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Original article

The effect of $MgSO_4$ addition and the increasing doses of calcium and phosphorus during ending drying period on the occurrence of hypocalcaemia and hypophosphataemia in dairy cows

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Abstract

The aim of the presented study was the estimation of optimal Ca and P levels applied before calving together with anionic salt addition, as an element of hypocalcaemia and hypophosphataemia prevention.

The experiment was carried out during the dry period on 48 cows with similar milk yield in the previous lactation. Cows were divided into four groups. In group I (control) the amount of minerals was in accordance to NRC standards. In experimental groups (groups II-IV), two weeks before calving, cows received 140 g/day/head of hydrated magnesium sulphate to achieve dietary cation-anion difference at the level of about 50 mEq/kg DM. In groups II and III cows received calcium carbonate (100 g/day) 10 days a.p. (antepartum) (group II), or 5 days a.p. (group III), while cows in IV group received dicalcium phosphate (100 g/day) for 5 days a.p. Application of $MgSO_4 \times 7H_2O$ significantly affected the urine pH of cows from group III and IV 4-5 d. before calving – 6.45 and 6.81, respectively. The acidification of urine was observed after calving in group IV (7.13). In cows from group II (100 $CaCO_3$ 10 days a.p.) urine pH decline was not found (7.97-7.75). In that group the incidences of hypophosphatemia were noted (blood serum inorganic P level 1.41-1.46 mmol/l). Addition of magnesium sulphate prevented hypocalcaemia occurrence – 4-5 d. before calving the concentration of ionized Ca in blood serum was 1.11, 1.13 and 1.16 mmol/l (respectively for group II, III and IV). Reproductive functions were significantly improved after the application of $CaCO_3$ and $CaHPO_4$ for 5 days a.p. in comparison with control and group II – progesterone concentration in the blood serum on the 45th day of lactation was 1.396 – 1.409 vs 0.799 – 0.401. The correlation between progesterone and inorganic P level in serum was almost significant. Based on the obtained results a treatment optimal in prevention of hypocalcaemia and hypophosphataemia is the application of 50 g $CaCO_3$ and 50 g of $CaHPO_4$ for the last 5 days of the dry period together with $MgSO_4 \times 7H_2O$ given for 14 days a.p.

Key words: dairy cows, hypocalcaemia, hypophosphataemia, fertility, anionic salts

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Introduction

According to Bell et al. (1995), the occurrence of metabolic disorders during periparturient period, including Ca and P deficiency, are caused by a rapid increase in the cows' needs for nutrients at the onset of lactation. These components are absorbed from feeds or the organism's stock, but could be insufficient for high-producing diary cows, because hypocalcaemia leads to a decrease of dry matter intake (Goff and Horst 1997, Chappi 2003, Goff 2006). Hypocalcaemia appears in two forms: subclinical, when blood serum Ca level decreases below 2 mmol/l or clinical (known as periparturient paresis, milk fever) when blood serum Ca level decreases below 1.375 mmol/l. Milk fever is associated with great decrease of dry matter intake and with other disorders including muscles function reduction, retained placenta, displacement of abomasum, ketosis and fat liver, immunosuppression and mastitis (Goff 2006, Gray et al. 2007, Grummer 2008).

The physiological level of inorganic phosphorus in cows' blood serum varies in range of 1.00-2.71 mmol/l, however, sometimes a narrow scope (1.5-2.1 mmol/l) is accepted. Phosphorus deficiency (hypophosphataemia) frequently appears in cows after calving accompanied by ketosis, fertility disorders as well as hypocalcaemia diseases (Gelfert and Staufenbiel 2008). Phosphorus is associated with adenosine triphosphate (ATP) metabolism and its deficiency could cause disturbances of all life functions of an organism. Phosphorus absorption in ruminants is high and amounts to about 70-80% but half of rumen phosphorus originate from endogenic saliva phosphorus (Kinal et al. 2004). Absorption is not high in dry cows (Kinal et al. 1996). Practical observation shows that periparturient paralysis could also be caused by hyphophosphatemia only, while calcium and magnesium level in blood serum is normal. High daily ration of Ca and Mg decrease the absorption of P (Kinal and Preś 1995).

The amount of phosphorus in high milking dairy cows diet during dry period should be determined as about 0.4% of DM, but should not exceed 80 g per animal per day, because it could block production of vitamin D precursors and cause milk fever occurrence (Kinal et al. 1996, Goff and Horst 1997, Petersen et al. 2005, Twardoń et al. 2006, Ramos-Nieves et al. 2009, Bodarski et al. 2010).

Numerous authors (Goff and Horst 1997, Oetzel et al. 1988, Kinal et al. 2005) pointed out the strong anion (Cl^- i SO^{4-}) action in prevention of hypocalcaemia. The anions slightly acidify the cow's organism (decreasing blood pH by about 0.02-0.04 units) and eliminate physiological metabolic alkalosis which decreases Ca resorption from bones, synthesis of vitamin D_3 (1, 25 OH_2 D_3) as well as the absorption of Ca from the digestive tract. Because all of these processes are directly or indirectly regulated by parathyroid hormone (PTH), general conclusion is that metabolic alkalosis plainly reduce PTH activity. The anion in the form of $MgSO_4$ prevents the immune suppression (Goff 2006, Grey et al. 2007).

Dietary cation-anion balance (DCAB) is a practical measure of anions in dry cows' rations. Decreasing of DCAB values cause higher Ca excretion in urine (Beening 1998, Vagnoni and Oetzel 1998) therefore scientists have suggested to increase the amount of Ca in cows' ration 2 weeks ante partum (a.p.) to 100 – 150 g daily, when anion salts were applied.

The aim of the presented study was the estimation of the optimal Ca and P rations (both the amount and the length of administration) applied before calving together with anionic salt addition, as an element of hypocalcaemia and hypophosphataemia prevention.

Materials and Methods

The experiment was conducted in a tie-stall cowshed on 360 dairy cows, lowland Black- and-White breed upgraded with about 90% of HF genes and an average milk yield for previous lactation of about 9500 kg of milk. The fertility of the herd (based on fertilization after the first insemination, the insemination index, period between pregnancies) fitted the accepted norms. In feed samples, the basic nutrients were estimated according to AOAC (2006) methods as well as minerals – Ca, Mg, K, Na using atomic spectrophotometry (ASA, Varian). Phosphorus was analysed after previous wet mineralization with nitric acid (HNO_3) and perchloric acid ($HClO_4$) by the ammonium vanadomolybdate method (Fick at al. 1979) using spectrophometer Specol 11 (Carl Zeiss, Jena) at a wavelength of 470 nm, sulphur – nephelometrically according to Bardsley and Lancaster (1960) method and Cl according to Polish standard PN-81/R-64780.

Obtained analytical data allowed calculation of nutritive value of diets for cows according to INRA standards (1988), balance of minerals according to NRC (2001) as well as DCAB calculation according to the following equation DCAB = (Na % DM/0.0023+ K % DM/0.0039)-(S % DM/0.0016+Cl % DM/ /0.00355) – Table 2.

The experiment was started during the dry period on 48 multiparous cows (in the 3rd and 4th lactation) with similar milk yield for the previous lactation. Cows, according to the analogue method, were divided into four groups (12 heads per each). In group I (control) the amount of minerals was in accordance

Table 1. Experimental design.

Kind of applied calcium and magnesium salts	Feeding groups			
	I – control	experimental		
		II	III	IV
$MgSO_4$ 140 g/day/head	–	2 weeks before calving		
$CaCO_3$ 100 g/day/head	–	10 days a.p.	5 days a.p.	–
$CaHPO_4$ 100 g/day/head	–	–	–	5 days a.p.

Table 2. Daily rations for dry cows acc. to INRA, mineral supplementation acc. to NRC.

Feedstuffs	Amount in ration (kg)
Corn silage (31.06% DM)	11
GPS (barley) (36.02% DM)	6
Alfalfa silage (36.33% DM)	6
Sugar beet pulp silage (23.07% DM)	3
Brewers' grains pulp (26.03% DM)	2
Meadow hay	2
Wheat straw	1

Nutritive value of ration (pro head and day)

	DM (kg)	UFL	PDIN (g)	PDIE (g)	Ca (g)	P (g)	Mg (g)	Na (g)	K (g)	Cl (g)	S (g)	DCAD (meq/kg DM)
	10.6	7.56	611	690	68	38	10	19	159	65	23	154
After application of additives				gr. II	108	38	24	19	159	65	41	47
				gr. III	108	38	24	19	159	65	41	47
				gr. IV	97.5	61	24	19	159	65	41	47

to NRC standards (2001). In experimental groups (groups II-IV) two weeks before calving, cows received a hydrated magnesium sulphate ($MgSO_4 \times 7H_2O$) in amount of 140 g/day/head, in aim to achieve dietary cation-anion difference on the level of 47 mEq/kg DM.

The feeding groups (II, III and IV) were diversified with respect to calcium source and the length of its supplementation to the cows diet. In groups II and III cows received calcium carbonate ($CaCO_3$) in amount of 100 g/day/head for 10 days a.p. (group II) or for 5 days a.p. (group III) while cows in IV group received calcium phosphate ($CaHPO_4$) in amount of 100 g/day/head for 5 days a.p. (Table 1). Dry period lasted 8 weeks. The basic diet was identical in all of the groups. From the 3rd week to week 0 before calving concentrate mixture was added to the ration in the amount of 1.5 to 4.5 kg and increased by 1.5 kg per each week before calving. All cows have received basic mineral-vitamin mixture in amount of 100 g/day/head.

Two weeks before calving, 4-5 days before calving and on the 1st or the 2nd day after calving the twenty-four hours urine production was collected from each cow. The pH of urine was determined by a manual pH-meter, minerals: Ca, Mg, Na and K using atomic spectrophotometer, P photometrically as well as Cl using Humana kits (Humana, Wiesbaden, Germany) were determined.

Four to five days before calving jugular vein blood samples were taken before the morning feeding from all of the cows. In these samples pH as well as indices of acid-base balance were determined according to the Astrup method using Corning -286 apparatus. Moreover, in blood serum, the concentration of Ca, $P_{inorganic}$ and Mg was determined using biochemical kits EMAPOL (E10011, E10027, 10010, respectively). On the 1st or 2nd day after calving, the content of the mentioned minerals was assayed in the blood serum of all of the cows.

Between the 15th and 21st day and on the 45th day of lactation, before morning feeding, blood samples from the jugular vein were taken and the concentration of progesterone in the blood serum was determined using Enzyme Linked Fluorescent Assay (mini-VIDAS instrument, bioMérieux, Inc, USA).

On the 45th day after calving some biochemical indices in the blood serum were determined for estimation of liver condition and metabolic pathways – concentration of NEFA, betahydroksybutiric acid, AlaT and AspAt – with an instrumental method using

Table 3. Urine pH values and minerals excretion (g/day/head) in cows' urine.

Item	Feeding groups				P=
	I – control	II	III	IV	
			pH		
2 w. before calving	8.01 ± 0.44	7.97 ± 0.75	8.09 ± 0.34	8.00 ± 0.87	0.2156
5-4 d. before calving	8.06^{Aa} ± 0.45	7.97^{Aa} ± 0.22	6.75^{Bb} ± 0.96	6.81^{Bb} ± 0.84	0.0089
1-2 d. after calving	7.66^{ABab} ± 0.68	7.75^{ABa} ± 0.49	7.98^{Aa} ± 0.52	7.13^{Bb} ± 0.86	0.0076
			Calcium excretion g/day/head		
2 w. before calving	2.10 ± 0.32	2.70 ± 0.88	1.65 ± 0.42	1.95 ± 0.51	0.4560
5-4 d. before calving	1.80^{Aa} ± 0.24	6.90^{Bb} ± 1.25	8.85^{Bc} ± 1.18	8.10^{Bc} ± 1.47	0.0042
1-2 d. after calving	8.10 ± 7.35	9.60 ± 8.72	7.95 ± 9.34	7.95 ± 6.31	0.0802
			Phosphorus excretion g/day/head		
2 w. before calving	0.97 ± 0.17	1.08 ± 0.27	0.96 ± 0.26	1.08 ± 0.15	0.1025
5-4 d. before calving	0.96 ± 0.28	0.96 ± 0.15	0.99 ± 0.26	0.98 ± 0.24	0.6583
1-2 d. after calving	0.99 ± 0.22	0.87 ± 0.10	0.94 ± 0.23	0.96 ± 0.13	0.4599
			Magnesium excretion g/day/head		
2 w. before calving	5.25 ± 1.31	5.55 ± 1.07	4.95 ± 1.63	4.95 ± 1.54	0.0839
5-4 d. before calving	5.40 ± 2.70	5.55 ± 1.05	5.40 ± 1.50	5.54 ± 1.20	0.7767
1-2 d. after calving	4.80 ± 1.35	4.65 ± 1.35	4.65 ± 1.20	4.20 ± 1.05	0.1333
			Sodium excretion g/day/head		
2 w. before calving	6.03 ± 3.15	6.53 ± 3.15	5.55 ± 4.50	6.32 ± 4.80	0.0988
5-4 d. before calving	6.15 ± 4.65	6.75 ± 3.90	7.80 ± 3.45	7.50 ± 3.45	0.2690
1-2 d. after calving	7.50 ± 6.90	11.10 ± 6.15	9.00 ± 5.85	8.70 ± 1.80	0.1476
			Potassium excretion g/day/head		
2 w. before calving	114.40 ± 28.90	121.50 ± 33.60	124.60 ± 27.45	115.2 ± 36.15	0.3206
5-4 d. before calving	117.00 ± 35.25	115.20 ± 37.50	106.10 ± 29.40	120.10 ± 41.40	0.2873
1-2 d. after calving	100.20 ± 41.85	114.90 ± 38.85	121.65 ± 35.70	99.60 ± 39.30	0.1986
			Chloride excretion g/day/head		
2 w. before calving	32.6 ± 11.23	43.59 ± 13.71	44.37 ± 10.55	36.63 ± 15.41	0.1006
5-4 d. before calving	40.13 ± 12.51	46.81 ± 18.35	43.63 ± 17.62	37.41 ± 6.66	0.3475
1-2 d. after calving	39.11 ± 12.25	42.47 ± 19.10	45.55 ± 14.69	47.44 ± 15.96	0.0966

Differences in rows signed with $^{a, b, c}$ – significant by P<0.05, signed with $^{A, B}$ – significant by P<0.01

the automatic biochemical analyzer ABX Pentra 400 (HORIBA, Ltd, Japan).

All data obtained in the experiment were statistically analyzed with a one factor variance analysis using Statistica 9.2. software (StatSoft, Poland) and significance of differences was estimated by means of Duncan's multiple interval test.

Results

Two weeks before calving urine pH of all cows was similar and ranged between 7.97-8.09 (Table 3). However, on the 4th or the 5th day before calving (Table 3) urine pH of experimental cows' decreased significantly (P≤0.01), especially in cows from III and IV groups (6.75 and 6.81, respectively). Urine pH values on 1st and 2nd day after calving (Table 3) were significantly lower (P≤0.01) for cows from group IV than urine pH in cows from group III as well as significantly (P≤0.05) lower than urine pH of cows from group II. Calcium excretion in urine of cows on the 4th – 5th day before calving ranged between 6.90-8.85 g daily with the exception of cows from the control group (Table 3). Moreover, on 1st and 2nd day after calving, cows from group II had a slightly higher calcium excretion – 9.60 g/day (Table 3).

Urine phosphorus excretion before calving was similar and ranged from 0.96 to 1.08 (2 weeks a.p.) and from 0.96 to 0.99 g/day/head (5-4 days a.p.) – Table 3. However, 1-2 days after calving, cows from group II excreted the lowest amount of phosphorus in urine – 0.87 g/day/head, whereas urine phosphorus excretion in the remaining animals ranged from 0.94 to 0.99 of P/day/head (Table 3).

Two weeks before calving and 4-5 days before calving cows from all groups excreted similar amounts of magnesium (Table 3). After calving urine mag-

Table 4. Blood gasometry indices (4-5 days before calving).

Indice	Feeding groups				P=	References according to Baumgartner (2009)
	I – control	II	III	IV		
pH	7.38 ± 0.03	7.35 ± 0.04	7.38 ± 0.04	7.37 ± 0.03	0.8732	7.38-7.32
pCO_2 mmHg	46.1 ± 4.85	50.2 ± 4.07	46.9 ± 4.56	45.1 ± 6.04	0.7621	38-45
pO_2 mmHg	45.8 ± 10.12	38.2 ± 7.12	38.7 ± 5.36	43.3 ± 10.28	0.3245	35-46
Hb g/dl	11.65 ± 0.82	12.03 ± 0.98	11.76 ± 0.67	11.30 ± 0.95	0.2476	8-14
Base (BE) mmol/l	1.28 ± 1.24	1.15 ± 2.40	1.60 ± 1.57	0.30 ± 2.23	0.0987	1-4
Base (Ecf) mmol/l	1.65 ± 1.34	1.87 ± 2.23	2.06 ± 1.42	0.63 ± 2.39	0.4631	–
HCO^{3-} mmol/l	26.3 ± 1.56	26.9 ± 1.89	26.7 ± 1.21	25.3 ± 2.54	0.8973	25-30

Table 5. Minerals concentration in blood serum samples.

Item	Feeding groups				P=
	I – control	II	III	IV	
Calcium mmol/l					
4-5 d. before calving	$2.25^{a} \pm 0.14$	$2.42^{ab} \pm 0.32$	$2.47^{ab} \pm 0.21$	$2.51^{b} \pm 0.31$	0.0418
1-2 d. after calving	$2.16^{a} \pm 0.34$	$2.12^{ab} \pm 0.26$	$2.12^{ab} \pm 0.25$	$1.92^{b} \pm 0.25$	0.0456
Ionized calcium mmol/l					
4-5 d. before calving	$1.04^{a} \pm 0.07$	$1.11^{ab} \pm 0.15$	$1.13^{ab} \pm 0.10$	$1.16^{b} \pm 0.14$	0.0398
1-2 d. after calving	$0.99^{a} \pm 0.15$	$0.97^{ab} \pm 0.12$	$0.97^{ab} \pm 0.12$	$0.88^{b} \pm 0.12$	0.0405
Inorganic phosphorus mmol/l					
4-5 d. before calving	$1.58^{Aba} \pm 0.14$	$1.41^{Aa} \pm 0.22$	$1.74^{Bb} \pm 0.22$	$1.70^{Bb} \pm 0.28$	0.0068
1-2 d. after calving	1.51 ± 0.47	1.46 ± 0.34	1.60 ± 0.36	1.69 ± 0.55	0.1987
Magnesium mmol/l					
4-5 d. before calving	0.86 ± 0.10	0.97 ± 0.20	0.92 ± 0.13	0.91 ± 0.08	0.7890
1-2 d. after calving	$0.77^{Aa} \pm 0.06$	$0.88^{Bb} \pm 0.14$	$0.85^{ABb} \pm 0.08$	$0.81^{ABab} \pm 0.08$	0.0089

Differences in rows signed with $^{a, b, c}$ – significant by P<0.05, signed with $^{A, B}$ – significant by P<0.01

nesium excretion was similar to that obtained 4-5 days before calving and amounted to 4.20 – 4.80 g/day.

The amount of the urine-excreted potassium was several fold higher than sodium, which was caused by the amount of minerals that were ingested in the fodder. Two weeks before calving the highest urine potassium excretion was found in cows from group II and III, whereas 4-5 days before calving that parameter was the highest in animals from group IV (Table 3). However, on days 1-2 after calving, urine potassium excretion was higher in cows from group III and amounted to 121.65 g/day (Table 3).

The amount of excreted chloride in the urine in cows 2 weeks before calving and 4-5 days before calving was on the similar level for all the cows. On the other hand, 1-2 days after calving, excretion of this element ranged between 42-47 g per day and was higher for cows from the experimental groups than for the control ones (Table 3).

Blood gasometry data has shown that gasometry indices – blood pH (7.35-7.38), amount of bicarbonate HCO_3 (25.3-26.9 mmol/l) as well as the base concentration BE (1.15-1.60 mmol/l) remained on the similar level (Table 4). In the blood samples of cows from group IV a decrease of the base levels was observed (0.30 mmol/l).

A slightly higher value of the partial pressure of carbon dioxide (pCO_2) was found in the blood samples of cows from group II (50.2 mmHg). In the blood samples all of the experimental cows high values of partial pressure of oxygen (pO_2) were found, especially in animals from group I and IV (45.8 and 43.3 mmHg).

Calcium concentration in the blood serum samples from experimental cows 4-5 days before calving was higher than its concentration in the blood serum samples of control ones, but remained within the reference values (Winnicka 2008). Similar dependence as mentioned above was found regarding ionized calcium. A slightly higher level of phosphorus was found in the blood serum of cows from group III and IV than in control animals (1.74 and 1.70 vs.

Table 6. Progesterone concentration (ng/ml) in blood serum.

Day of lactation	Feeding groups				P=
	I – control	II	III	IV	
15-21	$0.171^{a} \pm 0.094$	$0.324^{ab} \pm 0.344$	$0.856^{b} \pm 1.481$	$0.396^{ab} \pm 0.423$	0.0371
45	$0.799^{ABa} \pm 0.531$	$0.401^{Bb} \pm 0.973$	$1.396^{A} \pm 1.209$	$1.409^{A} \pm 0.968$	0.0079

Differences in rows signed with $^{a, b}$ – significant by P<0.05, signed with $^{A, B}$ – significant by P<0.01

Table 7. Indices of liver condition and liver metabolic processes in blood serum of cows in the 45th day after calving.

Day of lactation	Feeding groups				P=
	I – control	II	III	IV	
NEFA mmol/l	0.32 ± 0.04	0.34 ± 0.07	0.32 ± 0.08	0.36 ± 0.07	0.8871
BHB mmol/l	0.50 ± 0.18	0.48 ± 0.18	0.49 ± 0.28	0.46 ± 0.21	0.6542
AlaT (U/l)	21.40 ± 0.25	19.24 ± 3.79	20.85 ± 3.98	17.72 ± 4.63	0.2004
AspAt (U/l)	76.67 ± 11.79	72.46 ± 8.94	77.41 ± 20.45	74.98 ± 16.7	0.6062

1.58 mmol/l, respectively). The lowest concentration of this element was found in cows from group II (1.41 mmol/l) and might indicate the presence of hypophosphataemia. Magnesium level in the blood serum of cows from all groups was similar (from 0.86 to 0.97 mmol/l) and remained within the references values by Meyer and Harley (1998).

One to two days after calving both calcium and ionized calcium concentration in the blood serum of cows from group II, III and control were similar (Table 5) and were at the physiological levels. On the other hand, in blood serum of cows from group IV the concentration of calcium and ionized calcium were significantly lower (P≤0.05) in comparison with the control group and remained at the level that might indicate the risk of hypocalcaemia. The amount of phosphorus in the blood serum 1-2 days after calving was quite low (Table 5) especially in animals from the control group and in animals from group II. Magnesium content in the blood serum of cows from experimental groups 1-2 days after calving was similar and was higher in comparison with the control group. Obtained data were within physiological values (Winnicka 2008).

Between the 15th and 21st day of lactation, the average progesterone level in the blood serum of cows from particular groups varied (Table 6), but it did not reach concentration of 1 ng/ml. On the 45th day of lactation the average progesterone level in the blood serum of cows that were receiving calcium carbonate since the 10th day a.p. was significantly (P≤0.05) lower than in the control group and it was also significantly lower (P≤0.01) than in the remaining groups. Average progesterone levels within groups III and IV (over 1 ng/ml) are the evidences of correct estrous cycle of cows from these groups.

Concentration of the blood serum non-esterified fatty acids – NEFA (0.32-0.36 mmol/l), beta-hydroxybutyrate – BHB (0.46-0.50 mmol/l) as well as the values of enzyme activity of alanine transferase – AlaT (17.72-21.40 U/l) and aspartate aminotransferase – AspAt (72.46-77.41 U/l) were similar for all the animals regardless of the group (Table 7) and were in physiological ranges (Winnicka 2008).

Discussion

The decrease of urine pH after application of sulphate was observed in studies of Goff et al. (2004), Peterson et al. (2005) and Chan et al. (2006). Chiappi (2003) suggested, that application of the high amount of calcium (100 g of Ca) could limit anions acidifying effect. Those observations had been confirmed in studies of Horst et al. (1997) where the addition of the high amount of chalk had raised anions action. Therefore, Gelfert and Staufenbiel (2008) had recommended the highest doses of calcium given as sulphates or chlorides, and only part of calcium should be given as carbonates. Recommendation for very high calcium doses (150-180 g/daily) should be found as inappropriate or applied when DCAB is negative (from -100 to -150 meq/kg of DM).

In German and American studies (Fürll et al. 1996, Beening 1998, Vagnoni and Oetzel 1998, Chan et al. 2006) the increasing of urine Ca excretion by 6-7 g/day after anions application was confirmed. In these studies concentration of calcium in rations was not increased, what demonstrated that the additional pool of calcium in urine originated from bone resorption. This is an indirect proof confirming that synthesis of PTH increased and influenced Ca resorption from

bones. After bringing the cows into mild metabolic acidosis Goff and Horst (1997) observed a decrease of PTH concentration in blood with a simultaneous increase of hydroxyproline concentration. An introduction of anionic salts into the diet reduced incidence of hypocalcaemia and milk fever. This preventing action of anionic salts could be intensified by simultaneously supplying Ca in the daily ration (Chiappi 2003).

Application of $CaCO_3$ and phosphate could improve the level of $P_{inorg.}$ in serum, ovary function and had an effect on the increase of blood progesterone on the 45th day post partum (Kawashima et al 2012). But the suggested addition of P a.p. in amount of 35 g/d (Peterson et al. 2005) could be accepted only under the condition that the increase of Ca in the ration occured several days before calving. It is important that the ratio of Ca:P remains in the range of 1.5 – 2:1, as P absorption levels are then optimal. Thus, hypophoshataemia may still occur a.p. with the large amount of dietary Ca and have a negative effect on the process of ovulation.

Goff et al. (2004) suggested that hydrated magnesium sulphate could be used not only as acidifying agent, but also as a source of magnesium. Among the anion salts, this compound has the least effect in decreasing appetite in cows (Goff 2006). Chiappi (2003) suggested that cows receiving anionic salts were exposed to hypomagnesemia and required increase the of magnesium levels even to 4 g/kg of DM. The incidence of postcalving immunosuppression was also lower in cows sufficiently supplied with magnesium, which could improve fertility indices in cows receiving $MgSO_4 \times 7H_2O$ (Goff and Horst 1997, Goff 2006).

In the present studies application of magnesium sulphate during 14 days a.p. favorably decreased pH of urine. High calcium excretion in the urine of cows from group III and IV on the 4^{th} – 5^{th} day before calving, indicated that calcium resorption from bones before calving had started. However, it is worth to note that acidifying action of sulphate was reduced by long application of calcium carbonate (for 10 days a.p. – group II). On the other hand, lowest urine pH that was observed in the group of animals receiving calcium phosphate could be explained by additional (besides sulphate) acidifying action of this anionic salt. Calcium given to cows for the 5 last days before calving, in amount of 100 g as $CaHPO_4$, increased the phosphorus level to the 6 g/kg DM and could be stated as an effective method of hypocalcaemia and hypophosphataemia prevention. The opinion that phosphate anion induces hypocalcaemia concerned the situation when P concentration in the daily ration amounted to a very high level – over 15 g/kg DM (McNeill et al. 2002, Peterson et al. 2005).

The higher concentration of calcium in the blood serum of cows from group II, III and IV could indicate a possibility of hypocalcaemia occurrence in cows from the control group. The higher concentration of phosphorus in the blood serum of cows from group III and IV (Table 5) could indicate the higher body acidifying action (Table 3) and more effective Ca and P mobilization from bones. The obtained data suggested that the best strategy for hypocalcaemia prevention is to slightly acidify the cow's organism with the moderate administration of $MgSO_4 \times 7H_2O$ (DCAB about +50) for 14 days a.p., combined with an increase of calcium supplementation to 100 g/day/head, during the last 5 days of dry period. The longer application of chalk (for 10 days a.p.), as well as application of phosphate only, were less effective in prevention of the calcium levels reduction in the blood. The use of phosphate as a second source of calcium improved Ca:P ratio of daily ration and effectively counteracted hypophosphataemia.

A high level of progesterone in group II and IV on the 45^{th} day of lactation was a result of the high levels of P and an adequate level of Ca in serum. The addition of Ca 5 days before calving (in contrast to the group receiving the higher amounts of this element for 10 days), did not decrease the absorption of P from the digestive tract, although a negative effect of Ca and Mg on P absorption in the body was found in earlier studies (Kinal et a. 1996). Between 15^{th} and 21^{st} day of lactation ovaries, in all treatments, were not activated what is assumed as physiologically correct phenomenon in such a short time after calving. However, a low level of progesterone on the 45^{th} day of lactation in the blood serum of cows from group II suggested that in those cows ovulation was inhibited and *corpus luteum* was not developed.

After application of 140 g of $MgSO_4 \times 7H_2O$, the magnesium concentration in daily ration increased from 0.09 to 0.23% of DM, the concentration recommended in USA (Peterson et al. 2005). Thus, the obtained data regarding the magnesium concentration in the urine, as well as in the blood serum, could indicate meeting the cows requirements for that element.

Blood gasometry indices corresponded well with the references according to Baumgartner (2009), with the exception of BE for group IV, which was visibly lower.

Activity of AlaT and AspAt were found within physiological ranges (Meyer and Harley 1998). These data indicated a good condition of the liver.

Taking into consideration all of the obtained results, it could be stated that in the prevention of hypocalcaemia and hypophosphataemia the application of 100 g $CaCO_3$ or 100 g of $CaHPO_4$ for the last 5 days of dry period together with $MgSO_4 \times 7H_2O$ given 14 days a.p. would be optimal.

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