Original Article

Influence of electromagnetic stimulation on blood macro and micro elements in dairy cows

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Abstract

The aim of this study is to determine the influence of EM stimulations on macro and micro element concentrations in the blood, to determine if changes in ion concentrations depends on their basal values and to determine the usefulness of this kind of stimulation in the maintenance of the blood macro and micro element levels in cows. 17 cows were included in the experimental group (affected by a unique EM stimulation protocol of three weeks duration) and 9 cows were included in the control group. Significantly higher (P <0.01) concentrations of Ca, P, Na and Cu and lower (P <0.05) concentrations of Zn were found in the experimental group after EM stimulation. Dynamic changes of macro and micro element concentrations were inversely proportional to their basal value before the beginning of the experiment which means that animals with lower basal concentrations had greater changes of value during stimulation. Dynamic changes of Ca, P, Mg, Fe, K and Cu were more notable in the experimental group that was exposed to EM stimulation compared to the control group. However, dynamic changes of Na and Zn concentrations were identical in the experimental and control groups. Exposure of cows to EM stimulation had a positive effect at Ca and P concentrations and improved calcaemia and phosphatemia in all the exposed animals.

Keywords: EM stimulation, Dairy cows, Macro-elements, Micro-elements

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Introduction

Macro and micro-elements have a major role in the preservation of functionality, health and productivity of dairy cows. These nutrients play a critical role in physiological processes related to health, growth and reproduction and the adequate functioning of the immune and endocrine systems (Galyean *et al.,* 1999; Sharma, *et al.*,2006; Soetan *et al.*,2004; Djokovic *et al.*, 2014).

Blood levels of calcium, inorganic phosphorus and magnesium in dairy cows during the lactation period reflect their metabolism or the supply of these macronutrients through feed and their utilisation by peripheral tissues, the mammary glands in particular. Any decline in their blood level below the physiological limit, any deficiency of these nutrients and their unfavourable ratio in lactating cows generally leads to subclinical and clinical manifestations that adversely affect animal health and fertility (Sharma *et al.*,2006; Puls, 1998; Duffield *et al.*, 2005; Goof 2006; Starič and Zadnik, 2010; Cincović, *et al.*, 2017).

Micronutrient deficiency such as iron, zinc, manganese, copper, cobalt , selenium, iodine and chromium in dairy cows results in reduced animal performance, such as impaired reproduction, a high incidence of mastitis, reduced milk yield, impaired immunity and an increased degree of lameness due to laminitis (Puls,1998; Galyean *et al.*,1999; Spears, 2003; Soetan *et al.*,2004; Staric and Zadnik, 2010; Djokovic *et al.*, 2014).

During the past decades, there has been a marked increase in the evaluation of the effects of EM field on biological systems. The chamber characteristics allow the use of a wide range of exposure such as electric fields (0–30 kV/m) and magnetic fields (0–100 μT) at frequencies ranging from 45 to 3000 Hz (Nguyen *et al.*, 2005).

Kavaliers and Ossenkopp (1985) have suggested that magnetic fields (MF) affect the functioning of the Ca channels. This disruption can result in an alteration of the effectiveness of the opiate system to cope with pain (Teskey *et al*., 1988). In vitro evidence exists that electric fields (EF) have an effect on the mobilisation of Ca from brain cells in freshly isolated avian and feline brains (Blackman, 1989). Some researchers (Bawin and Adey, 1976) have found that sinusoidal EF decreases the efflux of Ca from nervous tissues.

Blackman (1989) has demonstrated an increase in the efflux of Ca from brain preparations. Other ions, such as Cu and Zn, have multiple functions as cofactors of enzyme activity and their variations have been suggested as part of the biological effects caused by EM field (Brugere *et al*., 1995). EM stimulation has shown that exposure in cows can cause changes in blood plasma values of macro and micro elements (Burchard *et al*., 1999). The EM stimulation affects the oxidative status of the organism by influencing different enzymatic systems that can be related to micro element values in blood (Kıvrak *et al*., 2017).

The aim of this study is to determine the influence of EM stimulations on macro and micro element concentrations in the blood to determine if changes in ion concentrations depends on their basal values and to determine the usefulness of this kind of stimulation in the maintenance of blood macro and micro element levels in cows.

Materials and Methods

Experimental conditions on the farm: The experiment was conducted on a herd of the Holstein Friesian breed of cows (Backa Palanka, Vojvodina, Serbia). For the purposes of this experiment dairy cows (n = 26) in full lactation (150+50 days) were chosen, aged 2-6 years, with an equal daily production of milk, divided into two groups: Group 1 consisted of experimental (EM exposed of three weeks duration) dairy cows (n = 17) and Group 2 included control (nonexposed) dairy cows (n = 9). The experiment and control cows were confined in the same chamber. The chamber was 20 m long, 10 m wide and 3 m high.

Preparation and standardization of the daily meal was done in accordance with the nutritional and energy needs of dairy cows in full lactation (NRC, 2001). The current daily production average on a farm is 27 kg (fat corrected milk yield, FCM) of milk or 8235 kg of milk for 305 days of lactation.

Electromagnetic stimulation: The Delta Sigma (DS) stimulation principle of activation was used. The DS stimulator produced electromagnetic oscillation at a frequency of about 400 kHz. This bike was connected to an antenna coil 0,39 mH. This coil acting as an antenna sent an electromagnetic signal into space, circular about 10 m. One or more coils were placed at the chosen animal (Fig.1). If there were several animals in the vicinity, the effect of EMF interference was used when the phenomenon of interference was used to cover as much space as possible by EM field stated characteristics. A timer was used to regulate the dosage of EM radiation, which included periods of radiation and periods of pause. During the stimulation process, 15 devices, distributed uniformly along the exposure chamber and attached to a metal rod at about 1 m above the animals' heads, were used in the experimental group (Fig.1).

Experimental animals (n=17) were affected by a unique EM protocol of three weeks duration, at the following pace: 8 hours of stimulation and 8 hours of pause. This kind of stimulation cycle lasted to the end of the experiment. The results of measured values of magnetic induction during different frequencies (Electrotehnical Institute „Nikola Tesla", Belgrade) are showed in Figs 2 and 3.

The control group of animals had identical conditions with regard to accommodation, care and diet but they were not subjected to the experimental EM stimulation protocol.

Blood sampling: The blood samples were collected from all experimental and control groups of cows before (0. day) and after the end of the experiment (day 21) at 8:00 h immediately after milking and feeding, by puncturing the jugular vein into sterile disposable test tubes without anticoagulant. After clotting for 3 hours at 4 °C and centrifugation (1500 g, 10 minutes, 4° C), sera were carefully harvested and stored at -20 °C until analysis. From the experimental group of animals, determination of minerals in blood serum was conducted in order to track their value before and after the experimental period. Concentrations of sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn) and copper (Cu) in blood serum were determined by atomic absorption spectrophotometry (Perkin Elmer 2830). Inorganic phosphorus (P) was determined by the ammonia molybdate method of spectrophotometry (AOAC, 1980).

Figure 1 EM stimulators positioned in the chamber (marked by arrows)

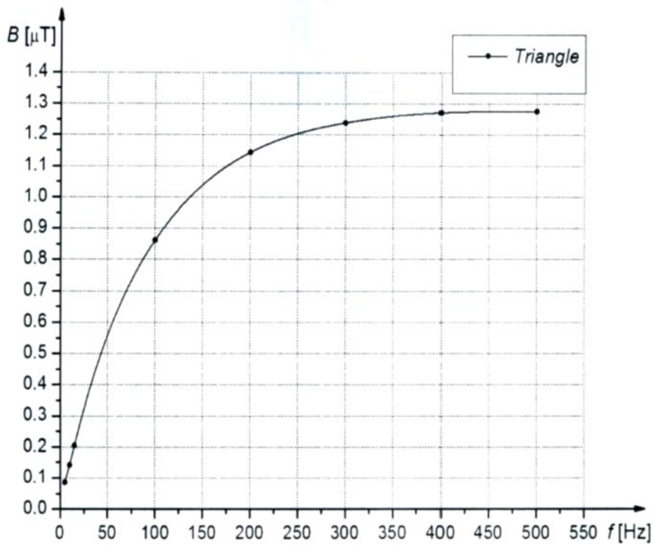

Figure 2 The measured magnetic induction during different frequencies for form signal Triangle.

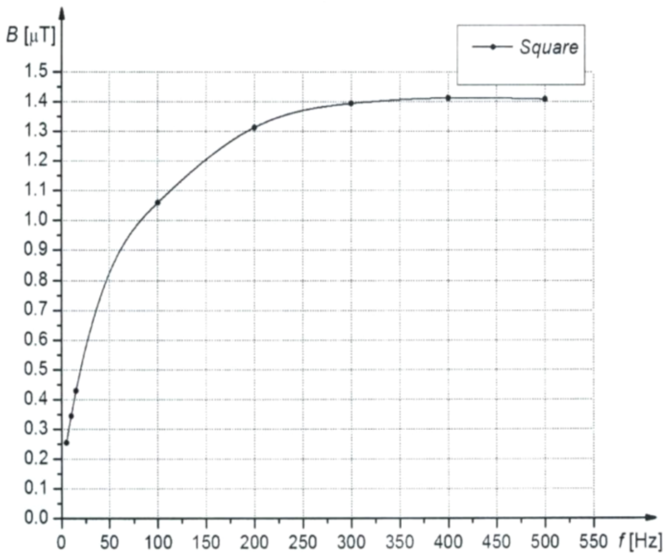

Figure 3 The measured magnetic induction during different frequencies for form signal Square.

Statistical analysis: The influence on the experimental and control group and the moment of blood sampling, just like the interaction group × moment on values of micro and macro elements, were determined by the GLM model: $Y_{ijk}=m+a_i+b_j+(ab)_{ij}+e_{ijk}$.. Explanation: Y is the sum of five components: m the mean; *ai* the contribution of the i-th level of a factor a (group); *bj* the contribution of the j-th level of a factor *b* (moment); (ab)ij the combined contribution of the i-th level of a factor *a* and the j-th level of a factor *b*; eijk is the contribution of the k-th individual, called the "error". Given values were represented graphically in the form of mean values. Correlation of basal concentration (concentrations before electromagnetic stimulation) with dynamic changes of ion values (delta = concentration after-concentration before) was determined by linear regression and Pearson's correlation and graphically represented for each group (general formula $r=\sigma_{xy}/\ \sigma_x\sigma_y$; r-correlation, σ_x and σ_y as the population standard deviations, and σ_{xy} as the population covariance).

Ethical approval: The Ethics Committee on animal use at the University of Novi Sad (number III-2018-07) approved this study.

Results

Exposure to electromagnetic stimulation in dairy cows can cause changes in concentrations of macro and micro elements. Significantly higher (P <0.01) concentrations of Ca, P, Na and Cu and lower concentrations of Zn (P <0.05) were found in the experimental group in the period after EM stimulation compared with before stimulation. The Fe concentration was not changed (P >0.05) in any of the groups during the time. There were no determined differences in Ca, P and Zn concentrations before and after stimulation in the control group (P >0.05), but the concentrations of Mg, Na and K were changed (P <0.01). Statistically significant was the interaction time and group (P <0.05). After EM exposure higher (P <0.05) concentrations of Ca were found in the experimental group than in the control group. Increases in P concentration was non-significantly higher (P >0.05) in the experimental group compared to the control group. Cu concentration was higher (P >0.05) in the control group than in the experimental group of cows. Mg value was increased (P <0.001) in the blood of the control animals but was decreased (P >0.05) in the blood of the experimental group. Other ion concentration changes in blood during the time were similar in the control and experimental groups (P >0.05). Results are shown in Table 1 and Figs. 4-11.

Dynamic changes of macro and micro element concentrations were reversely proportional to their basal values before exposure which means that animals with lower basal values had greater change of values during exposure. Dynamic changes of Ca, P, Mg, Fe, K and Cu concentrations were more notable (P <0.05) in the experimental group of cows that were exposed to EM stimulations compared to the control group. However, dynamic changes of Na and Zn concentrations were identical in the control and experimental groups (comparing coefficients of determination and regression parameter b in formulas in each (Figs. 12-19).

EM stimulation showed a positive effect on calcium and phosphorus concentrations in the blood of cows. In the control group change of Ca concentration during the time was low (0.2 mM/L) while in the experimental group it was increased (1.0 mM/L) leading the cows to form subclinical hypocalcaemia in a state of normocalcaemia. Research results for all 26 cows in the experiment are shown at Fig. 20.

Table 1 Influence of EMS on values of macro and micro elements in cows

Ions	Group	Before	After	Paired sample t-test	Effects in GLM model		
					Group	Time	Group× time
Ca (mM/l)	Experimental	1.80±0.27	2.28±0.26	P<0.001	P<0.05	P<0.001	P<0.05
	Control	1.84±0.19	1.91±0.24	NS			
P (mM/l)	Experimental	1.25±0.30	1.53±0.22	P<0.001	NS	NS	P<0.05
	Control	1.45±0.19	1.36±0.33	NS			
Mg (mM/l)	Experimental	0.79±0,11	0.72±0.14	NS	P<0.05	NS	P<0.05
	Control	0,83±0.10	1,00±0,07	P<0.001			
Fe (μM/l)	Experimental	18.51±5,21	15.86±4,65	NS	P<0.001	NS	NS
	Control	23.03±6.14	23.06±4.78	NS			
Na (mM/l)	Experimental	117.82±13.12	128.82±11.94	P<0.05	NS	P <0.001	NS
	Control	119.00±9.87	135.56±3.68	P<0.01			
K (mM/l)	Experimental	3.52±0,49	3.99±0,57	NS	NS	P <0.01	NS
	Control	3.63±0.34	4.07±0.37	P <0.01			
Zn (μM/l)	Experimental	18.11±4,76	15.09±2,37	P <0.05	NS	P <0.01	NS
	Control	22.16±9.53	16.51±1,34	NS			
Cu (μM/l)	Experimental	6.44±1.90	7,75±1.45	P <0.05	NS	P<0.001	P<0.01
	Control	5.32±0,99	9.52±1.09	P<0.001			

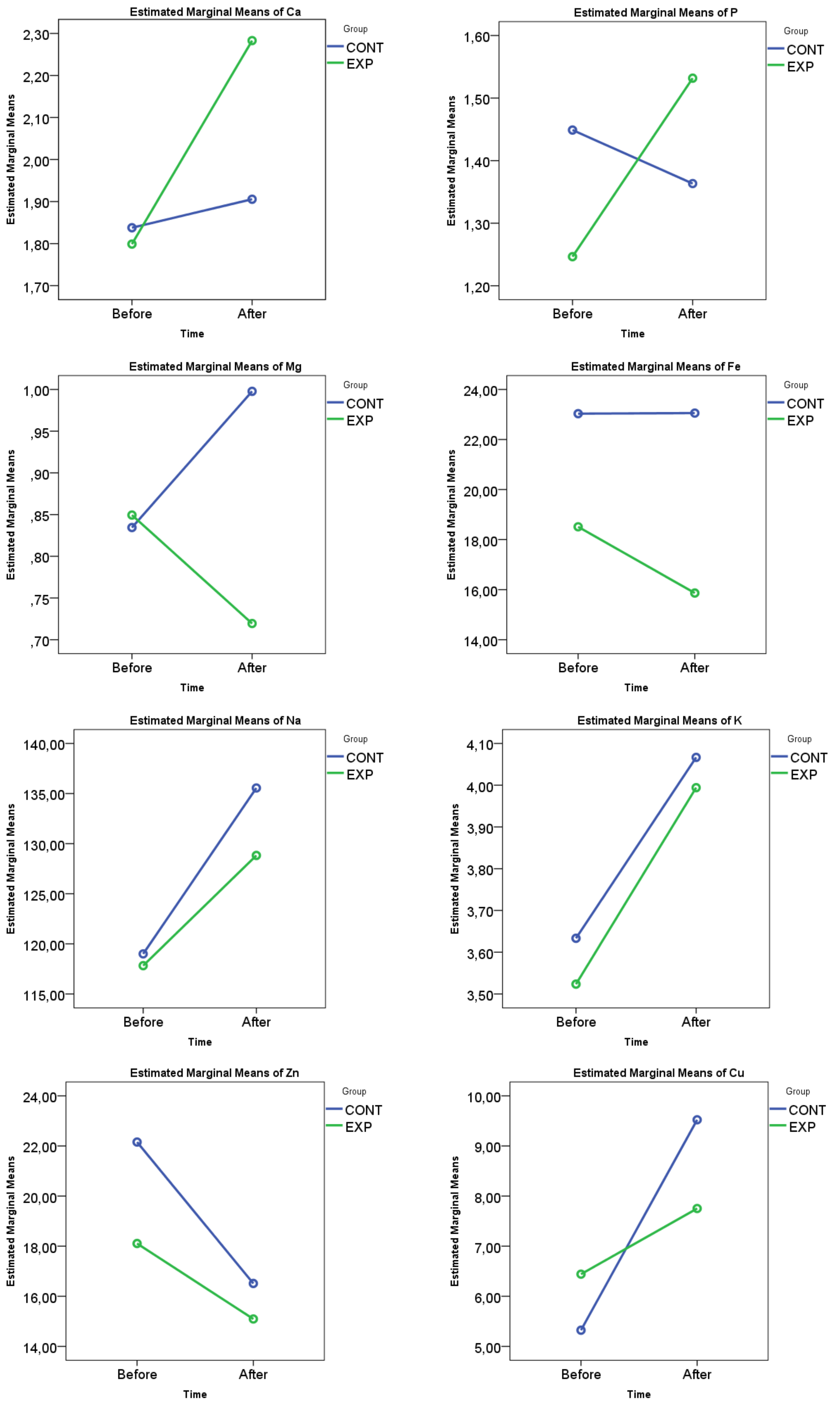

Figure 4-11 Group × time interaction influence at values of macro and micro elements

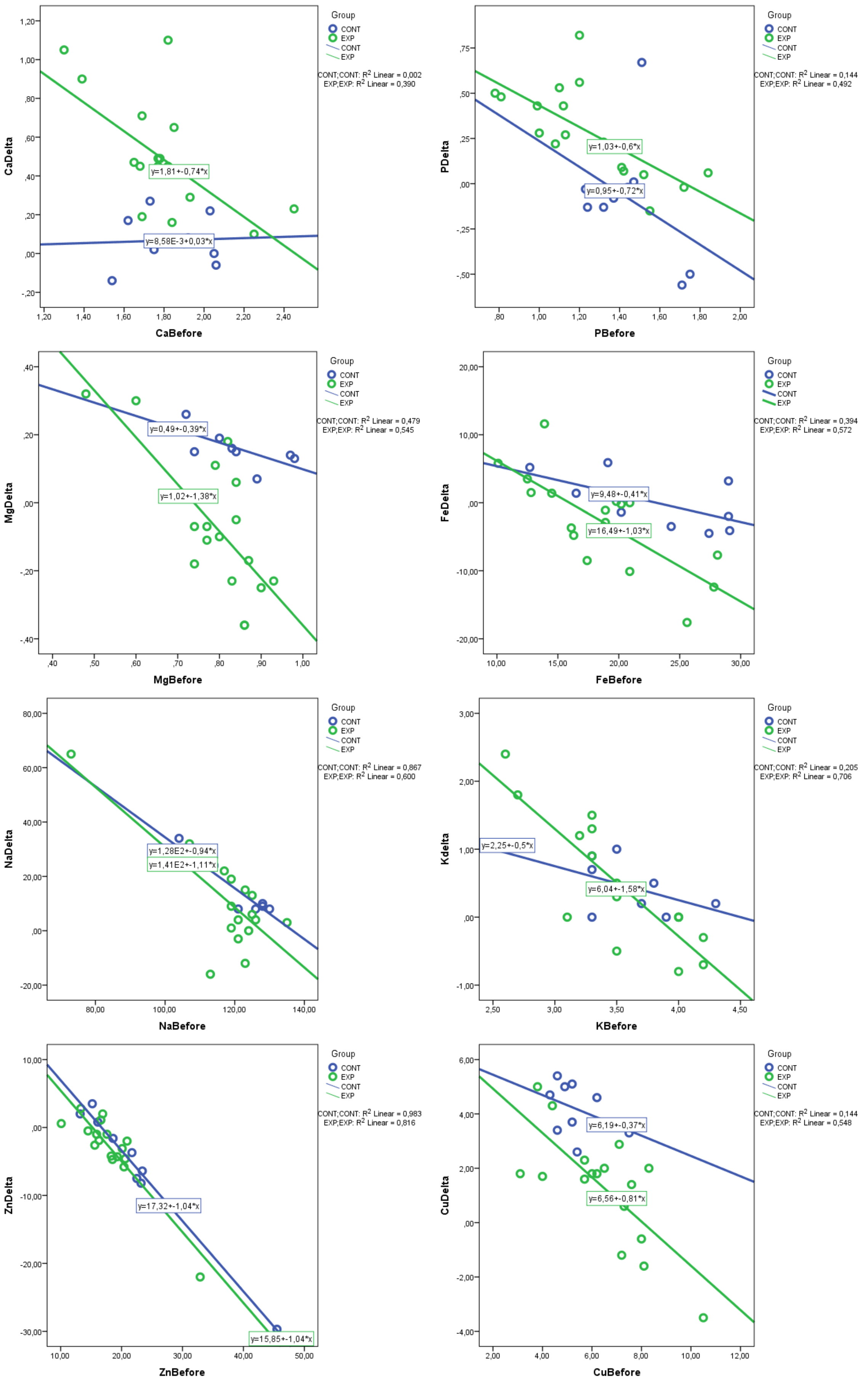

Figure 11-19 Influence of basal concentration of macro and micro elements on dynamic change of their concentrations in the experimental (EXP) and control group (CONT) after exposure to EM stimulation.

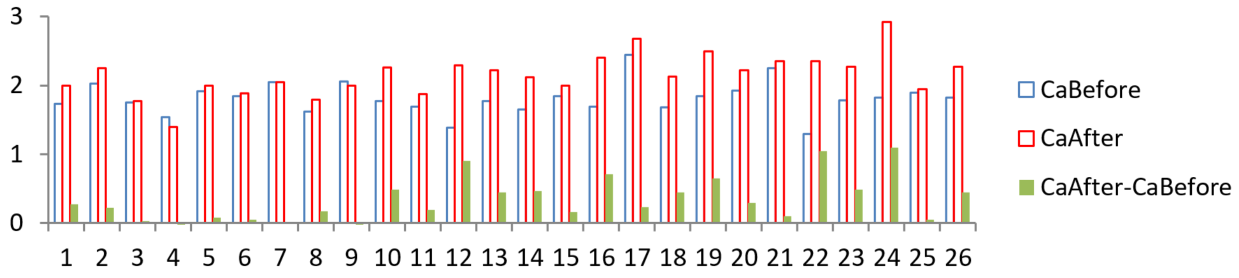

Figure 20 Calcium concentration before experiment (CaBefore), after experiment (CaAfter) and differences in Ca values (CaAfter-CaBefore) in the control (1-9) and experimental group (cows 10-26).

Discussion

The influence of EMF stimulation on the physiological, endocrinological and reproductive status of dairy cows has been researched for a while now (Nguyen *et al.*, 2005).

In the current study, concentrations of Ca, P, Na, Zn and Cu in blood sera significantly increased during EM exposure in the experimental group of cows, suggesting the effect of EM stimulation on values in the blood of these macro and micro elements. The values of Na, K and Fe in the blood sera did not significantly change during EM stimulation compared to the values before stimulation. The experimental lactation cows exhibited significantly increased (P <0.05) values of Ca and significantly decreased values of Mg and Fe in the blood than the non-explored (control) group of dairy cows, suggesting a possible effect of EM stimulation. Dynamic changes of Ca, P, Mg, Fe, K and Cu concentrations were more notable in the experimental group of cows that were exposed to EM stimulation compared to the control group. Reaction to EM stimulation was more intense in dairy cows with lower basal levels of ions.

Literature data shows that the exposure EM stimulation of lactating cows can cause changes in the concentration of macro and micro elements in blood plasma and cerebrospinal fluid (CSF) (Burchard *et al.*, 1999). Under the influence of EM field Mg, Fe and Cu concentrations dropped in the blood of exposed animals. Ca and P concentrations were increased in exposed animals in the blood and Fe and Mn were decreased in CSF of the experimental animals. No significant changes were noted in Na and Mn concentrations. Significantly greater variations in macro-element concentrations in the blood plasma were noted compared to micro elements (Burchard *et al.*, 1999). This is similar to the effect of EM stimulation on concentrations of macro and micro elements in the blood sera we have found in current study because EM stimulation had a positive effect on Ca and P concentrations in blood and improved calcaemia and phosphatemia in all exposed animals.

EM fields may have had positive or negative implications for a marine organism within the nearby vicinity. Varying reactions were observed in embryo development, depending on species. Research shows B-fields delay embryonic development in sea urchins and fish, while several studies have found that EM fields alter the development of cells; influencing circulation, gas exchange and the development of embryos; and alter orientation (Fisher and Slater, 2010).

Low-frequency electric fields (16 Hz, 18,3 mV/cm2) cause increased consumption of Ca ions from medium and this increases in cells (in vitro) (Weaver *et al.*, 1997). Also, low-frequency electric and magnetic field have the same influence on intracellular Ca ion concentrations (Lindstrom *et al.*, 1993) and magnetic fields can affect the function of Ca canals just like the distribution of Ca ions (Kavaliers and Ossenkopp, 1985).

Recent studies have shown that increased concentrations of Ca are developed as a consequence of Ca mobilisation from bones during EM stimulation and this can be related to oxidative stress and parathyroid gland function(Kunt *et al.*,2016; Lei *et al.*, 2017).

Also, Cu and Zn have multiple roles as co-factors of enzymatic activity so their variations can be related to the influence of EM fields on biological systems and that these changes are caused by direct interaction interaction of the EM field and cell membrane (Brugere *et al.*, 1995) .

Fe is essential for many physiologic processes in the brain and it is actively transported across the blood-brain barrier (Blackman, 1989; Goodman *et al.*,1989; Thompson *et al.*,2001). Under the influence of EM stimulation the permeability of the blood-brain barrier is increased (D'Andrea *et al.*,2003; Nittby *et al.*, 2008) and that causes accumulation of Fe in the brain (Thompson *et al.*,2001; Zheng *et al.*, 2003) .

Compared to the control (unexposed) group, concentrations of Na, K, Ca, Zn, Cu, Fe, Se and Mn in blood sera of rats had not been changed 48h after exposure to EM fields of 50, 100 and 200 kV/m2 but reduction K and Zn and increase of Ca were noted as the intensity of EM was increased. No influence of pulsating EM fields at macro and micro elements in blood sera were noted (Li *et al.*, 2014).

The concentrations of Mg, Fe, K, Zn and Cu in the blood sera were not significantly changed after EM stimulation in the experimental group of cows in the current study. This is in accordance with research (Burchard *et al.*, 1999).

In conclusion, the EM stimulation of cows leads to changes in concentrations of macro and micro elements in the blood. Different elements react differently to EM stimulation. This needs to be further studied. Reaction to EM stimulation is more intense in dairy cows with lower basal levels of ions. Exposure of cows to EM stimulation had a positive effect on Ca and P concentrations and improves calcaemia and phosphatemia in all exposed animals.

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