

## Diagnostics of Hens Stresses in Poultry Industry

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**Abstract:** In this article, literature data about the methods of hens' stress diagnostics under laboratorial and production conditions are reviewed. There are data about own developments, permitting to diagnose stresses without direct influence on the poultry.

**Key words:** Stress in Hens • Diagnostics of Stresses

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### INTRODUCTION

Stresses in poultry farming are the issue of the day. In accordance with the prevalent conception stressors through the neural and the endocrine systems cause morphological and functional changes in organs and tissues and intensive synthesis and secretions of adjustment hormones, enforcing resistance of the organism to the stressor impact and stimulating renewal of reversible abnormalities, taken place in it.

Diagnostics of stresses in poultry farming is an important issue, intended to solve some adjacent tasks. The main point of scientific application of the diagnostics of stress state is study of stresses in hens under the conditions of industrial technologies and laboratorial physiological experiences and evaluating of the extent of the external factors' influence on the physiological state of the poultry. Monitoring physiological state of poultry while working out new methods of the stress prevention, working out and choosing of the optimal systems of poultrykeeping and feeding.

Nowadays there is no single approach to stress diagnostics in poultry farming [1]. Methods worked out have advantages and disadvantages that demand very careful choice of the stress markers from the researcher [2].

To our mind and in the opinion of many scientists, as the overall stress indicators, which are non-quantifiable, can be classified: young birds thymic and bursa atrophy, increase of the mass of anterior lobe of hypophysis and adrenals; depletion of cholesterol in the adrenals; increase

of the level of corticosterone, insulin and glucagon in plasma; increased consumption of glucose. Decrease of live weight growth and muscle atrophy, outcome of acute inflammatory process markers, cytokines into blood; disturbance of growth of cartilages and bones; anorexia; elevated body temperature. Excessive fat deposition in the abdominal cavity and ascites are marker criteria of stress at the time of intensive fattening of broiler chickens.

Productivity of hens is always an objective indicator of stress, because compensating mechanisms of the organism allow keeping productivity and health on the certain level for some time. Blokhuis *et al.* [3] has proved that as the resources of adaptation systems are depleting, increase of reactivity and resistance is observed and it is resulting in sharp drop of productivity, development of diseases and large-scale death of hens. High mortality can be an important indicator of acute and chronic stresses in poultry farming.

### MATERIALS AND METHODS

In our opinion, it is possible to mark out 'direct methods' and 'indirect methods' of determination of stress state in hens.

'Direct methods'. 'Direct methods' characterized quantitative expression of 'stress' hormones in blood.

Mormede *et al.* [4] claimed, that methods, based on direct measurement of hormones of the hypothalamic-hypophyseal-paranephric system and their metabolites, are effective enough.

Detection of dopamine in the blood is a valuable method of diagnostics of stresses and stress sensibility [5,6]. In the brain of hens, inclined to aggressive behavior, compared with hens, not inclined to deviant behavior, higher concentrations of dopamine are found [7].

Detection of adrenalin and noradrenalin in blood is used in poultry farming for determination acute technological stresses, caused by criteria, being sudden and causing high mortality and for diagnostics of cannibalism reasons [8, 9]. Indicator of adrenalin concentration in blood is used for creation of lines of birds with high and low levels of adrenalin induction, defining different sensibility to stresses [10].

Serotonin carries out different physiological functions and influences the adaptation to the factors of the social sphere, sexual behavior, emotions and reproduction. Detection of serotonin metabolites, mainly 5-hydroxyindolacetic acid, in blood and brain, is used for analyzing different genetic lines by the aggression extent and predilection to deviant behavior [11, 12].

Detection of corticosterone in blood, according to the data of numerous researches, is a method, commonly used for diagnostics of stress state in hens [13-16].

In our mind, nowadays stress diagnostics by determining the 'stress hormones' does not find a wide application on practice and it can be effectively used just for complex laboratorial experiments. Moreover, the main problem for stress diagnostics by the detection 'stress hormones' concentration in blood is activation of stress-realizing mechanisms during blood sampling, what affects the results of the researches, leads to distortion of the results and does not permit to get valid data.

Our opinion was conformed by Rushen [17] and Moneva *et al.* [18], who indicated at variance of the interpretation of the results obtained and absence of wide normative base for correlation obtained results during detecting stress hormones in blood.

According to the results of our research, some groups of indications, which have marker value for stress study, with the use of discriminant analysis, concentration of adrenalin and corticosterone in hens in the state of relative tranquility and after activation of stress-realizing mechanisms can be used just as verifying indicator while studying of stress and stress sensibility of hens [19].

Ingrid *et al.* [20], Savory and Mann [21] and Gross and Siegel [22] aiming to stress avoidance while blood

sampling and obtaining of more reliable data, use catheterization of large blood-vessels of hens- axillary vein, humeral vein or artery.

Our opinion conforms to scientists [23, 24], claiming, that different modifications of the method of detection stress hormones in blood are widely used in experimental researches, but under the conditions of industrial production their use is complicated due to labouriousness of hormones detection, complicity of result interpretation and activation of stress-realizing adaptation mechanisms of the organism in consequence of manipulations and soreness of blood sampling for poultry.

One of the indirect physiological tests- kinetics of the value of heterophiles and lymphocytes (H/I) correlation in peripheral blood, is widely used in researches, devoted to studying of stress and stress sensibility of hens. It was discovered, that during development of stress state this indicator is growing due to abortive emission of the heterophiles immature cells from the bone marrow to the bloodstream and lymphocytes migration from the latter to the tissues. Changes of the correlation H/I value correlate with the shift of corticosterone concentration in poultry blood and are proportional to the extent of impact of the stress-factors of different nature [25].

The correlation H/I indicator can characterize three levels of physiological state of hens: low level has the value of the indicator 0.2; medium level has the value of the indicator- 0.5 and at high level of stress the indicator can be more than 0.8 [26].

To the mind of Moneva *et al.* [18] and Edens [24], the disadvantage of this method is the fact that at the action of over-liminal stimulus H/I is not always changing equally to physiologically state of poultry and that is why it cannot be taken as accurate measure of stress impact in poultry, at the same time calculation of formed element in the blood films of poultry is a laborious process and demands high qualification from the specialist, carrying out the blood cells counting.

Post *et al.* [26] indicated at the possibility of using automatic hematologic analyzers and their high precision for counting H/I correlation.

Detection of eosinophils and monocytes quantity in blood films is used as the exacting indicators to diagnose stress state in hens. According to Maxwell and Robertson [27] increase of eosinophils and monocytes quantity is a marker of acute stress and according to Atlan *et al.* [28] this mechanism is not always realized and cannot work as a reliable stress marker.

One of the stress consequences is the immune system inhibition; this phenomenon in different modifications is used for diagnosing stresses. It is known, that cytokines perform an important role in interaction between immune and endocrine systems at the stress realization in the organism [29]; immunological research have discovered stress effects, declaring itself in increased expression of pro-inflammatory cytokines in liver and spleen of poultry, this phenomenon is used for working out stress diagnostics [30].

All methods listed above assume blood sampling for examination, that leads to additional stress and distortions of results obtained.

'Indirect' methods. It is rational to consider the other 'indirect' methods of stress diagnostics.

It is known, that one of the components of hens stress is fear, in its turn, the time of tonic immobility is the physiological indicator of fear. Tonic immobility is little-studied state, showing itself in decreased reactivity to external influences under the action of compulsory fixation. The TI time is connected with aggressive behavior, pecking and cannibalism among hens under the conditions of industrial keeping. According to Jones [31], Blokhuis [32] and Vestergaard [33] given indicator can serve as the criteria of chronic stresses, individual stress sensibility and inclination to aggressive behavior.

One of the indicators of stress in hens is loss of feathers. Stress state in hens is judged by the extent of feather loss in the period of productivity peak. Loss of feathers is connected with pecking and cannibalism and with increase of corticosterone in blood [34, 35]. Most advanced methods allow to conduct complex evaluation of feathering on the basis of calculation of feather amount in the different parts of the hens bodies and presentation the analysis results in points [36].

One of the experimental methods, passing through stage of approbation, is determination of stresses by detecting proteins of acute phase of inflammation in organism, alpha 1-acid glycoprotein (Orozomuroid) [37, 38].

Have analyzed different methods of diagnosing stresses in hens, it is possible to claim that nowadays non-invasive 'indirect' methods of detecting hormones in biological subjects are invented for avoidance any external influence while stress and stress sensibility of poultry is diagnosed.

For example, Downing [39] indicated the possibility of using eggs for stress diagnostics. Steroid hormones are accumulating in the egg during its formation [40]. Royo [41] found that approximately 80% of egg corticosterone is in the yolk and 20% - in the albumen.

In our opinion, the methods of determination of stress state and stress sensibility of hens by the contents of corticosterone in egg are not used on practice yet, due to variance during interpretation of the results of the research and extraordinary narrowness of this method usage, connected with time of egg forming [42].

Under the conditions of laboratorial experiments, methods of determination of stress state in hens, based on detecting glucocorticoids and their metabolites by the methods of radio-immunological analysis, mass-spectrometry and gas-liquid chromatography. However, mentioned methods are not available for conducting under the conditions of poultry farms and are very expensive for plan industrial using.

## RESULTS

For diagnosing of stress state in hens of parental flock of meat direction of productivity, we have worked out a method, allowing to monitor physiological state of hens on the basis of specific markers of stress reaction without direct impact on them. The method is based on detecting of corticosterone in dung specimen, taken in 1-3 hours after action of the factor, presumably causing stress. Extraction of corticosterone from the dung is carried out by the means of ethyl alcohol. Determination of hormone concentration in the dung is realized by the method of competitive solid-phase enzyme immunoassay, which is available in laboratories of most of poultry farms in Russia. The applied method has high specificity to corticosterone at the level of 100%, crossed activity to progesterone at the level of 7.4 %, dioxycorticosterone at the level of 3.4 %, to 11-dehydrocorticosterone - 1.6 % and to other steroids. Immunoenzyme method of detecting corticosterone has high sensibility and wide spectre of corticosterone detection from 0 to 240 nmol/l. Corticosterone, as other steroids, is stable in the external environment and well soluble in organic solvents.

Concentration of corticosterone in dung, more than 50 nmol/l, defined by extraction of corticosterone by using the given method, indicates activation of hypothalamic-hypophyseal-paranephric system of organism and serves as the indicator of stress state in hens of meat direction of productivity.

## DISCUSSION

Nowadays, numerous methods, permitting diagnosing stress state in hens, are worked out. Under the industrial conditions, for evaluating of the state of adaptation mechanisms and individual stress sensibility,

using methods, based on detecting stress markers in blood, is not a simple task due to necessity of using special equipment, presence of high-qualified and narrow-specialized staff and difficulty of interpretation of the results of the research [43].

The correlation of heterophiles to lymphocytes of blood is the informative method of diagnosing stress state in hens [44].

Indicators of overall feathering of hens possess high diagnostic value for chronic stresses diagnostics. As exacting indicator for these aims it is rational to use detection of stress hormones concentration in blood and time of hens tonic immobility [45].

One of the most important conditions of obtaining reliable results during diagnosing stress state is the minimal impact of the researcher on the object of study that is why all parameters influencing on poultry should be considered during working out these methods [46].

We have worked out a method, which allows diagnosing stress state by detecting corticosterone in dung specimen without impact on organism. Suggested method can be used for evaluating of appropriateness of application of anti-stress therapy schemes for defining of optimal compactness of settling the production space with hens, for analyzing the extent of impact of technological factors and veterinary processing on the hens organisms [47].

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