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ENDOCRINE CHANGES IN TWO BEHAVIORAL TYPES OF COWS EXPOSED TO HEAT[1]

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Abstract: Cows at the farm were divided into 3 behavioral groups according to their emotional response to the experimenter. Holstein cows from I^{-st} (n=3) and III^{-rd} (n=3) behavioral types at I^{-st} lactation were transported to climatic laboratory (20 miles) and exposed to heat (35^{0} C) for 7 hours. Blood and milk cortisol, epinephrine, norepinephrine, growth hormone and prolactin were measured before transportation (7.30 h) and during exposure to heat (9.30 h, 12.30 h, 13.30 h, 15.30 h and 16.30 h,). Physical and emotional responses resulted in a significant elevation in plasma and milk cortisol levels immediately after placement of the cows in the climatic laboratory (9.30 h). Heat exposure caused a significant decline in plasma and milk cortisol levels. The enumerated environmental stressors resulted in plasma norepinephrine elevation in III^{-rd} type (P>0.05). Plasma norepinephrine level in III^{-rd} type declined significantly under heat but tended to be higher compared to that in I^{-st} type. The emotional arousal caused a significant rise (P<0.01) in plasma prolactin levels, which remained high under heat in both behavioral types, but prolactin levels tended to be higher in III^{-rd} type of cows. Plasma GH level in III^{-rd} type was significantly higher at 15.30 h and 16.30 h, as compared to that at 7.30 h. The presented results suggest that the specific response to heat stimuli has overwhelmed the non-specific "psychological" response in order to reduce the level of calorigenic hormones and to counteract heat-induced inhibition of the immune response. Besides, these results show that plasma cortisol, epinephrine and norepinephrine are related to the behavioral type.
Key words: behavior, cortisol, epinephrine, norepinephrine, growth hormone, prolactin

Introduction

Continued selection for high milk yield is accompanied with elevation of endogenous heat production. Therefore the unilateral selection may aggravate the ability of the animal to cope with the adverse effect of the environmental heat. The endocrinological events in exposed to heat cows have been examined relatively well (*El Nouty et al*., 1980; *Hodate et al.*, 1982). However we did not find any data related to the endocrinological aspects in different behavioral types of cows under heat stress. *Mason* (1971) has shown that heat exposure do not activate the pituitary - adrenal axis if emotional arousal is carefully avoided. Therefore the magnitude of the emotional response may influence the rate of endogenous heat production.

We have now investigated the extent of the emotional arousal cows subjected to transport, new environment and acute heat exposure.

Material and Methods

Cows were divided into 3 groups according to their behavior characterized by cows avoidance response to man (*Lankin et al*., 1979). Food responses dominated in the behavior of I^{-st} type of cows, while avoidance responses to man were absent. In contrast III^{-rd} type demonstrated pronounced avoidance response to man. The cows were transported from the farm (at 7.30 h) to the climatic laboratory (at 9.30 h) and were immediately exposed to heat (35^{0} C) at relative humidity 50 % for 7 hours.

Blood samples were obtained from the tail vein at 7.30, 9.90, 11.30, 13.30, 15.30 and 16.30 h. Milk samples were taken at the same time intervals. Growth hormone levels (GH) were measured using RIA method (*Friesian and Car*, 1976), prolactin levels (PRL) were determined by the method described by *Barnes et al*. (1985); blood and milk cortisol levels (C) - by the method of *Kanchev et al*., (1976) and *Butler and Bordes* (1980) respectively. Plasma epinephrine (E) and norepinephrine (NE) were quantitated using reverse-

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phase high pressure liquid chromatography with electrochemical detection (*Keller et al*., 1976; *Kissinger*, 1977). Rectal temperature (RT) was measured with thermister probe (*Fisher Scientific, Pittsburg*); respiratory rate (RR) - by counting *Flank* movement.

Statistical method: Differences between groups means were analyzed by *Student*`t test.

Results

Exposure of cows to transportation and novelty induced a marked increase ($p<0.001$) in plasma C levels in both behavioral types (Figure 1). However plasma C levels in III^{-rd} type tended to be higher, compared to those in I^{-st} type. Acute exposure to heat (at 9.30 h) produced a significant decrease ($p<0.001$) of plasma C levels in both behavioral types. Plasma C levels under heat remained within the normal range in I^{-st} type. However plasma C levels in III^{-rd} type fluctuated and were significantly lower ($p<0.05$) at 16.30 h than those in I^{-st} type. The pattern of milk C dynamics was similar to that of plasma C dynamics (Fig. 2). However blood C concentrations were around 10 times higher than in milk.

The basal levels of blood E tended to be higher ($p>0.05$) in III^{-rd} type (59.68±12.53 vs. 32.66±11.085). Following exposure to heat blood E declined in III^{-rd} type (34±15.95) and was under sensitivity of the method in blood of I^{-st} type cows (at 9.30 h). During the rest of the heat treatment period E levels were not within sensitivity limits of the method. Milk E levels were under the sensitivity of the method either. Both basal and stress-induced NE levels in blood of III^{-rd} type cows tended to be higher, compared to those in I^{-st} type (F3). The combination of transport, novelty and heat (at 9.30 h) elicited diverse NE response in the two behavioral types. There was insignificant enhancement of NE levels in III^{-rd} type and unchanged NE levels in I^{-st} type cows. Blood NE levels under heat were within the normal range. The combined effect of transport, novelty and heat (at 9.30 h) resulted in a marked increase of blood PRL levels in both behavioral types (F4). Blood PRL levels remained high during the exposure to heat. There was no effect of the behavioral types on PRL response to heat. Neither transportation nor the heat exposure had any effect on milk PRL levels (F4), which remained unchanged in control time intervals. Combined stressor treatment (at 9.30 h) caused a slow elevation of blood GH levels in both behavioral types (F5). Heat exposure produced further GH enhancement, which was significant in III^{-rd} type at 15.30 h and 16.30 h ($p<0.05$) relative to control levels (7.30 h). The pattern of milk GH response to heat (F5) in both behavioral types was quantitatively similar but there were no significant changes in the response to heat. There was no significant difference in the heat-elicited rectal temperature rise between the two types of cows (Table 1). Heat exposure produced significant elevation of respiratory rate in both type of cows (Table 2). The behavioral type had no effect on respiratory rate under heat.

Table 1. Rectal temperature in two behavioral types of cows before and during exposure to heat

		I^{-st} type		III^{-rd} type	
Time	n	x	Sx	x	Sx
Farm – 7.30 h	3	38.60	±0.290	38.70	±0.320
Climatic laboratory 9.30 h	3	39.53	±0.035	39.70	±0.120
Climatic laboratory 11.30 h	3	40.10	±0.430	40.30	±0.350
Climatic laboratory 13.30 h	3	39.96	±0.120	40.10	±0.340
Climatic laboratory 15.30 h	3	39.70	±0.170	40.00	±0.240

Table 2. Respiratory rate in two behavioral types of cows before and during exposure to heat

		I^{-st} type		III^{-rd} type	
Time	n	x	Sx	x	Sx
Farm – 7.30 h	3	46.0	±5.600	44.0	±5.600
Climatic laboratory 9.30 h	3	141.0	±5.014	129.0*	±21.062
Climatic laboratory 11.30 h	3	138.0	±22.060	148.0*	±7.520
Climatic laboratory 13.30 h	3	139.0	±3.001	132.0*	±22.065
Climatic laboratory 15.30 h	3	147.0	±2.507	128.0*	±8.029

*p<0.001 control value

Discussion

The pattern of blood C dynamics (Figure 1) is consistent with the view of *Dantzer and Mermode* (1983) that removing the emotional arousal reduces stress response to heat. The elevated adrenal response to the combined stimuli, elicited by transportation, novelty and heat exposure during the accomodation of the cows in the climatic laboratory (at 9.30 h) could be considered as a nonspecific "general response" of the animal. During the exposure to heat the physical quality of the thermic stimuli overcame the effect of the emotional stimuli and caused a marked decline in C level (Figure 1). The reduction in plasma C level under heat could be due to the specific endocrine changes directed toward suppression of endogenous heat production. Cortisol has been shown to increase endogenous heat. Besides, glucocorticoids inhibit immune response directly through inhibition of cytokine production and indirectly by inhibition of pro-inflammatory transcription factors. Furthermore the lower level of C is expected to be related with lower level of corticotrophin releasing hormone and the latter has been found to mediate interleukin-1β induced acute phase protein synthesis. Thus the lack of C elevation under heat could contribute for attenuation of the heat induced immune suppression. The observed higher blood C level ($p>0.05$) in III^{-rd} type of cows suggests that adrenal response to stress is related to the behavioral type.

The lower milk content of C (F2) could be explained with the fact that the higher amount of C taken by a calf via milk could suppress calf`s hypothalamic–pituitary adrenal axis. Catecholamines response to stress unlike C occurs in seconds relative to perceived threats. Therefore the lack of E enhancement to the specific physical quality of heat stimulus directed towards reduction of endogenous heat production. Epinephrine unlike NE has a potent calorigenic effect, which necessitates a quick decline in response to heat. Beside the short half–life of E contributes for the rapid disappearance of E from the peripheral circulation. The higher baseline level of E in III^{-rd} type and the fact that E was still present in he blood of the same behavioral type at 9.30 h suggest that E is possibly related to the behavioral type. The factors responsible for the observed insignificant change in blood NE level to stressor treatment are uncertain (F3) the higher NE level in III^{-rd} type, observed as a trend, suggests that NE like E (F4), could be related to the behavioral type. The marked blood PRL response to the combined stressors at 9.30 h (F5) in the present experiment was expected, since it has been demonstrated, that physical and emotional stress potentate both PRL and adrenocorticotropin secretion. However blood PRL level, unlike C remained high under heat, showing once again that after the emotional arousal the endocrine changes correspond to the physical quality of the stimulus. This appears to be an important adaptive mechanism directed against the heat-induced heat shock transcription factor inactivation (*Mathew et al*., 2001) and against the heat provoked activation of apoptosis.

The increased respiratory rate under heat (Table 2) is related with markedly increased reactive oxygen species. Heat shock proteins are efficient protectors against reactive radicals. Therefore heat-evoked elevation of PRL level is directed against heat-induced immunosuppression. Moreover PRL has also been found to suppress adrenal steroidogenesis and to antagonize C-induced immune suppression. Corticosterone in turn suppresses PRL response to psychological and physical stress. Prolactin and C dynamics under heat are consistent with their antagonistic effect on the immune system. Furthermore it has been demonstrated that PRL is associated with body temperature regulation. Because of its specific temperature reducing effect PRL seems to be reliable indicator for the magnitude of the heat stress load. Cortisol, as a conventional indicator of stress cannot indicate the rate of the metabolic impairment under heat. Prolactin levels in both behavioral types of cows were quantitatively and qualitatively similar. It is not clear why milk PRL level remained low (F6) under heat since milk PRL is digested in the gastro-intestinal tract of the calf and could not disturb the endocrine function of the calf.

Exposure to the heat evoked an increase ($P>0.05$) in blood GH levels (F7). Nevertheless the increase in the III^{-rd} type was significant ($p<0.05$). The observed slight release in GH levels under heat could be related with its specific physiological role during exposure to heat. It has been shown that GH stimulates sweat secretion rate (*Sneepen et al*., 2000). Besides, GH promotes proliferation of lymphocytes and protects cells from high temperatures. The lack of marked increase in GH levels under heat is probably due to the fact that the control of GH secretion under heat is more complicated. Potassium deficiency has been found to cause GH and insulin like growth factor-1 suppression. Furthermore lower serum potassium has been reported in cows under heat (*El Nouty*, 1980), because of the higher potassium content in cattle perspiration. Therefore the lower serum potassium under heat could suppress GH secretion. There is also evidence favoring the modulation of GH secretion by C. It has been found that glucocorticoids exert a positive control on GH gene expression. On the other hand glucocorticoids inhibit GH effects at target tissue. Milk GH levels under heat were within the range of blood GH levels (F8). Our data suggest that heat exposure does not change the ratio between blood and milk GH levels. Although mean RR values under heat (Table 2) were not significantly different between the measurement (from 9.30 to 15.30 h), RR of each individual cow fluctuated within the range of 6 to 44 % from one measurement to next one.

These data suggest that the cows employed a selective brain cooling during the exposure to heat. According to *Jessen* (1998) high sympathetic activity suppresses the selective brain cooling. Therefore RR data in our case are consistent with the low level of E and NE.

Conclusion

Heat-induced changes in the studied hormones are directed towards suppression of endogenous heat production and elevation of heat dissipation. Besides, endocrine changes under heat could be considered as a means to alleviate heat-evoked immune suppression.

ENDOKRINE PROMENE U PONAŠANJU KOD KRAVA IZLOŽENIH TOPLOTI

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Rezime

Krave na farmi su podeljene u 3 grupe tipova ponašanja prema njihovim emocionalnim reakcijama. Holštajn krave I^{-st} (n=3) i III^{-rd} (n=3) tipa ponašanja u prvoj laktaciji su prebačene u klimatsku laboratoriju (20 milja) i izložene toploti (35^0 C) u trajanju od 7 sati. Kortizol iz krvi I mleka, epinefrin, norepinefrin, hormon rasta I prolaktin su mereni pre transporta (7.30 h) tokom izlaganja životinja toploti (9.30 h, 12.30 h, 13.30 h, 15.30 h and 16.30 h,). Fizičkei i emocionalne reakcije su rezultirale u significantnom povećanju nivoa kortizola u plazmi i mleku odmah nakon stavljanja krava u klimatsku laboratoriju (9.30 h). Izlaganje toploti je izazvalo singifikantan pad u nivoima koritzola u mleku i plazmi. Stresori iz okoline su rezultirali povećanjem norepinefrina u plazmi kod III^{-rd} type (P>0.05). Nivo norepinefrina u plazmi kod krava tipa III^{-rd} je signifikantno opao pod uticajem toplote ali je bio viši u poređenju sa nivoom utvrđenim kod tipa I^{-st}. Emocionalna reakcija je izazvala signifikantno povećanje (P<0.01) nivoa proteina u plazmi, koji se zadržao i pod uslovima toplote kod oba tipa ponašanja, ali nivoi prolaktina su bili nešto viši kod krava tipa III^{-rd}. GH nivoi u plazmi kod krava tipa III^{-rd} su bili signifikantno veći u 15.30 h i 16.30 h, u poređenju sa 7.30 h. Predstavljeni rezultati sugerišu da su specifične reakcije na toplotni stimulans nadvladali ne-specifične "psihološke reakcije" kako bi se smanjio nivo kalorigenskih hormona i suprotstavilo inhibiciji imuno reakcije izazvanoj toplotom. Pored toga, ovi rezultati pokazuju da kortizol u plazmi, epinefrin i norepinefrin su povezani sa tipovima ponašanja.

Ključne reči: ponašanje, kortizol, epinefrin, norepinefrin, hormone rasta, prolaktin

Figure 1. Plasma cortisol level in two behavioral types of cows before and during exposure to heat (35^o C)

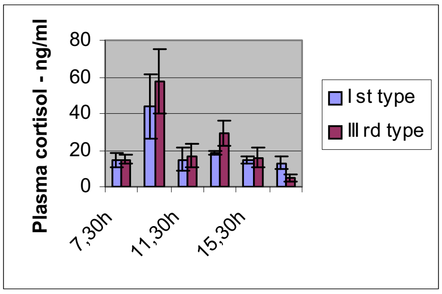


Figure 2. Milk cortisol level in two behavioral types of cows before and during exposure to heat (35^o C)

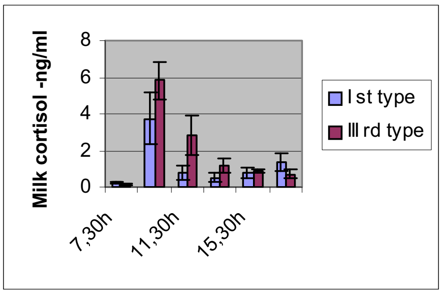


Figure 3. Plasma norepinephrine level in two behavioral types of cows before and during exposure to heat (35^o C)

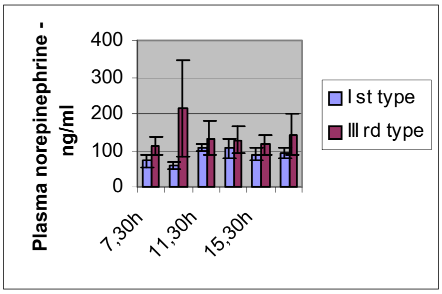


Figure 4. Plasma epinephrine level in two behavioral types of cows before and during exposure to heat (35^o C)

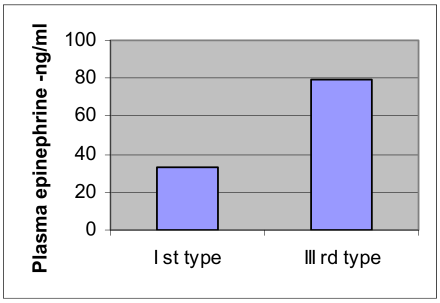


Figure 5. Blood prolactin level in two behavioral types of cows before and during exposure to heat (35° C)

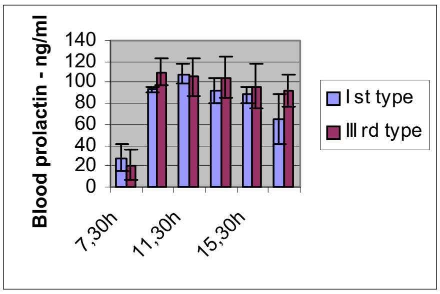


Figure 6. Milk prolactin level in two behavioral types of cows before and during exposure to heat (35° C)

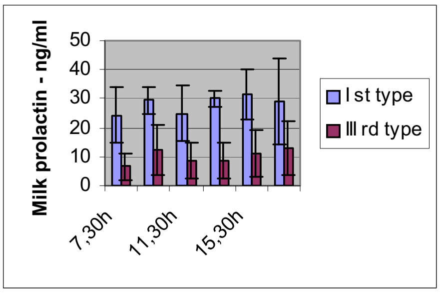


Figure 7. Blood growth hormone level in two behavioral types of cows before and during exposure to heat (35° C)

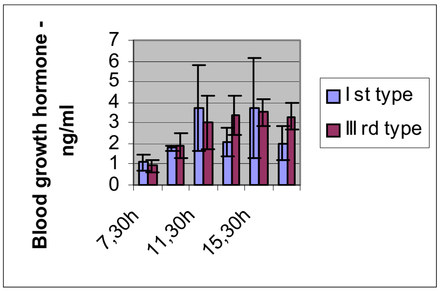
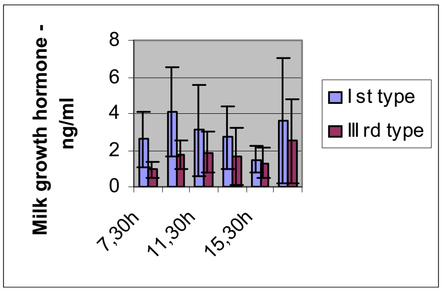


Figure 8. Milk growth hormone level in two behavioral types of cows before and during exposure to heat (35° C)



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