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INVASIVE AND NONINVASIVE DETECTION OF ADAPTIVE RESPONSE IN MEAT POULTRY AFTER PREVENTIVE APPLICATION OF A STRESS-PROTECTIVE ANTIOXIDANT COMPOSITION

V.I. FISININ[1], A.V. MIFTAKHUTDINOV[2], E.M. AMINEVA[2]

[1]*Federal Scientific Center All-Russian Research and Technological Poultry Institute RAS,* Federal Agency of Scientific Organizations, 10, ul. Ptitsegradskaya, Sergiev Posad, Moscow Province, 141315 Russia, e-mail fisinin@land.ru;
[2]*South Ural State Agrarian University, Department of Physiology and Pharmacology,* 13. ul. Gagarina, Troitsk, Chelyabinsk Province, 457100 Russia, e-mail nirugavm@mail.ru (corresponding author A.V. Miftakhutdinov)
Fisinin V.I. orcid.org/0000-0003-0081-6336 Miftakhutdinov A.V. orcid.org/0000-0001-8496-2810
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Abstract

Prevention of technological stress among chickens at commercial faring is an important task but the methods for evaluating effectiveness of these measures in using pharmacological agents have not yet been developed. A method has been developed to diagnose the stressful conditions in commercial stock herds that makes it possible to control physiological state of chickens via corticosterone detection in poultry manure. The method is used to assess development of nonspecific adaptive response in chickens under the influence of various technological factors without direct impact on the body. Here, in farm trails of pharmacological prevention of stresses under intensive poultry production we first proved that the non-invasive assay of corticosterone level in manure extracts may estimate an adaptive response in chickens after technological stress. The SPAO complex (Stress-Protector Antioxidant, a pharmacological composition developed at the Department of Physiology and Pharmacology, South Ural State University) was used as an anti-stress agent. SPAO active complex contains lithium citrate, vitamins, vitamin-like substances etc. that affect metabolism. Blood and poultry manure samples were collected from the Hubbard F15 crossbred chickens during the transfer from the section of rearing flocks to the adult herd at 120 day-old poultry (group 1 of 12371 females and 1246 males, and control group of 12865 females and 1255 males). SPAO complex increased viability in the herd up to 4.6 %, egg production up to 2.53 %, average hatching up to 2.28 % and decrease feed costs per broiler chick on average by 16.0 % (presumably due to the direct effect of the components of the complex on the hypothalamic-pituitary-adrenal system). Pharmacological prevention of stresses allowed to extend economic use of hens in the experimental group for 2 weeks, to increase the gross yield of eggs up to 12.4 pcs whereas an earlier depletion of the productive performance occurred in the control group. In use of SPAO complex blood corticosterone level decreased 2.7 times and corticosterone in extracts of poultry manure was 22.3 % lower. Corticosterone levels in hen blood and the extracts from manure showed statistically significant correlations both before stressing ($r = 0.89$, $p < 0.05$) and after stressing ($r = 0.68$, $p < 0.05$) which indicates a high reliability of the proposed noninvasive method of stress diagnostics to estimate the effectiveness of pharmacological prevention of stress in industrial poultry.

Keywords: stress in chickens, pharmacological prophylaxis of stress, industrial poultry production, SPAO-complex, diagnostics of stress, non-specific adaptation reactions

Recognition and prevention of stresses in chickens remain an actual challenge of modern poultry farming [1, 2] in consideration of commercial efficiency coupled with requirements of humane technologies of animal rearing, welfare of animas raised for food and their protection [3]. In practice, laboratory methods for diagnosing stresses are practically not used [4, 5], and approaches which are based on monitoring of productivity, mortality and external signs of poultry adaptation to stress are not effective enough to diagnose stress reactions prior to the onset of pathological processes [6, 7].

Blood leukocyte indices [8] and level of hormones, mainly those of hypothalamic-pituitary-adrenal system [9], are characteristic indicators of the activity of adaptation systems. However, they vary widely under the influence of various factors, including individual's features [10], are associated with circadian rhythms [11] and therefore are limitedly applicable in industrial conditions [5]. Blood sampling causes additional activation of bird's hypothalamic-pituitary-adrenal system [12] similarly to that under technological stresses. This diminishes practical effectiveness of stress monitoring though in laboratory studies these methods are acknowledged as the most accurate and reliable [13]. Indicators of bird's immune system activity, particularly, expression of the proinflammatory cytokines IL-1β, IL-6, heat shock proteins HSP-70 and lysozyme [14] has not been sufficiently studied and are not applicable in commercial poultry [15].

Non-invasive recognition of stresses in chicken based on estimates of hypothalamic-pituitary-adrenal-adrenal hormones in biomaterials collected without direct effects on animals, e.g. eggs [16], feathers [17] and litter [18] is a perspective direction in stress control. A.V. Miftakhutdinov [19] developed method based on specific markers of poultry physiological state that makes it possible to identify stress in meat chickens under commercial farming without direct exposure to a bird. This method is used in the analysis of nonspecific adaptation reactions under the influence of various technological factors.

In the present study, we at first time showed that the accuracy of the non-invasive determination of the amount of corticosterone in the litter extracts after the stress is sufficient to use this test when assessing the effectiveness of anti-stress therapy in commercial poultry.

The aim of the work was to estimate the reliability of the developed non-invasive method in detection of chickens' adaptive reactions in order to control the effectiveness of pharmacological prevention of stresses.

Techniques. Blood and litter samples collected from the chickens of the Hubbard F15 cross were studied at transfer of the replacement stock youngsters from the workshop for growing to the adult flock. The transfer was accompanied by intramuscular injection of Nobilis vaccines (MSD Animal Health, USA) against infectious rhinotracheitis, chicken infectious bronchitis, infectious bursal disease, Newcastle disease, chicken rotovirus tenosynovitis as per recommendations of the manufacturers.

Test group of 12,371 females and 1,246 males and control group of 12,865 chickens and 1,255 males were selected during growing of replacement stock. Poultry aged 120 days was transferred to adult flock. Observation was carried out until the end of the productive period, i.e. for 55 weeks in the test group and for 53 weeks in the control group. Survival rate, egg production, egg fertilization, hatchability, egg weight, and feed consumption were assessed. In the test group, the birds received a pharmacological complex for animals, a stress-protective antioxidant SPAO (white water soluble powder; developed in South Ural State Agrarian University), which includes lemon-acid lithium salt, vitamins, vitamin-like and other substances that affect metabolism [20]. Chickens drank the drug with water through water medicators at a dose of 185 mg/kg of body weight two days before the transfer and vaccination, and a day and for 2 days after transfer and vaccination. The control group did not receive SPAO at transfer and vaccination.

Blood was collected within 30-45 minutes after the stressing impact (transfer to another workshop and vaccination). Litter was collected 3 hours after manipulations. To avoid effect of circadian rhythm, samples were collected in the test and control groups at the same time.

Corticosterone was extracted according as per the protocol [19]. Blood and litter corticosterone level was assessed by a competitive enzyme immunoassay (ELISA) in a Tecan Sunrise ELISA analyzer (Tecan, Austria) at λ = 450 nm, with DRG kits Corticosterone ELISA KIT (DRG, Germany) and corticosterone-conjugated peroxidase competing for binding to polyclonal antiserum to corticosterone which was used to cover wells of microplate. The smallest measurable concentration of corticosterone in the test is 1.63 nmol/l. The cross reactions for corticosterone reached 100 %, for progesterone were 7.4 %, for deoxycorticosterone were 3.4 %, for 11-deoxycorticosterone were 1.6 %, for cortisol were 0.3 %, and for other steroids were less than 0.1 % (according to the instructions attached to the kit). In accordance with the procedure [19], the corticosterone concentration in litter extracts more than 50 nmol/l indicates activation of the hypothalamic-pituitary-adrenal system and stress in meat chickens.

The table shows the arithmetic mean (X) and the squared deviations from the means ($\pm S_x$). Differences between the groups were estimated using the non-parametric Mann-Whitney U test (MW-U). The figure shows an average with a standard error (squares) and the minimum and maximum values. The Kruskal-Wallis test (KW-H) was used for intergroup comparisons. Non-parametric Wilcoxon test (W) was applied to compare the dependent variables within the group before and after exposure to factors presumably causing stress. Analysis of the statistical relationship between the experience and control before and after the stress was performed by the Kendall method of rank correlation.

Results. Chicken placement is preceded and accompanied by catching, estimating habit, transporting, intramuscular vaccination and preparing the body for egg production, breaking and forming new hierarchies of subordination in the herd, changing the lighting regime and diet. Due to a combined impact of several technological factors, this period is, in our opinion, one of the critical ones for the future productivity and reproductive qualities of the parent herd [21, 22], so we chose period to evaluate the antistress activity of the drug.

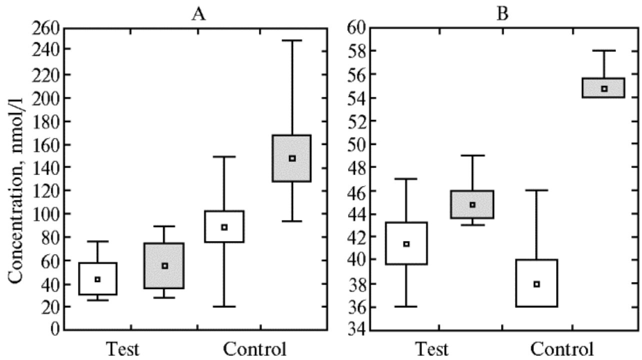
In total, the hens of the parent flock from the test group received the SPAO complex 5 times. As a result, in the test group (Table), as compared to control, survival for the whole period was 4.6 % higher, egg production was 2.53 % higher, egg yield per initial layer increased by 12.4 pcs., the average hatchability was 2.28 % higher, while the cost of feed per reproduction of one broiler chicken decreased by 16.0 % on average. The control hens were withdrawn from the production cycle 2 weeks earlier than the test birds because of an earlier depletion of the productive potential, which is manifested in a decrease in egg production and fertility. This may serve as one of the proofs of the expediency of pharmacological prevention of stress in commercial poultry farming.

Productivity and reproductive parameters in Hubbard F15 cross in farm test ($X\pm S_x$)

Indicator	Test (n = 13617)	Control (n = 14120)	P
Finial survival in total, %	89.0	84.4	
Average survival of parent flock, %	93.92±3.64	91.69±4.65	P = 0.0439
Average egg-laying, %	70.34±10.35	67.81±9.59	P = 0.2219
Total egg output per laying hen, psc.	139.0	123.6	
Average number of eggs fertilized, %	80.56±7.60	77.65±4.64	P = 0.0174
Average hatching, %	77.30±4.14	75.02±3.93	P = 0.0178
Average egg weight, g	59.58±5.46	61.69±5.46	P = 0.1120
Average feed consumption per reproduction of one broiler chicken g	833.51±743.78	993.39±795.72	P = 0.0016

Glucocorticoids are commonly used to assess the extent of impacts on and adaptive responses of the body [15]. Prior to transfer and vaccination, the concentration of corticosterone in chickens from both groups was statistically equivalent (44.00±8.68 and 89.0 ±17.06 nmol/l, respectively).

After using the antistress agent in the test group, an increase in the concentration of blood corticosterone (up to 55.50±7.41 nmol/l) was not statistically significant (P = 0.3105). However, the chickens of the control group experienced a sharp increase of corticosterone synthesis in response to stress (up to 148.14±26.52 nmol/l, P = 0.0425).



Corticosterone concentration in blood (A) **and litter extracts** (B) **of Hubbard F15 chickens prior** (left) **and post vaccination** (right) **with the use of a stress protector antioxidant preparation** (test) **and without stress protector** (control) (farm trials). A: prior to vaccination, Kruskal-Wallis test KW-H(1; 14) = 3.4554, p = 0.0630; post vaccination KW-H(1; 14) = 9.8216, p = 0.0017. B: prior to vaccination, Kruskal-Wallis test KW-H(1; 10) = 2.0979, p = 0.1475; post vaccination KW-H(1; 10) = 7.4503, p = 0.0063.

When recognizing nonspecific adaptation response by concentration of stress hormones, a significant problem is the activation of stress mechanisms at blood sampling, which affects the outcomes, leads to a distortion of the results and often does not allow obtaining reliable data [5]. Given this, we performed a noninvasive assessment of corticosterone in the litter extracts in the test and control groups (Fig.) to confirm the data obtained.

Before the transfer and vaccination, the amount of corticosterone excreted with litter (see Fig.) in test was the same as in control (P = 0.1475). After the vaccination, statistically unreliable (P = 0.0800) elevation of corticosterone level, from 41.40±4.04 to 44.80±2.68 nmol/l, occurred in the test birds. In the control, on the contrary, there was a statistically significant (P = 0.0431) increase in corticosterone excretion, from 38.00±4.47 to 54.80±1.79 nmol/l. Comparing the indices (see Fig.) indicates on a statistically significant increase in the amount of corticosterone in litter (by 18.2 %, P = 0.0063) of the chickens from the control group after a technological impact, as compared to the test birds.

In comparing corticosterone in blood and litter extracts in the test and in control birds, the Kendall rank correlation coefficient prior to stressing was statistically significant and high (r = 0.89, p < 0.0500), and post stressing value was statistically significant and medium (r = 0.68, p < 0.0500). The revealed decrease in the correlation coefficient after the manipulations can indirectly indicate that blood collection as an irritant has less effect on birds in a state of relative rest than on poultry exposed to this technological stress factor. The observed regularity agrees with the theory of nonspecific adaptive response according to L.Kh. Garkavi et al. (23) and other findings pointing to the potential effects of stressors of different strengths and duration [24]. At the same time, the observed high and medium coefficient of statistically significant correlations indicates the practicality of the method of noninvasive stress recognition in chickens by the corticosterone in litter extracts. Simple manipulations, clear results and the wide availability of ELISA make it possible to use the method we suggested in commercial poultry farming to recognize stress impact and to evaluate the effectiveness of the anti-stress therapy. When using the diagnostic method under consideration, it must be taken into account that the older the chickens, the less pronounced changes in blood glucocorticoids [6]. These differences, apparently, can be considered as a result of gradually progressing physiological and biochemical changes in the hypothalamic-hypophyseal-adrenal system, which facilitate a more rapid restoration of glucocorticoid level [25].

Thus, the pharmacological prevention of stresses during the growing of replacement stock youngsters and keeping adult parental flock makes it possible to increase the survival and fertility of the poultry and extend the period of its economic use. The use of a stress-protective antioxidant complex at vaccination and transfer of chickens slows down the stress development, as followed from a 2.7 times decrease in blood corticosterone level and a 22.3 % lower corticosterone in litter extracts. Decreased induction of corticosterone under the action of stressors is an important indicative parameter of the effectiveness of a pharmacological antistress agent. The corticosterone concentrations in blood and litter extracts statistically significantly correlate, which indicates the expediency of using the developed noninvasive method to determine the effectiveness of pharmacological prevention of stress in commercial poultry farming.

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