Experimental Models of Study and Prevention of Stress in Birds in Industrial Poultry Farming

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***Abstract*—In birds, acute stress (a three-day 12-hour inversion of the photomode) is associated with the dominant activity of the sympathetic hypothalamus and hippocampus, which suppress the tone of the parasympathetic section and the reticular formation of the midbrain.**

The effect of hyperglycemia on the background of a negative chronotropic effect indicates a mismatch of the functions of the autonomic sections of the hypothalamus on the 30th day. Chronic stress in birds (30-days crowding) causes persistent pathological stress of the functions of the anterior hypothalamus and reticular formation of the middle brain with the suppression of papasympathetic tone of the posterior hypothalamus and high functional activity of the hippocampus, determining the switching of the ventricles of the heart to a more economical mode of functioning by the 30th day.

Using central cholinoblockers or tranquilizers, having a cholinoblocking component in the mechanism of its influence during neurogenic stress and in the post-stress period is excluded, but it is possible to use stressprotektors.

Keywords—realistic models of acute and chronic desynchronosis, neurogenic stressors, glycemia, electrical activity, adaptation

I. INTRODUCTION

The problem of stress prevention is one of the actual problems in physiology and veterinary medicine [1- 3]. The research on experimental neurophysiology presented in the scientific literature is mainly related to the study of the mechanisms of neurogenic stresses and adaptation to various stress factors in mammals [4- 6]. Such works are performed at different levels of phylogeny and ontogenesis and are aimed at increasing knowledge about the mechanisms of structural and functional adaptations [7, 8]. The results of the study of the impact of modern technologies of keeping and feeding in the conditions of industrial poultry farming on the productivity of domestic chickens are widely presented in scientific publications [9]. Much less of the research is devoted to the study of central and peripheral mechanisms of adaptation and maladjustment of domestic chickens [10].

The aim of the research: to present the results of experimental study of the development of neurogenic stress mechanisms in artificially simulated realistic models of acute (of photolesions) and chronic (crowding) stress in poultry breed Russian White on the dynamics of glycemia, habit and behavior, quantitative and qualitative parameters of electrocardiography (ECG) and electroencephalography (EEG) of deep structures of the birds` brain.

II. EXPERIMENTAL

In preparing the animals for the experiment, much attention was paid to handling – accustoming them to the conditions of the experimental environment and the experimenter. At all stages, the experiments were carried out at the same time of day: the birds were weighed before feeding, blood was taken for analysis - from 8 to 9 a.m, ECG and EEG recording - from 10 to 14 p.m. Blood was obtained driply out of the comb. The level of glycemia was determined in the peripheral blood of birds by color reaction with ortho-toluidine reagent. Habitus was assessed by live weight, density of feather cover, the state of flight feathers of the first order as objective indicators of the severity of the stress state in chickens. The ECG was recorded for 3-5 minutes in a horizontal plane-both wings, the left leg in three standard leads on the EGCT-02 in 25 minutes after placing the animals into the machine [11].

The preparation of roosters for the experiment included: relief of their crest, introduction of bipolar electrodes into deep brain structures: anterior hypothalamus (Hptn), posterior hypothalamus (Hptз), reticular formation (RF) of the midbrain and hippocampus (Hip) using stereotactic micromanipulator for rats [11]. In accordance with the scheme, the study analyzed the dynamics of the parameters of habitus and behavior of birds, the level of glycemia, quantitative and temporal parameters of ECG and EEG in birds in a state of calm wakefulness before their inclusion in the conditions of acute neurogenic stress (12-hour 3-day inversion of the photo mode, followed by a transfer to a 30-

day natural photo mode). The model of acute neurogenic stress fotodesynchronous, and a model of chronic stress – transfer birds from individual cages into the group ones i.e. crowding for 30 days with the increasing of the density of birds to 120 cm^2 per animal vs 270 cm^2. The status of birds was assessed according to the above parameters, taking into account the manifestation of somatovegetative and behavioral reactions before stress and after the restoration of the light regime and in conditions of crowding on the 1st, 3rd, 7th, 14th, 23rd and 30th days [12]. The resulting digital material was processed by conventional methods of variation statistics using programs "Statistica 6.0". The obtained results were considered as reliable starting from the value $p \le 0.05$.

III. Results and discussion

A comparative analysis of the impact of synchronous shifts of the temporal organization of behavior and physiological functions of experimental cocks in the framework of the simulated desynchronosis (DS) and crowding showed their differences in speed and nature of changing the parameters studied [12].

The criterion of completion of the process of individual adaptation were indicators of restoration of the studied functions of the organism to the initial level [11]. It allowed to point out conditionally 4 phases of adaptation of experimental cocks to desynchronizing factors of the environment. Their effects are considered from the position of occurrence in the body the common temporal functional system, determining their adaptation to the action of external synchronizers and inside of desynchronosis.

Chronophysiological load caused the development of emotional tension within 1-3 days inversion of daily photoregime, and the three phases of adaptation: resistance, with a predominance of catabolic processes – 1-3 days, compensatory-adaptive phase with the presence of anabolic processes – 7-23 days, incomplete adaptation - 30 days. Overcrowding caused the phase of emotional stress during the 1st day, from the 1st to the 3d day – phase of resistance with the dominance of catabolic processes, 7-15 days – compensatory-adaptive with a predominance of anabolic processes, 23-30 days –fragile (incomplete) adaptation [12].

The inversion of photoregime and overcrowding in conditions of cage birds placement cause internal desynchronosis with phase changes of glycemia levels, electrical activity of the cardiac cycle, both divisions Hpt, Hip, RF midbrain, stereotyped behavior, locomotor activity, and habitus. The ECG parameters of heart in terms of photodesynchronosis noted the predominance of tone Hpt and voltage functions Hip, suppressive function Hptз and the RF of the midbrain. Hyperglycemia on the background of a negative chronotropic effect notes the mismatch of the functions of the vegetative parts of HPT. Fast casting birds`feathers, hypersynchronous processes in the brain structures with enhanced activity Hptз and RF of mid-brain pointed to the fact that the 30-days period is not sufficient to stabilize body functions [12].

Overcrowding caused in birds sustained pathological stress of the Hptn and RF mid-brain functions during the suppression of the tone Hpt3 and high functional activity of the Hip, determining the switching of the electrical conductivity of the heart ventricles to a more economical mode of operation.

The results of statistical and correlation analysis of vegetative and electrophysiological indicators of overall functional status of experimental cocks in every model of DS showed that in terms of the cage placement the model of photodesynchronosis ensured the development of acute neurogenic stress, and overcrowding – its chronic form. A comparative analysis of the synchronization effect shifts the temporal organization of the functions of experimental animals under conditions of desynchronization revealed differences in the speed and nature of the restructuring of electric activity (EA) of the investigated structures of the brain and heart, revealing possible ways and mechanisms of adaptation to the state of photodesynchronosis and crowding [11].

The criteria of completeness of individual physiological adaptation were the restoration indicators of the studied body functions to the initial level [8]. This allowed us to distinguish the duration of 4 phases in the process of development of adaptation of experimental birds to desynchronizing factors.

Four phases were identified in terms of experimental model of photodesynchronosis: emotional stress – 1-3 days of inversion of daily photoregime, resistance to the dominance of catabolic processes – 1-3 days of adaptive period, compensatory-adaptive with anabolic effect – 7-23 days, incomplete adaptation – 30 days.

In the state of crowding: the phase of emotional stress was observed during the first day of stress, the phase of resistance with the implementation of catabolic processes - from 1 to 3 days, the phase of the intermittent adaptation from 23 to 30 days.

The effects of each phase of body adaptation were considered from the point of occurrence in the body the common temporal functional systems that provide functional and energy homeostasis, and therefore, the adaptation of experimental birds to the action of external synchronizers and internal state of DS. The phase of emotional stress is marked by its stereotypical manifestation during 3 days of inversion of the photoregime and the first day of crowding [12]. Desynchronizing environmental factors caused excitation in the activating systems of the central nervous system (CNS) of experimental birds and, first of all, Hptз, whose activity is aimed at quickly overcoming the negative stress impact by implementing stereotypical forms of protective behavior [9, 10]. But the cage placement, limiting the living space and motor activity of birds, did not allow them to avoid it. Initial increasing of roughly-search reflexes, not having achieved set goal, defined the transition of the organism to negative emotions (fear, fearfulness, alertness, aggressive voice response) and aggressive-defensive reactions, changed the nature and the mode of locomotor and food activity [10, 13, 14]. According to the literature data, we assume that the emotional-aggressive behavior of the bird corresponded to the cholinergic mechanism of excitation of the activating structures of the central nervous system [6, 15, 16]. The distribution of acetylcholinesterase in the CNS departments of chickens confirms the possibility of such triggering activation of the brain [17, 18].

The simultaneous development of two centers of excitation was identified in having a high reactivity the structures of the CNS of birds: caused by desynchronization factor previously formed in ontogenesis and the determining the initial reinforcement of the positive emotiogenic mechanisms for overcoming negative effects [1, 5]. This increased the mismatch of the main endogenous manifestations of daily behavior and vegetative processes with external cyclic processes, causing external desynchronosis, which through the implementation of negative emotions caused internal desynchronosis in birds [6, 19].

Cholinergic activation system, not realizing behavioral reactions, could cause mobilization of adaptive ergotropic processes by activating the sympathetic nervous system and catecholamine system of the brain [4, 20]. Direct confirmation of the ability of this mechanism in terms of photodesynchronosis is hyperglycemic effect observed in the first two days of inversion of daily photoregime, and a high degree of manifestation of negative emotions and aggressive behavior under conditions of both models of DS, and indirect confirmation is the reduction of living mass in all experimental birds [14, 21].

The increase of the amplitudes of the waves of all ranges in the studied structures of the CNS of experimental cocks on the 1st day in the state of quiet wakefulness and slumber, especially on the background of desynchronizing frequencies, reflects their high reactivity to desynchronizing factors of the environment and drawing in the stress response [4].

Pronounced changes in the parameters of electrical activity of the studied brain structures and behavior of experimental birds are objective criteria of internal desynchronosis, which determines the nature of subsequent adaptive reactions of the heart to shifts in daily synchronization factors – natural photoregime, mode of motor activity and behavior [14].

The transfer of experimental animals to a natural daily photoregime should be refered to an additional stress effect, which, despite the restoration of the rhythms of the external synchronizer with the endogenous one, only increased their emotional tension, causing the state of uncertainty. A sharp increase in the intensity of negative emotional and behavioral reactions in conditions of crowding from the first hours was due to the restriction of motor activity and the establishing of a hierarchy of zoo-social relations among birds.

Therefore, chronophysiological load and crowding as strong stressors, creating a situation of uncertainty, caused in birds the development of the stress of waiting based on advance notice – the primary form of adaptation of an organism to changes of the spatial-temporal structure of the environment [2, 5]. We believe that this caused the overstrain of the integrative and, above all, synthesizing brain function – alteration of unconditional reinforcement and mismatch of the action result acceptor due to changes in the biological parameters of the result [1, 4].

The manifestation of hypersynchronization reaction in the studied brain structures at the stage of expressed emotional tension testified to deep neurogenic shifts in nerve centers and mechanisms of emotion regulation, which was noted in experiments with laboratory animals by other researchers [1]. Cholinergic activation, causing in experimental birds overstrain of the systemic mechanisms of emotion formation and motivated behavior, could create a stable pathological focus of excitation in the higher structures of the CNS [6]. It is proved that the reciprocal mechanisms of this focus through the activation of metabolic pathways regulating the tone of nerve structures generates central inhibition, which prevents overexcitation and depletion of the functions of the activating systems of the brain [16]. The presence of a large amount of norepinephrine in the neurons of the blue spot and basal nuclei in chickens allows us to assume the possibility of such activation of central inhibitory mechanisms [18]. A sharp decrease in the flow of reverse afferentation, especially in crowding conditions, probably determined the high tension of the tone of the basal nuclei and contributed to the inhibition of the ascending activating systems of the brain – the sympathetic division of the posterior hypothalamus and the reticular formation of the middle brain.

The transition from the first phase – emotional tension to the second one -resistance is provided by the general non-specific protective mechanisms of the general adaptation syndrome [5], therefore, as the researchers note [8], in chickens, the activation of the developed neurotransmitter systems – noradrenergic regulation of metabolism and dophaminergic mechanisms of differentiation inhibition is possible. Thus, the conditions are created for the well-known subtle rearrangements of analytical and synthetic brain activity in accordance with the nature of the stressor. Reciprocally activating extinctive inhibition mechanisms at the level of neurons coeruleus and basal nuclei, mediator systems of the brain could provide the restructuring of ascending activatory influences, thereby breaking pathologically increasing activation of the brain. Thus, under severe stress, the suppression of the activity of the sympathetic part of the autonomic nervous system and the strengthening of the parasympathetic part is usually associated with a sharp increase in the level of catecholamines first in the blood, and then, according to the feedback mechanism – in the neurons of the hypothalamus and reticular formation. So, as it is proved by several researchers [15], the release of catecholamines into the blood could cause the inhibition of the functions of the visceral structures and to promote "saving of the body protective potency" according to the mechanism similar with the exorbitant inhibition in the CNS.

Deep revealed neurogenic changes – hypersynchronization of electrical activity in all studied brain structures and the manifestation of hypoglycemia in birds in a day from the start of crowding and on the 3d day after restoration of natural photoregime were evaluated as objective criteria of the transition phase of emotional stress (anxiety) to the phase of resistance (trophotropic, cholinergic).

The analysis of the established shifts of electrical activity of the investigated structures of the brain shows that the development of resistance stage is largely due to their dominance in the CNS of parasympathetic tone of the Hpt and high activation of Hip in suppressing the reactivity of the RF of the midbrain and sympathetic activity of Hptз in experimental birds in the state of photodesynchronosis. In conditions of crowding, it was more characterized by inhibition of activating functions of Hptз at high functional tension of the anterior hypothalamus, Hip and pathological-RF of the midbrain. The marked decrease in slow θ-and δ-activity in Hpt3 can be evaluated as the generation of the inhibition process in it-suppression of sympathetic tone, and due to the activation of the feedback mechanism - functions of the hypothalamic-pituitary-adrenal system [12] and cellular metabolism [14].

Increased θ-activity in Hip noted its high functional activity aimed at the development of internal inhibition processes in the activating structures of the brainstem – RF of midbrain and Hpt3. Strengthening of θ-rhythm is associated with a decrease in emotional tension and, possibly, disinhibition of the indicative reaction. An increase in Hip δ-waves on the background of pronounced θ-rhythm, especially on day 3, day 7 of the experiment pointed to the emotional tension in the CNS in the context of high conflict and uncertainty, the stress of waiting and pronounced negative emotiogenic condition that defines the search of successful adaptive responses. Desynchronizing Hip activity, suppressing corticosteroid levels in the blood of birds, could hinder the development of pathological processes of the CNS in experimental birds [1, 9]. This point was installed in photodesynchronosis cocks, anabolic effects associated with the stress functions of Hptn– hypoglycemia, prolongation of the total pause of cardiocycles, flattening of the R-waves, slowing electrical conduction of the sinus node of the heart with the development of severe bradycardia [4, 21].

Parasympathetic tone of Hpt dominated in birds in the state of crowding, and it was revealed by the decrease in glycemia level, flattening of T-waves, heart rate reduction mainly due to the slowing of the electrical systole of the heart ventricles. Hpt EA correlated with Hip activation and abnormal RF tension of the midbrain. Observed in birds in a state of crowding anabolic effects (increase of R-wave and shortening of total pause of cardiocycles) were associated with activation of Hip on the 1st day, and RF of mid-brain on the 3d day. These data allow us to note the high reactivity and synergism in the interaction of divisions of the ANS (autonomic nervous system) during the first 3 days of crowding – the suppression of the activity of CNS centres and increased PNS (peripheral nervous system), defining different character and degree of their influence on the activity of the heart and its power supply, Hip and RF during pathological stress Hptз [12].

The stage of resistance in birds was marked by the breakdown of vegetative synergies in order to enhance vagal influences of Hpt as the most phylogenetically ancient mechanism of adaptation [1], which determines the growth of body resistance to existing stressors due to the switching of visceral systems to a more economical mode of activity [5]. According to the literature [7] this adaptive mechanism could ensure the implementation of the principle of minimizing energy costs, which are the universal "price" of adaptation of living systems at the background of suppression of the ascending activating influences of the RF of the midbrain and the activity of the sympathetic Hptз of the evolutionarily younger nonspecific structure of the brain.

Revealed dynamics of EA (electric activity) of the studied structures of the brain and heart, glycemia levels and corresponding behavioral reactions showed that 7-days roosters in terms of desynchronization marked a transition from the second phase of resistance to the third, compensatory-adaptive (ergotropic, adrenergic). It was more prolonged and was manifested on 7-23 days of photodesynchronosis and on 7-15 days of crowding. Its beginning was marked by a weakening of the stress state of the bird, the restoration of glycemia to background levels testified it as usual and by the mismatch of the orientation and severity of vegetative indicators. The effect of bradycardia and normalization of glycemia levels with its increase corresponded to the activation of functions of the reciprocally conjugated autonomic divisions Hpt [6]. Simultaneous activation of cholinergic systems of the brain, including indicative mechanisms of behavior, and adrenergic mechanisms of homeostasis maintenance had a negative impact on stabilization of natural rhythms of various body functions [1, 4].

The detected changes of the electric activity of hypothalamus in terms of photodesynchronosis and crowding confirmed the fact that parasympathetic innervation as a universal depressant, acting on the principle of partialness could cause emotional depression, extinctive and local differentiation inhibition in the cortex and subcortical structures of the brain in being stressed birds [15]. It is possible it identified reducing the reactivity of the somatic and visceral systems of poultry and the transition of its body to a more favorable mode of operation [21].

High desynchronizing activity of experimental birds in terms of photodesynchronosis and crowding on the 7-15 days contributed to the manifestation of stagnant excitation in the activating systems of the brain. Synchronization of the electrical activity of the Hip on the 23rd day in a state of photodesynchronosis testified about the elimination of the inhibition effect of Hip on the structures of the brain stem and its core. This process enhanced the functional influence of the posterior hypothalamus and the reticular formation of the midbrain on visceral processes with the mobilization of metabolic resources of the body.

Increased desynchronization of electrical activity in the RF of the midbrain of experimental birds on days 7-30 of crowding, on days 23-30 in the state of photodesynchronosis indicated its upward activating effect on the limbic-cortical structures of adaptive behavior formation [6].

Roosters in the state of photodesynchronosis from 7 to 23 days increased the activity of Hptз and sympathetic effects of hyperglycemia, flattening of T-waves [21] and rapid shedding connected with high energy costs [9].

The steady decline in the frequency of heart rate from the 1st to the 23rd day of experiment and the changing ratio of the components of the temporal structure of cardiocycles in experimental birds indicated the activation of vagal regulation EA of the heart and activation of a more economical energetically mode of functions that determine the improvement of blood circulation of its conductive system [13].

Pronounced bradycardia corresponds to the manifestation of an indicative reaction in terms of high emotional stress of the organism [15]. Obviously, bradycardia is an adequate compensatory reaction of the heart of birds to the shift of daily rhythms of illumination in conditions of restriction of their motor activity.

At the background of high glycemia levels on the 7-15 days of crowding experimental birds showed improved R-waves and the flattening of T-waves, increased negative chronotropic effect by slowing down the electrical systole of the ventricles. The temporal structure of cardiocycles did not differ from the initial one. In birds the level of hyperglycemia was less pronounced than in terms of photodesynchronosis. Perhaps these results to some extent indicate the presence in birds innate mechanisms of adaptation to stressors, including certain components of crowding. However, the EA parameters of the studied brain structures indicated high functional stress Hpt,, Hip and RF and pathological stress Hptз. Some decrease in tension in these structures - the presence of synchronization EA on the 15th day of crowding - was associated with the stabilization of glycemia levels of roosters, despite the large energy costs associated with their rapid molt. The increasing in the Hip during this period of θ-activity had a facilitating effect on the implementation of the dominant activities of the current FS and the inhibition of its secondary manifestations in the period of formation of the orienting reaction on the basis of a comparison of various sensor components into a single behavioral response [1, 4].

The 30th day of adaptive period corresponded in birds the fourth phase of incomplete adaptation, as the increased tone of the activating systems of the brain – sympathetic Hptз and RF, and their influence on visceral functions of the body helped to stabilize the birds behavior and energy homeostasis, but the ratio of the severity of temporary components of the cardiocycles was significantly different from the original. On day 30, the ratio of the basic frequencies of EA of all studied brain structures approached the background level while maintaining a high value of the potential amplitudes of all ranges. This fact noted the improvement of emotional and somatic status in birds, which allowed to overcome the negative consequences of desynchronosis and contributed to the development of sustainable adaptation. But, for full adaptation experimental birds need more than 30 days.

The 23-30th days of crowding corresponded to the fourth phase-unstable adaptation with pathological changes in heart activity. During this period, the EA of the studied structures of the brain of experimental birds was desynchronised according to the frequency with the expression on its background of high amplitude waves of all ranges, indicating a high level of emotional stress with the manifestation of hypoxic processes in the CNS.

Stereotypical behavior of birds could not be fully implemented in conditions of crowding, determining their rapid moult, high emotional stress with a decrease in heart rate, and a decrease in their body weight. Taken together, this indicated the instability of the state of the experimental birds. The restoration of the temporary structure of the cardiocycles to the initial allowed to suppose that from the 7th day of crowding the lengthening of all phases of the cardiac cycle corresponded to the negative compensatory-adaptive reaction of the roosters` heart to stress. This indicated the leading role of Hpt in the creation of homeostatic conditions for adaptive restructuring of the functions of the main animal systems of the body under chronic stress. From the 1st to the 15th days of high abnormal tension Hpt manifested with the suppression of the activity of Hptз, but high activity of the Hip and the RF. We believe that increased functional activity of Hptз on the 23-30th days of crowding contributed to the transition of the heart activity to a qualitatively different adaptive mode based on the stabilization of glycemia level within the initial one.

Since the full adaptation of birds, initially highly emotional,is impossible in the crowded conditions, the model of chronic neurogenic stress created on the basis of chronophysiological approach can be used to study the functional characteristics and the role of ancient structures in the CNS mechanisms of adaptation to various stressors.

IV. Conclusion

Thus, both models of experimental desynchronosis can be used to study industrial desynchronosis in birds – photoregime inversion (acute form) and placement in crowded conditions (chronic form). Factors of stressing - photoregime inversion and overcrowding in conditions of cage placement cause inner desynchronosis, which is manifested by the phase changes of glycemia levels, electrical activity of the cardiac cycle, anterior and posterior hypothalamus, hippocampus, reticular formation of the midbrain, as well as stereotyped behavior, locomotor activity and habitus. The parameters of the electrical activity of the heart indicating the adaptive reaction of the organism of birds in terms of photodesynchronosis are due to the dominant activity of the anterior hypothalamus and high functional tension of the hippocampus, suppressing the functions of the posterior hypothalamus and reticular formation of the midbrain. Hyperglycemia on the background of a negative chronotropic effect notes the mismatch of the functions of the vegetative parts of the hypothalamus. Therefore, the 30-days period is insufficient for restoration of the functional state of the organism, modified by the inverse of photodesynchronosis and this is testified by hypersynchronous processes in the brain with increased activity of the posterior hypothalamus and reticular formation of the midbrain and fast moulting of birds. Crowding causes stable pathological tension of functions from the anterior hypothalamus and reticular formation of the midbrain in birds with suppression of the tone of the posterior hypothalamus and high functional activity of the hippocampus, determining the switching electrical conductivity of the ventricles of the heart to a more economical mode of functioning.

Consequently, data obtained show that the central anticholinergics or sedatives, having anticholinergic component in the mechanism of its influence should not be applied to birds during neurogenic stress and post-stress period, but the application of stress-protection means is not excluded.

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