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Age-related Changes of Selected Blood Biochemical Indicators in Dairy Calves during Their First Week of Life*

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The aim of this study was to determine the influence of age and ingested food (colostrum and mature milk) on the concentrations of selected blood biochemical components connected with nitrogen and mineral metabolism in dairy calves during their first week of life. The experiment was carried out on 13 Polish Black and White breed dairy calves. The animals were fed colostrum within the first 3 days of postnatal life and thereafter the mature milk of their dams until the end of the experiment (7 days). The obtained results showed that intensive catabolic and anabolic changes in nitrogen occur in the first week of life. These changes were particularly intense during the first 24-48 hours of life and may reflect dynamic tissue remodelling. The results of this experiment also show that healthy calves efficiently regulate water and electrolyte homeostasis.

Key words: Calves, neonatal period, blood plasma, biochemical indicators.

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The most intense morphological and functional changes occur during the first week of life of calves (SKRZYPCZAK & DRZEŻDŻON 2001; OŻGO *et al*. 2009). The efficiency of homeostatic mechanisms of an organism is crucial for survivability and also for later growth and development. The first colostrum intake influences not only morphological development but also exerts metabolic and endocrine changes in blood of newborns (HAMMON & BLUM 1998; BLUM 2006).

Early postnatal adaptation of calves is often accompanied by a high rate of neonatal morbidity and mortality, up to 30%, especially in the first week of life. The causes of neonatal mortality are complex and often polyethiologically based. The analysis of blood biochemical indicators is a fast and reliable method for the evaluation of animal health. The blood concentrations of many constituents in calves change dramatically with age, particularly during their first week of life (BIRGELE & ILGAZA 2003; MOHAMMAD 2009). Therefore it is necessary to determine the clinical norms of blood biochemical indicators which will contribute to more precise interpretation of laboratory results, early and accurate diagnosis of disease, and thus may contribute to the limitation of neonatal mortality.

The aim of this study was to determine the influence of age and ingested food (colostrum or mature milk) on concentration of selected biochemical components in blood associated with nitrogen and mineral metabolism in dairy calves during their first week of life.

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Material and Methods

The experiment was carried out on 7 male Polish Black and White dairy calves of 75% share of the Holstein-Fresian (HF) breed during the first seven postnatal days. Immediately after birth a "zero" blood sample was collected and subsequently calves were fed approximately 1 l of colostrum. For the duration of the experiment, the animals remained in individual pens under unified environmental conditions. During the first 3 days of life calves were fed colostrum (4 l/head/day) and from the 4th until the 7th day they were fed the mature milk of their dams (6 l/head/day).

Blood was drawn from the jugular vein into heparin tubes immediately after birth (before the first colostrum feeding) and at the 3rd, 6th, 12th, 24th, 36th, 48th and 72nd hours of life. Subsequently 4 blood samples were collected at 24 hour intervals until the 7th day of life always three hours after feeding. The samples were centrifuged (10 minutes, 4°C, 3000 rpm) and the harvested plasma was stored at -80°C until processing.

The concentrations of total protein, albumin, urea and endogenous creatinine in blood plasma were determined spectrophotometrically (PowerWave XA, BioTek) with the aid of already manufactured colourometric test kits (BioRad, Cormay, Hydrex). Blood plasma Na+, Ca2+, Mg2+, Zn2+ and Cu2+ values were measured using an Atomic Absorption Spectrophotometer (AAnalyst 400, Perkin Elmer) following the manufactures guidelines for sample preparation. Plasma osmotic pressure was determined by means of the cryoscopic method (osmometer A 0300, Knauer) and plasma chloride concentration using a chloride meter (chloride meter 50 cl, TridentMed).

Mean values and standard deviations were calculated. The resulting data were analysed by an ANOVA with repeated measurements and Tukey's multiple range post hoc test (software: Statistica 8.0™) in order to test the significance of differences.

Results

Mean concentrations of total plasma protein, albumin, urea and endogenous creatinine for all calves are shown in Table 1. The concentration of total plasma protein during the first week of life ranged between 75.10 and 107.80 g/l. The lowest value of this indicator was observed at birth which was followed by a statistically significant ($P \leq 0.01$) increase during the first 24 hours of life. The plasma protein concentration was relatively stable in the following hours until the end of the experimental period. At birth, plasma albumin concentration was 31.70 g/l and decreased until the 6th hour of life (30.00 g/l). From the 12th hour till the end of the first week of life a statistically significant ($P \leq 0.05$) increase in concentration of this protein to a value of 34.30g/l (168 h of life) was demonstrated. During the first 12 hours of life, plasma urea values were stable (3.11-3.24 mmol/l). On the second day (48 h) a significant ($P \leq 0.01$) increase in concentration of this metabolite (3.84 mmol/l) was found and was followed by a significant ($P \leq 0.01$) decline to 2.31 mmol/l at the 168 hour. The concentration of endogenous creatinine in the plasma of calves during the first three hours of life was high and ranged between 234.91 μmol/l (at birth) and 235.65 μmol/l (3 h of life). From the 3rd until the 24th hour of life a statistically significant ($P \leq 0.01$) decrease of creatinine to 146.01 μmol/l was demonstrated. Thereafter from the second (48 h) until the seventh day (168 h) of life the concentration of this indicator was stable.

The mean blood plasma concentration of the main extracellular fluid electrolytes (Na+, Cl-) and blood plasma osmotic pressure for all calves are shown in Table 2. Immediately after birth, the sodium concentration in blood plasma was 142.93 mmol/l and decreased to 135.79 mmol/l in the second day of life. Until the 72nd hour, a moderate increase in sodium (143.05 mmol/l) was detected, which was followed by a decline to 134.68 mmol/l on the 7th day (168 h). The observed changes were not statistically significant. The highest chloride concentration was demonstrated at birth (92.91 mmol/l) and decreased until the end of the experimental period. Also the highest values of osmotic pressure of plasma was observed immediately after birth (300.93 mmol/kg H2O), the lowest value was observed on the 7th day of life (292.14 mmol/kg H2O).

Mean blood plasma concentrations of macroelements (Ca2+, Mg2+) and trace elements (Zn2+, Cu2+) for all calves are shown in Table 3. Total calcium concentration in blood plasma of calves during the first 24 hours of life was 3.06 mmol/l. From the 48th till 96th hour, a moderate increase in concentration of this electrolyte up to3.22 mmol/l was observed. It was followed by a decrease to 3.02 mmol/l at 120 h. Plasma Ca2+ levels gradually increased towards the end of the experimental period. The highest magnesium concentration was found on the 1st (1.25 mmol/l) whereas the lowest on the 6th (1.00 mmol/l) day of life. It is noteworthy that from the 24th hour until the end of the first week of life, a statistically significant decline ($P \leq 0.01$) of this macroelement in blood plasma

The use and handling of animals for this experiment was approved by the Local Ethical Committee (no. 4/2008 of 24.01.2008).

Table 1

Blood plasma concentration of total protein (g/l), albumin (g/l), urea (mmol/l), endogenous creatinine (μmol/l) and significant differences between values in the following hours of calves life

Indicator		Hours of life												Significance of differences	
		0	3	6	12	24	36	48	72	96	120	144	168	P≤0.01	P≤0.05
Total Protein	$\bar{x}$	75.10	79.10	88.70	97.40	103.80	103.50	105.00	105.60	107.80	105.60	105.80	104.10	0→6-168*, 3→ 12-168 6→24-168, 12→96	3→ 6
	SD	6.00	5.40	9.90	11.30	12.80	13.10	12.60	12.70	16.70	14.30	10.90	10.50		
Albumin	$\bar{x}$	31.70	3.60	30.00	29.50	29.90	32.10	32.10	33.90	34.70	34.10	34.70	34.30	no differences	12→96,144-168
	SD	3.20	2.90	2.60	1.80	3.10	3.30	3.20	3.10	3.50	4.10	3.30	5.50		
Urea	$\bar{x}$	3.11	3.17	3.31	3.20	3.84	4.63	4.65	4.06	3.43	2.93	2.65	2.31	36,48→144-168	36,48→120 72→168
	SD	0.99	1.01	1.10	1.24	1.61	2.38	2.17	1.98	0.86	0.69	0.82	0.36		
Creatinine	$\bar{x}$	234.91	235.65	210.32	183.45	140.01	140.74	128.05	122.47	115.01	113.74	112.86	115.94	0,3→ 12-168 6→24-168 12→36-48	12→24
	SD	50.26	3.35	26.06	27.19	16.52	13.57	14.80	18.84	17.51	16.23	15.02	18.73		

*Significance of differences on the level P≤0.01 between zero blood sample (before colostrum administration) and from 6th till 168th hour of calves life.

Table 2

Blood plasma concentration of sodium (mmol/l), chloride (mmol/l), osmotic pressure (mmol/kg H_2O) and significant differences between values in the following hours of calves life

Indicator		Hours of life								Significance of differences	
		0	24	48	72	96	120	144	168	P≤0.01	P≤0.05
Na^+	$\bar{x}$	142.93	135.79	140.20	143.05	137.95	138.50	138.24	134.68	no differences	
	SD	8.75	3.32	5.01	9.77	4.38	7.07	6.71	5.13		
Cl^-	$\bar{x}$	92.91	91.64	91.67	90.02	88.38	91.92	90.76	91.40	no difference	
	SD	2.97	4.50	6.53	6.21	6.34	3.62	3.94	4.82		
Osmotic pressure	$\bar{x}$	300.93	292.50	295.21	295.28	292.57	293.64	295.93	292.14	no difference	
	SD	5.42	13.94	6.52	7.49	8.99	8.09	7.47	10.43		

Table 3

Blood plasma concentration of total calcium (mmol/l), magnesium (mmol/l), zinc (μmol/l), cooper (μmol/l) and significant differences between values in the following hours of calves life

Indicator		Hours of life								Significance of differences	
		0	24	48	72	96	120	144	168	P≤0.01	P≤0.05
Ca^{2+}	$\bar{x}$	3.06	2.94	3.14	3.20	3.22	3.02	3.07	3.18	no differences	
	SD	0.27	0.15	0.19	0.30	0.24	0.21	0.15	0.14		
Mg^{2+}	$\bar{x}$	1.16	1.25	1.11	1.06	1.01	1.06	1.00	1.05	24→72,96,144,168	0→144 24→120
	SD	0.05	0.16	0.22	0.08	0.07	0.11	0.06	0.07		
Zn^{2+}	$\bar{x}$	15.90	11.05	17.26	22.56	21.98	19.43	19.52	23.17	24→72-168	0→168
	SD	7.66	2.93	4.10	6.78	4.63	3.79	4.90	5.22		
Cu^{2+}	$\bar{x}$	5.62	7.69	9.86	11.53	12.40	13.21	14.28	14.78	0→24-168, 24→72-168, 48→96-168, 72→144-168	0→24, 24→48 96→168
	SD	0.69	0.73	0.98	1.64	2.00	2.58	2.51	2.61		

was observed. The concentration of zinc in the blood plasma of calves during the first week of life ranged between 11.05 and 23.17 μmol/l. The lowest value was found on the 24th hour (11.05 μmol/l) but during the next two days significantly ($P \leq 0.01$) increased. At the 96th hour the Zn2+ concentration reached 21.98 μmol/l and remained at a similar level until the end of the experiment (average 21.33 μmol/l). At birth, the copper concentration in blood plasma was 5.62 μmol/l and gradually increased ($P \leq 0.01$) in the following hours to 14.78 μmol/l at 168 h.

Discussion

The low concentration of total plasma protein observed in calves at birth followed by its increase after the first colostrum feeding is consistent with previous reports (FRANKLIN *et al*. 2003; BATCHELDER *et al*. 2007). Undoubtedly the reason for the demonstrated effect was intestinal absorption of proteins (particularly IgG) from colostrum by the newborn calves. The increase in blood plasma protein level during the first 24 hours of life probably reflects an enhanced period of intestinal permeability for immunoglobulins (HAMMON *et al.* 2002).

Our experiment showed a decline in blood plasma albumin concentration at the 6th hour of calf life. A similar pattern in changes in the level of this protein in calves was described by other authors (HAMMON *et al*. 2002; NUSSBAUM *et al*. 2002). The observed plasma albumin decline was probably due to ingested food, which contributed to a transient increase in blood plasma volume (ZANKER *et al*. 2000). Results of an experiment conducted on neonatal piglets demonstrated that an increased dietary amino acids supply enhances hepatic albumin synthesis (DAVIS *et al*. 1998). These authors stated that a high plasma level of this protein in the early neonatal period helps to maintain the metabolic balance in newborns. Albumin is not only a transporter that binds and carries amino acids (AAs), but it also provides for temporary storage of AAs, which prevents their oxidation. In the case of low protein intake or increased protein demands of the growing organism, free AAs are released from AA-albumin complexes (VAN DEN AKKER *et al.* 2007).

The concentrations of endogenous creatinine and urea in blood plasma are often used as indicators of nitrogen metabolism and renal excretion efficiency. Intensive protein, carbohydrate and lipid metabolism occurs during the first week of life. Thus, analysis of changes of these two indicators in calf blood plasma enable the monitoring of the course of postnatal adaptation processes in this critical period.

The blood plasma urea changes noted in this study were in accordance with the report of KUHNE *et al*. (2000). RUFIBACH *et al*. (2006) state that plasma urea concentration is highly associated with supply, synthesis and degradation of proteins. A dynamic increase in its concentration in calf blood plasma from birth till the 3rd day of life probably reflects enhanced protein degradation and deamination of amino acids after protein-rich food ingestion and may also indicate a high rate of tissue remodelling (NUSSBAUM *et al*. 2002). According to TAKAGI *et al*. (2008), the increase in hepatic urea synthesis is a consequence of increased activity of urea cycle enzymes and also enhanced ammonium production. RAUPRICH *et al*. (2000) reported that blood plasma urea concentration during the first week of life is notably higher in calves fed milk replacer in comparison to calves fed natural food. These authors postulate that non-nutrient factors (mainly IGF-I and insulin) which are present in high quantities in cow colostrum are responsible for causing the anabolic effect and thus lowering the plasma urea concentration.

The decrease in endogenous creatinine level in blood plasma during the first seven days of the life of calves in this experiment confirms the observation of NUSSBAUM *et al*. (2002) and BIRGELE & ILGAZA (2003). KURZ and WILLET (1991) claim that a radical decrease in blood plasma creatinine concentration reflects the increase in glomerular filtration rate (GFR). Creatinine is not reabsorbed in renal tubules and 10-20% of its total amount which is excreted with urea originates from tubular secretion. Therefore, an increase or decrease in GFR always leads to adequate changes in plasma creatinine level (STREVENS *et al*. 2001). Moreover, MOHRI *et al*. (2007) state that a decrease in plasma creatinine level may also be the result of dynamic changes in calves muscle mass.

In the current study a high concentration of sodium in calf blood plasma at birth followed by a moderate decrease was demonstrated. These data accord fully with other investigations (SKRZYPCZAK & DRZEŻDŻON 2001; BATCHELDER *et al.* 2007). OŻGO (2001) states that a high concentration of plasma Na+ during the first day of life is caused by a high aldosterone level in plasma during this period.

Chloride, a major extracellular anion, closely follows the metabolism of sodium. Thus, an increase or decrease of its concentration in plasma results in parallel chloride changes. The results of the present study are in accordance with previous reports (OŻGO 2000; BATCHELDER *et al*. 2007).

The concentration of the main extracellular electrolytes (Na+, Cl-) were stable during the entire experimental period, thus plasma osmotic pressure was also stable.

The calcium levels in blood plasma of calves demonstrated in the present experiment were much higher than reported by other authors (BIRGELE & ILGAZA 2003; JAIN *et al*. 2007). Since milk is the main source of Ca2+ a possible cause of the observed phenomenon is a higher amount of ingested food. It should be emphasized that the calcium concentration of plasma in newborn calves is not directly dependent on maternal plasma Ca2+ concentrations. SZENCI *et al*. (1994) reported a higher level of plasma calcium in newborn calves when compared with their dams. However, the mechanisms responsible for maintenance of a constant Ca2+ level are not yet completely understood. High plasma concentration of this macroelement is probably the result of the high requirements of growing calves, since calcium is responsible for stimulation of osteoblast growth and thus whole skeletal development.

A significant decrease in blood plasma magnesium concentration in calves from the 24th hour till the end of the experimental period observed in this experiment agrees with the results of other researches (JAIN *et al*. 2007; MOHAMMAD 2009). It is hypothesized that these changes are a consequence of enhanced magnesium utilisation (along with calcium and phosphorus) for bone mineralisation and also decreased Mg availability from ingested food (MOHAMMAD 2009).

During the first 24 hours of life the Zn2+ concentration dropped from 15.90 μmol/l to 11.05 μmol/l. These data are consistent with previous reports (KUMAGAI *et al*. 1991; KINCAID 1999). According to KINCAID (1999) stress after birth and low Zn2+ content in ingested food results in a marked decrease in plasma zinc concentration in calves. Additionally, PODHORSKY *et al*. (2007) state that tends to zinc accumulate in the liver during foetal and early postnatal development of calves and subsequently is bound to the protein – metallothionein (MT). The concentration of glucocorticoids and cytokines in blood plasma in calves is very high during the first week of life. These substances enhance formation of Zn-metallothionein complexes, which results in plasma zinc concentration decrease, but it does not indicate its deficiency (KINCAID 1999; PAVLATA *et al.* 2005).

The observed low concentration of copper in calves at birth followed by its increase after first colostrum feeding until the end of the first week of life was consistent with previous reports (PAVLATA *et al*. 2004; SKRZYPCZAK *et al*. 2010). ENJALBERT *et al*. (2006) reported a progressive increase of Cu2+ concentration in fetal calf liver during uterine development, even in situations when a dam was copper deficient. In spite of this, the level of this trace element in the blood plasma of newborn calves is lower when compared to their dams. According to SLAVIK *et al*. (2006) during the early postnatal period in calves an inadequate amount of the main copper transport protein, ceruloplasmin (Cp), is produced. Blood plasma concentration of Cp in calves increases within three postnatal days and reaches similar values to those measured in mature animals. The progressive increase of Cu2+ concentration during the first seven days of life may be related with both an increase of plasma ceruloplasmin and an adequate supply of this trace element in ingested food.

In conclusion, our findings indicate that intensive catabolic and anabolic nitrogen changes occur in the following hours of the first week of life in calves. These changes are particularly intense during the first 24-48 hours of life and may reflect dynamic tissue remodelling. The results of this experiment have shown that healthy calves efficiently regulate water and electrolyte homeostasis. An age-related increase of intestinal absorption of macro-and microelements is a key mechanism to maintain the balance of these nutrients.

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