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Effects of mineral mixture addition on the level of selected macroelements in the hair of Polish Holstein-Friesian cows

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ABSTRACT

Studies aimed to determine the usefulness of mineral mixture Bovifosfomag® in bovine nutrition were carried out in southern Podlasie, Poland, over a period of two years. The criterion of its usefulness was the level of particular minerals measured in the hair of dairy cows. The concentrations of calcium, phosphorus and magnesium were measured by the ICP (Inductively Coupled Plasma) method using the ICP spectrometer Philips SC-PU 7000 attached to the Cetac U-5000 AT ultrasonic nebulizer. Hair samples were taken twice, 10-14 days before parturition and after the first month of lactation. Bovifosfomag® mineral mixture, which had been developed on previous macromineral deficiency monitoring studies, in most cases improved the supply of minerals to cows in the surveyed farms. Hair of cows differed significantly in calcium, phosphorus and magnesium content. The reasons for this probably lay in the individual characteristics of the cows. Bovine coat hair proved useful in the diagnosis of mineral deficiencies and revealed a short supply in phosphorus and magnesium in the control group. The study of the mineral composition of hair partially confirmed previous trends in the patterns of mineral levels observed in blood serum. An increase in Ca, P and Mg in the hair of the animals fed the supplement was found.

Key words: Dairy cows, Hair, Macroelements, Mineral mixture.

INTRODUCTION

The important problem in cattle feeding is to balance the chemical composition of the ration in terms of organic compounds and minerals. The list of essential minerals include also calcium, phosphorus and magnesium. The mineral supply of ruminants is largely dependent on the concentration of micro- and macroelements in the soil and the plants (Jones, 2002). Using feeds that are poor in minerals often leads to conditions referred to as production diseases (Kondracki and Bednarek, 1996). It has been widely recognized that farm feeds only partly satisfy the needs of livestock animals for minerals. Hence the necessity to fight any deficiency directly - by supplementing the diet with mineral mixes (Górski *et al*., 2006). It must also be kept in mind that both preventive and remedial measures against mineral deficiencies should be preceded by a survey to fully identify the mineral supply in the soil-plant-animal trophic system (Marques *et al*., 2013; Maan *et al*., 2013). There are various opinions on the usefulness of the determination of minerals in hair. The most reliable opinion seems to be that of Lamand (1972), who argues that mineral levels in the hair should be seen as complementary to blood and milk examinations.

According to Anke and Risch (1989) as well as Brochart (1971), the gradual, rather slow deposition of minerals in hair reflects well their real levels in the animal body. The usability of hair to diagnose the supply of dietary minerals results from the specificity of hair structure, which makes it resistant to biological changes and having a constant chemical composition (Chojnacka *et al*., 2010). The method is applied to determine the body levels of minerals as, unlike blood serum, the material for examination can be obtained easily and non-invasively (Combs 1987, Lloyd *et al*., 2009).

A number of scientists (Anke 1966, Neseni 1970, Combs *et al*., 1983; Fischer *et al*., 1985, Kempson and Lombi, 2011; Suliburska *et al*., 2011; Namkoong *et al*., 2013, Chikawa *et al*., 2014) suggest that there is an association between the content of mineral components in the hair and the both applied nutrition and disorders in the body functioning. An increased inflow of Ca^{2+} ions to cells is believed to contribute to many diseases, and hair mineral analysis enables diagnosis on cell ion channel gating (Chikawa *et al*., 2014). Neseni (1970) noticed that heifers whose hair phosphorus level was less than 200 ppm developed less rapidly compared to those with a concentration of the element exceeded 200 ppm. Cattle

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grazing pastures where vegetation contained low magnesium levels were found to contain less magnesium compared to animals grazing pastures with higher levels of Mg (Fischer *et al*., 1985).

Based on the results of the previous research, we carried out an assessment of the status of mineral supply of cows, which was based on an analysis of soil, feeds, and blood serum in selected facilities in the southern part of Podlasie (Górski *et al*., 2005). As a result of the research, a mineral mixture was composed.

The aim of this study was to test the nutritional usefulness of Bovifosfomag® mineral mixture. As the criterion of its usefulness, we measured the level of particular minerals in the hair of the cows.

MATERIALS AND METHODS

The experiment took two years and was carried out in four dairy farms, A, B, C and D, located in the south of Podlasie. Herds of Polish Holstein-Friesian cows, Black-and-White variety, each numbering 24 cows, attaining an average milk yield ranging 4500-5000 kg, were divided into a control (K, 12 cows) and experimental (E, 12 cows) groups. The groups were composed of animals similar in terms of age, productive performance, and physiological status. The control group cows did not receive the mineral supplement, whereas the experimental animals were fed with an addition of Bovifosfomag® mineral mixture (Table 1). The mixture was formulated taking into account the biogeochemical location of the farms (Górski *et al*., 2005). It was manufactured by Herbemin S.C. in Pu³awy. The daily dose of the mixture was 150 g per cow. The cows were clinically healthy, free from contagious diseases and covered by preventive program to combat invasive diseases. The studies included the periods of summer and winter feeding. In winter, the cows were housed in barns that met the required hygienic standards for dairy cows. Feeds fed during this period were corn silage, hay, and barley straw. In summer, the ration was composed of green forage from pastures and field crops, supplemented with hay and barley straw. Concentrates were added in amounts reflecting the individual yield of the cow. Nutritional demands of the cows were established on the NRC feeding standards for dairy cows (NRC, 2001). Material for laboratory analysis was collected in the second year of the feeding trial. Hair samples were taken twice, 10-14 days before parturition and after the first month of lactation.

A single-color (white) hair, freshly regrown (at a place previously shaved) was collected from the back of a cow, according to instructions given by Brochart (1971). The hairs were thoroughly cleaned using detergents and alcohol. Dried and weighed samples were placed in Teflon® vessels and concentrated, spectrally pure nitric acid and hydrogen peroxide (Merck, Germany) were added. Following microwave digestion, the samples underwent spectrometric analysis. The concentrations of macroelements were measured by the ICP (Inductively Coupled Plasma) method using the ICP spectrometer Philips SC-PU 7000 attached to the Cetac U-5000 AT ultrasonic nebulizer (Bodak and Dobrzañski, 1997; Górecka, 1995).

The results of the experiment were analyzed statistically by three-way ANOVA with cross classification, with more than one observation in the subclass, according to the following linear model:

Table 1: Composition of modificated material of mineral mixture Bovifosfomag®

		Content of pure element			
Specification	(g)	Ca (g)	P (g)	Mg (g)	Na (g)
$Ca(H_2PO_4)_2$ (calcium phosphate)	350	60	95	-	-
$Ca_3(PO_4)_2$ (tricalcium phosphate)	100	39	20	-	-
MgO (magnesium oxide)	175	-	-	105	-
$CaCO_3$ (ground limestone)	200	80	-	-	-
NaCl (forage salt)	175	-	-	-	70
Total macroelements	1000	179	115	105	70

		Content of pure element						
Specification	(g)	Zn (g)	Cu (g)	Fe (g)	Mn (mg)	Se (mg)	I (mg)	Co (mg)
$ZnSO_4$ $7H_2O$ (zinc sulfate)	22.000	5.0	-	-	-	-	-	-
$CuSO_4$ $5H_2O$ (cupric sulfate)	4.000	-	1.0	-	-	-	-	-
$FeSO_4$ $7H_2O$ (ferrous sulfate)	5.000	-	-	1.2	-	-	-	-
$MnCO_3$ (manganese carbonate)	0.020	-	-	-	10.0	-	-	-
Na_2SeO_4 (sodium selenate)	0.050	-	-	-	-	20.0	-	-
KI (potassium iodide)	0.040	-	-	-	-	-	30.0	-
$CoSO_4$ $7H_2O$ (cobalt sulfate)	0.015	-	-	-	-	-	-	3.0
Total microelements	31.125	5.0	1.0	1.2	10.0	20.0	30.0	3.0
Total	1031.125							

$$Y_{ijlp} = m + a_i + b_j + c_l + ab_{ij} + ac_{il} + bc_{jl} + abc_{ijl} + e_{ijlp}$$

Where:

Y_{ijlp}: level of trait (concentration of calcium, phosphorus and magnesium in hair)
m: population mean
a_i: effect of *i* th level of the factor A (farm)
b_j: effect of *j* th level of the factor B (group)
c_l: effect of *l* th level of the factor C (sampling)
ab_{ij}: effect of interaction: A x B
ac_{il}: effect of interaction: A x C
bc_{jl}: effect of interaction: B x C
abc_{ijl}: effect of interaction: A x B x C
e_{ijlp}: random error.

The means were post-hoc compared using Tukey's test at $P<0.05$. The data were processed statistically using the Excel 2010 spreadsheet and the Statistica® 10 PL package.

RESULTS AND DISCUSSION

The studies revealed that both the farm and the group had an effect on the level of calcium, phosphorus, and magnesium in the hair of dairy cows of the analyzed region. Sampling, on the other hand, had an impact on the concentration of calcium and magnesium in the cows' hair (Table 2).

Differences in calcium concentrations between farms, between groups, and between samplings, as well as a significant interaction: sampling x farm were found. In farms C and D, phosphorus content in both samplings was statistically the same and significantly higher compared to farms A and B. Higher levels of calcium were found in the experimental group cows compared to the control (Table 3).

Differences were also found in phosphorus content between farms and between groups, as well as a significant interaction: farm x group. In farm A, C and D, phosphorus levels were statistically equal and significantly higher than in farm B. Higher levels of phosphorus were found in the experimental group than in the control. In the control group, phosphorus levels were statistically equal regardless of the farm. In group E, significantly less phosphorus were recorded in farm B. In the remaining farms phosphorus content was at a comparable level (Table 4).

Differences in magnesium concentrations between farms, between groups, and between samplings, as well as a significant interaction: sampling x group were oberserved. A higher content of magnesium was found in the first sampling. Higher levels of magnesium were also recorded in the experimental group, as compared with the control (Table 5).

Table 2: The impact of examined factors, ie. farm, sampling and experimental group at the level of calcium, phosphorus and magnesium in the hair of cows

Macroelements		Farm	Sampling	Group	Interaction			
					Farm x sampling	Farm x group	Sampling x group	Farm x sampling x group
Calcium	F_{emp}	43.92*	8.93*	549.46*	8.45*	1.16	1.81	1.43
	level of significance	0.00	0.00	0.00	0.00	0.32	0.18	0.23
Phosphorus	F_{emp}	11.90*	0.49	283.05*	2.64	8.63*	1.45	1.85
	level of significance	0.00	0.48	0.00	0.05	0.00	0.23	0.13
Magnesium	F_{emp}	17.41*	3.62*	168.77*	1.75	1.17	18.26*	0.14
	level of significance	0.00	0.03	0.00	0.15	0.32	0.00	0.93

* Statistical significance at $P<0.05$

Table 3: Calcium content of cow's hair depending on the farm, sampling and experimental group (mg/kg d.m.)

Calcium			
Farm	Sampling		Mean for farms
	1	2	
A	1854.0 b	1796.6 b	1825.29 b
B	1778.9 c	1805.4 b	1792.16 c
C	1885.6 ab	1885.8 a	1885.70 a
D	1916.1 a	1861.4 ab	1888.75 a
Mean for sampling	1858.65 *	1837.32 *	1848.0
	Group		
	K	E	
Mean for group	1764.31 *	1931.65 *	

Means in columns denoted by the different letters differ significantly at $P<0.05$
Means in lines marked *differ significantly at $P<0.05$
K - control group, E - experimental group

Table 4: Phosphorus content of cow's hair depending on the farm and experimental group

Phosphorus			
Farm	Group		Mean for farms
	K	E	
A	174.50 a	199.92 a	187.21 a
B	171.83 a	184.17 b	178.00 b
C	177.50 a	197.50 a	187.50 a
D	171.00 a	201.25 a	186.13 a
Mean for group	173.71 *	195.71 *	184.71

Means in columns denoted by the different letters differ significantly at $P<0.05$
Means in lines marked * differ significantly at $P<0.05$
K - control group, E - experimental group

Table 5: Magnesium content of cow's hair depending on the sampling and experimental group (mg/kg d.m.)

Group	Magnesium		
	Sampling		Mean for group
	1	2	
K	187.46 *	176.38 *	181.92 b
E	202.50 *	206.17 *	204.33 a
Mean for sampling	194.98 *	191.27 *	193.13

Means in columns denoted by the different letters differ significantly at P<0.05
Means in lines marked * differ significantly at P<0.05
K - control group, E - experimental group

The highest levels of magnesium were found in farm C, significantly lower in farm A and lowest in farm B. Magnesium content in farm D was similar to the concentration of this nutrient in farm A and B (Figure 1).

In the previous studies (Górski *et al*., 2005) carried out in farms A, B, C, and D, located in the analysed region, abnormalities were found in the mineral supply of cows, which was expressed by an elevated content of Ca accompanied by deficiencies of P and Mg in the soil-plant-animal trophic system. Based on the analysis, a mineral mixture was developed that would have a nutritional usefulness in the given geochemical conditions.

It is possible to evaluate the mineral supply of an animal basing on the mineral composition of its hair, which is considered complementary to a blood test result (Anke and Risch, 1989). The method is used in the diagnosis of deficiencies in animals, since the sampling of biological material is easy and non-invasive (Kosla *et al*., 2011). It is believed that a gradual and slow deposition of minerals in hair reflects their actual levels in the animal body (Trupa *et al*., 2000). The usefulness of animal hair to diagnose the demand for macro- and microelements stems from the nature of the hair, which makes it resistant to biological changes and remaining a constant chemical composition (Gabryszuk *et al*., 2008).

According to Anke and Risch (1989), the concentration on Ca in the hair of ruminants should not drop below 750 mg/kg DM. In the light of these recommendations, calcium levels measured in this study should be considered normal (Table 3). In the hair of the control group cows, calcium content exceeded 1764 mg/kg DM and were similar to those observed by Wnuk *et al*. (2003). Application of the mineral supplement resulted in a significant increase in hair calcium levels (P<0.05).

Phosphorus levels in the hair of control cows did not exceed 175 mg/kg DM, which in relation to the reference value, 200 mg/kg DM, as recommended by Puls (1998), is an evidence of a phosphorus deficiency (Table 4). The results confirm earlier observations concerning phosphorus deficiencies in all the levels of the trophic system in southern Podlasie (Górski *et al*., 2005). The use of the mineral mix significantly increased the concentration of phosphorus in the hair of cows from the experimental group, as compared with the control group (P<0.05). There was no significant improvement in the supply of the cows in phosphorus. Its level in the experimental group fluctuated around 200 mg/kg DM.

The hair of the cows of the control group was characterized by levels of magnesium not exceeding 188 mg/kg DM (Table 5). In relation to 750 mg/kg DM, which according to Anke and Risch (1989) is a physiological level, the values we found should be considered deficient. Given the low levels of magnesium in the soil, feed and blood serum (Górski *et al*., 2005), it may be presumed that the reduced concentration of Mg in the hair was a consequence of its shortages on various levels of the trophic chain. According to Gabryszuk *et al*. (2008), a low magnesium content in soil is reflected by a deficiency of this element in the body of animals. The applied mineral mixture resulted in a significant increase in the level of Mg compared with the control cows (P<0.05). Regained levels of magnesium, however, were still significantly lower than those recommended (Anke and Risch, 1989) and a deficiency of the macroelement in the body was evident. In this case, changes in the hair levels of magnesium as a result of mineral supplementation should be monitored for a longer period of time.

Despite many similarities between the assessed farms (geographic location, breed, similar age of the cows, feeding system) hair of cows differed significantly in calcium, phosphorus and magnesium content. The reasons for this probably lay in the individual characteristics of the cows. Bovine coat hair proved useful in the diagnosis of mineral deficiencies and revealed a short supply in phosphorus and magnesium in the control group. The results support recommendations that multi-mineral mixes should be used

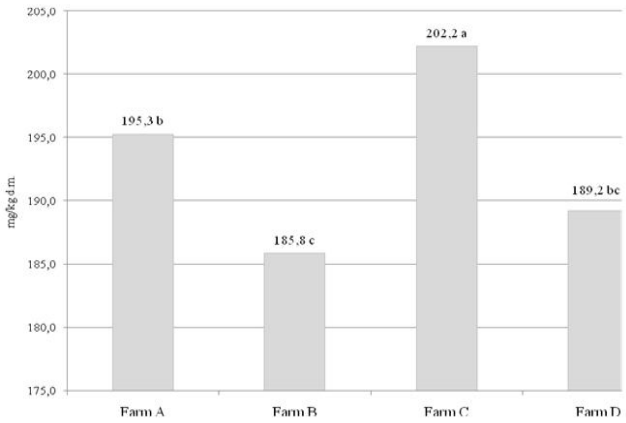

Fig 1: Magnesium content of cow's hair depending on the farm, from which the animals originated (means denoted by the different letters differ significantly at P<0.05)

in feeding cattle in this particular geochemical region. The administration of the mineral mixture of the proposed composition, which had been developed on the basis of our previous monitoring studies on macromineral shortages, contributed to an increase in the levels of Ca, P and Mg in the hair of cows.

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