Diagnostics of Hen Individual Stress Sensitivity in Poultry Farming

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Abstract. One of the approaches that helps prevent poultry stress can be the selection based on the degree of sensitivity to stress. To assess the stress sensitivity there is proposed a turpentine test, allowing to divide poultry into two groups - sensitive and resistant to stress – by modeling local adaptation syndrome. The most important role to assess the stress sensitivity is played by the H / L ratio, determined 30 minutes after the intradermal injection of a turpentine solution. Depending on the number of poultry in the group and the pursued tasks this criterion can serve as a basis to range parameters and identify sublevels of hen stress sensitivity. The indicators of hen general plumage and egg production are of high diagnostic value. To refine indicators there can be determined the concentration of stress hormones in the blood and the hen tonic immobility.

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1. Introduction

One approach contributing to an increase in the productivity of agricultural animals and poultry is the diagnosis of stress sensitivity and prevention of stress effects. Research in this direction is covered in some works [1, 2, 3]. Despite the relatively deep study of stress sensitivity of animals and poultry in the commercial herd, the phenomenon of stress sensitivity is not clearly defined. Stress sensitivity of farm animals and poultry can be defined as a different level of reaction of the body systems, where the functions and properties in sensitive and resistant to stress individuals will vary selectively, affecting the beneficial adaptive result, which is more or less useful for life activities and productivity.

In the study of the physiological characteristics of hens with different stress sensitivity we have found differences in parameters due to the peculiarities of the neuro-endocrine system. In this case an important issue is the data representativeness and the objectivity of the study parameters. The question of how the studied indicators affect stress sensitivity is also of certain scientific and practical importance. The answers to these questions reveal the possibilities of dividing hens into subgroups depending on the level of stress sensitivity or resistance within the selected turpentine method that allows separating hens only into two groups. The purpose of the research is to study the physiological characteristics of broiler hens with different stress sensitivity by assessing the representativeness of individual indicators and developing a model describing indicators for a deeper study of stress sensitivity.

2. Material and methods

Experiments were carried out in the conditions of Argayashskaya poultry farm on the broiler hens of ISA F15 productivity and roosters - M99. On the poultry farm there is used floor housing.

To determine the stress sensitivity we have proposed a method that comprises simulating a local adaptation syndrome by intradermal injection of 70% turpentine solution to the barb in a dose of 0.1 ml, and evaluating the reaction results based on the intensity of acute aseptic inflammation signs 24 hours after the test version.

The assessment of stress sensitivity was performed on 1,600 parent flock poultry aged 48 weeks. 24 hours after exposure, during the reaction readings, hens and roosters were divided into 2 groups – with positive and negative reaction, poultry with a questionable reaction result were not included in the experimental groups. The groups were formed according to the sensitivity: negatively reacting hens with negatively reacting roosters and positively reacting hens with positively reacting roosters. Each group totaled at 330 poultry, 30 roosters and 300 hens. The poultry were observed for 28 days.

To assess the physiological features of hens with different stress sensitivity there was selected a number of key indicators reflecting the state of stress implementing body systems and overall body tension, the state of plumage and productivity.

To assess the state of stress implementing body systems there were used indicators of adrenaline and corticosterone concentration in the blood plasma in a state of relative rest and 30 minutes after turpentine test version, which is an irritant for hens, causing the development of adaptive responses. Adrenaline and corticosterone concentration was determined by solid-phase competitive enzyme immunoassay.

To assess the state of general body tension there was used a ratio of heterophils to lymphocytes (H / L) as determined in a state of relative rest and 30-60 minutes after turpentine test. According to [4, 5] within the specified time period after exposure there is observed a vivid development of adaptive responses and the highest concentration of corticosterone in the poultry plasma. To count lymphocytes and heterophils blood smears were stained by Romanovsky-Giemsa, the blood cells were counted based on standardized methods . To assess the state of body stress associated with the hen emotional sphere and fear there was used the time of tonic immobility. Tonic immobility is indicative of undesirable behavioral responses that lead to the flock aggression and cannibalism [6]. Tonic immobility was evaluated by the method [7] in our own modification that consists in increasing the hen hold time to 15 seconds.

Plumage condition was evaluated by the method [8]. The method involves plumage point scoring of six body parts: neck, chest, cloacal opening, back, wings and tail. Points are given depending on the number of feathers on the body; the total number of points is added and serves as an indication of hen plumage.

To assess the productivity of hens with different stress sensitivity there were used the indicators of egg production, fertility and egg hatching.

The statistical analysis was performed using Statistica 6.1. To develop a model that describes the significance of the main indicators reflecting the degree of hen stress sensitivity there was used a discriminant analysis. To assess the statistical difference between the performance of hens with different stress sensitivity there was used a one-way ANOVA test.

3. Results

After evaluating turpentine test of parent flock 24 hours after the reaction, it was found that the 1072

poultry had a negative reaction, 360 poultry - a positive reaction and 168 poultry - a doubtful reaction, which made up 67%, 22.5 % and 10.5% respectively.

The results of variance analysis of indicators used to develop the model are presented in Table 1.

In the state of rest the content of adrenaline and corticosterone in the plasma of hens with different stress sensitivity does not have significant differences. 30 min. after turpentine test there is a sharp increase in hormone levels. An increase in blood hormones in the stress-sensitive animals is more pronounced that indicates, on the one hand, a higher degree of stressing, on the other hand, that the stress-sensitive hens adequately react to stimuli, and they have no depletion of the hypothalamic-pituitaryadrenocortical system characteristic of chronic stress.

$M \pm m$				
Indicator	Stress-	Stress-		
	sensitive hens	resistant hens		
Concentration of	9,36±0,25	11,80±1,31		
adrenaline in the state of rest, ng / ml	P=0,924565			
Concentration of	152,60±24,30	60,80±5,95		
adrenaline 30 minutes after turpentine test, ng / ml	P=0,001755			
Concentration of	33,59±2,72	33,89±1,41		
corticosterone in the state of rest nmol / l	P=0,924565			
Concentration of	132,00±15,74	81,20±12,41		
corticosterone 30		•		
minutes after turpentine	P=0,020878			
test, nmol / l				
H / L in the state of rest	0,29±0,02	0,23±0,02		
	P=0,056055			
H / L 30 minutes after	0,63±0,05	0,35±0,02		
turpentine test	P=0,000155			
Tonic immobility, sec	139,70±14,26	50,40±6,51		
	P=0,000001			
Plumage at 48 weeks,	12,90±0,22	15,95±0,28		
score	P<0,001			
Plumage at 52 week,	9,80±0,21	13,15±0,20		
score	P<0,001			
Egg production,%	61,66±1,32	70,79±1,95		
	P=0,000325	•		
Fertilized eggs,%	74,55±2,64	89,55±2,40		
	P=0,005644			
Hatch,%	71,08±1,21	84,23±0,79		
	P<0,001			

Table 1. Indicators of hens with different stress sensitivity $M\pm m$

Assessing changes in H/L indicator, it should be noted that this figure varies depending on the degree of stress sensitivity. After the test version the stresssensitive hens have a higher indicator than the stressresistant, which also indicates a more pronounced response.

Under the action of an external stimulus, there take place significant changes in the levels of adrenaline and corticosterone in the hen blood plasma, as well as an increase in the ratio of heterophils to lymphocytes. The intensity of changes is higher in stress-sensitive hens compared with the stress-resistant, indicating a higher activity of stressimplementing systems within 30 minutes after the turpentine test.

The differences identified in tonic immobility parameter indicate a higher stress level in stresssensitive hens compared with the stress-resistant. An increase in tonic immobility time confirms a greater tension of functional systems implementing adaptive processes in stress-sensitive hens. Those are presumably expressed in changing time of protective inhibition and longer orientation state preceding the development of stress response. According to [9] there is a direct relationship between fear and pecking. The higher the hen fear is, the more it is exposed to pecking, or vice versa initiates pecking, because of changes in behavior due to the mismatch of excitation and inhibition processes, which is known to be the main mechanism for implementing stress damage to the body functional systems, which ultimately affects the efficiency of hens.

Stress sensitive hens have fewer feathers, the difference becoming more noticeable during molting, which at 52 weeks reaches 40-50%. Given that the molt is accompanied by simultaneous growth of new feathers, stress sensitive hens have slower processes of plumage replacement and restoration compared with the stress-resistant. Activation of molting mechanisms occurs against a higher corticosterone content and other glucocorticoid hormones in the blood of stress-sensitive hens, under the influence of which the deplumation takes place. Comparing statistically significant differences in key performance indicators of hens with different stress sensitivity it should be noted that the difference in the number of laid eggs makes up 9.1%, 15% - in fertilized eggs and hatching -13.2%.

Thus, the studied indicators reflect the physiological and productive peculiarities of hens with different stress sensitivity; as a consequence, they were used to develop a mathematical model to assess the degree of indicators discrimination when assessing the hen stress sensitivity.

The discriminant analysis showed that all the studied indicators are within the developed mathematical model. The classification matrix corresponds to 100% correct recognition of group data, which indicates that all the indicators for the analysis were chosen correctly and match the

grouping variable - stress sensitivity. Table 2 shows the results of the discriminant analysis.

Table 2. Discriminant analysis results

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Indicator	Wilkes lambda	Private lambda	Р	Tolerance	
Plumage at 48 weeks, score	0.097	0.687	0.002	0,506	
Egg production,%	0.089	0.751	0.006	0.623	
Plumage at 52 weeks, score	0.086	0.777	0.010	0.568	
H / L 30 minutes after turpentine test	0.083	0.802	0.016	0.240	
Hatch,%	0.082	0.815	0.020	0.531	
Adrenaline concentration 30 minutes after turpentine test, ng / ml	0,072	0,917	0,130	0,309	
Adrenaline concentration in the state of rest, ng / ml		0.925	0.149	0.536	
Concentration of corticosterone 30 minutes after turpentine test nmol / 1	0.068	0.973	0.397	0.458	
Concentration of corticosterone in the state of rest, nmol / 1	0.068	0.976	0.423	0.754	
H/ L in the state of rest	0.068	0.980	0.467	0.452	
Tonic immobility, sec	0.067	0.993	0.663	0.669	
Fertilized eggs,%	0.067	0.999	0.873	0.752	

The figures are presented in the table from top to bottom in accordance with the significance of the indicators in the model structure investigated. In this regard, according to the mathematical calculations the greatest value in the stress sensitivity study may have indicators of hen plumage and egg production as well as the H / L ratio in the activated state of stress-implementing mechanisms of the hen body. Other indicators, despite the fact that they fit the model may be used only to clarify the data.

Egg production - is an important indicator reflecting the productivity of parent flock hens and according to the results of our studies may not always be an indicator of stress sensitivity, due to the nature of hen feeding and social dominance hierarchy. In the further study of productive features the studied indicators of egg production for hens aged 48-52 weeks did not have statistically significant differences in hens with different stress sensitivity. In this regard, to assess the level of stress sensitivity we propose to use egg production indicator only for clarifications.

The study of indicators was conducted during molting, which was at 20-40% during the experiment. Previous studies on hens and chickens outside the molting period indicate that the technological stresses

do not affect the condition of plumage, in contrast to experimental stress, having a significant impact on the plumage at any time during the life of the poultry. Therefore, to prove the role of plumage indicators in the diagnosis of stress sensitivity, it is necessary to conduct more detailed studies in different periods of a hen life. Despite this, these figures should be considered as clarifications, allowing to determine the level of stress sensitivity within the studied group features.

The H / L ratio is a reliable indicator of the adaptive reactions development. The relationship of stress sensitivity, dynamics in H / L ratio, poultry productivity and safety is stated in the works of some modern scientists .

4. Discussion

According to the classification of non-specific adaptive reactions the changes identified in the ratio of heterophiles to lymphocytes correspond to the stress orientation stage. It was found out that stresssensitive hens have a higher intensity of changes in H/L ratio in comparison with stress-resistant hens that indicates a higher activity of stress-implementing systems during 30 minutes after turpentine test. Tension of functional systems implementing adaptive processes in stress-sensitive hens is confirmed by the increased time of tonic immobility and is characterized by changes in the protective inhibition time and longer orientation state that precedes the development of stress response.

Increased secretion of corticosterone as a result of excitation of stress implementing systems along with other hormones causes molting. The studies found that the stress-sensitive hens aged 52 weeks have 40-50% fewer feathers.

As a result of mathematical processing of the research findings it was stated that the greatest value have the indicators of hen plumage, egg production as well as the H / L ratio in the activated state of stress-implementing mechanisms of the hen body.

5. Conclusion

1. The H / L ratio, determined 30 minutes after the intradermal injection of a turpentine solution is the most important indicator to assess the stress sensitivity. Depending on the number of hens in the group and the pursued tasks this criterion can provide a framework for ranging indicators and identifying sublevels of hen stress sensitivity.

2. Indicators of poultry general plumage and egg production are of high diagnostic value.

3. The concentration of stress hormones in the blood and poultry tonic immobility can be used as clarifying indicators.

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