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SELECTED HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF THE BLOOD PLASMA OF CALVES DURING THE FIRST MONTH OF LIFE BEFORE AND AFTER THE ADMINISTRATION OF MILK REPLACER SUPPLEMENTED WITH LACTOSE

WYBRANE PARAMETRY HEMATOLOGICZNE I BIOCHEMICZNE OSOCZA KRWI CIELĄT W PIERWSZYM MIESIĄCU ŻYCIA PRZED PODANIEM PREPARATU MLEKOZASTĘPCZEGO Z DODATKIEM LAKTOZY I PO JEGO PODANIU

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Streszczenie. Przeanalizowano wybrane parametry hematologiczne i biochemiczne osocza krwi cieląt w pierwszym miesiącu życia przed podaniem wraz z preparatem mlekozastępczym dodatkowej ilości laktozy i po jego podaniu. Doświadczenie przeprowadzono na 8 buhajkach rasy polsko-fryzyjskiej odmiany czarno-białej. Krew pobierano przed porannym karmieniem w okresie od 6 do 8, od 13 do 15 i od 20 do 22 dnia życia. W 6, 13 i 20 dniu życia podczas wieczornego karmienia oraz w 7, 14 i 21 dniu życia podczas porannego karmienia do preparatu mlekozastępczego dodawano jednowodną laktozę w ilości $1g \cdot kg^{-1}$ masy ciała. Na podstawie uzyskanych wyników stwierdzono, że u wszystkich cieląt w 3 grupach wiekowych dwukrotne podanie preparatu mlekozastępczego z dodatkiem laktozy spowodowało najprawdopodobniej przyspieszony pasaż treści jelitowej i dodatkowe straty wody wraz z kałem, których objawem były częste i luźne stolce o charakterystycznym ciemnozielonym zabarwieniu. Pomimo obserwowanych zaburzeń pokarmowych u cieląt nie stwierdzono zmian w stężeniu białka całkowitego (TP), albuminy (Alb), glukozy, sodu (Na), potasu (K) chlorków (Cl), miedzi (Cu), fosforu nieorganicznego (iP), magnezu (Mg) oraz hematokrytu (Ht) i średniej objętości krwinek czerwonych (MCV). W pierwszym tygodniu życia po podaniu cielętom dodatkowej porcji laktozy istotnie obniżyło się stężenie żelaza (Fe) oraz cynku (Zn).

Słowa kluczowe: cielęta noworodki, laktoza, wskaźniki hematologiczne i biochemiczne.
Key words: dietary lactose, haematological and biochemical indicators, newborn calves.

INTRODUCTION

The greatest economic losses in large herd cattle breeding occur during the rearing of calves. Particularly difficult are the first 2–3 weeks of life. According to statistical data of many countries, from 12.5 to 30% of the animals die during this period. It is assumed that the main cause of high mortality in neonatal calves is diarrhea. It usually occurs in the first

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two weeks of life, and it affects on average of 15% of the calves (Singh et al. 2009). Despite the progress in preventive treatment, diagnostic methods and new concepts of antibiotic and supportive treatments, many cattle herds both beef and dairy, are still struggling with this problem. The cause of failure in reducing the morbidity and loss of animals because of this problem is a great complexity of invasive and infectious agents that cause diarrhea. Polyetiological nature of this disorder makes the prevention and treatment of diarrhea in the herd a very difficult task that requires consideration of multiple factors. It is well known that the main causes of diarrhea in neonatal calves are enterotoxins of *E. coli*, rotaviruses, *Cryptosporidium parvum*, and salmonellas (Lorenz et al. 2011). However, apart from infectious factors in the etiology of diarrhea in newborn calves, an important role is also played by environmental and dietary factors. Poor zoohygienic conditions and incorrect feeding of young animals, significantly contribute to an increase of the incidence of diarrhea (Singh et al. 2009). Milk and milk replacers are a separate group, often underestimated by breeders, that triggers digestive disorder among calves after colostrum feeding. A mistake often made by farmers, is overfeeding and associated excessive administration of lactose. It is generally known that lactose is an osmotically active disaccharide present in the milk of all mammals. When calves are overfed or improperly fed with milk replacers, undigested lactose accumulation occurs in the intestinal lumen and osmotic pressure increases, which leads to the accumulation of water and formation of osmotic diarrhea (Olchowy et al. 1993; Mattar et al. 2012). This additional water loss caused by an excess of lactose in the diet can lead to disturbed homeostasis, reflected in changes in hematological and biochemical indicators of blood plasma. There are many published studies that discuss the cause of the diarrhea in newborn calves (Gutzwiller 2000; Cambie et al. 2001; Seifi et al. 2006; Singh et al. 2009). However, there are no studies that would fully consider influence of excess of lactose in the diet on digestive disorders. Therefore, the current study presents the analysis of selected hematological and biochemical parameters of the blood plasma in calves during the first month of life that were administered additional portion of lactose in the milk replacer.

MATERIAL AND METHODS

Eight male Polish-Friesan var. Black-and-White calves were used for the present experiment. They were fed colostrum until 3 days of age at dairy farm, transported to the Faculty of Biotechnology and Animal Husbandry at West Pomeranian University of Technology in Szczecin (Poland) at the 3rd day of life. The animals were held in single pens and were fed with the commercial milk replacer – Mlekovit Imupro®, manufactured by Polmass, Poland. Milk replacer contained 23% crude protein, 16% crude fat, 0.1% crude fibre, 45% lactose, 7.5% crude ash, 1.7% lisine, 0.42% methionine, 0.9% calcium, 0.7% phosphorus. The calves were fed milk replacer at 10% of their body weight, twice a day. During the experiment the calves did not have access to water. Blood samples were collected each day before the morning feeding, in three experimental periods: from 6th to 8th, from 13th to 15th and from 20th to 22nd day of life. In the evening on the 6th, 13th and 20th day of experiment and in the morning on the 7th, 14th and 21st day of experiment, monohydrate lactose (Pharma Cosmetic) in amount of 1g per 1 kg of body weight were added into the milk replacer. Hemoglobin (Hb), hematocrit (Ht) and mean corpuscular volume of erythrocytes (MCV) was determined in the whole blood using a haematology analyzer Sysmex F-80000. The concentration of total protein (TP), albumin (Alb) and glucose in blood plasma was determined spectrophometrically (Power-Wave XA, Bio Tek) with the aid of already manufactured colourometric test kits (BioRad, BioMaxima). Blood plasma was analyzed for iron (Fe), zinc (Zn), copper (Cu), magnesium (Mg), inorganic phosphorus (iP), sodium (Na), potassium (K) (inductively coupled plasma optical emission spectrometry, Optima 2000 DV spectrometer, Perkin Elmer Inc). Mean values and standard deviations were calculated. The resulting data were analysed by an ANOVA with repeated measurements and Dunkan multiple range post hoc test (Statistyca, 10.0™) in order to test significance of differences. In this paper attention was especially paid to an effect of lactose doses on given parameters within highlighted life period.

The use and handling of animals for this experiment was approved by the Local Commission of Ethics for the Care and Use of Laboratory Animals (No. 3/2010 of 14.01.2010).

RESULTS

The results obtained from an experiment are shown in Table 1. In all calves, after two administrations of additional quantity of lactose with milk replacer, loose stools have been observed with a distinctive dark green color. In addition, a small amount of mucus have been detected in feces. Increased excretion of looser stools was more prominent in the 2nd and 3rd week of life of calves.

There were no significant differences in the average concentrations of TP, Alb, glucose, Na, K, Cl, Cu, iP, Mg as well as Ht, Hb and (MCV) (Table 1). In the first week of life, after two administrations of milk replacer with the addition of lactose, despite the large interindividual differences, a statistically significant ($P \leq 0.05$) reduction of Fe concentration was shown in the blood plasma of the tested calves (Table 1). In the next two weeks, the level of this ion remained relatively stable and averaged 23.35 $\mu mol \cdot l^{-1}$. After double administration of milk replacer supplemented with lactose in the 7th and 8th day of life, statistically significant ($P \leq 0.05$ after 1st dose of lactose and $P \leq 0.01$ after 2nd dose of lactose) reduction was observed in the level of zinc in blood plasma (Table 1). On the 2nd and 3rd week, the concentration of this microelement did not change before or after the supply of an additional quantity of lactose and averaged 21.67 $\mu mol \cdot l^{-1}$.

DISCCUSION

We speculate that the dark, loose stools with addition of mucus in calves, which have been observed during the experiment, were most likely the result of the accelerated passage of digestive tract content caused by an excess of lactose in the diet. We assume, that the dark green color could be associated with increased level of bilirubin in the feces, which due to a faster flow of digestive tract content has not been converted to urobilinogen.

Table 1. Haematological and biochemical parameters of blood plasma in calves during the first three weeks of life before and after administration of milk replacer with the addition of lactose

Tabela 1. Hematologiczne i biochemiczne parametry osocza krwi cieląt w pierwszych 3 tygodniach życia przed podaniem preparatu mlekozastępczego z dodatkiem laktozy i po jego podaniu

Parameter Parametr	1st week of life – 1 tydzień życia			2nd week of life – 2 tydzień życia			3rd week of life – 3 tydzień życia		
	before lactose addition przed podaniem laktozy	after lactose addition po podaniu laktozy		before lactose addition przed podaniem laktozy	after lactose addition po podaniu laktozy		before lactose addition przed podaniem laktozy	after lactose addition po podaniu laktozy	
	day – dzień 6 (A)	day – dzień 7 (B)	day – dzień 8 (C)	day – dzień 13 (A)	day – dzień 14 (B)	day – dzień 15 (C)	day – dzień 20 (A)	day – dzień 21 (B)	day – dzień 22 (C)
Total protein – Białko całkowite (TP) [$g \cdot l^{-1}$]	5.49 ± 0.90	5.30 ± 0.91	5.07 ± 0.58	5.11 ± 0.78	5.30 ± 0.30	5.43 ± 0.34	5.39 ± 0.62	5.45 ± 0.84	5.47 ± 0.71
Albumin – Albumina (Alb) [$g \cdot l^{-1}$]	3.56 ± 0.08	3.53 ± 0.21	3.50 ± 0.20	3.54 ± 0.14	3.59 ± 0.17	3.50 ± 0.21	3.51 ± 0.11	3.42 ± 0.21	3.39 ± 0.14
Glucose – Glukoza [$mmol \cdot l^{-1}$]	4.47 ± 0.60	4.45 ± 0.67	4.23 ± 0.79	4.10 ± 0.47	4.12 ± 0.43	4.33 ± 0.32	4.06 ± 0.42	4.42 ± 0.43	4.04 ± 0.88
Hematocrit – Hematokryt (Ht) [$l \cdot l^{-1}$]	0.295 ± 0.041	0.229 ± 0.048	0.296 ± 0.038	0.285 ± 0.029	0.289 ± 0.043	0.283 ± 0.042	0.280 ± 0.036	0.289 ± 0.032	0.275 ± 0.028
Hemoglobin – Hemoglobina (Hb) [$mmol \cdot l^{-1}$]	6.10 ± 1.44	5.91 ± 0.76	5.86 ± 0.87	5.79 ± 0.77	5.65 ± 0.77	5.63 ± 0.64	5.88 ± 0.43	5.852 ± 0.55	9.34 ± 0.83
MCV [fl]	38.2 ± 2.3	38.3 ± 2.2	37.1 ± 1.6	37.7 ± 1.6	38.4 ± 3.1	38.0 ± 3.6	37.3 ± 3.9	37.6 ± 3.6	37.4 ± 3.5
Sodium – Sód (Na) [$mmol \cdot l^{-1}$]	133.0 ± 7.4	131.0 ± 4.8	134.0 ± 5.3	129.4 ± 4.4	132.7 ± 7.6	129.7 ± 6.5	137.3 ± 3.2	136.4 ± 4.7	138.7 ± 6.2
Potassium – Potas (K) [$mmol \cdot l^{-1}$]	4.09 ± 0.31	4.20 ± 0.31	4.00 ± 0.38	3.96 ± 0.22	3.96 ± 0.33	3.80 ± 0.23	4.04 ± 0.18	4.13 ± 0.14	4.12 ± 0.30
Chloride – Chlorki (Cl) [$mmol \cdot l^{-1}$]	96.83 ± 5.75	97.43 ± 3.53	96.82 ± 3.34	96.83 ± 2.66	97.25 ± 3.65	96.00 ± 4.68	98.12 ± 2.50	98.00 ± 3.27	98.29 ± 2.68
Iron – Żelazo (Fe) [$\mu mol \cdot l^{-1}$]	29.01 ± 6.80^{c}	24.7 ± 10.84	18.9 ± 9.28^{a}	23.88 ± 6.72	22.74 ± 3.65	24.80 ± 5.68	21.91 ± 6.24	21.94 ± 10.25	24.80 ± 12.67
Zinc – Cynk (Zn) [$\mu mol \cdot l^{-1}$]	22.47 ± 4.21^{b,C}	17.62 ± 3.63^{a}	17.10 ± 6.47^{A}	21.59 ± 5.35	21.80 ± 5.26	21.87 ± 2.24	22.58 ± 3.78	21.82 ± 4.50	20.37 ± 4.74
Copper – Miedź (Cu) [$\mu mol \cdot l^{-1}$]	13.71 ± 2.56	14.50 ± 2.53	14.69 ± 2.89	13.62 ± 1.34	14.17 ± 1.79	14.70 ± 2.01	14.59 ± 1.55	14.11 ± 1.98	13.70 ± 2.66
Inorganic Phosphorus Fosfor nieorganiczny (iP) [$mmol \cdot l^{-1}$]	3.56 ± 0.13	3.57 ± 0.04	3.67 ± 0.21	3.76 ± 0.33	3.77 ± 0.29	3.69 ± 0.24	3.69 ± 0.35	3.66 ± 0.32	3.62 ± 0.30
Magnesium Magnez (Mg) [$mmol \cdot l^{-1}$]	0.901 ± 0.027	0.917 ± 0.028	0.909 ± 0.077	0.938 ± 0.043	0.949 ± 0.038	0.932 ± 0.032	0.934 ± 0.042	0.927 ± 0.035	0.923 ± 0.047

[a,b,c] Significance of differences ($P \leq 0.05$) of means particular days of live in the experimental periods – Różnice statystycznie istotne ($P \leq 0.05$) pomiędzy średnimi w poszczególnych dniach w danym okresie eksperymentalnym.

[A,B,C] Significance of differences ($P \leq 0.01$) of means particular days of live in the experimental periods – Różnice statystycznie istotne ($P \leq 0.01$) pomiędzy średnimi w poszczególnych dniach w danym okresie eksperymentalnym.

The looser stool consistency was most likely caused by the increased water content, which was absorbed into the intestinal lumen because of remaining undigested lactose. More frequent and looser stools observed in calves in 2nd and 3rd week of life were most likely caused by lower digestibility of lactose in this period. It is known that the activity of lactase, enzyme hydrolyzing lactose into glucose and galactose, is highest in the first days of life of calves and then decreases with age (Guy et al. 1990; Gutzwiller 2000).

In the calves tested, during the first 3 weeks of life, total protein concentration and albumin did not change prior and after administration of an additional quantity of lactose with milk replacer. In calves with diarrhea, statistically significant increase of TP and Alb levels was observed in blood plasma due to large losses of water and associated dehydration (Paré et al. 1993; Seifi et al. 2006). In the experimental calves, supply of additional quantity of lactose did not increase any of these indicators, which might indicate the lack of a significant increase in water excretion in feces, which in turn, might have a significant impact on the change in volume of blood plasma. Total protein values obtained in the present study are consistent with the results of other studies and typical of calves fed milk replacer (Mohri et al. 2007; Lepczyński et al. 2011; Klinkon and Ježek 2012). According to Mohri et al. (2007), in calves during the neonatal period, levels of TP and Alb depend on, among others, the type and quantity of food intake and digestibility of protein in the diet. In suckling calves, compared with calves fed with milk replacer, both of these indicators in blood plasma stay at a higher level (Hammon et al. 2002; Al-Shami 2007). The relatively low concentration of total protein and albumin observed in calves in the current experiment was probably associated with a lower digestibility of protein derived from milk replacer. Our supposition confirms the results obtained by Coppo et al. (2003).

The concentration of glucose in the blood plasma of calves prior and after the administration of additional quantity of lactose remained relatively constant and fell within the reference standards for this species (Knowles et al. 2000). In the period analyzed, there were also no changes noticed in hematocrit, hemoglobin concentration and the mean corpuscular volume of erythrocytes. One of the most straight forward hematological parameters both in execution and interpretation is Ht, which allows a preliminary assessment of dehydration. During diarrhea and progressive dehydration, this parameter in calves may reach more than 50% (Seifi et al. 2006). During hypertonic dehydration that accompanies diarrhea in calves, MCV is significantly reduced (Dărăbus et al. 2009), while hemoglobin level is increased (Cambie et al. 2001). In the experimental calves in all three periods of the study, there was no increase in hematocrit and hemoglobin or a decrease in the MCV, which could indicate dehydration. It is worth noting that the observed MCV levels throughout the experiment were lower than that observed in a study by Klinkon and Ježek (2012) at the same time points.

Sodium, potassium and chlorides are the main electrolytes of aqueous spaces of the organism. The concentration of these ions and their relative proportions constitute the osmotic pressure of blood plasma. Calves during diarrhea lose large amounts of Na, Cl and K. The loss of sodium is relatively equal or greater compared to the amount of water loss, resulting in reduced concentration of Na in the blood plasma. The level of Cl is also decreased (Seifi 2006). However, despite the large losses of K in feces and urine of diarrhoeic calves, the level of this cation is often elevated in the plasma. An increase in K concentration occurs in response to a progressive acidosis during diarrhea and transport of K from the intra- to the extracellular fluid (Seifi 2006). In the experimental calves, despite the frequent and loose stools observed after the supply of additional quantity of lactose with milk replacer, there were no significant changes in levels of Na, Cl and K, which may indicate the lack of a significant loss of these electrolytes and water with feces.

The Fe concentration in the blood plasma in neonatal calves is the result of many factors. Among them we should mention iron accumulated in the liver during fetal life, the intensity of the erythropoiesis, absorption capacity of the intestinal mucosa (duodenum in particular) as well as feeding calves with milk replacer or mother's milk. According to the studies on piglets and rats, it is known that the molecular mechanisms of neonatal Fe absorption are not fully developed and the expression of key iron transporters such as DMT1 (divalent metal transporter 1) and ferroportin is very low (Leong et al. 2003; Lipiński et al. 2010). During the first days of life of calves, many authors observed a decreasing level of Fe in the blood plasma followed by a gradual increase in the concentration of this microelement with age (Knowles et al. 2000; Klinkon and Ježek 2012). These authors suggest that the reduction in the concentration of Fe in the first days of life of calves is primarily associated with increased erythropoiesis and non-suffcient supply of Fe with food. It is difficult to assess unambiguously the influence of excess of lactose in the diet on a significant decrease of iron observed in the 1st week of life of calves in the present experiment. Most likely it was a result of reduced Fe absorption in the intestines due to accelerated intestinal passage and intensified process of erythropoiesis in the first days of life of calves. Lack of change in the concentration of iron after the supply of additional amounts of lactose in the 2nd and 3rd week of life may be associated with an increasing with age ability to absorb this microelement from the intestines.

According to Ranjan et al. (2006), Zn level in the blood plasma of diahorrhoeic calves was significantly reduced, while Cu concentration was elevated. According to these authors, a decrease in the concentration of zinc in the blood plasma was due to the low absorption of this element from the intestine, large losses of Zn in feces and an increased demand for zinc of the immune system. Moreover, it was also caused by the utilization and sequestration of zinc at the tissue level for the synthesis of antioxidant enzymes as a compensatory mechanism to counter the excessive free-radical production (Ranjan et al. 2006). Significant reduction of the zinc concentration in the tested calves in the first week of life may also be associated with reduced absorption of this element from the intestine, due to the increased motility of the digestive tract as well as increased excretion of Zn in feces. Lack of changes in the concentrations of zinc in the blood plasma in the 2nd and 3rd week of life of calves is difficult to explain. Possibly, similarly to the concentration of Fe, calves also develop better ability to absorb intestinal Zn.

The plasma concentration of iP found in the present study was higher than that observed by Underwood and Suttle (2001). According to these authors, normal plasma concentration of iP in calves should be within 1.3–1.9 mmol $\cdot$ l^{-1}. However, iP levels determined in the current study are consistent with the results of Kraft et al. (1999). The latter authors stated that the average iP concentration till the 2nd month of life was 2.6–3.5 mmol $\cdot$ l^{-1}. According to Rosol and Capen (1997), a higher concentration of plasma iP in young calves is due to increased reabsorption of phosphate in the kidney.

RECAPITULATION

In conclusion of the conducted experiment, it can be said that in all calves in three age groups (in the 1st, 2nd and 3rd week of life), two-time administration of milk replacer with the addition of lactose, most likely caused rapid passage of intestinal content and additional water loss in feces, which manifested in frequent and loose stools of distinctive dark green color. Although digestive disorders were observed in calves studied, no changes were detected in the concentrations of total protein, albumin, glucose, sodium, potassium, chloride, copper, inorganic phosphorous, magnesium as well as hematocrit, haemoglobin and mean corpuscular volume red blood cells. Lack of change in these hematological and biochemical blood plasma indicators after double administration of lactose (1 g · kg^{-1} body weight) in milk replacer, suggests no significant effect of this modified diet on homeostasis of calf organisms in the 1st month of life. Calves in the first week of life, after the supply of additional portion of the lactose, had significantly reduced concentration of iron (Fe) and zinc (Zn). However it is difficult to unambiguously assess the effect of the addition of lactose to the diet on changes in these biochemical parameters of blood plasma. Most likely, the observed decrease in the level of Fe and Zn could be a result of reduced absorption of these microelements from the intestine resulting from accelerated intestinal passage, caused by the excessive lactose supply.

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Abstract. The current study presents an analysis of selected hematological and biochemical parameters of the blood plasma of calves during the first month of life, before and after administration of a milk replacer with additional quantity of lactose. The experiment was performed on 8 male Polish-Friesan calves var. Black-and-White. Blood samples were collected before the morning feeding from 6th to 8th, from 13th to 15th and from 20th to 22nd day of life. In the evening on the 6th, 13th and 20th day of the experiment and in the morning on the 7th, 14th and 21st day of the experiment, monohydrate lactose in an amount of 1g per 1 kg of body weight was added into the milk replacer. In all calves in three age groups, two-time administration of milk replacer with the addition of lactose, most likely caused rapid passage of intestinal content and additional water loss in feces, which manifested in frequent and loose stools of distinctive dark green color. Although digestive disorders were observed in the calves studied, no changes were detected in the concentrations of total protein (TP), albumin (Alb), glucose, sodium (Na), potassium (K) chloride (Cl), copper (Cu), inorganic phosphorous (iP) magnesium (Mg) as well as haematocrit (Ht) and mean corpuscular volume of erythrocytes (MCV). Calves in the first week of life, after the supply of additional portion of lactose, had significantly reduced concentration of iron (Fe) and zinc (Zn).

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