MORPHOLOGY OF BLOOD CELLS AND CONCENTRATION OF CORTISOL IN PLASMA OF GILTS DEPENDING ON POSITION IN THE SOCIAL HIERARCHY

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In farm animals stress often occurs in chronic form. This problem is very important because the hormonal changes connected with social hierarchy affect the metabolic status.

The experiment was carried out on 28 Polish Landrace x Polish Large White crossbreds gilts. Gilts were housed in straw bedded 240 x 350 cm pens in groups of 7 animals each. The two repeats of four day behavioural observations were made using video camera and time lapse video recorder. The ranking of gilts to the proper social hierarchy level on the basis of behavioural observations was made. Ranking of gilts was made on the basis of lying arrangement (taking lying places) and by the number and results of agonistic behaviour. The blood samples were taken from the vein and centrifuged directly after collection during 10 minutes and 3500 r.p.m. The plasma was stored at –18 ºC.

The highest cortisol level was found in low ranking animals and the lowest in dominants. The lowest values of blood cell parameters were observed in submissive animals. Stress response connected with social hierarchy establishment lead to the changes in hormone concentration Besides cortisol the indicators of stress may be some morphological parameters of blood such as WBC (number of leukocytes) RBC (number of erythrocytes), HGB (hemoglobin), HCT (hematocrite) and PLT (number of trombocytes).

Keywords: GILTS, SOCIAL HIERARCHY, CORTISOL, HEMATOLOGY

МОРФОЛОГІЯ КЛІТИН КРОВІ І КОНЦЕНТРАЦІЇ КОРТИЗОЛУ В ПЛАЗМІ КРОВІ РЕМОНТНИХ СВИНОК ЗАЛЕЖНО ВІД ПОЛОЖЕННЯ У СОЦІАЛЬНІЙ ІЄРАРХІЇ

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У сільськогосподарських тварин стрес часто зустрічається в хронічній формі. Ця проблема дуже важлива, тому що гормональні зміни, пов'язані з соціальною ієрархією, впливають на метаболічний статус організму тварин. Дослід проведено на 28 свинках (помісь польський ландрас х польська велика біла). Свинок утримували в клітках розміром 240 х 350 см по 7 голів у кожній. Виконано два чотириденні спостереження поведінки за допомогою відеокамери з реєстрацією часу. Проведено ранжування свинок за позицією у соціальній ієрархії на домінантних, субмісивних та низькорангових. Рейтинг свинок зроблено на основі тривалості лежання і за кількістю і результатами агоністичних відносин. Брали зразки венозної крові, які центрифугували безпосередньо після відбору при 3500 обертах за хвилину протягом 10 хвилин. Плазму крові зберігали при –18 °С.

Найвищий рівень кортизолу був знайдений у низькорангових тварин, а найнижчий — у домінантних. Найнижчі значення параметрів клітин крові спостерігалися в субмісивних тварин. Отже, пов'язана з соціальною ієрархією стресова

реакція призводить до змін у концентрації кортизолу в крові. Крім гормональних показників, маркером стресу можуть бути деякі морфологічні показники крові: кількість лейкоцитів, еритроцитів та тромбоцитів, вміст гемоглобіну, гематокрит.

Ключові слова: СВИНКИ, СОЦІАЛЬНА ІЄРАРХІЯ, КОРТИЗОЛ, ГЕМАТОЛОГІЧНІ ПОКАЗНИКИ

МОРФОЛОГИЯ КЛЕТОК КРОВИ И КОНЦЕНТРАЦИИ КОРТИЗОЛА В ПЛАЗМЕ КРОВИ РЕМОНТНЫХ СВИНОК В ЗАВИСИМОСТИ ОТ ПОЛОЖЕНИЯ В СОЦИАЛЬНОЙ ИЕРАРХИИ

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У сельскохозяйственных животных стресс часто встречается в хронической форме. Эта проблема очень важна, так как гормональные изменения, связанные с социальной иерархией влияют на метаболический статус организма животных.

Опыт проведен на 28 свинках (помесь польский ландрас х польский большая белая). Свинок содержали в клетках размером 240 х 350 см по 7 голов в каждой. Выполнены два четырехдневные наблюдения поведения с помощью видеокамеры с регистрацией времени. Проведено ранжирование свинок по позиции в социальной иерархии на доминантных, субмиссивних и низкоранговых. Рейтинг свинок сделан на основе продолжительности лежания и по количеству и результатам агонистических отношений. Брали образцы венозной крови, которые центрифугировали непосредственно после отбора при 3500 оборотах в минуту в течение 10 минут. Плазму крови хранили при –18 °С.

Наивысший уровень кортизола обнаружен у низкоранговых животных, а наименьший — у доминантных. Снижение параметров клеток крови наблюдались у субмиссивных животных. Итак, связанная с социальной иерархией стрессовая реакция привела к изменениям в концентрации кортизола в крови. Кроме гормональных показателей, маркером стресса могут быть некоторые морфологические показатели крови, такие как количество лейкоцитов, эритроцитов и тромбоцитов, содержание гемоглобина, гематокрит.

Ключевые слова: СВИНКИ, СОЦИАЛЬНАЯ ИЕРАРХИЯ, КОРТИЗОЛ, ГЕМАТОЛОГИЧЕСКИЕ ПОКАЗАТЕЛИ

One of the consequences in hormonal changes caused by stress is reproductive disturbances. They are visible especially in oestrus process, decrease in ovulation rate and conception rate, increased embryo death rate as well as in lower number of piglets born. That loss is caused by simultaneous effect of hormonal changes connected with the growth of young females and changes caused by environmental stimuli. These events may lead to the reduced reproductive abilities. The factors causing stress in sows and gilts may be different: thermal, technological (change of housing environment, change of diet), social (alterations in group composition) as well as human-animal interactions [1].

In animal breeding the chronic stress most often occurs. It is not acute stress, however long-lasting. This problem is very important because the hormonal changes connected with social hierarchy relations affect in some way the development of reproductive traits. The cortisol concentration is the most often used indicator of stress reaction. However, the problems with blood samples collecting in relatively short time are

really hard to overcome and cause the unclearness of the results and failures.

It is the reason why the hematological and biochemical indicators of stress in blood plasma are taken into account (glucose, urea, protein, Ca, Na, P concentration, number of erythrocytes, leukocytes, and white blood cells image). However, the changes in blood mainly represent the endocrine changes, so the biochemical and hematological indicators seem to be complementary for the recognition of these processes [2]. Other researchers [3] suggest that better indicator of stress is the concentration of Ca ions in blood plasma. Besides mentioned above parameters indicating stress reaction Zanella et al. [4] showed also endogenous opioids like ß-endorphin and dynorphin (1–13). Their concentration changes in chronically stressed animals.

Taking above information into account it was decided to investigate the profile of hematological parameters in blood as well as cortisol level in gilts from different hierarchic structures.

Material and methods

The researches were carried out on 28 gilts, crossbreds of Polish Landrace x Polish Large White. Gilts were housed in straw bedded 240 x 350 cm pens in groups of 7 animals each. The gilts were fed the compound feed.

The two repeats of four day behavioral observations were made using video camera and time lapse video recorder. These observations made it possible to rank each animal into the various hierarchic structures. Ranking of gilts was made on the basis of lying arrangement (taking lying places) and on the ground of number and result of agonistic behavior [5].

The blood samples were taken from the vein into heparin containing test tubes in very short time to avoid the corticoids increase connected with stress [6]. The blood samples were centrifuged directly after collection during 10 minutes and 3500 r.p.m. After centrifuging the plasma was separated and stored at –18 ºC until analysis. The concentration of cortisol, number of erythrocytes, leukocytes (including percentage of lymphocytes and granulocytes), trombocytes hemoglobin amount and hematocrite were analyzed.

The obtained data was statistically analyzed. Means, standard deviation, percentage values were counted and the comparison of the parameters coming from different social hierarchy levels was made.

Results and discussions

Stress reactions connected with social hierarchy establishment lead to the hormonal changes [7]. In opinion of Mendl et al. [8, 9] the social rank is correlated to the activity of HPA axis. The animals taking middle positions in social hierarchy, which are not successful in social interactions have elevated cortisol level in comparison to dominants and low ranking animals. Similar results were obtained by Zanella et al. [4]. The results of the researches by Hicks et al. [10] show the relation between the social rank of an animal and cortisol level in blood. In submissive animals the higher concentration of mentioned hormone was found (198.12 nmol/L) than in dominants (162.30 nmol/l) and middle ranking animals (151.25 nmol/L). Kowalski [11] shows the close correlation between the social rank and the dynamics of stress hormones changes. The most reactive were subdominants, then dominants and low ranking individuals. Such differences were explained by different psycho-physiological abilities of animals from various hierarchic structures to adapt in new environment. The explanation of such phenomenon is that submissive animals have hard social situation (there is no possibility to appease the domination need) as well as resistance and strength of dominants which showed low activation of HPA axis and defensive adaptation to environment in low ranking animals.

The cortisol level in blood plasma was evaluated in 28 gilts (9 dominants, 10 submissive and 9 low ranking). In observed gilts the mean cortisol concentration was 90.54 nmol/L (ranged from 7.38 to 316.61 nmol/L).

The highest cortisol level was found in low ranking gilts — 107.09 nmol/L the lowest in dominants — 79.06 nmol/L (table 1). These differences were not statistically significant.

Table 1

The cortisol concentration in gilts blood plasma (M±SE)

Hierarchy rank	n	The cortisol concentration (nmol/l)
Dominants	9	79.06±50.89
Submissive	10	85.57±69.44
Low ranking	9	107.0±97.85
Mean	28	90.54±72.73

The troubles in evaluation of the influence of stress on the changes of cortisol are connected with the problems with very quick blood samples collection [2]. Borman et al. [3] opined that history of the researches concerning stress is mainly the history of success and failures in evaluation of catecholamines and corticosteroids and the interpretation of these data which is far from being univocal. That is why the more useful indicators are needed to evaluate the stress effect.

Some hopes concerned morphological blood parameters because some of them are dependent on stress reaction however; in this case also not everything is clear.

In investigated gilts the basic morphological parameters of blood were evaluated: WBC (number of leukocytes), RBC (number of erythrocytes), HGB (hemoglobin), HCT (hematocrite) and PLT (trombocytes). The mean values and the profile of these parameters according to the social rank was presented in table 2.

Table 2

Blood morphological parameters of gilts

Hierarchy rank	n	WBC (10^9/L)	RBC (10^{12}/L)	HGB (g/L)	HCT (L/L)	PLT (10^9/L)
Dominants	9	26.24 (8.7–95.4)	10.13 (5.10–22.35)	166.0 (108–327)	0.51 (0.32–1.05)	666.30 (243–2004)
Submissive	10	16.05 (12.3–20.1)	7.04 (5.88–8.31)	117.2 (99–126)	0.34 (0.30–0.39)	357.00 (198–534)
Low ranking	9	49.11 (14.7–101.1)	13.35 (6.72–21.81)	218.6 (126–354)	0.69 (0.37–1.12)	861.43 (249–1818)
Total	28	30.47 (8.7–101.1)	10.17 (5.10–22.35)	167.3 (99–354)	0.51 (0.30–1.12)	628.24 (198–2004)

The highest differences were observed in number of leukocytes: the lowest number in submissive animals (16.05×10^9/L), the highest in low ranking — 49.11×10^9/L. The number of erythrocytes was less various among individuals classified to different social ranks and were 10.13, 7.04, 13.35×10^{12}/L in dominants, submissive and low ranking gilts, respectively. The lowest hemoglobin concentration in blood was observed in submissive animals — 117.2g/L. In these animals also other indicators were the lowest: hematocrite (0.34 L/L) and the number of trombocytes (357.0×10^9/L). The characteristic were also the highest values of all investigated parameters in low ranking gilts.

In blood samples the percentage of lymphocytes, and granulocytes was evaluated (table 3). The differences in lymphocyte percentage were small and very close to the mean which was 45.71 %. The content of granulocytes ranged from 27.78 % in dominants to 31.27 % in low ranking gilts.

The results of the researches by Morrow-Tesch et al. [12] show that the position in hierarchic structures may influence also the morphologic parameters of swine blood i.e. lymphocyte proliferation induced by mitogen. Submissive animals showed stronger proliferation response than dominants and low ranking animals, however in these groups the changes in immune functions i.e. the reduced antibodies production in comparison to submissive animals. The results obtained by McGlone et al. [13] showed that there is the relation between the social rank of each

individual and cortisol concentration as well as the morphological parameters of blood.

Table 3

The percentage of lymphocytes and granulocytes in gilts blood

Hierarchy rank	n	Lymphocytes (%)	Granulocytes (%)
Dominants	9	49.61 (34.40–91.40)	27.78 (0.80–37.50)
Submissive	10	45.87 (34.40–76.20)	28.30 (3.0–38.10)
Low ranking	9	41.64 (34.30–49.90)	31.27 (23.20–37.80)
Mean	28	45.71 (34.30–91.40)	29.12 (0.80–38.10)

However, in opinion of Hicks et al. [14] the number of leukocytes and hemoglobin concentration do not depend on the social rank of animal. In own researches the differences in leukocytes number in gilts from different social hierarchy levels were found. The highest number was found in low ranking animals then in dominants and the lowest in submissive gilts. In this last mentioned group the highest percentage of lymphocytes and granulocytes was found what may suggest better developed specific immune response. In researches by Hessing et al. [10] it was proved that the immune system of dominants is more effective than in other animals taking lower social positions — they were more resistant to Aujeszky disease.

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