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# EXAMINATIONS OF HEMATOLOGICAL AND METABOLIC CHANGES MECHANISMS OF ACUTE STRESS IN TURKEYS

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## ABSTRACT

In the poultry breeding, the negative side of selection aimed at gaining higher and higher body mass growths is the increase of birds sensitivity to the stressors action. In consequence, it leads to losses resulting from the infringement of balance, particularly in the range of co-operation between the endocrine and the immunological systems.

In the current examinations, the assessment of the reactivity of peripheral blood leukocytes in turkeys exposed to transport stress and turkeys subject to immobilisation was made. An attempt was also made to check whether and how the catecholamines, relieved during stress, influence the blood leukocytes. For the purpose of examination turkeys at the age of 6 – 9 months were used. 24 hours before immobilisation, some of the test birds received beta blocker - propranolol in the dose of 1 mg/kg of body mass in the drinking water.

It was observed that the transport of birds as well as their immobilisation caused a shift in the proportional share of heterophils and lymphocytes of peripheral blood, characteristic of stress and manifested in the increase of H/L ratio. It was also found that propranolol administered to turkeys does not neutralise, but only mitigates the changes in blood occurring during stress. Moreover, it was found out that during the course of acute stress the concentration of glucose increases in blood. The hyperglycaemic activity of stressors was intensified by the propranolol administration to birds. These examinations confirmed the complex character of stress in birds, and also encourage to make further attempts aimed at clarifying the mechanisms of stress and to seek the possibilities of reducing its negative consequences.

**Key words:** stress, turkeys, transport, immobilisation, leukocytes, propranolol

## INTRODUCTION

Carry of animals to closed compartments, thus depriving them of their natural environment, created many threats, not only epizootic ones. The unfavourable stress consequences constitute a problem here. In poultry production it concerns particularly turkeys, in case of which the breeding work has been mainly directed at achieving maximal muscle mass. The evaluation of sensitivity to the activity of environmental factors of stressor character, thus the innate adaptation ability, was disregarded. In practice it leads to considerable losses, resulting from high sensitivity and irritability to manipulation stress occurring during technological and veterinary procedures. It favours the falls and the appearance of pathological changes of unknown etiopathogenesis [3, 27]. The mechanisms of stress formation in animals, especially in poultry, as well as reducing its negative consequences remain an open issue [20]. The reasons for it may be found in not fully recognised mechanisms of systemic change formation, resulting from complex mechanisms and functional interaction between the neuroendocrine and lymphatic systems [4,18,23,24,29].

Most of research papers related to stress in birds and elaborated so far, concentrate on the relations within hypothalamus – pituitary –adrenal axis (HPA), playing a primary role in adaptation processes. Thus, they concern systemic changes resulting from the increased concentration of glycocorticosteroids. The role of catecholamines and other neuromediators is frequently disregarded. The release of catecholamines in the initial phase of psychogennic stress causes the occurrence of not only cardiac-vascular and metabolic effects (glycogenolysis) [2,6,10,14,28]. The influence of these substances on the activity of lymphatic system cells and organs is proved [1,7,9,11,19]. High sensitivity to the changes occurring during stress is demonstrated by leucocytes, and the index expressing the ratio of heterophils to lymphocytes is considered to be a sensitive criterion of stress [12,15,16,22,26,32].

Changes occurring under the influence of relieved catecholamines can be totally or partially neutralised by blocking the adrenergic receptor [5,6,8,17,25]. These circumstances were taken into consideration when undertaking research, which aim was to examine the reactivity of the leucocyte in turkeys exposed to transport stress, as well as stress caused by immobilisation. Some of the test birds were administered the beta adrenergic receptors blocker to evaluate in what extent the haematological and metabolic changes in the course of acute stress depend on released catecholamines. The determination of glucose concentration in blood serum of birds was an attempt of the indirect evaluation of metabolic changes occurring in the course of acute stress.

## MATERIAL AND METHODS

The testing material consisted of BIG 6 line turkeys, at the age of 6 – 9 weeks. The experiment were carried out in two phases.

In phase I, blood smears which served as referential material, were taken from 30 randomly chosen turkeys, kept in standard farming conditions.

Then, the subsequent 32 birds chosen from the herd were placed in cages. After 3-hour transport and 1-hour rest, blood was taken from all transported birds, and the smears were made.

Prior to conducting next experiment, all birds were subjected to acclimatisation in new compartments.

After 14-day acclimatisation, the turkeys were divided into two groups and placed in boxes “A” and “B”, and were provided the identical feeding and zoohygienic conditions. All the birds were weighed before the test. The turkeys kept in “B” box were administered propranolol (Uterotonic-Polfa) in the drinking water, 12 hours before the test. A dose of 1 mg/kg of body mass was accepted in the test. A dose of propranolol, recalculated for total body mass of turkeys in a particular group, was dissolved in a precise volume of water. When determining the water volume, previous own findings of water consumption were taken into account, as well as norms specifying mean daily water requirement for birds at this age [21]. The accepted procedure, not allowing for detailed control of propranolol dose, was to simulate its potential dosing in farming conditions.

After 12 hours of free access to the water with propranolol dissolved, blood smears were taken from all birds.

After that, 6 turkeys from box “A” and 6 birds from box “B” were immobilised for the next six hours by tying up their legs and wings. The rest of the birds were deprived of fodder and water access during this period. After 6 hours, blood for smears was taken again.

The following 4 test groups were formed.

| Box | | Box | |
|---|---|---|---|
| A | A | B | B |
| Group - "I"<br>Control | Group - "II"<br>*Immobilisation* | Group - "III"<br>*Propranolol* | Group - "IV"<br>*Propranolol + immobilisation* |

Blood smears stained according to May-Grünwald-Giemsa were used for leucograms making. The percentage of particular leukocyte forms was determined by counting in the optical microscope 200 subsequently encountered cells and differentiating them into lymphocytes, heterophils, eosinophils, basophils and monocytes [15].

Simultaneously, glucose concentration was determined in blood serum by means of a colorimetric enzymatic method (gluco-oxidase + peroxidase) using the “Glukoza PAP” kit of Analco company.

## RESULTS AND DISCUSSION

The percentage of leukocytes in blood of turkeys staying in farming conditions, and birds exposed to transport stress is presented in the table 1 and Fig. 1.

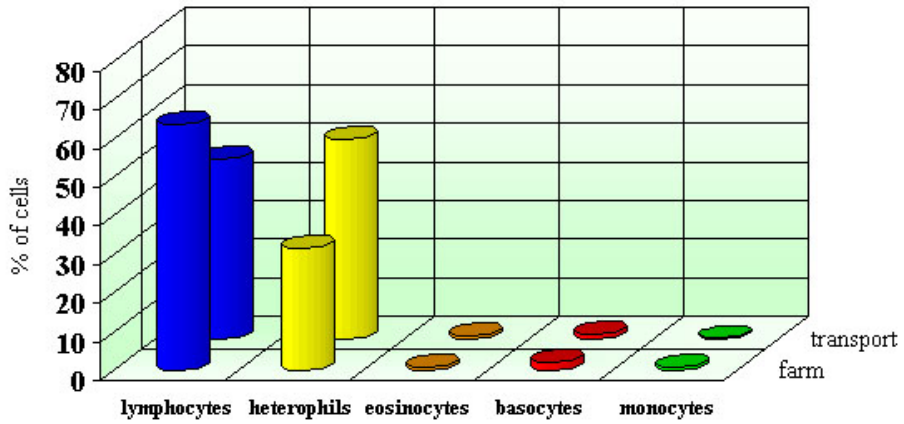
The data presented indicate that in turkeys, similarly to other bird species, lymphocytes constitute over 60 % of all leukocytes. The second population represented in a great number is constituted by cells, functionally corresponding to neutrophil granulocytes, in birds defined as heterophils. This pool of cells sets approximately 30 %.

The stress caused by the transport of turkeys caused a clear shift in the blood picture. Heterophils prevail in the leucogram (about 51 %), while the proportional share of lymphocytes was reduced to approx. 46 %.

**Table 1. Leucogram of farm turkeys and birds after 3-hour transport**

| Birds | Lymphocytes<br>%<br>L | Heterophils<br>%<br>H | Eosinocytes<br>% | Basocytes<br>% | Monocytes<br>% | Index<br>H/L |
|---|---|---|---|---|---|---|
| *Farm control* | *63.85* | *31.9* | *1.0* | *2.21* | *1.0* | *0.5* |
| After transport | 46.45 | 51.72 | 0.64 | 1.16 | 0.16 | 1.11 |

**Fig. 1. Leukogramm of turkeys staying in farming conditions and turkeys after 3-hour transport**




symbols:
transport - turkeys after transport,
farm -turkeys staying in farming conditions.

The descriptions presenting the share of particular leukocytes in blood of birds are numerous [4,12,15,16,32]. However, the comparison of own research results and data from literature is difficult. The reason lies mainly in different and diversified determination methods of the discussed cell share. Therefore, the value of index expressing the ratio of heterophils to lymphocytes (H/L) turns out to be helpful in the interpretation of haematological changes in birds. The values of mentioned H/L ratio noted in turkeys staying in farm conditions are at the level of 0.5 and are approximate to the values noted by other authors [3, 4].

In birds exposed to transport stress the value of H/L ratio exceeds 1.1, what emphasises and highlights the shift in the proportional share of particular leukocytes presented before. The change of H/L ratio confirms also the observations of many authors claiming that in the course of stress the ratio of heterophils to lymphocytes increases, which constitutes a perfect measure of the response on stressors activity [15,16,22,32].

In reference to little data concerning the blood picture in turkeys exposed to the stressors activity, the obtained results indicate that also in these birds evident changes in the course of stress are related to blood. Data collected in table 2, presenting the blood picture of birds after 2-week acclimatisation in new compartments, constitute confirmation of these observations. It appears that H/L ratio in birds of all four test groups acquires middle values between those noted straight after transport, and those observed in birds kept on the farm. This profile of the index mentioned may prove regression of the stress reaction caused by transport, and also adaptation to new living conditions.

According to the preliminary assumption, in the next testing phase an attempt was made to evaluate the catecholamine role in the reactivity of blood cells and the formation of haematological and metabolic changes in the course of acute stress.

The participation and role of adrenal medulla hormones in the functional regulation of the organism, which takes place in the course of stress, are well known [1,2,7,13,14,18,25,29,31]. These hormones, when exerting an effect on cell beta receptors, initiate the formation of secondary transmitters (cAMP, diacyloglycerol, phosphoinositols) [28,30].

It was proved that beta receptors blocking prevents communication between receptors and the enzymatic system of cell, leading to the reduction or even suppression of the secondary transmitters formation, changing this way the cell reply. The existence of catecholamine receptors is most frequently associated with the central nervous system and nerve endings, mainly of the muscular coat of vessels and heart [6,8,14,28]. It appeared that their occurrence is common. The catecholamine receptors were also observed on blood cells [1,7,9,11,19]. Benshop et al. [5] suggest that changes in the immunological system in man observed in the course of stress are subject to adrenergic beta control. It was proved that the activation of the symphatic system in stress leads to the increase of NK cell number in blood. These changes suppress propranolol, what indicates that they remain under the beta adrenergic control. The catecholamine concentration was not determined in the discussed test.

Yet, when administering non-selective adrenergic beta blocker-propranolol to turkeys, an attempt was made to determine whether and what influence on particular leukocytes may have catecholamines relieved during stress.

**Table 2. Leucogram of turkeys before immobilisation**

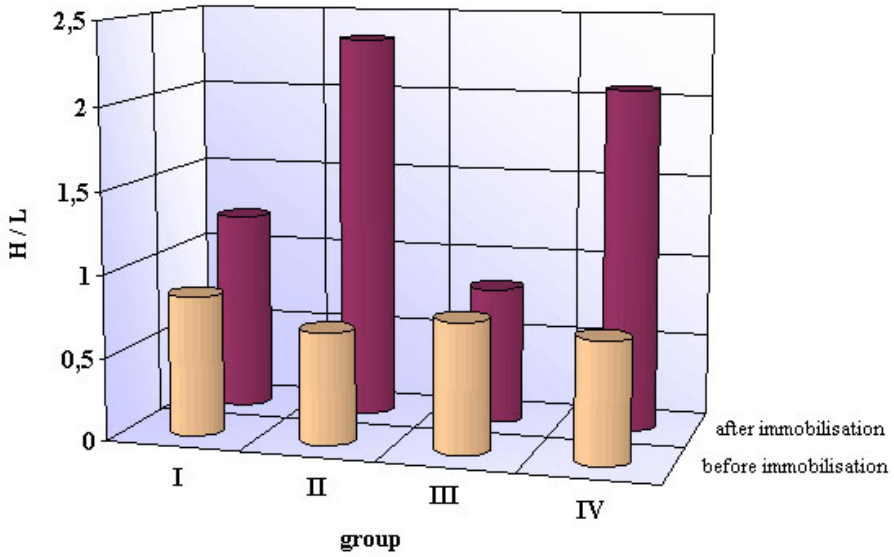
| Group | Lymphocytes % | Heterophils % | Eosinocytes % | Basocytes % | Monocytes % | H/L ratio |
|---|---|---|---|---|---|---|
| *I* | *52.83* | *45.0* | *0.83* | *1.33* | *0* | *0.85* |
| II | 57.16 | 39.16 | 2.66 | 1.0 | 0 | 0.68 |
| *III* | *54.0* | *42.83* | *2.0* | *1.16* | *0* | *0.79* |
| IV | 55.83 | 41.16 | 1.83 | 1.0 | 0.16 | 0.73 |

The ”input” leucograms presented in table 2, made from blood of turkeys before exposing them to the immobilisation stress, indicate that the administration of propranolol alone does not lead to any clear changes in the proportional share of particular leukocytes (compare group III and group IV to group I and group II, table 2). However, it appears that after 6-hour immobilisation there occur the significant changes in the peripheral blood picture of turkeys. There is a shift characteristic of stress, consisting in the decrease of lymphocytes percentage and the increase of heterophils share (table 3, Fig. 2).

**Table 3. Leukogramm of turkeys after 6-hour immobilisation**

| Group | Lymphocytes % | Heterophils % | Eosinocytes % | Basocytes % | Monocytes % | H/L ratio |
|---|---|---|---|---|---|---|
| *I* | *44.16* | *53.66* | *0.5* | *1.66* | *0* | *1.21* |
| II | 29.83 | 69.33 | 0.66 | 0.16 | 0 | 2.34 |
| *III* | *53.4* | *44.6* | *0.2* | *1.8* | *0* | *0.83* |
| IV | 31.66 | 65.66 | 0.83 | 1.83 | 0 | 2.07 |

**Fig. 2. Heterophils / lymphocytes ratio (H/L) in turkeys before immobilisation and in turkeys after 6-hour immobilisation.**




symbols:
I - test turkeys,
II - turkeys after immobilisation,
III - turkeys after Propranolol administration,
IV - turkeys after Propranolol administration and immobilisation.

A detailed analysis of the value of the mentioned index in turkeys, which were administered propranolol, reveals an interesting phenomenon. Propranolol administered to turkeys not being under stress does not seem to have any influence on the blood picture. Yet, the input values noted in control birds and in birds exposed to mild stress manipulation only by blood taking suggest that in the latter group of birds the value of the index increases (group I, table 2 to table 3). Such changes were not observed in birds receiving propranolol (group III, table 2 compare tab. 3). One may suppose that the administered propranolol neutralised haematological changes formed under the influence of mild stressors.

A similar tendency of changes in the blood picture can also be noticed in turkeys from groups subject to immobilisation. In the group of birds receiving propranolol, the H/L ratio value is lower than in immobilised birds not receiving propranolol (table 3, compare group II to group IV, Fig. 2).

The above comparison allows presuming that the hematological effects of propranolol administered to birds, depends mainly on the type and intensity of stressors acting.

Certainly, the described changes in the blood picture of test turkeys cannot be only associated with the changes of concentration and activity of endogenic catecholamines, all the more that the mechanisms through which diversified stressors can modify the reactivity of blood cells have not been fully recognised [7,9,13,14,29]. It is believed that the changes in blood, occurring in the course of stress, constitute a resultant of direct and/or indirect numerous factors activity, including glycocorticosteroids, adrenal catecholamines, and mainly cytokines produced by the lymphatic system cells [9,13]. Catecholamines, as stress hormones relieved during the activation of the nervous system, play a role of a regulator and modulator in the immunological system, which significant element is constituted by peripheral blood leukocytes [7, 9].

In the discussed experiment an attempt was made to clarify what is the role which catecholamines may play in the formation of blood changes during acute immobilisation stress. It appeared that independently of the mechanisms responsible for the formation of blood changes, propranolol as non-specific beta blocker does not prevent the occurrence of hematological changes in turkeys exposed to acute stress. It may, however, modify their intensity, particularly those occurring under the influence mild stressor activity.

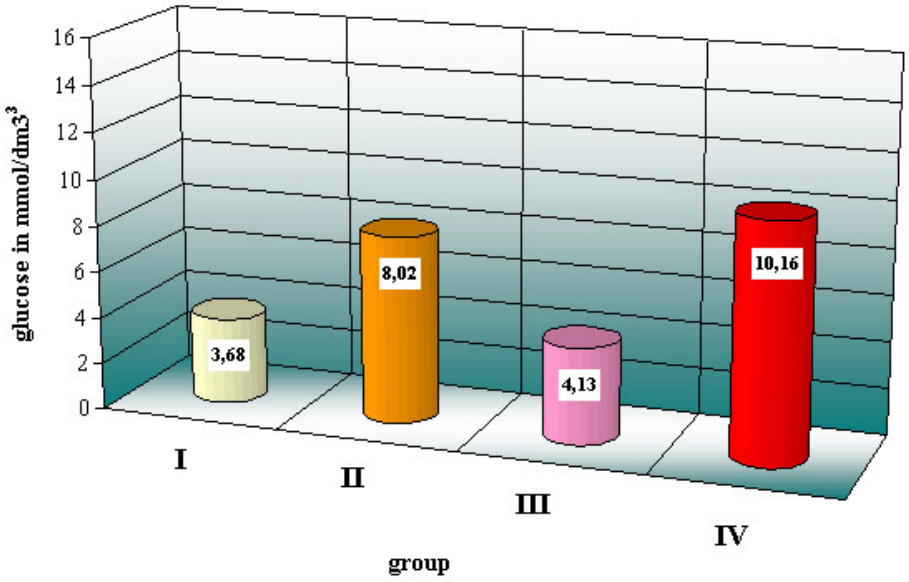
Also, propranolol administered to turkeys does not neutralise but only mitigates blood changes formed under the influence of intense stressor activity.

Despite the lack of direct proofs that it is the blocking of beta receptors that leads to described changes, the hematological changes may be only associated with the fact of propranolol administration to birds, as it was mentioned before. The analysis of glucose concentration in blood serum may indirectly prove the participation and role of relieved catecholamines table 4 and Fig 3.

**Table 4. Mean glucose concentration in the serum of turkeys of particular test groups after 6-hour immobilisation (mmol/dm$^3$)**

| Box "A" | | Box "B" | |
|---|---|---|---|
| Control | Immobilisation | Propranolol | Propranolol + Immobilisation |
| Group I | Group II | Group III | Group IV |
| 3.68 | 8.02 | 4.13 | 10.16 |
| ± 0.66 | ± 1.51 | ± 1.45 | ± 0.85 |

**Fig. 3. Mean glucose concentration in serum of turkeys after 6-hour immobilisation**




symbols:
I - test turkeys,
II - turkeys after immobilisation,
III - turkeys after Propranolol administration,
IV - turkeys after Propranolol administration and immobilisation.

Considerable increase of glucose concentration in turkeys subjected to immobilisation, confirms observations of other authors indicating that in the course of stress in turkeys hyperglycaemia occurs in blood [2]. It has been proved that in birds the increase of glucose concentration in blood is accompanied by the decrease of glycogen in liver [10]. This type of changes indicate a glycogenolytic activity of relieved catecholamines.
Hyperglycaemia proved in the discussed test cannot be treated only as a consequence of the relieved catecholamines activity. It should be also considered the potential glycogenic activity of adrenal glycocorticosteroids relieved during stress [13,18]. Caution in drawing conclusions and observations results not

only from the awareness of a complex mechanism of hyperglycaemia formation, but also from observations concerning glucose concentration in the serum of turkeys receiving only propranolol (group III).

It turns out that beta receptors blocking, does not suppress glycogenolysis, on the contrary, it leads to the increase of glucose concentration in serum. However, as it was mentioned before, the insignificant increase of glucose concentration takes place in test birds receiving propranolol (group III), but it is considerably higher in birds receiving the beta blocker prior to immobilisation (table 4 compare group II to group IV).

Concluding, it should be emphasised that the experiment conducted reveals a complex mechanism determining the reactivity of blood leukocytes and the formation of metabolic changes in turkeys exposed to intense immobilisation stress. The tests allowed also the statement that propranolol administration does not form an easy way enabling the prevention and reduction of losses in turkey breeding, created as a result of environmental stressor activity. They also encourage undertaking further research on the mechanisms and functional conditions of particular systems, developing in the course of stress in animals.

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