

# THE IMPACT OF ENVIRONMENTAL FACTORS ON THE MILK EJECTION AND STRESS OF DAIRY COWS

T. Bobić<sup>1</sup>, P. Mijić<sup>1</sup>, I. Knežević<sup>1</sup>, M. Šperanda<sup>1</sup>, B. Antunović<sup>1</sup>, M. Baban<sup>1</sup>, M. Sakač<sup>1</sup>, E. Frizon<sup>2</sup>, T. Koturić<sup>2</sup>

<sup>1</sup>Faculty of Agriculture, 31 000, Osijek, Republic of Croatia

<sup>2</sup>Croatia Agriculture Chamber, 31 000 Osijek, Republic of Croatia

Corresponding author: [tbobic@fos.hr](mailto:tbobic@fos.hr)

Original scientific paper

**Abstract:** Farm breeding of cattle is organized so that the animals are kept in a controlled environment (an closed corner, feeding time, milking time, workers), where daily animal encounter with familiar sounds, smells, movements and equipment. Any positive or negative change of environment causes certain physiological reactions of cattle on it. Negative stimuli (relocation, new technological activities, unidentified sounds and people) disrupt the homeostasis of the animals and lead them into a state of stress. During stress in cattle appears central inhibition of milk ejection from the alveoli of the udder, causing its incomplete emptying which could lead to pathological changes. In stressful situation, there is a connection between nervous (SA system), endocrine (HPA-axis) and the immune system. Stimuli from the outside were obtained through sight, hearing, smell and touch, leading to nerve impulses which then activate the endocrine system (increased levels of cortisol,  $\beta$ -endorphin, adrenaline, and reducing levels of oxytocin in the blood), whose actions over a long time lead to decline in immune response and susceptibility to disease. Many studies suggest an association between central inhibition of milk ejection and stressful situations (first milking of primiparous cattle, environment changes), but the very principle of inhibition is not fully understood. Some studies indicate an association between endogenous opioids ( $\beta$ -endorphin) and catecholamines (adrenalin) in the central inhibition of milk ejection, however, there is still no clear evidence and there is still plenty to do in this area.

**Key words:** stress, milk ejection, oxytocin,  $\beta$ -endorphin, cortisol

## Introduction

Despite thousands of years that have passed since the domestication of animals, animal behavior and animal today resemble their wild ancestors (McBride et al., 1969, Jensen, 1986). Because they were hunted in the past, fear of predators

taught them easy spotting threats and rapid escape from it (*Rushen et al., 1999*). *Boissy (1995)* states that fear is aversive emotional state, caused by environmental factors. Adjustment of farm animals to changes in environmental factors result from the interactions of neural and endocrine processes (*Weiss et al., 2004*). Adverse changes in the environment that disturbs homeostasis of animals can be called stressors, and they can refer to: unknown noises, strangers, injury, trauma, bad previous experience, inadequate housing, therapeutic interventions, transport, transfer, lower social rank (*Squines, 2003*). Stress in cattle can occur for many reasons, most are at primiparous cattle during the first milking (*Reenen et al., 2002; Bruckmaier et al., 1992*), relocation into an unknown environment (*Bruckmaier et al., 1993*), transition from suckling to milking (*Tančin et al., 1995*), the transition from conventional to automatic milking (*Weiss et al. 2004*) or because of rough handling animal by workers (*Rushen et al., 1999, 2001*). The physiological changes in animals are occurred as a consequences of stress (*Blokhuis et al. 1998*). Which could affect an animal in a manner that negatively affect health (*Hopster et al. 1998a,b*), reproduction or production, but can also affect the milk ejection, which is closely linked the nervous and endocrine regulation in the body.

In dairy cows, the largest amount of milk (about 80%), located in the alveoli of the udder (*Bruckmaier, 2005*). It is essential to enable the release of milk from alveoli during suckling or machine milking. The effect of environment (without of stress) on the psychological state of the cattle is crucial, because it allows undisturbed release of oxytocin during milking and complete emptying of the udder. Since the possibility of not releasing milk, leading to stagnation of milk in the udder and creating conditions for the development of pathological conditions, such as mastitis, which creates a great technological and financial difficulties of farmers, the aim of this study was to show the relationship between the endocrine and central nervous system during exposure cattle to adverse impacts of the environment in which it is located.

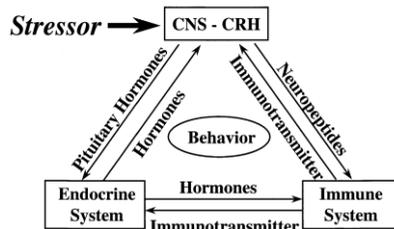
**Milk ejection.** Milk ejection is the active transport of alveolar milk into the cisternal compartment. It consists of: the contraction of the myoepithelial cells that surround the alveoli like a basket and the transfer of the milk through the milk duct system (*Bruckmaier, 2005*). The release of milk is an innate reflex, neuroendocrine origin, that is not influenced by the will of the animals and called a neuroendocrine reflex arc. Part of the nervous reflex arc begins with tactile stimulation of mammary glands and receive stimuli through neuroreceptors (*Lefcourt and Akers, 1984*), and transferred him to supraoptic and paraventricular nuclei of the hypothalamus (*Richard, 1972*), where begins the synthesis of the hormone oxytocin. Through the pituitary stalk, oxytocin is transported to neurohypophysis and stored there. His release from neurohypophysis represent the endocrine reflex arc and his journey to the target site, myoepithelial cells of alveoli (*Tančin and Bruckmaier, 2001*). It connects to specific receptors, causing conformational

changes in the cells and their contraction, and thus allows the release of milk from the alveoli into the drains and then into the udder cistern. Accumulation of milk in the tank increased intra mammary pressure and volume of the tank (*Bruckmaier and Blum, 1996*). Due to the limited capacity of tanks, it can not be full amount of alveolar milk squeeze out, if not simultaneously squeeze out of the udder, with suckling or milking.

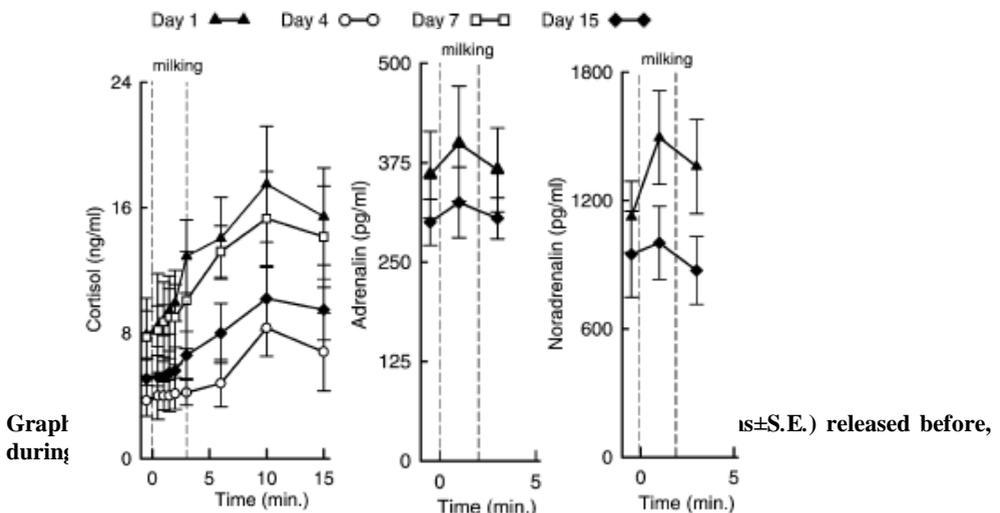
**Nervous-endocrine response to stress.** Response of animals to stress depends on its genetic inheritance, previous experience and physiological response. That is associated with the function of HPA axis (hypothalamic-pituitary-adrenal axis), SA system (the sympathetic nervous system) and the immune system (*Borell, 2001*). The animal receiving certain stimuli from its environment over: sight, hearing, smell and touch. In situations like the first milking or unknown environment, an animal that is seen as negative stimuli and that lead to stress (*Hopster et al., 1998b*). During stress occurring neural and hormonal changes in the animals that cause behavioral changes and changes in reproduction, production and immunity. Neurons of the autonomic nervous system belongs to sympathetic or parasympathetic way, who are acting contrary to one another. Parasympathetic dominate in a relaxed state. In situations where the animal is exposed to a negative stimuli from the environment triggers the sympathetic way of the central nervous system, activation of the adrenal glands, who release from the catecholamine (adrenalin and noradrenalin) which are important in the "fight or flight" situation when it increases blood pressure and heart rate, symptoms typical of stress situations. This response to stress occurs in seconds and affects the animal reacts to fight or to retreat. The answer is subsequent to that under the influence of the HPA axis and occurs more slowly (minutes to an hour) but has a far-reaching impact (*Squines, 2003*). Specifically, the hypothalamus releases CRH (corticotrophin releasing hormone), which is transmitted via the hypophyseal stalk to the anterior pituitary, causing release of ACTH (adrenocorticotrophic hormone),  $\beta$ -endorphin,  $\beta$ -lipotropin and  $\alpha$ -melanotropin (*Borell, 2001*). ACTH acts on the cortex of the adrenal glands, which then release glucocorticoids (cortisol and corticosterone), which have a wide range of effects in the body (increases blood sugar levels, in the body reduce protein synthesis, increases the activity of  $\text{Na}^+$ -  $\text{K}^+$  pump in cardiac cells, increases proteolysis in muscle, bones and skin). Long exposure of animals to stress, and to high levels of glucocorticoids in the organism can cause: diabetes, low immunity, increase hart rate, slowed growth in young animals, and reduced production.

**Disturbance of milk ejection.** In dairy cows, according to the *Bruckmaier (2005)*, can cause problems in the milk ejection, either due to braking the secretion of oxytocin from the posterior pituitary (central inhibition), whether due to disruption of action of oxytocin in the milk glands (peripheral inhibition). *Peeters et al. (1973)*, a statement *Wellnitz and Bruckmaier (2001)* point out that the  $\alpha_1$ -,  $\alpha_2$ - and  $\beta_2$ -adrenergic receptors are divided into three different regions of udder. The

highest level of expression of receptors, the authors found in the muscular layer of the teat, the less in the tissue of large milk drainage canals that surround the tank, and the lowest concentrations were found in the parenchyma of the udder. High concentrations of  $\alpha$ -adrenergic receptors within the large milk ducts that surround the tank, indicating that peripheral inhibition of milk can be placed precisely in that area, mainly due to contraction of smooth muscle surrounding the milk ducts (*Bruckmaier et al. 1997*). In the present of antagonists  $\alpha$ -adrenergic receptor and oxytocin receptor blocking agent, will perform peripheral inhibition of milk ejection. In this case, the amount of oxytocin is normal, but its effect on myoepithelial cells has been disabled and missing their contractions and the milk can not squeeze out alveoli in the drains. Central inhibition of milk ejection is caused by release of oxytocin from the pituitary gland, and occasionally occurs in cattle production as a result of various stresses, in which decreased levels of oxytocin, and increased levels of  $\beta$ -endorphin, cortisol, ACTH, and catecholamines in blood plasma.



**Shema 1.** Interaction between central neural system (CNS), endocrine and immune system (*Borell, 2001*)



**Table 1. Milk production characteristics, and behavioral and physiological measures recorded in primiparous cows (n = 23) during milking sessions on day 2, 4, and 130 of lactation (Van Reenen et al. 2002)**

Measure	Day of lactation		
	Day 2 Mean	Day 4 Mean	Day 130 Mean
Milk yield (kg)	8.4 <sup>a,b</sup>	7.2 <sup>a</sup>	10.6 <sup>b</sup>
Residual milk (% of total milk <sup>1</sup> )	34 <sup>c</sup>	18 <sup>b</sup>	9 <sup>a</sup>
Baseline heart rate (beats/min)	97.6 <sup>b</sup>	98.1 <sup>b</sup>	82.4 <sup>a</sup>
Heart rate change <sup>2</sup> (beats/min)			
During preparation	12.0 <sup>b</sup>	4.6 <sup>a</sup>	9.7 <sup>b</sup>
During milking	8.3 <sup>b</sup>	5.8 <sup>a,b</sup>	4.2 <sup>a</sup>
Oxytocin after preparation (pg/mL)	11.4 <sup>a</sup>	15.0 <sup>a,b</sup>	27.8 <sup>b</sup>
Oxytocin <sup>3</sup>	92.4	113.7	116.0
Cortisol <sup>3</sup>	174.6 <sup>b</sup>	134.5 <sup>a</sup>	138.2 <sup>a,b</sup>
Cortisol 10 min after attachment (ng/ml)	11.5 <sup>b</sup>	8.9 <sup>a</sup>	9.6 <sup>a,b</sup>

<sup>1</sup>Total milk (kg): milk yield plus residual milk obtained after administration of exogenous oxytocin; <sup>2</sup> Relative to baseline; <sup>3</sup>Area under time after cluster attachment vs. hormone curve; a,b,c Means in the same row without a common superscript differ significantly ( $P < 0.05$ ).

**$\beta$ -endorphin, cortisol and catecholamines.** During stress caused due to an unknown environment, the secretion of oxytocin are inhibited but plasma levels of  $\beta$ -endorphin and cortisol are increased. When the cows get used to the new environment, the concentrations of these hormones decrease while oxytocin release is gradually normalized (Bruckmaier, 2005). According to a study of Tančin et al. (2000), the release of oxytocin and milk ejection can halt with application of exogenous opioids morphine. This effect can be undone application of opioid antagonists Naloxon. However Wellnitz et al. (1997) and Mačuhova et al. (2002) emphasize that Naloxon it can not stop the inhibition of oxytocin in cows that are milked in unfamiliar surroundings or at primiparous cows that have recently calved, according to research Kraetzl et al. (2000). However, the role of endogenous opioids in regulating the release of milk and mechanisms of inhibition of oxytocin release in cows is not fully understood.

During routine milking, the concentration of plasma cortisol increases physiological in cows (Bruckmaier et al., 1993). Also, intravenous application of cortisol has no effect on the release of milk according to the results given by Mayer

and Lefcourt (1987), a statement Bruckmaier (2005), and from this to suggest that cortisol has no effect on the central inhibition of milk ejection.

In primiparous cows according to the Tančin and Bruckmaier (2001) in the first few days after calving, appear central inhibition of milk injection due to the disturbance of oxytocin release from neurohypophysis, because the first encounter with the milking, and they are see that as a kind of stress. The hypothesis was confirmed by Lefcourt and Akers (1991) who conducted a study on pregnant heifers, in which they found a weak increase in oxytocin levels during the first tactile stimulation of the udder, as compared with the same stimulation in older pregnant cows where it was released much higher levels of oxytocin.

Song et al. (1988) noted disturbance of oxytocin release in rats by activation of  $\beta$ -adrenergic receptors, which are activated by adrenalin from the core of the adrenal glands. In sheep and calf during milking and udder first tactile stimulation inhibits the release of oxytocin, while increasing the levels of noradrenalin (Lefcourt and Akers, 1991). However, Blum et al. (1989) noted that the machine milking does not lead to secretion of adrenaline.

To overcome central inhibition of milk ejection it is necessary to elevate oxytocin blood concentrations above the threshold level, either by exogenous oxytocin (by the injection) or by applying nervous stimuli strong enough to induce endogenous oxytocin release by vaginal stimulation (Wellnitz and Bruckmaier, 2001).

## Conclusion

During its production life, the cows were exposed to both positive and negative factors of their environment. In situations when they feel threatened, insecure and frightened, stress appear, which manifests itself through physiological changes in her body. Negative stimuli from outside activate the SA system and HPA axis, causing a hormonal change in the blood (increased levels of  $\beta$ -endorphin, cortisol, ACTH, and catecholamines), which on the one hand leads to elevated blood pressure and increased heart rate, on the other hand leads to disturbances in the release of milk, decreased productivity and decreased immunity. Studies suggest an association between central inhibition of milk ejection and stressful situations (first milking of primiparous cows, changes in the environment, etc.), but the very principle of inhibition is not fully understood. There was a connection between endogenous opioids ( $\beta$ -endorphin) and catecholamines (adrenalin) in the central inhibition of milk, however there is still no clear evidence and there is still plenty to do in this area.

## Uticaj faktora sredine na izlučivanje mleka i stres muznih krava

*T. Bobić, P. Mijić, I. Knežević, M. Šperanda, B. Antunović, M. Baban, M. Sakač, E. Frizon, T. Koturić*

## Rezime

Farmsko gajenje goveda organizovano je tako da se životinje drže u kontrolisanom okruženju (zatvoreni prostor, vreme obroka, vreme muže, radnici) gde se životinja svakodnevno susreće sa poznatim zvucima, mirisima, pokretima i opremom. Svaka pozitivna ili negativna promena okruženja uzrokuje izvesne psihološke reakcije goveda na nju. Negativni stimuli ( promena lokacije, nove tehnološke aktivnosti, nepoznati zvuci i ljudi) ometaju homeostazu životinja i uvode ih u stanje stresa. Tokom stresa kod goveda dolazi do centralne inhibicije ejakcije (ispuštanja) mleka iz alveola vimena, prouzrokujući njihovo nepotpuno pražnjenje što može dovesti do patoloških promena. U stresnim situacijama postoji veza između nervnog (SA sistem), endokrinog (HPA-axis) i imunog sistema. Spoljašnji nadražaji dobijeni čulima vida, sluha, mirisa i dodira prenose se nervnim impulsima aktivirajući endokrini sistem (povećava se nivo kortizola,  $\beta$ -endorfina, adrenalina, a smanjuje nivo oksitocina u krvi), čija aktivnost tokom dužeg vremenskog perioda dovodi do pada imunog odgovora i sklonosti ka bolestima. Mnoge studije ukazuju na povezanost između centralne inhibicije ejakcije mleka i stresnih situacija (prva muža prvotelki, promene u okruženju), ali sam princip inhibicije nije u potpunosti objašnjen. Neke studije ukazuju na vezu između endogenih opioida ( $\beta$ -endorfin) i kateholamina (adrenalin) u centralnoj inhibiciji ejakcije mleka ali ipak ne postoje jasni dokazi i još mnogo toga treba uraditi u ovoj oblasti.

## References

- BLOKHUIS H. J., HOPSTER H., GEVERINK N. A., KORTE S. M., VAN REENEN C. G. (1998): Studies of stress in farm animals. *Comparative Hematology International*, 8, 94-101.
- BLUM J. W., SCHAMS D., BRUCKMAIER R. (1989): Catecholamines, oxytocin and milk removal in dairy cows. *Journal of Dairy Research*, 56, 167-177.
- BOISSY A., (1995): Fear and fearfulness in animals. *Quarterly Review of Biology*, 70, 165-191.
- BORELL E. H. (2001): The biology of stress and its application to livestock housing and transportation assessment. *Journal of Animal Science*, 79, E260-E267.

- BRUCKMAIER R. M. (2005): Normal and disturbed milk ejection in dairy cows. *Domestic Animal Endocrinology*, 29, 268-273.
- BRUCKMAIER R. M., SCHAMS D., BLUM J. W. (1992): Etiology of disturbed milk ejection in parturient primiparous cows. *Journal of Dairy Research*, 59, 479-98.
- BRUCKMAIER R. M., SCHAMS D., BLUM J. W. (1993): Milk removal in familiar and unfamiliar surroundings: concentrations of oxytocin, prolactin, cortisol and  $\beta$ -endorphin. *Journal of Dairy Research*, 60, 449-456.
- BRUCKMAIER R. M., BLUM J. W. (1996): Simultaneous recording of oxytocin release, milk ejection and milk flow during milking of dairy cows with and without prestimulation. *Journal of Dairy Research*, 63, 201-8.
- BRUCKMAIER R. M., WELLNITZ O., BLUM J. W. (1997): Inhibition of milk ejection in cows by oxytocin receptor blockade,  $\alpha$ -adrenergic receptor stimulation and in unfamiliar surroundings. *Journal of Dairy Research*, 64, 315-325.
- HOPSTER H., VAN DER WERF J. T., BLOKHUIS H. J. (1998a): Stress enhanced reduction in peripheral blood lymphocyte numbers in dairy cows during endotoxin-induced mastitis. *Veterinary Immunology and Immunopathology*, 66, 83-97.
- HOPSTER H., VAN DER WERF J. T. N., BLOKHUIS H. J. (1998b): Short- and long term consistency of behavioural and physiological stress responses in dairy cows during a novel environment test, Ph.D. Thesis, University of Wageningen, Wageningen, Netherlands, 67-84.
- JENSEN P. (1986): Observations on the maternal behaviour of free-ranging domestic pigs. *Applied Animal Behaviour Science*, 16, 131-142.
- KRAETZL W. D., TANCIN V., SCHAMS D. (2000): Significance of opioids for the inhibition of oxytocin release and milk let down in primiparous fresh lactating dairy cows. 5th Joint EAAP/ ASAS Workshop on Biology of Lactation in Farm Animals.
- LEFCOURT A. M., AKERS R. M. (1984): Small increase in peripheral noradrenaline inhibit the milk-ejection response by means of a peripheral mechanisms. *Journal of Endocrinology*, 100, 337-344.
- LEFCOURT A. M., AKERS R. M. (1991): Teat stimulation-induced oxytocin and catecholamine release in pregnant and lactating holstein heifers. *Domestic Animal Endocrinology*, 8, 235-243.
- MAČUHOVA J., TANČIN V., KRAETZL W. D., MEYER H. H. D., BRUCKMAIER R. M. (2002): Inhibition of oxytocin release during repeated milking in unfamiliar surroundings: the importance of opioids and adrenal cortex sensitivity. *Journal of Dairy Research*, 69, 63-73.
- MAYER H. K., LEFCOURT A. M. (1987): Failure of cortisol injected prior to milking to inhibit milk ejection in dairy cattle. *Journal of Dairy Research*, 54, 173-7.

- MCBRIDE G., PARER I. P., FOENANDER F. (1969): The social organization and behaviour of the feral fowl. *Animal Behaviour Monographs*, 2, 127-181.
- NEGRÃO J. A., MARNET P. G. (2003): Cortisol, adrenalin, noradrenalin and oxytocin release and milk yield during first milkings in primiparous ewes. *Small Ruminant Research*, 47, 69-75.
- PEETERS G., DE BRUYCKER R., QUINTELIER W., PAAPE M. (1973): Contractions of the teat muscles of lactating cows. *Acta Milk Endocrinology*, 177, 50.
- RICHARD P. (1972): The reticulo-hypothalamo pathalamo pathway controlling the release of oxytocin in the ewe. *Journal of Endocrinology*, 53, 71-83.
- RUSHEN J., DE PASSILLE A. M. B., MUNKSGAARD L. (1999): Fear of people by cows and effectes on milk yield, behavior, and heart rate at milking. *Journal of Dairy Science*, 82, 720-727.
- RUSHEN J., TAYLOR A. A., DE PASSILLE A. M. (1999): Domestic animals' fear of humans and its effect on their welfare. *Applied Animal Behaviour Science*, 65, 285-303.
- RUSHEN J., MUNKSGAARD L., MARNET P. G., DePASSILLÉ A. M. (2001): Human contact and the effects of acute stress on cows at milking. *Applied Animal Behavior Science*, 73, 1-14.
- SONG S. L., CROWLEY W. R., GROSVENOR C. E. (1988): Evidence for the involment o fan adrenal catecholamine in the  $\beta$ -adrenergic inhibition of oxytocin release in lactating rat. *Bain Research*, 457, 303-309.
- SQUINES E. J. (2003): *Applied Animal Endocrinology*. CABI Publishing, Cambridge, MA, USA.
- TANČIN V., HARCEG L., BROUČEK J., UHRINČAT M., MIHINA Š. (1995): Effect of suckling during early lactation and change over to machine milking on plasma oxytocin and cortisol levels and milk characteristics in Holstein cows. *Journal of Dairy Research*, 62, 249-56.
- TANČIN V., BRUCKMAIER R. M. (2001): Factors affecting milk ejection and removal during milking and suckling of dairy cows. *Veterinarni medicina*, 46, 4, 108-118.
- TANČIN V., KRAETZL W. D, SCHAMS D. (2000): Effects of morphine and naloxone on the release of oxytocin and on milk ejection in dairy cows. *Journal of Dairy Research*, 67:13-20.
- VAN REENEN C. G., VAN DER WERF J. T. N., BRUCKMAIER R. M., HOPSTER, H., ENGEL B., NOORDHUIZEN J. P. T. M., BLOKHUIS H. J. (2002): Individual differences in behavioral and physiological responsiveness of primiparous dairy cows to machine milking. *Journal of Dairy Science*, 85, 2551-2561.
- WEISS D., HELMREICH S., MÖSTL E., DZIDIC A., BRUCKMAIER R. M. (2004): Coping capacity of dairy cows during the change from conventional to automatic milking. *Journal of Animal Science*, 82, 563-570.

WELLNITZ O., BRUCKMAIER R. M., BLUM J. W. (1997): Naloxone and adrenergic blocking agents fail to abolish central inhibition of milk ejection in cows. *Journal of Dairy Research*, 64, 627–31.

WELLNITZ O., BRUCKMAIER R. M. (2001): Central and peripheral inhibition of milk ejection. *Livestock Production Science*, 70, 135-140.

Received 30 June 2011; accepted for publication 15 August 2011