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PARAMETERS OF PROTEIN AND IRON METABOLISM IN DAIRY COWS DURING PERIPARTURIENT PERIOD

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ABSTRACT

Knowledge of the changes in the concentrations of biochemical parameters gives the possibility to conclude about the health status of the cows, the quality of nutrition, or reproductive performance and milk productivity. The studies aimed to assess the health status of the primiparous dairy cows at the time of prospectus new fertilization and to screen the challenges of the preceding periparturient period through the analysis of protein metabolism and iron parameters in context of their potential role as markers of the reproductive status. Despite significant variations, the studies showed that homeostatic mechanisms and adaptational processes in cows organism are rather efficient during this period. Nevertheless, it should be underlined that at the time of prospectus new pregnancy higher concentration of urea, lower concentration of creatinine and iron parameters was noted, emphasizing the dysregulation of the organism condition and pointing the possible reason of/association with prospectus difficulties in further reproductive performance. If analyzed for the particular herd, these parameters may be useful in considerations about the selection of the time for new fertilization.

Key words: cows, periparturient period, blood plasma, protein, iron

INTRODUCTION

Increased milk yields and intensified reproductive performance are the key factors affecting profitability of breeding. However, if overdone, both increase the risk of homeostasis disruption and health problems. The course of periparturient period is crucial in this context, as the tremendous changes in the function of the organism are observed. At the end of pregnancy and the beginning of lactation an increase in maternal requirements for all nutrients is observed, with even higher demands at peaking lactation. The challenges are also associated with the regeneration of the reproductive system after pregnancy and preparation of the body for a new conception [Taylor et al. 2003, Nogalski and Górak 2008].

There are numerous biochemical indices which may be easily determined and reflect the health status of the herd, thus pointing the direction of changes in nutrition and rearing or showing probable health problems. The analysis of changes in selected indices may especially help in the selection of appropriate time for subsequent parturition and further success in breeding.

Knowledge of the changes in the concentrations of these components (e.g. urea, albumin), gives the possibility to conclude about the health of the cows, the quality of nutrition, or reproductive performance and milk productivity [Al-Mujalli 2008, Szeląg-Gruszka and Skrzypek 2009].

Many protein-related parameters are highly affected around parturition and may significantly impose to further reproductive performance [Kurpińska et al. 2015, Kurpińska et al. 2016]. Gonzales and Rocha [1998] reported that higher levels of urea and lower levels of protein are noted in cows with prolonged inter-pregnant period and Jorritsma et al. [2003] underlined that urea may impact the oocyte development, fertilization and embryo development. Cows with higher plasma concentrations of urea and lower creatinine concentrations were at risk of delayed resumption of postpartum ovarian cyclicity [Damptey et al. 2014].

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Parturition may significantly affect the iron concentration in blood. Due to the fact that iron may decrease in immunological challenges [Yatoo et al. 2013] and appropriate levels are necessary for ovarian activity [Qian et al. 2001] it would be highly recommended to monitor whether the imbalances in iron status occur.

Having in mind that parameters of protein metabolism [Jorritsma et al. 2003, Tóthová et al. 2008] are good markers for the assessment of health status and that iron status is very important for reproductive performance [Kumar et al. 2011] the studies aimed to assess the health status of the primiparous dairy cows at the time of prospectus new fertilization and to screen the challenges of the preceding periparturient period. Thus, the hypothesis was that the parameters of iron and protein metabolism may be valuable indicators of status and readiness for further reproductive performance, which might be easily compared to the reference values.

MATERIAL AND METHODS

The research was performed in 10 primiparous cows of Polish Holstein–Friesian Black-and-White variety (95%–100% HF, half-sisters, clinically healthy) in the last month of pregnancy and the first 2 months of lactation. Sampling points included: 30, 14, and 7 days before parturition and 1, 7, 14, 30, and 60 days after calving every time at 9.00 am. Animals were fed according to TMR system of feeding (INRA, 2007. Alimentation des Bovins, Ovins et Caprins, Besoins des Animaux – Valeurs des Aliments, Tables INRA. Editions Quae Paris, France 2007) and had free access to water. Blood was drawn from the external jugular vein into sterile tubes with anticoagulant (EDTA K3 – 1.6 mg/ml of blood) or heparine (0.5–2.0 IU/ml of blood) depending on the analytical procedures. Blood samples were centrifuged (3000 rpm, 4°C). Plasma samples were stored at –80°C until use. The local ethics committee for experiments on animals in Szczecin approved the design of the experiment (resolution no.: 22/2009, 10.07.2009).

In blood plasma the following parameters were determined: Total protein concentration – with the aid of Bradford method (Protein standard Assay, Bio-Rad); Albumin concentration – end-point method, bromocrezol green method (BioMaxima); Urea concentration – kinetic, enzymatic method with urease and glutamate dehydrogenase (BioMaxima); Creatinine concentration – Jaffe kinetic method, without deproteination (BioMaxima); Iron concentration – direct determination with ferene tiazine (BioMaxima); Total Iron Binding Capacity (TIBC) – by the magnesium carbonate method (reagent kit for determining total iron binding capacity, BioMaxima); Unsaturated Iron Binding Capacity (UIBC) – calculated according to the formula: TIBC – iron concentration; Transferrin saturation – calculated according to the formula: (Iron concentration/TIBIC) × 100.

The results are presented in figures as mean value with SD. Statistical analysis was performed with the aid of ANOVA with repeated measurements with posthoc Tukey's test (Statistica 10.0 software)

RESULTS AND DISCUSSION

The concentration of total protein in the blood is an indicator of the intensity of the nitrogen metabolism and may be an indirect indicator of animal nutritional status [Kupczyński and Chudoba-Drozdowska 2002, Lepczyński et al. 2011]. The concentration of total protein in blood plasma of the examined cows (Table, Fig. 1a) ranged within the reference standards for cattle [Winnicka 2015]. A slight reduction was noted in the last two weeks of pregnancy (the difference was not statistically significant) and a statistically significant increase in the concentration in the first two months of lactation ($P \leq 0.01$). It has been shown that in the last 14 weeks before calving, the concentration of total protein in blood serum of cows increases, reaching a maximum value of about 4 weeks before calving [Larson and Kendall 1957]. According to Mir et al. [2008], a high concentration of protein in late pregnancy is associated with the release of regulatory proteins modulating the function of the body of the pregnant cows and the developing fetus. Milinković-Tur et al. [2005] reported, that increased secretion of sex hormones (in mares), which stimulate the secretion of thyroxine and glucocorticoids contribute to the severity of metabolic processes, e.g. increased mobilization of amino acids from tissues and their increased transport to liver. Tóthová et al. [2008] suggested that lower concentration of the total protein in the final days before calving may be associated with decreased absorption, unbalanced nutrition, liver and kidneys dysfunction, as well as the transfer of gamma globulin and albumin to the mammary gland. According to Bell et al. [2000] this phenomenon may also be related to the fact that a large amount of amino acids is used for the synthesis of glucose in the liver. The lower concentration of total protein in the plasma/serum of cows may be maintained even in the first few days after parturition, and then increases up to about week 10 of lactation [McAdam and O'Dell 1982, Dolezel et al. 1991, Piccione et al. 2012]. Lebeda and Bus [1985] suggested that an increased concentration of total protein during lactation may be associated with increased protein synthesis, and better utilization of the protein from fodder.

Albumins represent approximately 60% of the total proteins in the plasma (above 30 $g \cdot l^{-1}$) and they are responsible for maintaining oncotic pressure and the transport of fatty acids, free bilirubin and some non-steroid hormones. Albumins are also the "negative" proteins of

Table 1. The level of analyzed biochemical parameters in blood plasma primiparous cows in the last month of pregnancy and the first two months of lactation (mean values and standard deviations, SD; D – days, asterisk * indicates time points before parturition, P)

Tabela 1. Koncentracja analizowanych wskaźników biochemicznych w osoczu krwi krów pierwiastek w ostatnim miesiącu ciąży i pierwszych dwóch miesiącach laktacji (wartości średnie i odchylenie standardowe SD; D – dzień, * gwiazdką oznaczono dni przed porodem P)

		Prepartum Przed porodem			Postpartum Po porodzie				
		30D*	14D*	7D*	P	7D	14D	30D	60D
Total protein, g · l^{-1} Białko całkowite, g · l^{-1}	x̄	62.49	64.24	61.55	60.63	63.51	64.97	68.09	67.37
	SD	2.76	7.58	10.72	7.22	5.98	6.56	5.24	4.45
Urea, mmol · l^{-1} Mocznik, mmol · l^{-1}	x̄	4.07	4.86	3.95	4.43	4,38	4.54	5.36	5.89
	SD	0.92	0.48	0.67	1.00	0.65	0.85	1.50	1.13
Albumin, g · l^{-1} Albuminy, g · l^{-1}	x̄	36.45	35.55	34.13	34.07	33.95	32.39	33.98	34.43
	SD	1.38	2.08	3.72	2.50	2.49	2.45	2.01	2.19
Creatinine , µmol · l^{-1} Kreatynina, µmol · l^{-1}	x̄	122.70	118.00	122.60	113.40	108.20	101.80	90.26	87.88
	SD	12.87	18.93	17.65	22.60	16.02	13.64	18.71	14.35
Iron, µmol · l^{-1} Żelazo, µmol · l^{-1}	x̄	28.5	25.73	24.96	14.92	15.84	18.18	17.77	19.66
	SD	4.36	5.97	4.66	4.73	3.45	2.65	3.68	2.91
TIBC, µmol · l^{-1}	x̄	74.74	69.33	67.64	63.51	60.92	59.25	66.27	65.38
	SD	4.57	4.89	7.89	4.83	8.47	7.37	6.60	4.77
UIBC, µmol · l^{-1}	x̄	46.23	43.60	42.68	48.60	45.07	41.07	48.50	45.72
	SD	4.64	6.16	6.91	6.14	7.20	5.41	3.70	5.42
Transferrin saturation, % Wysycenie transferyny, %	x̄	38.10	37.10	37.00	23.50	26.10	30.70	26.60	30.20
	SD	5.00	8.10	6.30	7.40	4.60	2.80	3.30	4.90

the acute phase [Tóthová et al. 2008]. Changes in the concentration of albumins in the blood of cows in the final weeks before calving and in early lactation, show similar trends to changes in the concentration of total protein (Table 1, Fig. 1b). The concentration of albumins in the blood plasma of the cows in this experiment ranged within standards for dairy cattle [Alberghina et al. 2011], nevertheless it showed a significant reduction during the last month of pregnancy and in the first two weeks after calving, and a slight increase up to 8 weeks of lactation (non-significant). A similar tendency was noted by Al-Mujalli [2008]. In the last month of pregnancy the author showed a decrease in albumin concentration by 57%. Reduction of the concentration of albumins, immediately before calving was also observed by Park et al. [2010]. The authors suggest that this may be associated with decreased synthesis in the liver, increased protein requirements during this period, the preparation of the mammary gland for lactation and the use of albumins as a source of amino acids for the production of colostrum/milk.

Urea is the final product of protein metabolism. Its concentration in the blood serum of bovine range from 3.60 to 9.30 mmol · l^{-1} [Winnicka 2015]. In this experiment, the concentration of urea in the blood plasma of the cows ranged within reference values (Table 1, Fig. 1c). Worth mentioning is a significant increase in the concentration of this indicator starting from 2nd week of lactation. In the antepartal period, Al-Mujalli [2008] observed a reduction in the concentration of urea in the blood plasma of Frisian cows and the increase in the concentration of this indicator in the blood at the beginning of lactation. In contrast to those results, in our studies there was no reduction of the concentration of urea in the blood in the last month of pregnancy, but both studies reported an increase in concentration of urea after calving. A growing trend of this component during lactation was also observed by Taylor et al. [2003] and Cernescu et al. [2010]. Wathes et al. [2007] indicate that the concentration of urea in blood of heifers is positively correlated with milk yield. The authors also pointed that in the antenatal period, the increased demand of the fetus for energy and protein increases protein catabolism, which inhibits the conversion of ammonia produced by microorganisms in proventriculus into protein. The excess is thus converted into urea, promoting increase in its concentration in the blood. Chládek [2002] and Nogalski and Górak [2008], suggest that an increase in the concentration of urea in the serum may be associated with increased amounts of the protein in the feed, easily degradable in the rumen.

Creatinine is an important product of protein catabolism. Its concentration in the blood of cows ranges from 80 to 135 mmol · l^{-1}. Despite significant changes in the concentration of creatinine in the blood plasma of

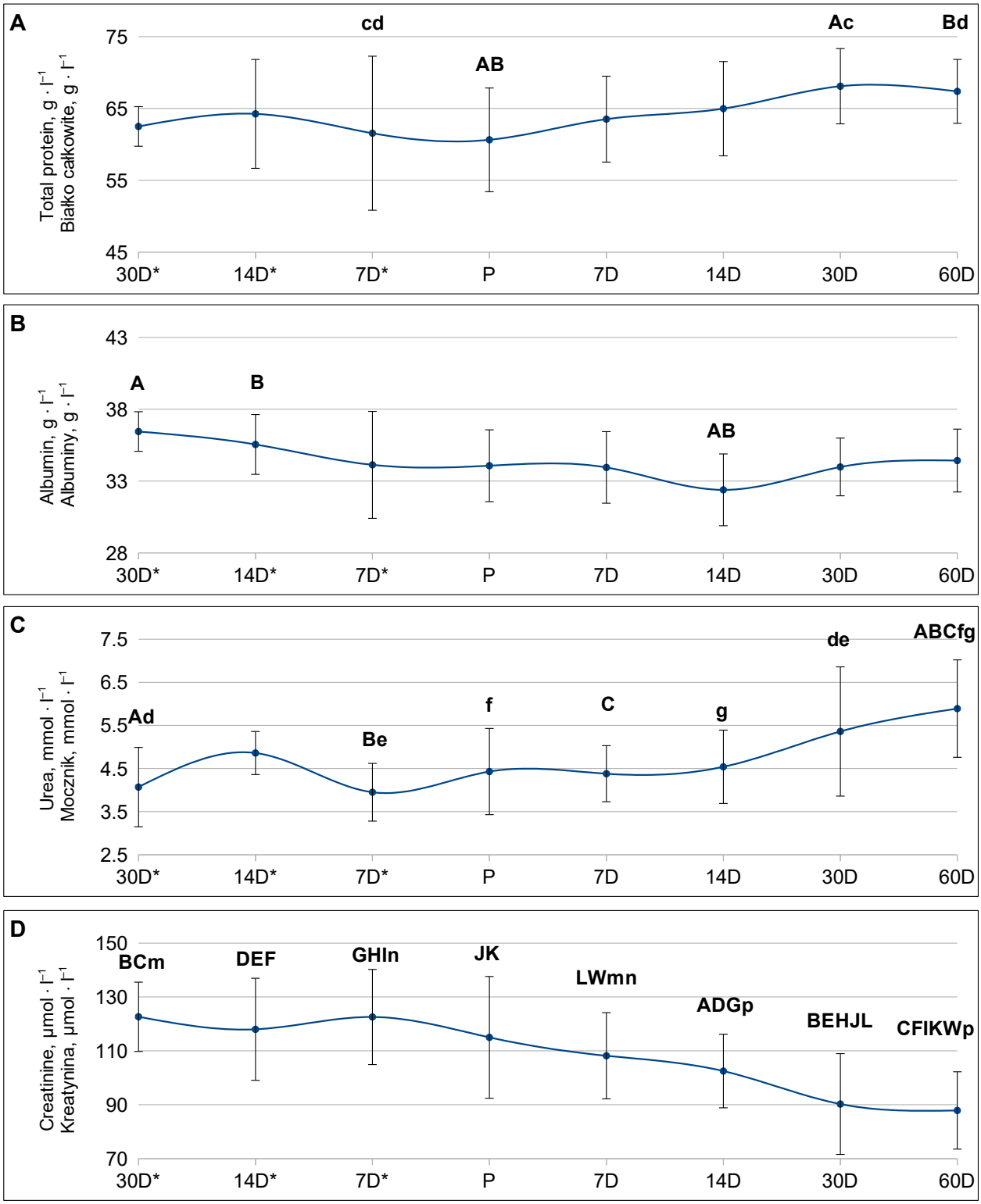

Fig. 1. The level of total protein (a), albumin (b), urea (c) and creatinine (d) in blood plasma of primiparous cows in the final month of pregnancy and the first two months of lactation (values showing significant differences marked with the same letter: capital letter $P \leq 0.01$, lower case letter $P \leq 0.05$; D – day, * asterisk indicates time points before parturition, P)

Rys. 1. Stężenie białka całkowitego (a), albumin (b), mocznika (c) i kreatyniny (d) w osoczu krwi krów pierwiastek w ostatnim miesiącu ciąży i pierwszych dwóch miesiącach laktacji (wartości, pomiędzy którymi wykazano statystycznie istotne różnice, zaznaczono tymi samymi literami: duże litery $P \leq 0.01$, małe litery $P \leq 0.05$; D – dzień, * gwiazdką oznaczono dni przed porodem P)

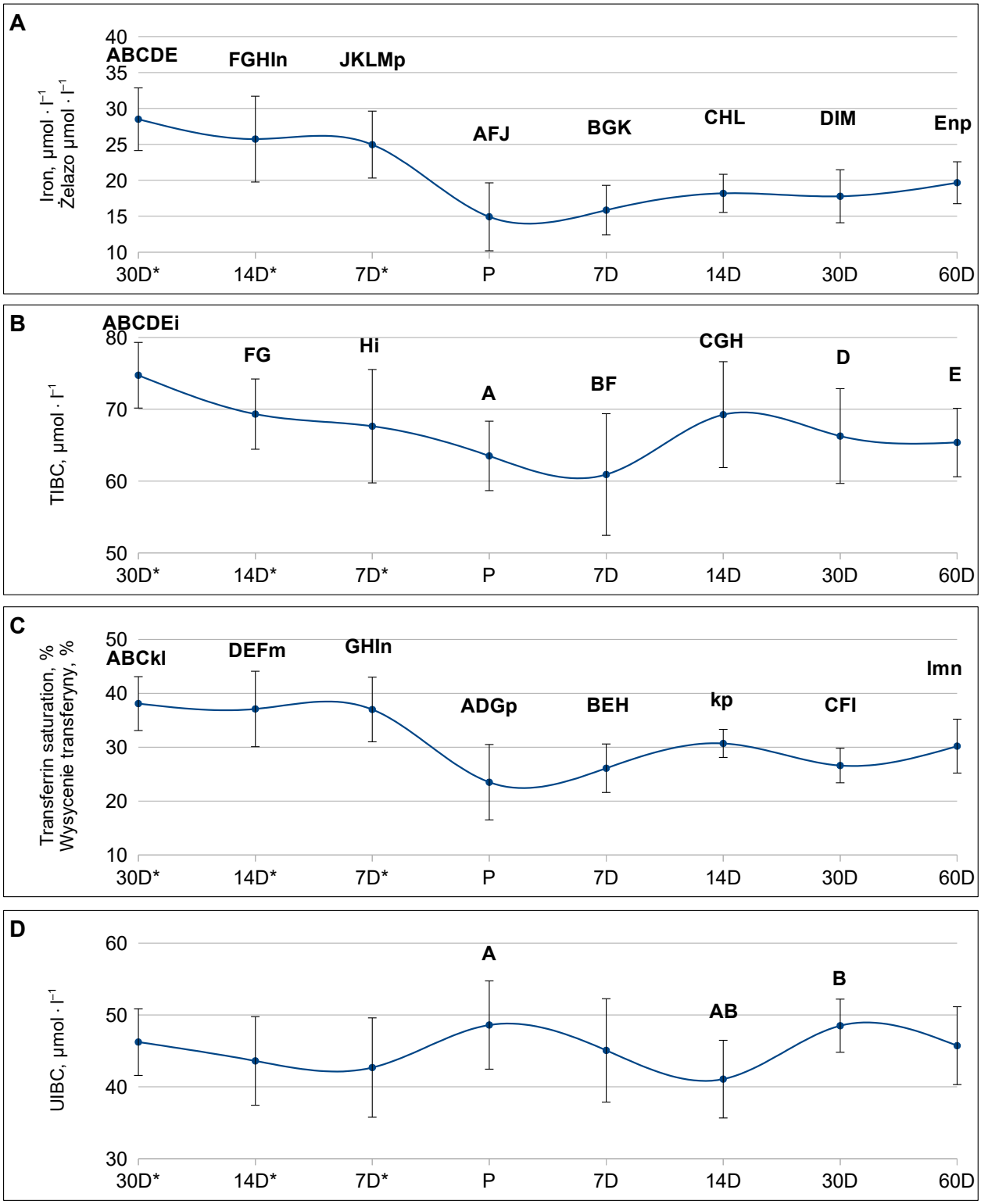

Fig. 2. Iron concentration (a), Total Iron Binding Capacity, TIBC (b), Transferrin saturation (c) and Unsaturated Iron Binding Capacity, UIBC (d) in blood plasma of primiparous cows in the final month of pregnancy and the first two months of lactation (values showing significant differences marked with the same letter: capital letter $P \leq 0.01$, lower case letter $P \leq 0.05$; D – days, * asterisk indicates time points before parturition, P).

Rys. 2. Koncentracja żelaza (a), całkowita zdolność wiązania żelaza – TIBC (b), wysycenie transferyny (c) i utajona zdolność wiązania żelaza – UIBC (d) w osoczu krwi krów pierwiastek w ostatnim miesiącu ciąży i pierwszych dwóch miesiącach laktacji. Wartości pomiędzy którymi wykazano statystycznie istotne różnice zaznaczono tymi samymi literami: duże litery $P \leq 0.01$, małe litery $P \leq 0.05$. D – dzień, * gwiazdką oznaczono dni przed porodem (P).

cows it was within the limits of reference values for cattle [Mordak 2008]. From the fourth to the last week before calving the concentration was stable and in the last week of pregnancy and the first two months of lactation decreased significantly (Table 1, Fig. 1d). It has been shown that changes in the concentration of this parameter depend on the diet and muscle metabolism, as well as environmental factors e.g. temperature. Abeni et al. [2004], Mordak and Nicpoń [2006] and Tóthová et al. [2008] indicate that the concentration of creatinine in the last two months of pregnancy in the blood plasma of cows significantly increased, and tended to be lower in the postnatal period. Abeni et al. [2004] indicate that the concentration of creatinine in the blood significantly increases during pregnancy, which may be associated with increased muscular effort in pregnant cows. According to Piccione et al. [2009] higher concentration of creatinine in the blood before calving may also be linked to an increased mobilization of proteins of skeletal muscles.

Iron is mainly related to energy metabolism and process of respiration – as a component of hemoglobin, myoglobin, cytochromes and several enzymes e.g. catalase, cytochrome oxidase [Swensson and Lindmark-Mansson 2007, Skrzypczak et al. 2009, Skrzypczak et al. 2010, Luchowska-Kocot 2014]. The reference values of iron in the serum of bovine range from 21.5 to 35.8 mmol · l^{-1} [Winnicka 2015]. In this experiment, the concentration of iron in the blood plasma of the cows ranged within 14.92 – 28.50 µmol · l^{-1} (Table 1, Fig. 2a). Iron is mainly transported in the blood by transferrin, but may also be in the form of chelates with albumin, citrates, amino acids and sugars [De Domenico et al. 2008]. Total iron binding capacity (TIBC) is three times higher than the concentration of iron in the blood plasma (Table 1, Fig. 2b). Saturation of transferrin in the weeks preceding delivery was high and stable (Table 1, Fig. 2c). From the day of calving it decreased statistically significantly. Changes in the UIBC varied greatly and in the blood plasma of cows and in the perinatal period were not significant (Table, Fig. 2d). Statistically have been confirmed only differences between the lowest (two weeks after calving) and the highest values – on the day of calving and the fourth week of lactation. Before calving the iron parameters in the blood plasma of the cows was significantly higher compared to the concentration in the postnatal period below the reference values [Miltenburg et al. 1991, Weiss et al. 2010, Winnicka 2015]. Kume et al. [1998] indicate that reduced concentration of the iron just before calving may be associated with the transfer of iron for the hemoglobin synthesis, which concentration increases during this period. It has been shown that the concentration of iron in the serum may be decreased as a result of increased erythropoiesis and insufficient provision of the element with food. In addition, lower concentration of iron in the blood may be associated with high concentrations of glucocorticoids. Africa et al. [1996] indicate that the reduction of the iron content may be related to the growth and development of the fetus and placenta, increasing blood volume and weight of the red blood cells and blood loss during delivery.

CONCLUSION

In the last month of pregnancy and the first two months of lactation the dynamic changes in the concentrations of all examined indicators was noted. Of particular interest are changes in the concentration of iron parameters, urea and creatinine. It is clearly readable that these parameters are greatly affected, and following their status gives us comprehensive answer about the status of the organism and remarks for further breeding. However, despite the intense morphological and functional changes in the body of the pregnant cow at the end of pregnancy and the first months of lactation, associated with the intensive development of the fetus, preparing the mammary gland for lactation, calving, regeneration of the reproductive system and intensive milk production, most studied biochemical indicators of plasma ranged within reference values for cattle. Thus, it appears that homeostatic mechanisms and adaptational processes in cows organism are rather efficient during this period. Nevertheless, it should be emphasized that at the time of prospectus new pregnancy higher concentration of urea, lower concentration of creatinine and iron parameters was noted, emphasizing the dysregulation of the organism condition and pointing the possible reason of association with prospectus difficulties in further reproductive performance. If analyzed for the particular herd, these parameters may be useful in considerations about the selection of time for new fertilization.

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KSZTAŁTOWANIE SIĘ WSKAŹNIKÓW METABOLIZMU BIAŁKOWEGO I GOSPODARKI ŻELAZEM U KRÓW PIERWIASTEK W OKRESIE OKOŁOPORODOWYM

STRESZCZENIE

Utrzymanie homeostazy organizmu, w końcowym okresie ciąży jest warunkiem zdrowia krowy, rzutującym na późniejszą jej użytkowość mleczną i rozrodczą, jak i urodzonego cielęcia, co decyduje o jego sprawności adaptacji neonatalnej. Monitorowanie stanu zdrowia w okresie okołoporodowym jest szczególnie istotne u pierwiastek, u których ujawnia się potencjał rozrodczy i potencjalna wydajność mleczna. Badania miały na celu ocenę zmian metabolizmu białkowego i gospodarki żelazem u krów pierwiastek w okresie okołoporodowym. w kontekście zmian morfologiczno-funkcjonalnych zachodzących w organizmie samicy, związanych z intensywnym rozwojem płodu, przygotowywaniem gruczołu mlekowego do laktacji, regeneracją układu rozrodczego po porodzie oraz przygotowywaniem organizmu do nowego zapłodnienia i ciąży. Wykazano dynamiczne zmiany badanych wskaźników, zwłaszcza stężenia żelaza oraz kreatyniny. Nie mniej, kształtowanie się większości badanych wskaźników w zakresie norm referencyjnych, świadczy o sprawności mechanizmów homeostatycznych i dużych możliwościach adaptacyjnych organizmu krów w tym okresie.

Słowa kluczowe: krowy, okres okołoporodowy, osocze krwi, białka, żelazo

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