

Comparative Study of Selected Blood Biochemical Components in Milk or Milk-Replacer Fed Calves during the Second Week of Life*

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The experiment was carried out on 13 male Polish Black and White dairy calves of 75% share of the Holstein-Friesian (HF) breed during the second week of life. The animals were divided into two groups. One group (n=7) was fed mother's milk and the second (n=6) milk replacer. The dynamics of changes in concentration of selected blood biochemical components connected with nitrogen metabolism (plasma total protein, albumin, urea, endogenous creatinine) and with mineral metabolism (sodium, chloride, calcium, magnesium, zinc, copper and plasma osmotic pressure) were analyzed in both groups. The results show that the type of ingested food influences the concentration of indicators reflecting nitrogen metabolism. Changes of these parameters in calves fed milk replacer are possibly connected with advantageous catabolic changes. Stable concentrations of main extracellular fluid electrolytes and blood plasma osmotic pressure were found in both groups of calves. Constant blood plasma calcium, magnesium, zinc and copper concentrations observed during this study might also indicate the relative maturity of mechanisms maintaining water and electrolyte balance. Nevertheless, it seems justifiable to monitor the copper concentrations in plasma of young calves.

Key words: Calves, milk, milk replacer, blood biochemical compounds.

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Proper calf feeding during their first weeks of life has a fundamental influence on the functional adaptation of many vital organs. The efficiency of these processes is directly linked with the maintenance of organismal homeostasis and normal development in later life. Cow colostrum which gradually changes into mature milk is a natural food which provides the calf with all necessary nutrients. It also contains many biologically active compounds which influence the functional development and maturation of the gastrointestinal tract and also has a systemic effect on metabolism (KLUTH *et al.* 2000; BLUM 2006).

The feeding of milk replacer instead of whole milk has become a common practice on dairy farms. However, the compositions of the formulas greatly differ from the composition of milk. For example, proteins and fats in milk replacers are partly replaced with components derived from plants. Moreover, the formulas are devoid of many essential bioactive substances in milk such as hormones, bioactive peptides, growth factors and cytokines which influence the growth and development of young calves (ZABIELSKI *et al.* 1999; GUILLOTEAU *et al.* 2009).

Blood biochemical components analyses are fast and constitute a reliable source for the evaluation

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of animal health. Morbidity and mortality rate may even reach 30% in calves during the first weeks of life. The causes of neonatal mortality are complex and often polyethiologically based. Therefore it is necessary to determine the clinical norms of blood biochemical components contributing to the early diagnosis of disease. The results of previous studies show that multiple factors may influence the concentration of blood biochemical components including breed, utility type and feeding system (CLINQUART *et al.* 1995; AL-SHAMI 2007).

This study was undertaken to determine the effect of different types of ingested food (milk or milk replacer) on concentrations of selected blood biochemical components associated with nitrogen metabolism (blood plasma total protein, albumin, urea, endogenous creatinine) and with mineral metabolism (sodium, chloride, calcium, magnesium, zinc, copper and blood plasma osmotic pressure) in dairy calves during the second week of life.

Material and Methods

Animals

The experiment was carried out on 13 male Polish Black and White dairy calves of 75% share of the Holstein-Friesian (HF) breed during the second week of life. Animals of both groups were fed with colostrum for the first 3 days (3-4 kg/head/day) and then milk (5-6 kg/head/day) throughout the first week of life. On the 8th day animals were divided into two groups. One group (n=7) was fed mother's milk (6-7 kg/head/day) and the second (n=6) milk replacer (6-7 kg/head/day). In the experiment a commercially available milk replacer (Sanolac Premium, Sano) was used (21% total protein, 17% crude fat, 0.2% crude fibre, 9.2% crude ash). The proteins in the formula comprised cow colostrum and milk-derived proteins. The fat was a mixture of coconut and palm oil.

Blood sampling

Blood was drawn from the jugular vein into heparin tubes once a day from the 8th day until 14th day of life, always three hours after morning feeding. The samples were centrifuged (10 minutes, 4°C, 3 000 rpm) and the harvested plasma was stored at -80°C until processing.

Biochemical analysis

Blood plasma osmotic pressure was determined by means of the cryoscopic method (osmometer A 0300, Knauer). Blood plasma total protein, albu-

min, urea and endogenous creatinine concentrations were measured spectrophotometrically (PowerWave XS, BioTek) by using manufactured colourimetric test kits (BioRad, Cormay, Hydrex). Blood plasma sodium, calcium, magnesium, zinc and copper levels were determined using an atomic absorption spectrophotometer (AAAnalyst 400, Perkin Elmer) according to the manufacturer's guidelines. The blood plasma chloride concentration was quantified with the aid of a chloride meter (chloride meter 50 cl, Trident-Med).

Statistical analysis

Mean values and standard deviations were calculated. The resulting data were analysed by ANOVA with repeated measurements and Tukey's multiple range *post hoc* test (Statistica 8.0TM software) in order to test the significance of differences of blood biochemical compounds according to the age of calves. Data were also analysed by ANOVA with repeated measurements and Duncan's test (Statistica 8.0TM software) in order to test the significance of differences according to calf feeding system.

Results

Mean total protein, albumin, urea and endogenous creatinine values in blood plasma for calves from two experimental groups are shown in Table 1. Total blood plasma protein concentration in calves fed mother's milk was stable throughout the duration of the experiment and was higher when compared to its concentration in calves fed milk replacer. A significant ($P \leq 0.01$) decrease of this indicator between the 8th (99.70 g/l) and 14th day (92.70 g/l) of life was observed in calves fed milk replacer. Blood plasma albumin concentration was stable in both experimental groups until the 12th day. Subsequently the differences in plasma albumin levels between calves fed mother's milk and milk replacer increased and achieved statistical significance ($P \leq 0.05$) on the 13th and 14th day of life. Blood plasma urea level in the group of calves fed mother's milk was stable during their second week of life. However, in the group of calves fed milk replacer, a significant ($P \leq 0.01$) increase in blood plasma urea level between the 9th (1.67 mmol/l) and 14th (3.80 mmol/l) day of life was observed. There were no statistically significant differences between groups with differing feeding systems. Endogenous creatinine concentration in blood plasma was stable in both groups throughout the entire experimental period.

Table 1

Blood plasma concentration of total protein, albumin, urea and endogenous creatinine in milk or milk-replacer fed calves during the second week of life

Indicator	Feeding system		Day of life						
			8	9	10	11	12	13	14
Total protein (g/l)	\bar{x}	milk	102.30	103.40	104.40	101.80	104.70	102.60	104.00
	SD		9.60	12.00	13.70	12.50	13.60	12.00	11.60
	\bar{x}	replacer	99.70 ^A	100.00 ^B	99.00 ^{Ca}	97.70	95.00	93.50 ^a	92.70 ^{ABC}
	SD		8.50	9.60	8.10	11.90	9.00	8.50	10.10
Albumin (g/l)	\bar{x}	milk	34.20	34.80	35.00	34.60	35.40	35.00*	36.00*
	SD		2.90	2.80	2.00	3.10	1.90	2.20	2.10
	\bar{x}	replacer	31.70	31.30	32.40	31.20	31.20	29.60*	29.20*
	SD		5.70	4.50	4.30	4.80	4.20	2.60	2.20
Urea (mmol/l)	\bar{x}	milk	2.39	2.43	2.25	2.53	2.92	2.90	3.06
	SD		0.56	0.66	0.75	0.76	1.17	1.19	1.13
	\bar{x}	replacer	2.77 ^{ab}	1.67 ^{ABCa}	2.13 ^{Dc}	2.57 ^E	3.20 ^{Ac}	3.13 ^B	3.80 ^{CDEb}
	SD		1.29	0.66	0.66	1.05	0.56	0.69	0.96
Creatinine (μ mol/l)	\bar{x}	milk	119.09	119.60	114.40	111.12	118.51	121.23	122.59
	SD		21.64	17.83	21.61	17.30	20.43	18.40	22.56
	\bar{x}	replacer	99.45	105.35	122.21	123.90	123.05	106.20	118.84
	SD		12.66	19.82	18.21	16.54	11.38	22.39	11.42

The single star (*) is used to show the statistical significance level of $P \leq 0.05$ between the groups of calves fed either milk or milk replacer.

The small letters are used to show the statistical significance level of $P \leq 0.05$ with age. The capital letters are used to show the statistical significance level of $P \leq 0.01$ with age.

Table 2

Blood plasma concentration of sodium, chloride and osmotic pressure in milk or milk-replacer fed calves during the second week of life

Indicator	Feeding system		Day of life						
			8	9	10	11	12	13	14
Na ⁺ (mmol/l)	\bar{x}	milk	138.41	139.86	135.05	137.12	135.28	136.45	139.08
	SD		3.44	10.71	2.40	8.68	6.52	2.90	13.75
	\bar{x}	replacer	132.38	132.08	133.11	132.74	137.11	134.19	130.37
	SD		6.22	4.56	3.16	2.73	5.53	7.43	5.89
Cl ⁻ (mmol/l)	\bar{x}	milk	91.18	90.59	92.29	90.36	91.58	89.25	91.03
	SD		4.13	3.87	2.18	4.72	4.53	9.37	5.87
	\bar{x}	replacer	91.17 ^a	94.77 ^{bc}	90.50	92.37	95.08	98.12 ^b	98.22 ^{ac}
	SD		2.69	4.73	4.33	4.89	3.32	4.87	4.66
Osmotic pressure (mmol/kgH ₂ O)	\bar{x}	milk	286.36	292.21	289.43	292.07	293.36	292.00	291.28
	SD		20.94	7.36	12.73	7.06	10.93	11.48	9.48
	\bar{x}	replacer	288.67	288.33	273.33	281.67	298.83	287.75	284.50
	SD		6.15	10.51	22.73	15.96	6.62	9.60	12.03

The small letters are used to show the statistical significance level of $P \leq 0.05$ with age.

Mean blood plasma concentrations of the main extracellular fluid electrolytes (Na⁺, Cl⁻) and blood plasma osmotic pressure for all calves are shown in Table 2. Sodium levels in blood plasma did not significantly change in either group of calves in the following days of the second week of life. Blood

plasma chloride concentration was stable in calves fed mother's milk during the experimental period. On the other hand, the values of plasma chlorides increased ($P \leq 0.05$) between the 10th (90.50 mmol/l) and 14th day (98.22 mmol/l) in calves fed milk replacer. In spite of the differences between

Table 3

Blood plasma concentration of calcium, magnesium, zinc and copper in milk or milk-replacer fed calves during the second week of life

Indicator		Feeding system	Day of life						
			8	9	10	11	12	13	14
Ca ²⁺ (mmol/l)	\bar{x}	milk	3.03	2.98	2.94	2.89	2.91	2.87	2.83
	SD		0.20	0.11	0.17	0.13	0.10	0.16	0.13
	\bar{x}	replacer	3.01	3.16 ^a	2.96	2.91	2.82	2.76 ^a	2.89
	SD		0.08	0.26	0.37	0.22	0.24	0.25	0.29
Mg ²⁺ (mmol/l)	\bar{x}	milk	1.10	1.05	1.06	1.06	1.07	1.04	1.04
	SD		0.13	0.10	0.11	0.13	0.20	0.10	0.20
	\bar{x}	replacer	1.20	1.16	1.14	1.05	1.32	1.12	1.12
	SD		0.13	0.07	0.09	0.08	0.30	0.13	0.11
Zn ²⁺ (μ mol/l)	\bar{x}	milk	22.32	24.47	24.82	22.77	21.57	22.21	23.74
	SD		10.14	12.22	11.24	10.68	7.93	8.17	8.94
	\bar{x}	replacer	17.31	16.88	16.55	17.82	17.21	17.33	18.20
	SD		1.58	2.33	2.95	4.74	4.31	4.13	3.27
Cu ²⁺ (μ mol/l)	\bar{x}	milk	15.04	15.84	15.83	16.11	16.19	16.11	15.72
	SD		2.59	3.34	3.37	3.43	3.80	4.58	5.35
	\bar{x}	replacer	14.74	15.96	14.83	14.61	14.40	14.14	14.13
	SD		3.22	3.68	3.29	3.44	4.14	3.64	4.05

The small letters are used to show the statistical significance level of $P \leq 0.05$ with age.

chloride values between these two groups no significant changes were observed. Blood plasma osmotic pressure in both groups of calves in the following days of the second week of life did not change significantly. It is noteworthy that values of plasma osmotic pressure in calves fed mother's milk were higher than in the second group of calves. However these differences were not statistically significant.

Mean blood plasma macro element (Ca²⁺, Mg²⁺) and trace element (Zn²⁺, Cu²⁺) profiles for calves fed mother's milk or milk replacer are shown in Table 3. Blood plasma calcium concentration in calves fed mother's milk was stable throughout the second week of life, whereas its concentration in calves fed milk replacer decreased ($P \leq 0.05$) from the 9th (3.16 mmol/l) until 13th day (2.76 mmol/l). The magnesium concentration in blood plasma in both groups of calves was stable during the entire experimental period. Blood plasma zinc concentration was higher in calves fed mother's milk when compared to its concentration in calves fed milk replacer. The values for copper concentration in blood plasma were stable and higher in the group of calves fed mother's milk in the following days of the second week of life than in the second group of calves. Blood plasma Cu²⁺ decreased from 15.96 μ mol/l (9th day) to 14.13 μ mol/l (14th day) in calves fed milk replacer. There were no statistically significant differences observed according to feeding system.

Discussion

In this study, differences in total blood plasma protein concentration as a result of different types of feeding were demonstrated. High stability of total blood plasma protein concentration in calves fed mother's milk is consistent with results obtained by other authors (ZANKER *et al.* 2000; HAMMON *et al.* 2002; AL-SHAMI 2007). The decrease in total blood plasma protein concentration in calves fed milk replacer reported in the present study was also observed by MOHRI *et al.* (2007). Blood plasma protein concentration in calves during their first weeks after birth correlates with plasma immunoglobulin G concentration absorbed from colostrum (HAMMON *et al.* 2002; KURIHARA *et al.* 2004). It was previously shown that a 24 hour delay in first colostrum feeding caused a marked decline in plasma protein concentration when compared to a group of calves receiving colostrum immediately after birth (ZANKER *et al.* 2000). Moreover, feeding rate during the first days of life also has a great influence on blood plasma protein changes. Feeding calves with a high amount of colostrum and milk greatly increases blood plasma protein concentration (EGLI & BLUM 1998; KÜHNE *et al.* 2000). COPPO *et al.* (2003) reported that animals fed with mother's colostrum and milk showed a progressive blood plasma protein concentration increase (until the 60th day), whereas a decrease of this indicator was observed after weaning and transition to milk re-

placer feeding. The decline of total blood plasma protein concentration in calves fed milk replacer documented in this study probably resulted from a lower digestibility of proteins from milk replacer. This observation and also the relatively lower secretion and activity of gastrointestinal proteolytic enzymes in young calves may contribute to limited growth rate. These suppositions were confirmed by COPPO *et al.* (2003; 2007a; 2007b).

In this study albumin concentration in blood plasma from calves fed mother's milk was stable throughout the entire experimental period. These results agree with the reports of HAMMON *et al.* (2002) and JE EK *et al.* (2006). BIRGELE and ILGAZA (2003) reported that the increase in albumin concentration of blood plasma observed in calves in subsequent weeks of life is a physiological process and may serve as an indicator of functional and morphological adaptation of an organism. Moreover, the authors pointed out that albumin synthesis is highly correlated with the amount and quality of ingested food. The decrease in blood plasma albumin concentration in calves fed milk replacer reported in this study was probably due to lower digestibility of proteins in milk replacer. These data are in accordance with previous investigations (FIEMS *et al.* 1998; COPPO *et al.* 2003).

In the group of calves fed mother's milk, a progressive increase of blood plasma urea concentration was observed in the present study. Similar results were reported by HAMMON *et al.* (2002). On the other hand SKRZYPCZAK *et al.* (1996) demonstrated that the blood plasma concentration of this metabolite in calves was stable during the first 3 weeks of life (mean concentration was 3.25 mmol/l). However, BIRGELE and ILGAZA (2003) reported that blood plasma urea level in calves fed mother's milk decreased between the first and second weeks of life. In the current research plasma urea concentration in calves fed milk replacer was much higher than in calves fed mother's milk. These results are in accordance with the report of RAUPRICH *et al.* (2000). These authors suggested that differences in urea concentrations were due to a lack of biologically active substances (e.g. IGF-I and insulin) in milk replacer which exerts a mitogenic effect and is also responsible for the domination of anabolic over catabolic processes. Predomination of catabolic processes, connected with protein changes, may be the main cause of progressive increase of blood plasma urea concentration in the group of calves fed milk replacer.

Endogenous creatinine levels in blood plasma were stable and similar in both experimental groups during the second week of life. These data were confirmed by other authors (SKRZYPCZAK & DRZEŹDŹON 2001; HAMMON *et al.* 2002; BIRGELE & ILGAZA 2003).

Results of the current study indicate that mechanisms responsible for water and electrolyte homeostasis regulation are efficient in calves in the early postnatal period. This is partly reflected by lack of marked changes of blood plasma osmotic pressure during the experimental period in both groups of calves. Blood plasma osmotic pressure depends mainly on electrolyte concentration in plasma (Na^+ , K^+ , Cl^-). It should be emphasised that blood plasma sodium concentrations in both groups of calves during the second week of life were stable. Similar results were reported by OŹGO (2000) and SKRZYPCZAK and DRZEŹDŹON (2001). Chloride, the major extracellular anion, closely follows the metabolism of sodium. An increase or decrease of blood plasma sodium concentration results in parallel blood plasma chloride changes. In the group of calves fed mother's milk chloride concentration in blood plasma was stable whereas in calves fed milk replacer the chloride values increased gradually but within the reference values. This indicates that kidney mechanisms responsible for sodium and chloride saving in this period of life are relatively mature (OŹGO 2000; OŹGO 2001; SKRZYPCZAK & DRZEŹDŹON 2001; JE EK *et al.* 2006; BRZEŹDŹON *et al.* 2007).

In this study blood plasma calcium concentration decreased in both groups of calves during the experimental period but the decline was much higher in calves fed milk replacer. This was in accordance with previous studies in neonatal calves (BIRGELE & ILGAZA 2003; MOHRI *et al.* 2007; MOHAMMAD 2009). Because blood plasma calcium concentration is closely related to its availability in food, it can be postulated that both milk and milk replacer are appropriate sources of this macro element for calves and the mechanisms that regulate calcium homeostasis are efficient. A decrease of blood plasma calcium values in calves fed milk replacer might be due to a lower blood plasma albumin concentration. These results agree with the report of JE EK *et al.* (2006) who found a significant correlation between total calcium and albumin concentration in the blood plasma of calves. The age-related decrease in blood plasma calcium concentration observed in the present study may be connected with high requirements for this macro element. This nutrient plays a fundamental role as a substrate for many processes, mainly in bone mineralization (HUNT *et al.* 2008).

High and stable blood plasma magnesium concentrations in both groups of calves indicate an adequate provision of this macro element from ingested food. These data are consistent with a previous report (MOHAMMAD 2009).

In the present study blood plasma zinc concentration was higher in the group of calves fed mother's milk in comparison to calves fed milk re-

placer. As seen in previous research, the mean blood plasma zinc concentration in veal calves fed mother's milk was 23.00 $\mu\text{mol/l}$ (ABI-RIZK *et al.* 2008) and 18.50 $\mu\text{mol/l}$ in dairy calves (BOMBIK *et al.* 2006). A decline in zinc level in calves fed milk replacer observed in the present study was in accordance with the report of JAGOŠ *et al.* (1981) who showed a marked decrease of blood plasma zinc concentration in calves fed milk replacer between the 7th (39.42 $\mu\text{mol/l}$) and 14th day (26.32 $\mu\text{mol/l}$). The authors postulate that high plasma concentrations of this electrolyte in calves on the 7th day was connected with intensive feeding during the first 6 days of life, whereas a decline in its concentration was caused by increased liver storage capacity for zinc and also lower bioavailability of Zn in milk replacer when compared to milk.

The results of this study indicate that calves fed milk replacer had lower blood plasma copper concentrations when compared to calves fed mother's milk. It is noteworthy that the blood plasma Cu concentration was within reference values in both groups of calves (WINNICKA 2008).

The relatively high blood plasma Zn and lower Cu concentrations demonstrated in the present study in both treatments could be the result of competition between these two trace elements for intestinal absorption (OESTREICHER & COUSINS 1985). Moreover, the low levels of blood plasma copper may be caused by substantial utilization of this trace element in metabolic processes, e.g. haemopoiesis (LÖNNERDAL 1996). The decrease in blood plasma copper concentration in calves fed milk replacer observed in the current study may be related with limited utilisation of Cu from milk replacer.

Results from this study suggest that differences in concentration of indicators reflecting nitrogen metabolism were due to different types of ingested food. Changes of these parameters in calves fed milk replacer are linked to advantageous catabolic changes. Stable concentrations of the main extracellular fluid electrolytes and blood plasma osmotic pressure were found in both groups of calves during the second week of their life. Constant blood plasma calcium, magnesium, zinc and copper concentrations during this study might also indicate the relative maturity of mechanisms which maintain water and electrolyte balance. Nevertheless, it seems justified to monitor the plasma copper concentrations in young calves.

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