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RELIABILITY OF SOME ENDOCRINE AND BEHAVIORAL INDICES OF STRESS

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Abstract

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This investigation accentuates on the reliability of some endocrine (cortisol and prolactin), behavioral (bleating frequency, prevalence of feed and defense response) and immune (heterophil/lymphocyte ratio) indices of stress. Our study comprised several experimental designs with laying hens, cows, does and kids.

We found sizable stress response to blood sampling procedures that was registered as early as the first minutes after catching of the bird. There were pronounced individual differences in stress-sensitivity demonstrated by plasma cortisol levels which varied from low to high values. Heterophil to lymphocyte ratio, unlike cortisol began to change after a latent period of 10-15 min. following the start of stress stimuli. When combined with cortisol this ratio allowed us to differentiate stress stimuli induced by the handling procedures from those induced by a certain environmental and technological factors.

Plasma cortisol level turned out to be reliable stress indicator in does, previously habituated to blood sampling procedures. However, kid separation at the time of weaning caused significant plasma cortisol enhancement. There was no stress when the kids were prevented from suckling but remained to their mothers. The frequency of bleating did not correspond to plasma cortisol dynamics indicating that it is not reliable stress indicator in this particular case.

Exposure of cows to heat stress elicited short-term increase of plasma cortisol followed by a quick decline to values that were within the normal range in spite of the elevated rectal temperature. Plasma prolactin increased and remained high throughout the heat load period. These results demonstrate that plasma cortisol level can not be used as a universal stress-indicator.

Introduction

Animal welfare is closely related with the presence or absence of stress. There are still many controversial and difficult areas concerning animal welfare that stems from sophisticated behavioral and physiological mechanisms used by the animals to deal with stress. Most of the currently used endocrine, behavioral and immunological criteria failed to produce reliable assessment of animal welfare. This is due mainly to the fact that the term "stress" is applied loosely to various situations that often has little in common. The attempts to monitor stress are further hampered by the fact that the very act of monitoring is stressful. Therefore, farm animal welfare has to be objectified and measured. This will help to reliably assess animal welfare and to formulate advice for farmers on possible improvement.

The object of this paper is to present our investigations on the reliability of some stress indicators.

Material and Methods

First experiment

Birds were raised on floor pens in stress free conditions. Feed and water were available *ad libitum*. Twenty five 55 weeks old, Loman laying hens were chosen at random for blood collection. After catching they were placed in a common crate and carried to a separate room in the same building. During the crating period the hens had no access to food and drinking water. Blood was collected from each hen only once in order to evade possible stress effect of multiple blood sampling. The exact time from the point of catching to the point of bleeding of each bird was registered. Blood samples were taken by puncture of wing vein, for 90 min. Plasma corticosterone dynamics is presented as average corticosterone values in birds that were sampled between 1-5 min (taken as 5 min), 5-15 min (taken as 15 min), 15-30 min (taken as 30 min), 30-60 min (taken as 60 min) and 60-90 min (taken as 90 min).

Plasma corticosterone was determined by the ELISA method, using enzyme immunoassay kit (IBL, Gesellschaft fur immunchemie und immunbiologie, MBH, D 22335 Hamburg, Germany).

Peripheral blood leukocytes subpopulations were counted microscopically in smears (Giemsa-Romanovsky-stain).

The results of one factor statistical analysis are expressed as means ±S.E.M. and were analyzed by ANOVA.

Second experiment

Four Bulgarian native milk type goats and their kids (n=5), were reared in a dry pen. Before weaning the kids were kept with their does for natural suckling and in addition to suckling they were given high quality meadow hay and a starter mixture offered *ad libitum* in a creep to which their mothers had no access.

The kids were abruptly weaned at the age of 45 days and kept in a room next to dams` pen with a possibility for vocal communication between kids and their does.

Blood samples were obtained by jugular vein puncture 5 days before weaning (-5d) and at regular intervals after the weaning (16th h, 5th and 10th d). All blood samples were taken in less than 5 min in order to minimize the effect of handling.

Third experiment

It had similar experimental design to that of the second experiment. The only difference was that after weaning the kids remained to their mothers but was prevented from suckling by an apron fitted to the udder of the mother.

Fourth experiment

Six cows were transported from a farm (7.30h) to a climatic laboratory (9.30h) and were immediately exposed to heat (35 ^{0}C) at relative humidity 50% for 7 hours (Table 1). Blood samples were obtained at 7.30, 9.30, 11.30, 13.30, 15.30 and

Table 1

Rectal temperature and respiratory rate in cows exposed to heat (35^0 C)

Hour, h	n	Rectal temperature		Respiratory rate	
		x	Sx	x	Sx
Farm					
7.30	6	38.65	0.3	45	5.6
Climatic laboratory					
9.30	6	39.61	0.077	135	21.03
11.30	6	40.20	0.39	143	18
13.30	6	40.03	0.23	135.5	22.06
15.30	6	39.85	0.21	137.5	8.029

16.30h. Cortisol level was measured by the RIA method of Kanchev et al. (1976) and prolactin level by the method described by Barns et al. (1985). Respiratory rate was measured by counting flank movement and rectal temperature was registered with thermister probe (Fisher Scientific, Pittsburg).

Results and Discussion

Plasma corticosterone levels increased steadily at 30 min. (P<0.01) after catching and crating and then declined at 60 min. (P<0.01) and 90 min. (P<0.01) relative to those at 5 min (Figure 1). However, corticosterone level at 90 min was a little bit higher than that at 15 min, indicating that hens were still under stress although less pronounced. Our results are not consistent with those reported by Kannan and Mench (1996) who found steady increase of corticosterone level up to 3 h following 2 min long handling and subsequent crating for 3h in 7 –week- old broilers. The enumerated contradictory results regarding the stress-inducing effect of catching and crating in hens and broilers were probably due to the effect of some concomitant stressful events, like crowding, social disruption, breed, age, previous experience etc. Psychological stress is fear stress. Fear responses in a particular situation are difficult to predict because they depend on how the bird perceives the handling or exposure to novelty. Fear elicited stress fits completely to the classical general adaptation syndrome. Physical stress, unlike psychological one, depends on the specific effect of the physical stimulus which may or may not require further activation of hypothalamic-pituitary-adrenal axis following the initial general adaptation syndrome.

When we scrutinized individual corticosterone levels we found individual hens with low corticosterone level (4-6 nmol/L), while other hens had 7 to 9 fold higher corticosterone levels within the second minute after catching. These data demonstrate that psychological stress stimuli induced by catching, social disruption and novelty cause quick adrenal response with variable magnitude amongst the individual hens. Following the peak values at 30 min plasma corticosterone levels gradually declined until the end of the crating period (90 min). Heterophil-to-lymphocyte ratio (H/L) unlike plasma corticosterone levels began to increase after a 15 min delay reached a peak at 60 min and then declined over the next 30 min (Figure 2). It reached level of significance (P<0.05) at 30 min relative to the ratio at the 5 min. These results indicate that H/L ratio follows corticosterone dynamics with a certain lag period. They are consistent with the finding reported by Dhabhar et al. (1995) which indicated that corticosterone is an important mediator of stress-induced changes in blood leukocyte distribution. The very fact that leukocyte kinetics is largely corticosterone dependent makes H/L ratio an important concomitant stress indicator, all the more so as it occurs 15-20 min after the initiation of the stress stimuli.

This peculiarity of the stress-induced leukocyte

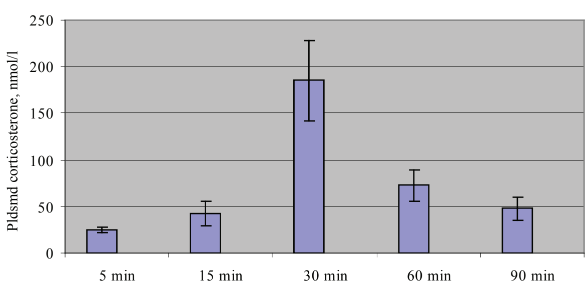

Fig. 1. Handling – induced corticosterone dynamics in hens

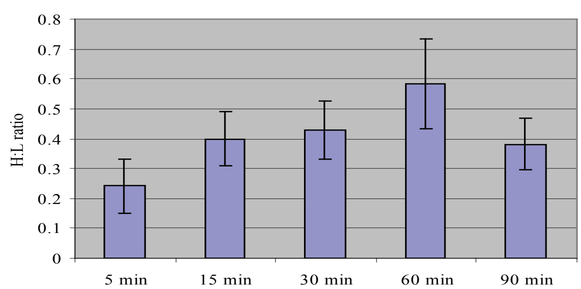

Fig. 2. Handling – induced heterophil-to-lymphocyte ratio in hens

profile can be used as a reliable source of information, when evaluating hen's well-being. Bearing in mind the fact that H/L ratio starts to change at least 15-20 min after the beginning of the stress event we can easily assess whether a possible increase of plasma corticosterone level at the first few minutes after catching of an individual hen is due to psychological stress caused by the catching or to some permanent technological stress- stimuli. Taken together our results demonstrate that H/L ratio is more reliable stress indicator than corticosterone itself. Blood corticosterone level is very sensitive indicator of stress but it can be seriously influenced by the handling procedure at the time of blood collection. It is desirable to measure both H/L ratio and plasma corticosterone levels as stress indices, since corticosterone level provides information on the magnitude of the stressor load.

Separation from the mother caused significant increase ($P<0.05$) of cortisol in kids by 16th h following the weaning. Cortisol levels remained high up to the 5th day (Figure 3). However, the absolute values of cortisol by 16th h and 5th day were not very high (only 2.5 times higher than baseline levels), and suggest that separation of kids from their mothers causes a moderate stress in kids. By the 10th day after the weaning plasma cortisol levels returned to the baseline levels. Therefore the weaning resulted in stress of short duration. No significant cortisol changes due to weaning were found in mothers (Figure 3).

The frequency of bleating (Figure 4) before weaning was higher in mothers (mean number of bleats 14.25) than in kids (mean number of bleats 0.2). Similar results have been reported by Smith (1963) who found higher level of bleats in ewes than in lambs. According to Smith (1965) ewe bleating is an instinct which allows the lamb to imprint the main vocal peculiarities of its mother. Plasma cortisol levels, measured before weaning, suggested that the elevated frequency of does' bleating was not provoked by stress and probably was an instinct. Therefore the use of the behavioral parameter "bleating" as a single stress-indicator may lead to an erroneous interpretation of the results obtained.

Separation from the mother resulted in a great increase of bleating frequency (BF) in kids which was highest by the 16th h and was maintained relatively high by the 5th day following the weaning (Figure 4). The enhanced BF by 16th h and 5th day is consistent with the augmented cortisol levels during the same time interval and suggests that the elevated BF was due to stress brought about by weaning. Three out of 4 does didn't bleat by 5th and 10th day following the weaning. The frequency of bleating in does increased by 16th h (60.25) and sharply declined

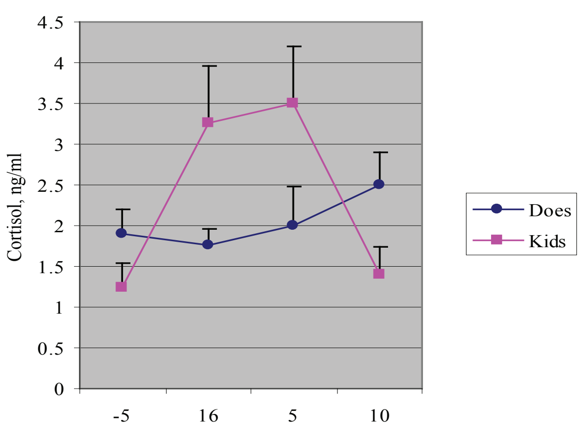

Fig. 3. Cortisol dynamics in does and kids before (-5th day) and after (16 h, 5th day, 10th day) weaning

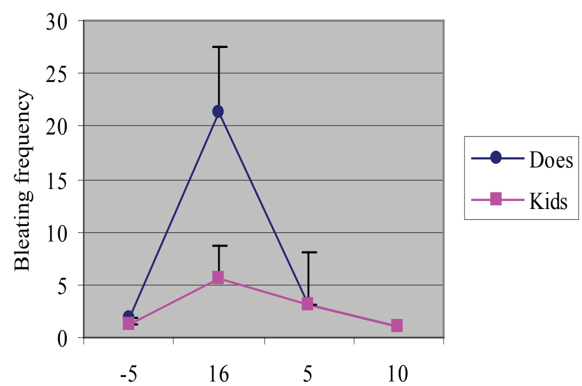

Fig. 4. Bleating frequency in does and kids before (-5th day) and after (16 h, 5th day, 10th day) weaning

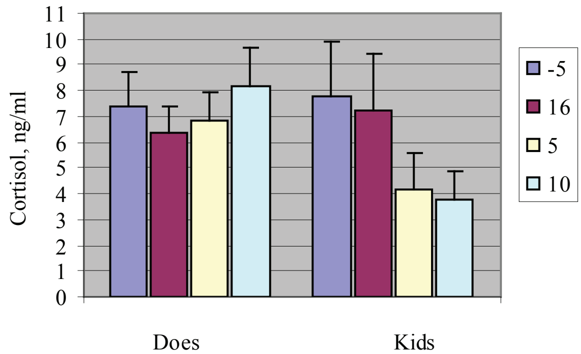

Fig. 5. Cortisol dynamics in does and kids before (-5th day) and after (16 h, 5th day, 10th day) weaning. Does and kids remained with their mothers after weaning

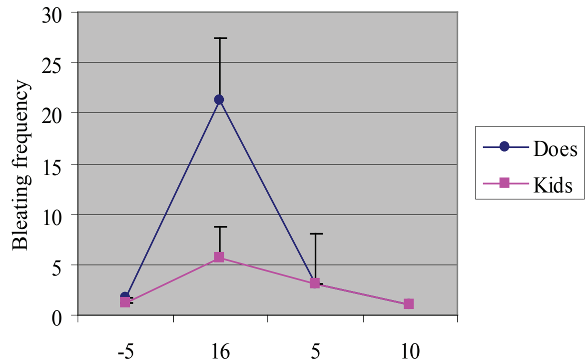

Fig. 6. Bleating frequency in does and kids before (-5th day) and after (16 h, 5th day, 10th day) weaning. Does and kids remained with their mothers after weaning

by 5th and 10th day following the weaning (0.25 and 0.75 respectively). However, the magnitude of bleating in does by 16th h following the weaning was lower than that in kids. The increased BF in does immediately after weaning (by 16th h) could be due to the fact that vocal communication was the only way through which mothers could get information for the welfare of their kids. These results indicate that bleating frequency can not be used as a single indicator of stress.

Plasma cortisol levels in does and kids did not change after the weaning during the second experiment when the kids were not separated from their mothers and had physical contact with them immediately after the weaning (Figure 5). These data demonstrate that the increased cortisol level in the kids during the previous experiment was due to disruption of the physical contact mother-kid and the new environment that was probably perceived by the kids as a potential threat. However, the ethological criterion of stress did not correspond to the unchanged level of plasma cortisol. Bleating frequency increased both in kids and does though it was 3 times lower in does and 30 times lower in kids relative to that in the previous experiment (Figure 6). The established discordance between plasma cortisol and bleating frequency was probably due to the temporary frustration of the kids caused by the prevented suckling. Does bleating was probably an innate reassuring response to the kids rather than stress-induced drive.

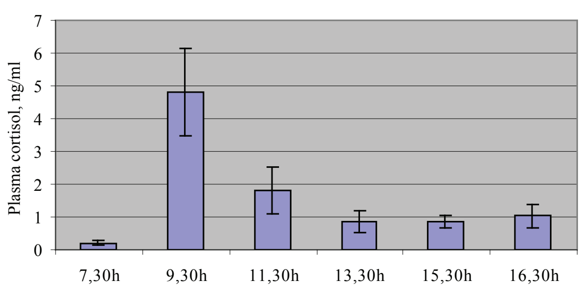

Fig. 7. Plasma cortisol dynamics in cows exposed to heat (35^{o}C)

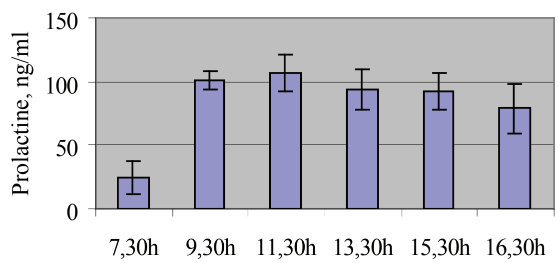

Fig. 8. Plasma prolactin dynamics in cows exposed to heat (35^{o}C)

Exposure of cows to transportation and novelty (Figure 7) induced a mark increase (P<0.001) in plasma cortisol levels, which declined sharply after exposure to acute heat load (P<0.001) in spite of the extreme stress load indicated by the elevated rectal temperature (Table1). Plasma prolactin levels increased sharply after exposure to heat an remained high throughout the heat treatment period. The reduction of plasma cortisol 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 production (Jousef and Johnson, 1967). Prolactin level in cows exposed to heat load increased and remained high throughout the treatment period (Figure 8). The increased prolactin level in cows under heat show once again that the emotional arousal induced by the new environment was quickly overcome by the specific physical quality of heat. Because of its specific temperature reducing effect (Sorbinho, 1993) prolactin seems to be reliable indicator for the magnitude of stress in cows under heat. Therefore cortisol can not indicate the rate of metabolic impairment in cows under heat. Prolactin, rectal temperature and respiration rate turned to be reliable heat stress indicators.

Conclusions

- Animal welfare assessment based on a single endocrine or behavioral criterion is not reliable and can mislead the experimenter.
- Animal welfare evaluation requires development of a verified set of endocrine behavioral and immunological indices of stress which can reliably identify animals exposed to various stress stimuli.

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