AGRICULTURAL BIOLOGY, ISSN 2412-0324 (English ed. Online)
2019, V. 54, № 6, pp. 1135-1143
(SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA) ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 636.52/.58:636.018

doi: 10.15389/agrobiology.2019.6.1135eng
doi: 10.15389/agrobiology.2019.6.1135rus

COMPARATIVE EVALUATION OF THE PECULIARITIES OF STRESS REACTIVITY OF THE RUSSIAN WHITE BREED CHICKEN WITH *sw+* MUTATION AND AMROX IN HYPOTHERMIA CONDITIONS DURING EMBRYONAL AND EARLY POSTNATAL PERIODS OF ONTOGENESIS

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The authors declare no conflict of interests
Acknowledgements:
The work has been carried out under the State Task AAAA-A18-118021590129-9.
Received June 21, 2019

Abstract

Modern programs of conservation of poultry genetic resources suggest the need to study specific characteristics of breeds for their further use in breeding. The ability of chickens to adapt and maintain productivity over a wide range of temperatures is an important economically significant feature, since maintaining a temperature optimum to realize the genetic potential of productivity requires significant energy costs. Regarding this, the unique long-term experiment of RRIFAGB on the population of Russian white breed chickens, started by A.N. Sokolova, is of interest. This population was created through selection for resistance to low temperatures in the first days of life (15-22 °C in the first 5 days with a gradual decrease to 14-11 °C to 21-30-day-old age) and the keeping of adult chickens in winter at a temperature below 0 °C. As a result, genotypes appeared, differing not only in thermal resistance, but also possessing an increased resistance to diseases of the leukemia-sarcoma complex and white embryonic down (so-called «snow whites»). The aim of this work was to investigate the degree of adaptive response of Russian white embryos (*Gallus gallus domesticus*) and neonatal chicks, homozygous for the gene *sw+*, to a lower ambient temperature compared to the breed Amrox, resulting in the first demonstration of interbreed differences in epigenetic adaptation of chickens in early ontogeny. The thermal stability of *sw+* homozygous Russian white chickens («snow white» population) and Amrox breeds in prenatal (12.5 days of incubation) and early neonatal periods (when removed from the incubator) was studied in a comparative aspect. Thermal stability in embryos (n = 70) was assessed by changes in the volume of extraembryonic fluid; in chickens (n = 60) — by changes in body temperature and characteristic behavioral reactions under hypothermia. Body surface temperature was determined using Thermal Expert FL 13mm f/1.0 thermal imaging camera, and rectal temperature was determined with an electric thermometer. Interbreeding differences in the response to low temperatures in embryos and 1-day-old chickens were revealed. Cooling of incubated eggs for 6 hours at +20 °C caused a slight expected decrease in the embryo weight in both breeds, but led to an increase in the volume of allantois-amniotic fluid (as a protective mechanism). The decrease in temperature caused an increase in the amount of fluid in embryos of both breeds, but in «snow whites» by 8.2 % and in Amrox embryos to a lesser extent, by 6.7 %. It is found that neonatal «snow white» chicks, as a result of a 2-hour exposure at 16 °C, lose body heat less intensively (the body temperature range of 32,4-17,0 °C) than Amrox chickens (the body temperature range of 28,2-14,5 °C) with no critical decrease in rectal temperature allowed. The rectal temperature of Amroxes reduced by 12.7 % or 5.1 °C ($p < 0.001$) compared to that at the moment of removal from the incubator, while in «snow whites» — only by 3.7 % or 1.4 °C. The «snow white» chicks are mobile, whereas the Amrox chicks are torpid. Thus, the chickens of the Russian white breed, homozygous for *sw+* gene, along with the snow-white color of the down at 1-day age, have more perfect mechanisms of thermoregulation (mainly physical, not chemical) and are better adapted to the conditions of low temperatures during embryonic and early postnatal periods.

Keywords: *Gallus gallus domesticus*, chickens, thermoregulation, embryos, extraembryonic fluid, *sw+* gene, neonatal chickens, hypothermic stress

Modern programs for the conservation of genetic resources of poultry require the study of specific characteristics of breeds for further use in breeding purposes. The ability to adapt and maintain productivity over a wide temperature range is an important economically significant feature, since maintaining a temperature optimum to implement the genetic potential of productivity requires significant energy costs. When solving the problem of thermal resistance of poultry, two approaches are considered, the first one is based on the study of epigenetic adaptation (including due to the dosed temperature effect in sensitive periods of embryogenesis) [1-3] and the second is genetic selection. The possibility of selection in order to obtain resistance to extreme housing temperatures has been proved [4-6]. Candidate genes associated with thermal resistance are identified both in birds of gene pool breeds and of industrial lines [7, 8].

A large number of studies all over the world are focused on the issues of chickens' thermal resistance. However, the overwhelming majority of them relate to bird resistance to tropical and subtropical climate conditions [2, 6], while the problem of adaptation to lower temperatures for a significant part of Russia is more relevant.

In this regard, a unique long-term experiment on a population of Russian White breed chickens, started by Sokolova in 1954 (All-Russian Research Institute of Farm Animals Genetics and Breeding, RRIFAGB) is of interest. The Russian White breed chickens were bred in the USSR on the basis of crossing the white Leghorn breed with local outbred chickens and approved in 1953. The population of Russian White chickens bred in the RRIFAGB Genetic Collection of Rare and Endangered Breeds of Chickens was created by selection for resistance to low temperatures in the first days of life (15-22 °C in the first 5 days with a gradual decrease to 14-11 °C to the age of 21-30 days) with adult animals kept in winter at temperatures below 0 °C [4, 5]. Sokolova [4, 5] found an uneven response of 1-day-old Russian White chickens to a decrease in ambient temperature. At 30 °C, the body temperature of all neonatal chickens was 39.6—40.0 °C. Under conditions of lowering the temperature to 21 °C, some individuals developed hypothermia, which led to death; in others, the temperature remained the same or decreased slightly (to 37-38 °C), and they maintained normal viability. It was also found that increased thermal resistance was inherited according to the dominant type, and thermal stability had monogenic inheritance. In generations of selection for thermal resistance, the role of chemical thermoregulation decreased, but the role of the physical one, aimed at reducing heat transfer, in particular by reducing the relative mass of the lungs, increased. As a result of selection under hypothermic stress, genotypes appeared that differed from the original bird not only by the thermal resistance of young animals but also by increased resistance to Marek's disease, leukemia (confirmed by experimental infection), as well as white embryonic down hair (the so-called "snow white"), the proportion of which was approximately 25% of the total number of chickens at hatch [4, 5]. The remaining chicks had predominantly ordinary yellow down hair, with the exception of a small number of individuals with an intermediate type of color. Possibly, the selection of neonatal chicks according to their degree of reactivity to such a stressful factor as sublethal low temperature influenced the mechanism of neuroendocrine regulation of melanogenesis, which is controlled by the pituitary, thyroid, steroid and sexual hormones [9], which resulted in a mutation in the color of the down hair of chicks.

The authors' assumption about the influence of the neuroendocrine status on the color of plumage in chickens is confirmed by the studies of Dmitriev [10, 11]. Thus, when assessing 10-week-old chickens from the Russian White population that had snow-white coloration of down hair at 1 day old, according to the index of functional reserves of the adrenal glands (FRAG), detected by the introduction of adrenocorticotropic hormone (ACTH), it was found that the average concentration of corticosterone in the blood of "snow whites" in response to the introduction of ACTH was 82.6 ng/ml. Within the same experiment, 10-week-old chicks from the Aurora Blue population obtained by selection of black-motley Australorps with a high FRAG index were evaluated (this selection led to the individuals with a blue plumage color). The average concentration of corticosterone in the blood of chickens in the Aurora Blue population was 84.8 ng/ml. In other breeds of the collection, it was significantly lower (24.0-52.3 ng/ml). It is known that the increase in oxidative processes associated with increased thyroid function causes decomposition of melanin (in particular, these results in white color of arctic animals) [12].

At present, selection for resistance to low temperatures is not carried out, but as a result of selection according to the snow-white color of the down hair, a population of Russian White chickens homozygous for the sw^+ gene was created (snow white down). From the authors' point of view, this experimental population is of significant interest as a model for studying the genetic and physiological mechanisms that determine the thermoregulation processes in chickens in the embryonic and early postnatal periods of ontogenesis. The effect of cold stress on the morphophysiological parameters of embryos in the second half of the incubation period, including in terms of epigenetic adaptation, was studied as early as the 1950s and 1960s [13, 14], but without taking into account the pedigree features of the embryos.

This paper for the first time shows interbreeds differences in epigenetic adaptation of chickens in early ontogenesis.

Our goal was to study the degree of the adaptive response to a low ambient temperature in embryos and neonatal chicks of the Russian White breed homozygous for the sw^+ gene in comparison with the Amrox breed.

Techniques. Studies were carried out in 2018 on embryos (n = 35) and neonatal chicks (n = 30) of Russian White breed chickens (*Gallus gallus domesticus*), homozygous for the sw^+ gene, and the Amrox breed (respectively n = 35 and n = 30), bred in the Genetic Collection of Rare and Endangered Breeds of Chickens (RRIFAGB). Hens of both breeds were kept in individual cages with individual registration of egg-laying capacity.

Eggs were obtained from chickens at the age of 49 weeks. Eggs, as well as embryos and chickens, were weighed using HL-400 EX electronic scales (A&D Company Ltd., Japan). The yolk diameter was determined without violating the integrity of the shell using an ultrasound portable scanner Raskan (Rateks NPP, Russia) [15]. The eggs were incubated in laboratory conditions at the accepted temperature conditions for hens of the gene pool (1-2 days − 38.0 °C, 3-10 days − 37.8 °C, 17-21 days − 37.2 °C) in the incubator and hatcher Remil-Ts (Ramil NPP, Russia).

In the experiment, eggs with 5.5-day-old embryos were cooled to 20 °C for 6 hours; in the control group, they continued to be incubated under the standard regimen. The volume of extraembryonic fluid (allantoic and amniotic) was determined at the age of 12.5 days, when it reached its maximum value [15] using a measuring cylinder; the result was taken into account in absolute terms and as an output relative to the egg mass (volume/mass).

In neonatal chickens from the control group, the external and rectal body temperature was measured immediately when removed from the incubator. Body temperature in the region of the head and paws was determined using a

Thermal Expert FL 13mm f/1.0 thermal imager (Thermal Expert, South Korea), and rectal temperature was determined using a Microlife MT 3001 electronic thermometer (Microlife, China).

To avoid undesirable behavioral response to lower air temperatures (crowding), 1-day-old chickens were placed in individual cells of hatcher trays. The experimental group I (n = 10) was there for 2 hours at 24 °C, the experimental group II (n = 10) — for 1 hour at 16 °C. In young animals of both groups, the external and rectal temperatures were measured, then they were placed in a common tray and behavioral reactions were evaluated at room temperature (24 °C). The control bird (n = 10) was kept at a temperature of 30-32 °C optimum for chicks of this age.

Statistical processing of the results was carried out in Microsoft Excel. The group means (M) and the standard error of the means (± SEM) were determined. The significance of differences was evaluated using Student's t-test. Differences were considered statistically significant at $p < 0.05$.

Results. Amrox chickens were used for comparison given the fact that their productivity is the closest to that of Russian White chickens, 17.0±0.7 eggs per the month preceding the experiment, with a weight of 60.0±0.5 g (in Russian White, 21.0±0.5 eggs per month and 59.6±0.3 g, respectively). In addition, Amroxes were not subjected to selection for resistance to low ambient temperatures. The age of 5.5 days was chosen for cooling, because it was found that during this period (before the start of functioning of the neuroendocrine system) the resistance of embryos to cold stress was especially high (data not shown).

Interbreed differences in the degree of response of embryos to cold stress were identified in comparison with the generally accepted incubation mode (Table 1). Since the volume of extraembryonic fluid is largely dependent on the weight of the egg, its absolute and relative values are presented.

1. Egg weight, egg weight loss, embryo development and extraembryonic fluid output in chickens (*Gallus gallus domesticus*) of *sw*$^+$ gene homozygous Russian "snow white" breed and Amrox depending on the incubation mode (M±SEM)

Порода	Egg mass, g	Weight loss, %	Embryo, g	Fluid	
				ml	ml/g
Russian "snow white" (n = 35):					
control	58.9±0.70	6.9±0.10	8.9±0.10	12.2±0.20	0.207±0.0030^{a}
experiment (cooling)	58.9±0.60	6.6±0.20	8.1±0.10	13.2±0.40	0.224±0.0040^{c}
Amrox (n = 35):					
control	60.6±0.70	6.1±0.20	8.8±0.10	11.8±0.20	0.194±0.0020^{b}
experiment (cooling)	60.7±0.70	6.0±0.20	8.4±0.10	12.6±0.30	0.207±0.0030^{d}

N o t e. For a description of the groups (control and experience), see the Techniques section. Extraembryonic fluid output and development were evaluated in 12.5-day-old embryos.
$^{ab, cd, ac, bd}$ Differences are statistically significant at $p < 0.001$.

Cooling caused a slight decrease in the mass of embryos of both breeds, but led to an increase in the volume of allantoic-amniotic fluid: in "snow white" by 8.2%, in Amroxes by 6.7%. The increase in fluid volume in itself is consistent with Romanoff [14], who found that lowering the incubation temperature caused an increase in the size of the amnion. Probably, this is how its protective function is implemented.

Interbreed differences are, from the authors' point of view, of greater interest, since at this stage of development, they were identified for the first time and testify to the particularity of the reaction of "snow white" embryos to temperature stress. This phenomenon can be explained, on the one hand, by the result of many years selection of the population for adaptation to cold stress conditions, and, on the other, by the known differences in the thermal tolerance of adult chickens of different breeds. According to the literature, Leghorns better adapt to heat stress compared to other breeds, and the Russian White breed was selected using Leghorns [14].

The theoretical prerequisites for conducting studies on neonatal chicks were the evidence that they are considered poikilothermic and only at an older age react with an increase in blood corticosteroids in response to exposure to low temperatures [16-18]. Chicks at the age of 1 day are not capable of this because of the imperfect mechanism of thermoregulation [19-21].

2. Reaction to temperature stress in neonatal chicks (*Gallus gallus domesticus*) of *sw*⁺ gene homozygous Russian "snow white" breed and Amrox depending on differences in egg quality (M±SEM)

Indicator	Breed	
	Russian "snow white" (n = 10)	Amrox (n = 10)
Live weight, g	37.8±1.2	39.4±1.0
When removed from the incubator, 37.2 °C		
Temperature, °C:		
head	39.2±0.05[a]	38.3±0.06[d]
legs	38.5±0.12	38.4±0.17
rectal	37.7±1.2	40.1±0.69
1-day-old chickens after 2 hours at 24 °C (group I)		
Temperature, °C:		
head	38.7±0.36	38.9±0.25
legs	34.2±0.38[a]	32.5±0.31[c]
rectal	37.3±0.42[a]	38.5±0.13[b]
Behavioral response	Actively moving, looking for feed	Crowd in the corner of the tray, clinging to each other
1-day-old chickens after 1 hour at 16 °C (group II)		
Temperature, °C:		
head	33.1±0.32[a]	30.7±0.50[d]
legs	21.9±0.24[a]	19.1±0.83[c]
rectal	36.3±0.42[a]	35.0±0.39[b]
Behavioral response	Active, despite muscle trembling, eyes open, running, looking for feed, trying to get out of the tray	Numbness, muscular tremors, eyes closed, chicks fall sideways
Egg quality parameters (30 eggs for each breed)		
Egg weight, g	54.7±0.80	57.6±0.50
Yolk diameter, cm	2.88±0.010[a]	2.95±0.020[c]

[ab, ac, ad] Differences are statistically significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

It is known that under conditions of low temperatures in neonatal chickens, compensatory reactions of the body are activated, primarily behavioral: young animals erect feathers, trying to keep warm. Muscle tremors appear, with muscle contraction additional heat is released to heat the body [22-24]. If the effect of lowered temperatures is maintained, the body temperature drops by 3-4 °C or more, the chickens fall on their side, they develop a coma, and then death occurs. In the authors' studies, interbreed differences in the response of chickens to hypothermic stress were found.

When removed from the incubator, there were almost no statistically significant differences between breeds in live weight and temperature in the chickens' body (Table 2). Young animals of "snow white" in comparison with Amrox chicks had a lower rectal temperature (2.4 °C) and a higher temperature in the head area (0.9 °C). When the neonatal chickens were kept for 2 hours at a temperature of 24 °C, the thermoregulation differences became noticeable: in "snow white", there was only a slight decrease in limb temperature while maintaining the values of the remaining indicators, while Amrox chickens more intensively lost heat through their paws, their rectal temperature also decreased by 1.6 °C ($p < 0.05$). In an attempt to maintain body temperature, the chickens crowded in the corner of the tray, while the young animals of the Russian White breed were active and comfortable.

After 1 h at 16 °C, the differences turned out to be even more significant (Fig. 1). Amrox chickens heat losses became critical: rectal temperature decreased by 12.7% (or 5.1 °C) ($p < 0.001$) compared to that measured at the time of removal from the incubator, while in "snow white" it was only 3.7% (by 1.4 °C); heat losses in the head and legs of the Amrox were also significantly higher.

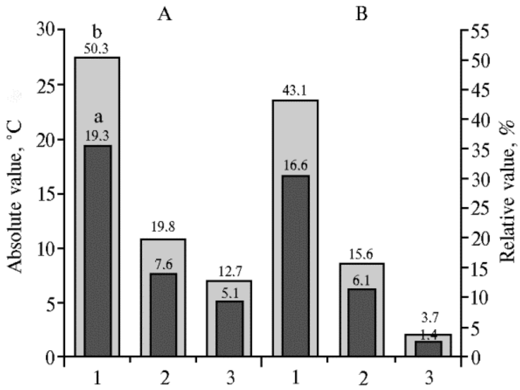

Fig. 1. Absolute (a) **and relative** (b) **decrease in temperature of neonatal chicks (*Gallus gallus domesticus*) of *sw*$^{+}$ gene homozygous Russian "snow white" breed and Amrox after 1 hour at 16 °C:** 1 — legs, 2 — head, 3 — rectal.

After 2 h at 16 °C, muscular tremors and drowsiness were observed in Amrox chickens, by the end of the period the young animals fell into a state of stupor. Russian White chickens reacted to cooling with muscle tremors, but were generally active (Fig. 2).

At a low temperature, Amrox chickens more intensively lost heat over the entire surface of the body (temperature range 28.2-14.5 °C) in comparison to "snow white", which had lower heat range (32.4-17.0 °C), and areas of intense heating had limited localization (Fig. 3). Probably, this is also an adaptation feature of Russian White chickens.

Fig. 2. Behavioral response of neonatal chicks (*Gallus gallus domesticus*) of *sw*$^{+}$ gene homozygous Russian "snow white" breed and Amrox after 2 hours at 16 °C.

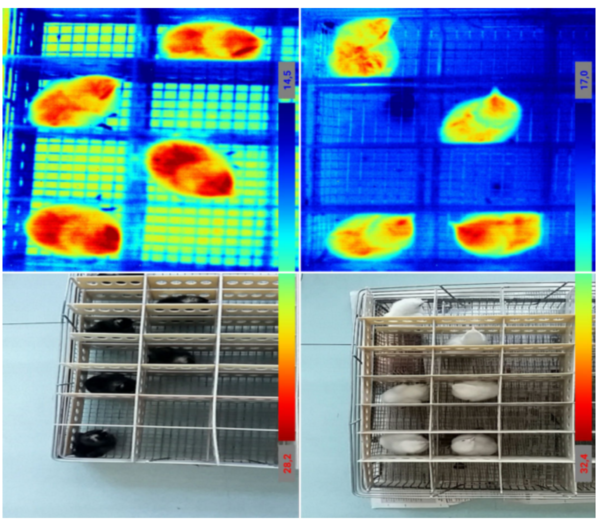

Рис. 3. Heat irradiation intensity neonatal chicks (*Gallus gallus domesticus*) of *sw*$^{+}$ gene homozygous Russian "snow white" breed (on the right; body temperature range 32.4-17.0 °C) **and Amrox** (on the left; 28.2-14.5 °C) **after 1 hour at 16 °C.**

According to the literature, at lower temperatures (20 °C for 12 h), the absorption of the yolk sac and the amount of fatty acids in the blood of neonatal chickens are increased [25]. Neonatal chickens are considered poikilothermic, the ability to maintain body temperature appears only at the age of 4 days (for example, 6-day-old chickens are able to tolerate the effects of 4 °C for 24 hours), they become fully homeothermic by the age of 10 days [26]. In chickens from 4 days of age, the content of corticosteroids in the blood plasma increases in response to exposure to low temperatures. In 1-day-old chicks, this does not happen [26]. At the age of 1 day, a response to a lower temperature is also an increase in lipolysis processes, which is accompanied by an increase in the content of free fatty acids in the blood [27-29].

Similar results were obtained in our studies on the Plymouth Rock chickens of the B2 line of the domestic cross-country Baros 123 when growing an experimental group of chickens up to 6 days of age at 24 °C. As compared to the control, chickens from the experimental group showed a statistically significant decrease in the content of triglycerides in the blood (by 38%) and protein in the dry matter of the pectoral muscle (by 5%) with the same amount of dry matter [30, 31]. However, it is obvious that the Amrox chickens, which had the starting advantage in the form of a larger yolk in the egg (see Table 1), were not able to compensate for heat loss due to this mechanism.

Thus, chickens of the Russian White breed homozygous for sw^+ gene, along with snow-white coloration of down hair at 1 day old, have more advanced thermoregulation mechanisms (mainly physical, rather than chemical) and are better adapted to low-temperature conditions in the embryonic and early postnatal periods. This feature can be used in further breeding of the line, as well as in the creation of a hybrid bird with increased adaptive abilities in conditions of hypothermic stress.

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