZNYCH W KRWI CIELĄT RASY LIMOUSINE WE WCZESNYM OKRESIE NEONATALNYM

Streszczenie. Badaniem objęto grupę cieląt bydła rasy limousine w pierwszych dziesięciu dniach okresu noworodkowego. U analizowanych zwierząt prześledzono zmiany wybranych wskaźników hematologicznych i biochemicznych krwi takich jak liczba czerwonych krwinek (erytrocytów), wskaźnik hematokrytu (HCT), stężenia hemoglobiny (Hb), całkowitą zdolność wiązania żelaza (TIBC), utajaną zdolność wiązania żelaza (UIBC), stężenie Fe i wartość potencjału antyoksydacyjnego. W analizowanym okresie stwierdzono w krwi jałówek zwiększoną wartość RBC niż u buhajków. Natomiast wartości HCT i Hb ulegały systematycznemu obniżeniu w czasie, niezależnie od płci. Wartość TIBC wzrastała, przy jednoczesnej redukcji stopnia nasycenia transferryny żelazem (TS%). Natomiast stężenie żelaza u analizowanych cieląt oscylowało blisko dolnych zakresów wartości referencyjnych. W osoczu analizowanych zwierząt w analizowanym okresie, odnotowano również tendencję spadkową wartości potencjału antyoksydacyjnego. Obserwowane zmiany analizowanych parametrów krwi wskazują na zwiększone wymagania cieląt rasy limousine dla podaży żelaza w pierwszych dniach życia.

Słowa kluczowe: cielęta, żelazo, FRAP, antyoksydanty

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