POULTRY WELFARE ASSESSMENT; IS IT POSSIBLE TO AVOID HANDLING-INDUCED MENTAL STRESS INTERFERENCE?

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Original scientific paper

Abstract: Monitoring and quantification of poultry welfare requires more studies on the pattern of adrenal response in hens, exposed to a large variety of stressors. The aim of this experiment was to investigate concurrent stress effects of catching, social disruption, crating and unfamiliar environment on the dynamics of plasma corticosterone level and heterophile/lymphocyte ratio in 55 weeks old, Loman breed hens. The hens were raised in a free range housing system. Twenty five 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. Blood samples were taken at 05, 15, 30, 60 and 90 min. after the crating. There was a steady increase in plasma corticosterone over the first 30 min. (P<0.01), followed by gradual decline by 60 and 90 min. (P<0.01) after the crating. Heterophile/lymphocyte ratio increased significantly at 60 min. after an initial lag period of about 15-20 min. Taken together our results suggest that corticosterone alone, can not be used for animal welfare assessment. Heterophile-to-lymphocyte ratio turns to be reliable indicator of poultry welfare status but it should be combined with measurement of plasma corticosterone in order to obtain a comprehensive view of any particular poultry welfare.

Key words: welfare, stress, H:L ratio, corticosterone, hens

Introduction

Animal welfare is a subject of rapidly increasing concern in most countries because of ethical reasons and new economic insights. Welfare can be defined in a way which incorporates ideas about need, feelings, stress and health (Broom, 2004). Monitoring and quantification of poultry welfare is frequently related to mental and/or physical stress. Research in physical or psychological stress, in order to monitor objective parameters of poultry welfare is usually investigated during experimental stress induction (Puvadolpirod and Thaxton, 2000; Post et al., 2003) and evaluated variety of parameters, including corticosterone (CS) and
heterophyl/lymphocyte ratios (H/L). However, the debate concerning the quantification of stress as it relates to poultry productivity and well-being is ongoing. Rushen (1991) has stated, that claims about animal welfare based on data regarding the pituitary-adrenocortical axis (the physiological stress response system) should be viewed with scepticism because of the lack of consistency between the results of different studies. A series of conclusions and recommendations were made on various aspects of housing systems, behaviour, health and mortality and other matters in relation to bird welfare. As part of its comprehensive assessment of the well-being of laying hens, one EU working group in the LayWel project (Blokhuis et al., 2007) compiled data on a variety of stress measures and found that measures were highly inconsistent, clearly demonstrating the fundamental problem with using a single measure of stress as the sole indicator of animal well-being. Blood sampling procedure can greatly influence corticosterone concentration (Mormede et al., 2007) and as a consequence, methodological differences can mask differences in reactivity and functionality of the HPA axis and thus worsen welfare assessment. Hence, monitoring and quantification of poultry welfare requires more studies on the pattern of adrenal response in hens, exposed to a large variety of stressors. It is not clear yet whether it is possible to avoid handling- induced mental stress interference.

The aim of this experiment was to investigate concurrent stress effects of catching, social disruption, crating and unfamiliar environment on the dynamics of plasma corticosterone level and heterophile/lymphocyte ratio in 55 weeks old, Loman breed hens.

Materials and Methods

This experiment was carried out in the Experimental poultry unit of the Institute of Animal science- Kostinbrod. Birds were raised in floor pens in stress free conditions. Feed and water were available ad libitum. Twenty five 55 weeks old, Loman egg 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 the stress potentiating effect of multiple blood sampling. The exact time from catching to 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) made immediately after each venipuncture throughout the 90 min. long sampling period. White blood cell differential count was determined by counting a total of 100 leukocytes, including heterophiles (H), lymphocytes (L), monocytes, basophils, and eosinophils under light microscopy. The H/L ratio was determined by Gross and Siegel (1983).

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

Results and Discussion

Plasma corticosterone levels increased steadily at 30 min. (P<0.01) after catching and crating and then decline declined at 60 min. (P<0.01) and 90 min. (P<0.01) relative to those at the 5 min (Figure 1). However, corticosterone level at 90 min was yet 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. Similarly, time of crating (4, 8, 12 h respectively) have been reported to correlate positively with plasma corticosterone levels in 42 day old Ross broilers under reduced floor space (Chloupek et al., 2008). Handling alone is known to cause immediate increase in corticosterone concentration (within 10 min.), followed by a return to normal values 30 min. after the stressful action (Harvey and Hall, 1990). On the contrary, plasma corticosterone level in hens has been reported to return to its baseline level within 12 h after handling (Downing and Bryden, 1999).

The enumerated contradictory results regarding stress potentiating effect of catching and crating in hens and broilers are probably due to the effect of some concomitant stressful events, like crowding, social disruption, breed, age, previous experience etc. Each stressor has mental and physical component and the relative ratio between these components is crucial for the duration of the provoked stress response.

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.

Figure 1. Effect of psychological stress on plasma corticosterone level in hens

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). These results show that crating – elicited fear at the beginning of the crating period declines over time probably because hens did not perceive the novelty and strange sights as threatening at the end of the crating period as at the beginning of the crating period when they were suddenly confronted with them. Individual differences in plasma corticosterone levels still existed at the last 30 min. of the crating period, but the levels in stress-susceptible birds did not surpass those in the stress-resistant birds more than 2-3 times. These results come to show that novelty and social disruption-induced fear declines over time.

Heterophile-to-lymphocyte ratio (H/L) unlike plasma corticosterone levels began to increase after 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 indicate that corticosterone is an important mediator of the 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.

Figure 2. Effect of psychological stress on heterophile-to-lymphocyte ratio in hens

This peculiarity of the stress-induced leukocyte 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. In our experiment we compared those hens which had high level of corticosterone up to 10 min. after catching with the corresponding H/L ratio in each hen and found that their H/L ratio was not changed at that time. Knowing that H/L starts to change 15 min after the onset of the stress event we can claim with certainly that hens with elevated plasma corticosterone level against the background of unchanged H/L ratio during the first 10-15 min. after the onset of the stress event have not been under stress conditions in the previous period (before the hens were taken away from the flock). Consequently, H/L ratio turns out to be indispensable research tool regarding poultry welfare assessment. Heterophile-to-lymphocyte ratio has been proposed as less variable but more reliable indicator of stress in birds than corticosterone level as early as the end of the last century (Maxwell, 1993).
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 and it can be seriously influenced by the handling procedure at the time of blood collection, yet 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.

**Conclusion**

Corticosterone alone is not suitable for welfare evaluation since it can mislead the investigator.

Heterophile-to-lymphocyte ratio is reliable welfare indicator but it doesn’t provide information about the varying levels of stress experienced by hens. For this reason H/L ratio must be used along with plasma corticosterone to specify correctly poultry welfare status.

**Ocena dobrobiti živine - Da li je moguće izbeći mentalni stres izazvan manipulacijom?**

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**Rezime**

Monitoring i kvantifikovanje stresa kod živine zahteva više ispitivanja i studija o obrascima adrenalne reakcije kod kokoši koje su izložene velikom broju raznovrsnih stresora.

Cilj ovog ogleda je bio da se ispitaju istovremenki uticaji stresa pri hvatanju, socijalne smetnje, stres pri stavljanju u sanduke i u nepozнатu sredinu na dinamiku nivoa plazma kortikosterona i odnos heterofila i limfocita kod Loman kokoši u uzrastu od 55 nedelja.

Kokoši su gajene u sistemu slobodnog držanja sa ispustom, 25 kokoši je odabrano slučajnim uzorkom za analizu krvi. Nakon hvatanja, stavljeni su u zajednički sanduk i odnete u odvojenu prostoriju u istom objektu. Uzorci krvi su uzimani 5, 15, 30, 60 i 90 minuta nakon stavljanja u sanduk.

Utvrđen je stabilan porast kortikosterona u plazmi u periodu od prvih 30 min. (P<0.01), nakon čega je nastupio pad u 60 i 90 min. (P<0.01) nakon stavljanja u sanduk.

Odnos heterofila i limfocita se povećavao signifikantno 60 minuta. Nakon početnog perioda mirovanja u 15-20 minuta.
Dobijeni rezultati ukazuju da se kortikosteron ne može koristiti kao jedini pokazatelj za ocenu dobrobiti živine. Odnos heterofila i limfocita je takođe pouzdan pokazatelj statusa dobrobiti živine, ali mora biti korišćen u kombinaciji sa merenjem kortikosterona plazme kako bi se dobio sveukupan pregled dobrobiti živine.

References


Received 31 May 2009; accepted for publication 15 August 2009