Influence of electromagnetic stimulation on secreting oxytocin and milk production in dairy cows under the heat stress

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SUMMARY

The release of oxytocin (OT) and milk ejection are most important for rapid and complete milk secretion and production. Inhibition of OT release and decrease in milk production has been observed in dairy cows during heat stress. The influence of electromagnetic stimulation (EMS) of whole body on the concentration of OT in the blood and milk yield in Holstein cows during a moderate heat stress has been tested. For the purposes of experiment dairy cows (n=60) in full lactation (150±50 days) were chosen, with an equal daily production of milk, divided into two equal groups: the experimental group of cows (n=30) and the control group of cows (n=30). Experimental animals were exposed by a unique bio-informational EMS protocol of three weeks' duration. OT concentration, milk production and temperature-humidity index (THI) were measured in the week before EMS exposure, during EMS exposure in the first, second and third weeks of the experiment and in the week after EMS exposure. Cows were exposed to heat stress during experiment (average THI 75-82 in all weeks). The concentration of blood OT was significantly different in the experimental group from the control group ($p<0.001$). In EMS cows OT concentration was higher in the 2nd and 3rd week of the study and the week after compared to the control group. The effect of the study week was not significant for the secretion of OT in the control group of cows ($P>0.05$). In control group milk production decrease during experiment, while in the EMS group milk production was stable, so that in EMS group milk production was significantly higher in the 2nd and 3rd week of the study and the week after compared to the control group ($p<0.05$). Dynamic changes in the concentration of OT and milk produced during the experiment show positive correlation ($R^2=0.31$; $p<0.01$), and that relation striving towards zero in control group. EMS can reduce the loss in milk production in cows in conditions of heat stress by stimulation of OT production. This needs further research with rigor experimental protocol.

KEY WORDS

Electromagnetic stimulation, dairy cows, oxytocin, milk yield, heat stress.

INTRODUCTION

The heat stress in cows is of great economical and health significance. During heat stress, there are behavioral, metabolic, endocrinological and productive changes which occur as a consequence of the body protecting itself from overheating. Cows experiencing heat stress consume less food, and the body uses larger amounts of glucose for adaptive mechanisms, so it redirects it from the udder towards other tissue which decreases milk production[1, 2]. Chronic stress can impact the welfare of dairy cows, and acute stress during milking can decrease milk yield[3]. Peripheral inhibition of mammary gland is induced by increased levels of catecholamines through stimulation of alpha-adrenergic receptors, likely via changes in ductal resistance.

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Inhibition of oxytocin (OT)release by the neurohypophysis has been observed in dairy cows during early lactation, during estrus period, and during milking under stress condition, blood levels of beta-endorphin and cortisol are increased in this periods[4]. The release of OT and milk ejection occurrence as an answer to teat stimulation are most important for rapid and complete milk secretion during milking and suckling[5].

The influence of electromagnetic stimulation (EMS) on physiological, endocrinological and reproductive status of dairy cows has been researched for a while now. Exposure to EM produced in an average, increased their feed intake, milk production, progesterone (P4) values in blood and estrous period length[6,7]. These EM have also been correlated with changes in the levels of macro- and microelements in blood[8], neurotransmitters in cerebrospinal fluid[9] and low variations in serum thyroxine (T4),[10] in dairy cows. Pregnant dairy cows exposed to electromagnetic field (EMF) (60 Hz, 10 kV/m, and 30 mT) had increased blood prolactin (PRL) and decreased melatonin (MLT) levels[11]. These EMSs have also been associated with changes in the milk

yield in dairy cows[6, 12-15]. Today, there are few papers about the influence of EMS on the secretion of OT and milk yield in dairy cows[16, 17]. The chamber characteristics allow use of a wide range of stimulation such as electric fields (0-30 kV/m) and magnetic fields (0-100 T) at frequencies from 45 to 3000 Hz[18].

EMS change morphogenesis and metabolic characteristic of cells in vitro[19]. The aim of this study was to examine the influence of morphogenetic EMS on synthesis and secretion of OT and milk yield in Holstein cows during moderate heat stress by special protocol of morphogenetic EMS.

MATERIAL AND METHODS

*Experimental conditions on the farm***:** The experiment was conducted on a farm of Holstein Friesian breed of cows (Ba ka Palanka, Vojvodina, Serbia). The animals were kept using a free range system. Preparation and standardization of a daily meal was done in accordance with the nutritive and energetic needs of dairy cows in full lactation. The cows were categorized in groups and the amount and contents of the meal depended on the stage of production and stage of lactation. On the farm, cows were fed twice daily using total mixed ration (TMR) and water was available *ad libitum*. The cows received a diet consisting of 7 kg lucerne hay, 20 kg maize silage (30% DM) and 5 kg concentrate (18% CP). Nutrient content of ration for experimental dairy cows in early lactation include: dry matter (DM) 21.5 kg; net energy of lactation 153.2 MJ; crude protein (CP) 18.3% DM; rumen-undegradable protein 39.69% CP; fat 4.92% DM; fiber 17.2% DM; acid detergent fiber (ADF) 22.6% DM; neutral detergent fiber (NDF) 37.16% DM. For the purposes of experiment dairy cows (n=60) in full lactation (150±50 days) were chosen, in a second and third parity with an equal daily production of milk in the termo-neutral period, divided into two equal groups: the experimental group of cows (n=30) and the control group of cows (n=30). The experiment and control cows were confined in the same chamber. The chamber was 20 m long, 10 m wide, and 3 m high. Current daily production average on a farm is about 28 L of milk. Milking is performed two times a day.

Heat stress index: Temperature and humidity index value (THI) was determined at 7 AM and 2 PM using the following formula: THI= (1.8×Temperature) - (1-Humidity) × (Temperature-14.3) +32. Air temperature was 19.5-24°C at 7AM and 31-36.5°C at 2PM, and humidity was 75-85% at 7AM and 45-55% at 2PM. The data was obtained by reading the closest weather station Republic Hydro-meteorological Service of Serbia. The results of the study show that during the experiment cows were exposed to mild-moderate to moderate-severe heat stress, with average THI from 74.5 to 81 (Fig. 1). (explanation: https://www.techmix-global.com/knowledge-center/dairy-heat-stress/).

Electromagnetic stimulation: Electromagnetic stimulation, protocol and equipment were described previously[20]. Before stimulation, recording of nerve impulses of dairy cows and their saving in the database was performed in a specific way in the first phase, with graphic representation of recorded signal on Fig 2b. Delta Sigma (DS) stimulation principle of activation is used (Fig. 2c). The signal includes a modulated digital information, previously recorder in the first phase (patent number: II - 2017/00107)[21].

Blood sampling and milk production: The blood samples were collected from all experimental and control groups of the cows before and in the first, second and third week of experiment, and in the week after the end of the experiment at 8:00 h im-

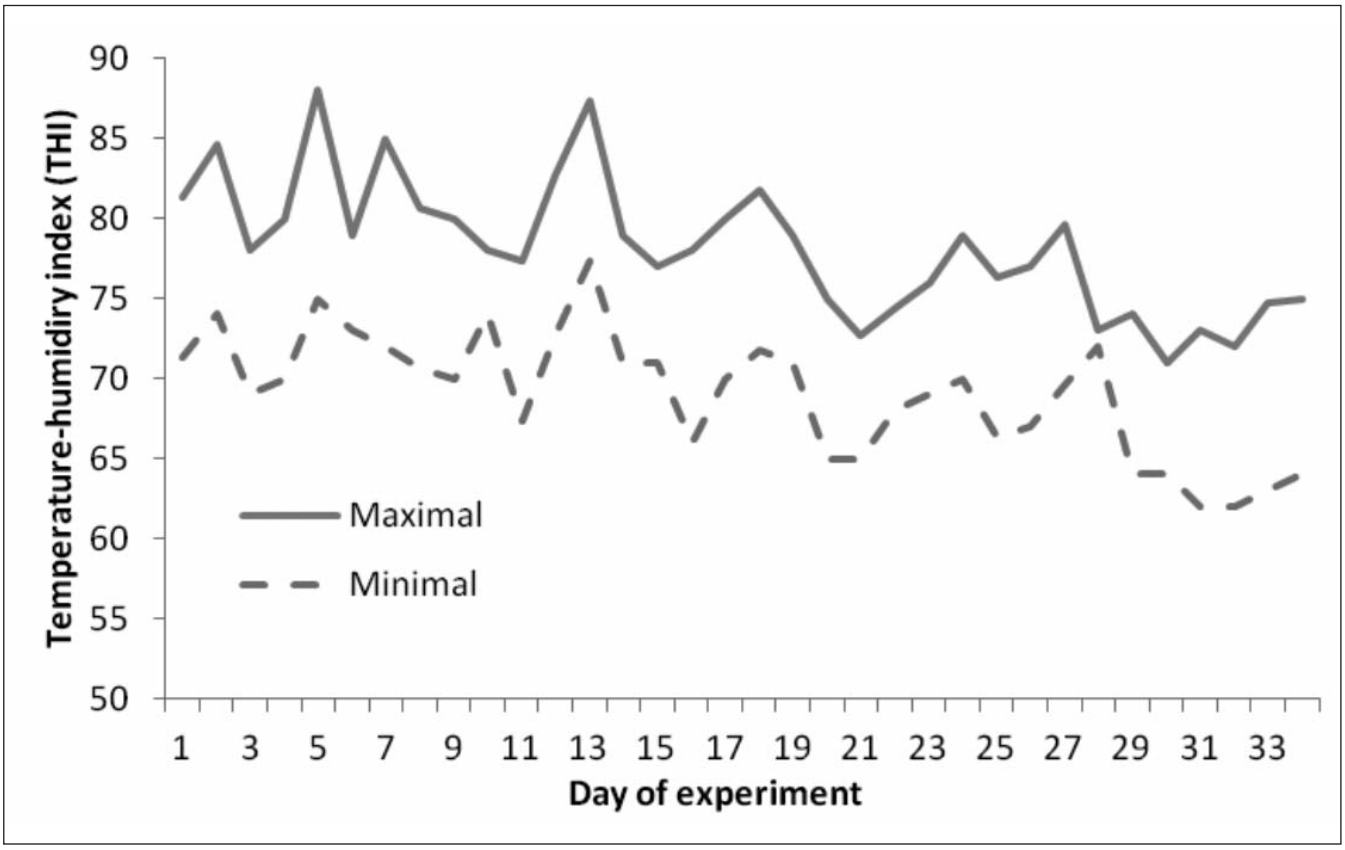

Figure 1 - Temperature-humidity index (THI) value during experimental period.

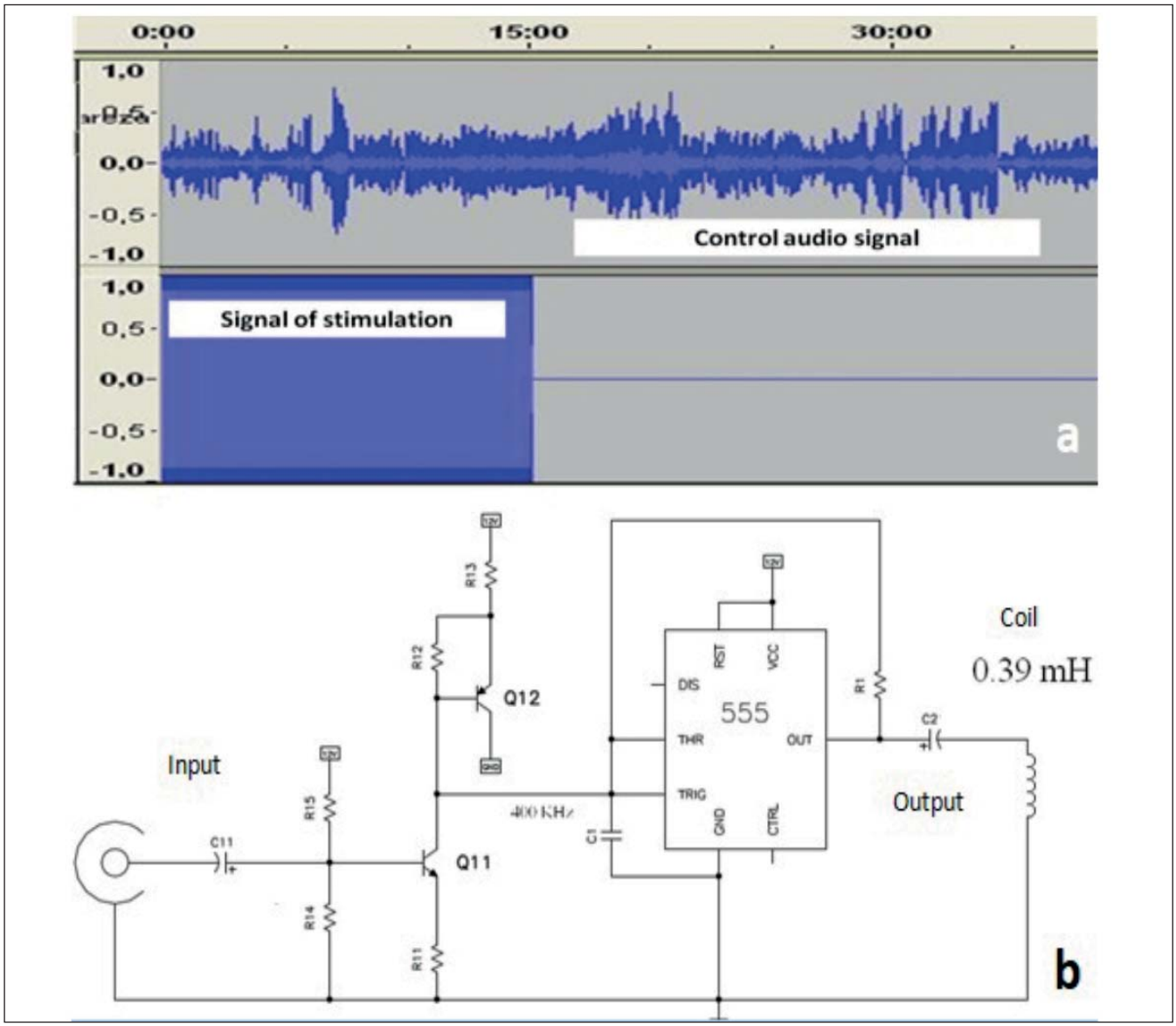

Figure 2 - Electromagnetic recorded stimulation signal in first phase (a); MS modulator for production of electromagnetic stimulation (b).

mediately after milking and feeding, by puncture of 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. OT concentrations in blood serum were measured by oxytocin ELISA Kit (Abcam, ab133050, UK) on ELISA reader (Rayto, PRC). At the farm, there was a fishbone milking machine (DeLaval), with individual milking machines and a software for monitoring milk production for each cow separately.

Statistical analysis: The influence of the group (EMs to control) and the effect of the week of the experiment (before, one, two, three, and one week after stimulation) on the value of OT and milk production using the two-factor ANOVA analysis, with the post-hock least significant difference test were examined. Correlation and regression between oxytocin and milk production and milk production and THI were determined using the Pearson coefficient correlation and analysis of the linear regression equation of the team Y=bX+a. The statistical package SPSS (IBM, USA) was used.

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

RESULTS

Influence of EMS on OT concentration and milk production was presented on table 1 and figure 3 and 4. The concentration of blood OT was significantly different in the experimental group from the control group ($P<0.001$). In EMS cows OT concentration was higher in the 2nd and 3rd week of the study and the week after compared to the control group. The effect of the study week was not significant for the secretion of OT in the control group of cows ($P>0.05$). In control group milk production decrease during experiment, while in the EMS group milk production was stable, so that in EMS group milk production was significantly higher in the 2nd and 3rd week of the study and the week after compared to the control group ($p<0.05$).

Dynamic changes in the concentration of OT and milk produced during the experiment show positive correlation

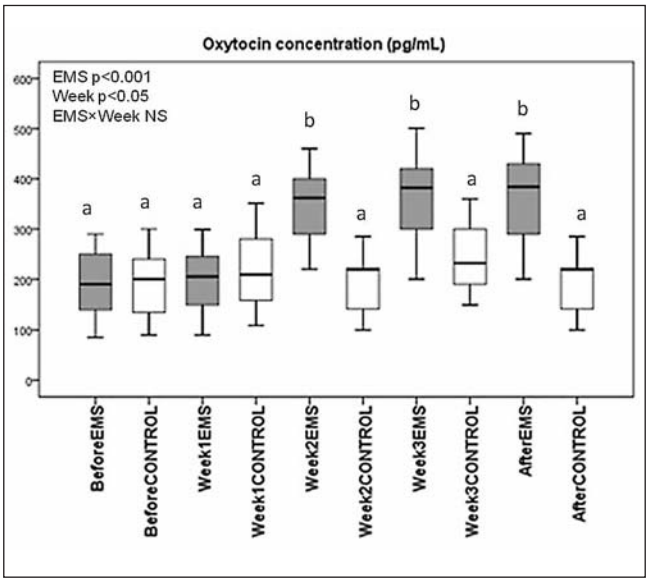

Figure 3 - Concentrations of oxytocin in blood (pg/mL) before, during and after the EM stimulation in the experimental (gray boxplot) and the control group (white boxplot) of cows (a, b- different superscripts mean a significant difference at the minimum level p<0.05).

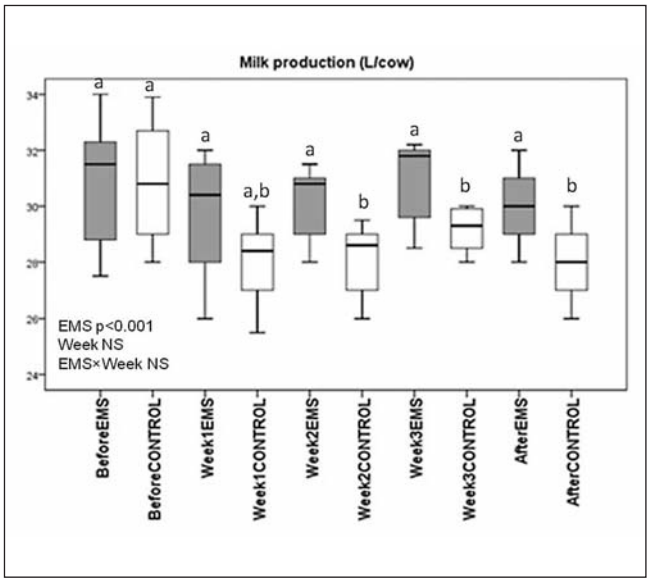

Figure 4 - Milk production (L/cow) before, during and after the EM stimulation in the experimental (gray boxplot) and the control group (white boxplot) of cows (a, b- different superscripts mean a significant difference at the minimum level p<0.05).

(R^2=0.31; p<0.01), and that relation striving towards zero in control group (Fig. 5).

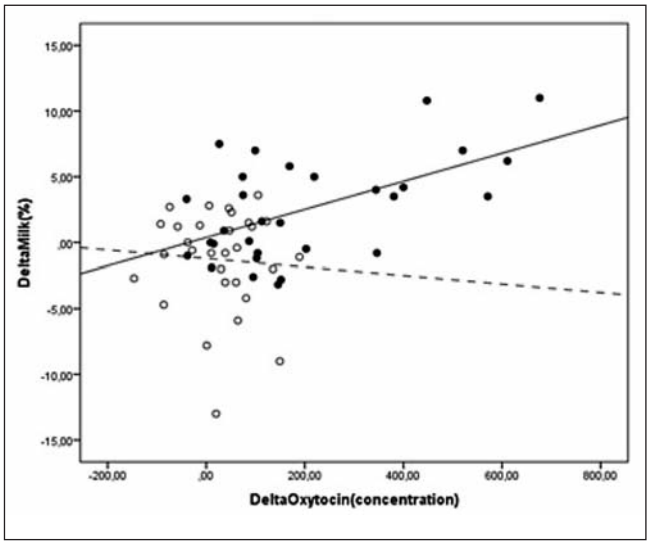

Figure 5 - Correlation of the oxytocin response and milk response over time (filled circle and solid line - EMS exposed, empty circle and dashed line - negative control).

DISCUSSION

Moderate heat stress of cows is a regular occurrence in the region of Vojvodina (Serbia) during the summer months, and the values of the maximum THI index showed a tendency to increase in the last decade[2]. All cows in experiment are in middle (full) lactation, when milk production is much more correlated with heat stress[22].

Electrophysiological recordings showed that, immediately before every milk extraction, the entire neurosecretory OT population creates a synchronized explosion of action potentials leading to the release of OT from the nerve endings of neurohypophysis[17, 21]. Thus, with morphogenetic stimulation, not only is the activation of the hypophysical oxytocin done, which causes the contraction of myoepithelial muscle cells around the milk canals, causing the release of milk, but also a

Table 1 - Influence of EM and exposure time on the concentration of oxytocin and milk production

	Source	Type III Sum of Squares	Mean Square	F	Sig.
Oxytocin concentration (pg/mL)	Corrected Model	1647607.6	235372.5	8.03	P<0.001
	Intercept	18928426	18928426.1	645.86	P<0.001
	EM	1296255.1	1296255.1	44.23	P<0.001
	Week	302463.7	100821.2	3.44	P<0.05
	EM ×Week	44018.9	14672.9	0.5	NS
Milk production (L)	Corrected Model	425.4	60.8	2.17	P<0.05
	Intercept	213351.8	213351.8	7617.57	P<0.001
	EM	329.1	329.1	11.75	P<0.01
	Week	22.4	7.5	2.27	NS
	EM ×Week	70.6	23.5	0.84	NS

conditioned reflex (biofeedback) is created, which explains the increase in the concentration of OT in the blood in the experimental group of cows even after the end of the experimental period in this study.

The average basal and dynamic OT values obtained in this experiment were slightly higher than OT values in the blood in other study[23, 24]. Other authors came to similar results, i.e. the tendency of the increase in OT levels was also established during occasional EMS, with maximum values of 350 pg/mL[17]. The obtained results in this experiment indicated that EMF stimulation has a positive influence on the release of oxytocin from neurohypophysis in dairy cows in conditions of moderate heat stress. In accordance with this are the data from other researchers which found that the increase in OT in the blood during the OT infusion administered at milking is necessary for the increase of intramammary pressure increases, flow of milk and complete extrusion of milk[25]. OT application immediately before or after the milking can increase milk production by 3%[26]. When dairy cows were treated with electric shock (3.6 mA, or 6.0 mA) (5 sec on, 25 sec off) in the morning and afternoon milking for 7 days, the total milk yield did not change significantly, but the yield of milk was significantly higher in the morning milking (15.87 kg) compared to the afternoon milking (13.90 kg). Furthermore, higher OT values in blood were recorded, for both basal OT (409 pg/mL) and maximum OT (761 pg/mL) in the morning, compared to the afternoon values, basal OT level (298 pg/mL), as well as maximum OT (515 pg/mL), in a group of 3.6 mA, which may indicate that animals have adapted to stimulation[16]. The concentrations of OT in our experiment agree with the reported values. The maximum concentrations of OT in our experiment were lower compared to the quoted result, which can be attributed to the influence of heat stress on the concentration of OT. Electric shocks as well as EMS represent sensory stimuli for OT secretion. In the latest editorial article[27] it was found that "oxytocin may be released by different kinds of sensory stimulation to induce well-being and restorative processes and to inhibit pain, stress and inflammation", which is consistent with the positive effect of EMS on OT secretion in our experiment.

In this study, the use of EMS in cows of the experimental group in moderate heat stress leads to the maintenance of milk production throughout the experimental period and in the week after the end of the experiment, while in the control group of cows there is a significantly lower milk production. This suggests that by the end of the experimental period, milk yield had declined less rapidly in the exposed than in the non-exposed animals. This observation is consistent with previous reports indicating that the milk yield decreased (non significant) as response to EMS[10, 11, 14]. In accordance with that Burchard et al.[28] found that exposure to EMS resulted in an average decrease of 4.97% in milk yield, and an increase of 4.75% in dry matter intake. In contrast, other authors determined a slight increase in milk production after EMS in experimental dairy cows by 0.3 kg[6], 0.5 kg[13] and 0.1-0,5 kg[12]. Avdeenko et al.[15] found that after application of electromagnetic radiation of extremely high frequency wave during lactation, increase in milk production in dairy cows by 18.45-22.0% and improvement of the functional state of the udder by 16.45-26.34%. In our experiment, there was a double effect of EMS, so that in the first week there was a decrease in milk production, and in the third week milk production was slightly higher (by 0.2L) than in the week before exposure to EMS. It is possible that there was an increase in food consumption during exposure to EMS, which led to these slight variations in milk production. In this experiment, cows were used in mid/full lactation, when milk production is most dependent on the feed intake. With the cessation of EMS, milk production decreased slightly, although the value of oxytocin remained unchanged, so it is possible that EMS also helps to maintain milk yield during heat stress in other ways, and the increase in dry matter intake could be an interesting theory for future research.

Electromagnetic stimulation showed different effect on endocrine status, milk production and behavior change in dairy cows[29-30], and our results are in accordance with previous results. The obtained results unambiguously indicate that EMS positively influences the secretion of OT, that is, in conditions of stress (heat or milking) it enables increased and complete milking (milk ejection and removal during milking) of the mammary gland in cows with low basal OT values in the blood, and thus achieving slightly higher milk production.

CONCLUSIONS

The use of EMS in cows in moderate heat stress leads to maintenance of milk production, while in the control group of animals there is significantly lower milk production. In cows under EMS, the concentration of OT increases during the time of experiment and is significantly higher compared to the control group. Changes in milk production and OT secretion show a positive linear correlation. EMS can reduce the loss in milk production in cows under heat stress by stimulation of OT production.

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