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NITRIC OXIDE (NO) CONTENT AND EXPRESSION OF GENES INVOLVED IN MYOGENESIS IN EMBRYONAL TISSUES OF CHICKENS (*Gallus gallus domesticus* L.)

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Abstract

Nitric oxide is known to be involved in myogenesis in birds. Previously, we established that in the embryos of poultry bred for meat, intensive oxidation of nitric oxide occurs while in the embryos of egg hens it is insignificant and NO accumulates in donor compounds. Within the breed, line and cross, the degree of oxidation of embryonic NO varied by no more than 15 %, and this trait is inherited. In this regard, a number of questions arise. If meat productivity is somehow related to the degree of oxidation of nitric oxide, then what is its role? Does NO itself or its oxidation products have an epigenetic effect during embryogenesis? How is NO oxidized in embryonic tissues and what is the direct physiological role of this process? Is it possible to regulate embryo development by influencing NO synthesis or stimulating its oxidation? The purpose of the work was to compare changes in the NO content in the tissues of meat and egg chicken embryos with changes in the expression of a number of genes involved in myogenesis, in order to elucidate the mechanisms of the possible epigenetic effect of NO as a factor in the regulation of embryonic development. We have shown that the expression of several genes responsible for myogenesis is associated with the concentration of NO in donor compounds. For the study, embryos of chickens (*Gallus gallus domesticus* L.) of the Hisex White egg cross and the mini-meat breed (line A77, group 2), characterized by high and low intensity of embryonic NO oxidation, respectively, were used. In tissues of the embryo homogenate on day 6 and in homogenates of pectoral and thigh muscles on day 14, the expression of seven genes involved in or influencing myogenesis was assessed. These are the genes for myocyte proliferation factor 2c (*Mef 2c*), myogenic differentiation 1 (*MyoD1*), myogenesis factor 5 (*Myf 5*), myosin (*Myh 1*), myogenin (*Myog*), myostatin (*MSTN*), and growth hormone receptor (*GHR*). The housekeeping gene *TBP* (TATA-binding protein gene) was a reference gene. During incubation, we assessed the concentration of embryonic NO, the intensity of its oxidation and the transcriptional activity of myogenesis genes as influenced by in ovo administered nitroarginine (NA), a blocker of NO synthesis, and green light that intensifies the oxidation of embryonic NO to nitrate but does not affects the intensity of NO synthesis. NA, when administered before incubation, on day 6, led to a 70 % decrease in the concentration of NO donor compounds in the embryo homogenate and increased the expression of the *MyoD1*, *Myog* and *Mef 2c* genes in the embryos of Hisex White egg cross. In the mini-meat chickens (line A77, group 2), which are characterized by a low level of deposited NO, the same trend occurred, that is, a decrease in the concentration of donor compounds and an increase in the expression of the *MyoD1*, *Myog* and *Mef 2c* genes, but the difference between the control and test embryos was less pronounced. The use of green light during incubation also contributed to an increase in the expression of the *MyoD1*, *Myog* and *Mef 2c* genes on day 6. Based on these data, it can be assumed that the expression of these genes is affected by the concentration of accumulated NO in the embryo tissues. Factors that cause a decrease in the concentration of accumulated NO increase gene expression, regardless of the method of NO reduction (due to less synthesis or more active oxidation). Therefore, the oxidation of NO in the tissues of the embryo may be a way of regulating gene expression. Mechanisms that ensure this oxidation, are inherited. Artificial regulation of the level of NO donors in embryonic tissues is problematic, since arginine, the source of NO in the avian embryo, is in a saturation concentration for NO synthase, and

the NO synthesis blocker nitroarginine effectively suppresses NO synthesis only during the first 7 days of incubation. Nevertheless, the rate of NO oxidation in the embryo is a highly sensitive parameter for selection.

Keywords: nitric oxide, gene expression, avian embryogenesis, myogenesis.

The fact that NO is intensively synthesized during embryogenesis has been revealed in many works [1-3]. Such conclusions were made based on an analysis of the effects of NO synthase blockers and exogenous NO donors. The role of NO as an endogenous epigenetic regulator of gene expression and cellular phenotype is examined [4, 5]. The effects of NO on the expression of certain genes in bacteria [6-8], tumor genes [9], genes responsible for myogenesis [10] are discussed. Various mechanisms of action have been suggested, in particular modification of the methylation of histone proteins [11-13]. However, these studies did not take into account the content of NO metabolites in tissues. Consequently, the data obtained did not provide insight into the intensity of both the synthesis and metabolism of NO and how these parameters are affected by exogenously administered NO donors, NO synthase blockers, and arginine, its substrate. Using a highly sensitive and highly specific enzyme sensor [14], we established that from the beginning of development, NO metabolites intensively accumulate in the avian embryo, a closed system. Their concentration in embryos of the same species is approximately the same. However, in some breeds, lines and crosses, NO metabolites accumulate predominantly in the form of NO donor compounds, while in others, in the form of a product of NO oxidation, the nitrate NO_3^-. Moreover, nitrate accumulates mainly in the embryos of meat breeds, lines and crosses. In them, the degree of oxidation of NO to nitrate is more than 90%. In egg breed embryos, mainly NO donor compounds were accumulated with the degree of NO oxidation to nitrate not exceeding a few percent. There were also intermediate forms on this trait. Within a breed, line and cross, the degree of NO oxidation varies by no more than 10-15% [15, 16]. Analysis of the inheritance in F_1 hybrids indicates that this trait is controled by several genes [16].

Green light, being a known factor promoting an increase in the rate of postembryonic growth, also increased the oxidation of embryonic NO during incubation [15, 16].

Since nitrate accumulates mainly in muscle tissue, it can be assumed that this is where NO oxidation occurs [15, 16].

However, the mechanism of the relationship between the degree of oxidation of embryonic NO and the characteristics of postembryonic development in birds is still unclear. Histological methods showed the absence of qualitative differences in myogenesis in embryos with high and low degrees of NO oxidation [16].

Data obtained by D. Cazzato et al. [10] indicated a connection between NO and the expression of genes involved in myogenesis. However, the authors did not evaluate tissue NO content, which is presumably associated with gene expression. There are other studies indicating the influence of NO on the development and condition of muscles [17-22], in particular on the differentiation and proliferation of myoblasts [17-19], on the activation of satellite cells [20, 21], and on the prevention of myofibril degradation [22]. And there was also no data on the content of NO metabolites in tissues.

In the present work, we studied the relationship between the accumulation of NO donor compounds in tissues and their oxidation to nitrate with the expression of seven myogenesis genes studied by D. Cazzato et al. [10]. This comparison made it possible to answer the question of whether embryonic NO has an epigenetic effect, how NO oxidation occurs in embryonic tissues, what is the direct physiological role of this process, and whether embryonic development can be regulated by influencing NO synthesis or stimulating its oxidation.

Our goal was to compare changes in NO content in the embryonic tissues of meat and egg chickens with changes in the expression of some genes involved in myogenesis, to identify the role and mechanisms of the possible epigenetic effect of NO as a factor in the regulation of embryonic development.

Materials and methods. The experiment was carried out on embryos of chickens (*Gallus gallus domesticus* L.) of the Hisex White egg cross and the mini-meat breed (line A77, group 2) with low and high degrees of embryonic NO oxidation, respectively.

Eggs were incubated in a vivarium (FSBI SGC Zagorskoye, EPH VNITIP, Sergiev Posad, Moscow Province, 2023-2024; incubators Stimul Inc-1000, Ramil, Russia) at 37.6 °C from day 1 to day 18 (incubation period), and at 37.2 °C from day 19 to day 21 (hatching period) [23]. The NO synthase blocker Nω-nitro-L-arginine (NA) for in ovo introduction was prepared in sterile saline; only saline was used as a control. The drugs were injected with a syringe through a hole in the shell from the side of the air chamber. A hole with a diameter of 1 mm was drilled with a micro drill. After the drugs were administered, the hole was sealed with BF-6 medical glue. The effect of green light was assessed in incubators IPH-10 (JSC Pyatigorskselmash, Russia) with energy-saving lamps Navigator NCL-SH10 (15 W with a green filter, luminous flux 975 lm). Eggs were kept in 24-hour lighting from day 1 to day 14, in the control, eggs were kept in the dark. The experiment was repeated 4 times; repetitions were performed with changing incubators. To determine the effects of light and Nω-nitro-L-arginine, eggs were taken from one hen.

Homogenates of egg contents without shells were obtained in a glass homogenizer (DWK Life Sciences GmbH, Germany; 6 °C, 8 min, 40 frictions/min); after 11 days of incubation, a grinder (Oster, Mexico) was used.

The concentration of NO metabolites in homogenates of the embryo or its tissues was determined using an enzyme sensor developed [14, 24].

To assess the relative expression of genes, on days 6 and 14 of incubation, in each group at least 5 embryos most developmentally appropriate for their age stage were selected. Tissue samples no larger than 5×5 mm in size were taken from each embryo (embryo trunk on day 6, pectoral and thigh muscle tissue on day 14). Samples were placed in 2 ml Eppendorf tubes with 1 ml of preservative reagent RNAlater RNA Stabilization Reagent (Qiagen N.V., Germany) and stored at –20 °C until analysis.

Total RNA from tissue samples was isolated according to the protocol of the manufacturer of the RNeasyMini Kit used (Qiagen N.V., Germany). The concentration and quality of RNA was assessed using a Qubit 3.0 desktop fluorometer (TermoFisher Scientific, USA) with a Qubit™ RNA HS Assay Kit (TermoFisher Scientific, USA). Contaminating genomic DNA in RNA samples was removed with the RapidOut DNA Removal Kit (TermoFisher Scientific, USA) as per the manufacturer's recommendations. Synthesis of single-stranded complementary DNA (cDNA) from total RNA was performed with the iScript RT Supermix kit (Bio-Rad, USA) in a programmable thermostat GNOM (DNA-Technology, Russia) according to the manufacturer's protocol.

Amplification reaction (real-time PCR, RT-PCR, detection with SYBR Green fluorescent dye) with primers of seven genes involved in or influencing myogenesis, the myostatin gene *MSTN*, growth hormone receptor gene *GHR*, myogenic differentiation factor 1 gene *MyoD1*, myogenesis factor 5 gene *Myf 5*, myogenin gene *Myog*, myocyte proliferation factor 2c gene *Mef 2c*, myosin gene *Myh 1*, and the reference housekeeping gene *TBP* (TATA box binding protein) (JSC Evrogen, Russia) were carried out with the Maxima SYBR Green/ROX qPCR Master Mix kit (2×) (TermoFisher Scientific, USA) in standard 96-well Semi-Skirted 96-

well PCR Plate. The plates were sealed with optically transparent UltraFlux RT-PCR film (SSIbio, USA) to prevent contamination and evaporation of the PCR mixture and placed in a Light Cycler® 96 System cycler (Roche, Switzerland). Buffer solutions and deionized water were used as negative controls. The expression of candidate genes in quantitative RT-PCR detection was calculated vs. the reference gene *TBP* using the $2^{-\Delta\Delta CT}$ method [25]. Gene expression analysis was performed at the International Laboratory of Molecular Genetics and Poultry Genomics (Danilova Department of Animal Hygiene and Poultry Science, Scryabin Moscow State Academy of Veterinary Medicine and Biotechnology).

The obtained data were analyzed using Microsoft Excel 2007. The results are presented as means (*M*) and standard errors of the means (±SEM). The differences between the compared values was assessed using Student's *t*-test. Differences were considered statistically significant at $p < 0.05$.

Results. The enzymatic sensor makes it possible to quickly assess the composition of nitro- and nitroso compounds. The enzyme sensor we use is based on the property of nitrite (NO_2^-), S-nitrosothiols (RSNO), dinitrosyl iron complexes (DNIC) and iron-free non-thiolate nitroso compounds (RNO) to reversibly inhibit catalase in the presence of halogen ions and lose the ability to inhibit under the influence of a number of compounds, different for each group [14, 24]. All nitro compounds and nitrate were reduced to nitrites with vanadium trichloride, as a result, they acquired the inhibitory properties of nitrite [14]. High molecular weight nitro compounds capable of producing DNIC (RNO_2) were defined as substances that acquire DNIC inhibitory properties in the presence of ferrous iron and thiols [14].

Catalase activity was determined by a calorimetric method based on monitoring the kinetics of heat production accompanying the decomposition of hydrogen peroxide [14]. There are no processes in tissues that have a comparable thermal effect and can lead to data distortion. This allows measurements to be carried out without any preliminary preparation and purification of the sample in a neutral environment, since there is no need to remove compounds that color the preparations and introduce turbidity. That is, the concentration of the test compounds can be determined without their modification. The instrument basis of the sensor is a highly sensitive calorimeter "Dithermanal" (Hungary) or VDK-1 Glavdiagnostika (Russia). The sensitivity in measuring concentration of nitroso compounds is up to 50 nM [14].

Other enzymatic sensors for determining nitrite concentration, also based on the principle of catalase inhibition, are described in the specialized literature [26, 27].

Thus, without preparing a sample or modifying an object, we can determine the concentration of all compounds that have a $NO(NO^+)$ group and the ability to transfer this group to other compounds, assigning them to RSNO, DNIC, RNO_2, and inorganic nitrate.

Factors influencing the intensity of NO synthesis and oxidation in the chicken embryo. As can be seen from the data presented in Table 1, NO metabolites accumulate in the tissues of the chicken embryo from the first day of incubation. Moreover, NO donors (RSNO, DNIC, RNO_2) accumulate first, and then their oxidation to nitrate occurs in the embryos of meat forms. Accumulation ends on day 3, reaching a total concentration in the homogenate of 130-150 μmol/l. From days 3 to 11, this indicator does not change significantly. From day 11, the concentration begins to increase, reaching 600-800 μmol/l by day 14 [15, 16]. It is logical to evaluate the relationship between gene expression and the content of NO metabolites in tissues in early embryogenesis between days 3 and 11, when the concentration of NO metabolites in tissues

is stable, as well as after day 11, when rapid accumulation of NO metabolites occurs.

1. Concentration (μmol/l) **of NO and nitrate (NO_3^-) donors in embryos of meat- and egg-type chicken (*Gallus gallus domesticus* L.) on days 7 and 14 of incubation as influenced by nitroarginine** (NA) **and green light** (*M*±SEM, vivarium of the SGC Zagorskoe, EPH VNITIP, Sergiev Posad, Moscow Province)

Treatment	Day 7		Day 14	
	donors NO	NO_3^-	donors NO	NO_3^-
Egg cross Hisex White				
Control (n = 6)	140.5±7.2	< 0.1	629.8±17.1	2.1±1.1
In ovo treatment:				
0.3 ml of saline before incubation (n = 6)	138.5±6.9	< 0.1	636.5±18.3	1.9±1.2
0.3 ml of saline on day 11 of incubation (n = 10)	141.6±7.7	< 0.1	641.4±19.1	2.1±1.3
0.3 ml 30 mM NA before incubation (n = 6)	39.5±3.1	< 0.1	631.5±18.9	1.9±1.3
0.3 ml 30 mM NA on day 11 of incubation (n = 7)	142.7±7.1	< 0.1	369.6±12.6	1.8±0.9
Green light from day1 to day 14 (n = 10)	52.8±3.4	88.3±4.5	235.4±9.9	402.6±13.9
Mini-mear breed (line A77, group 2)				
Control (n = 10)	1.9±0.9	143.3±6.8	10.2±2.4	762.1±18.6
In ovo treatmen:				
0,3 ml of saline before incubation (n = 6)	1.8±0.6	144.1±7.1	10.8±2.3	758.3±19.2
0,3 ml of saline on day 11 of incubation (n = 7)	1.8±0.6	142.8±7.3	11.1±2.2	759.4±18.8
0.3 ml 30 mM NA before incubation (n = 8)	1.4±0.4	38.8±3.1	11.5±2.2	751.5±18.7
0.3 ml 30 mM NA on day 11 of incubation (n = 6)	1.7±1.0	141.7±7.7	6.9±1.0	468.8±12.1

The NO synthase blocker Nω-nitro-L-arginine (NA) effectively suppresses embryonic NO synthesis only when administered before eggs are subjected to incubation, since the amniotic membrane is impermeable to it until day 11 [15, 16]. A significant decrease in the concentration of NO derivatives with the introduction of NA before incubation occurred on day 7, but not on day 14. A decrease in their concentration on day 14 was observed with the introduction of NA on day 11. However, the blocking efficiency is significantly less, by 40%, while when NA is introduced before incubation on day 7, a decrease of more than 70% is recorded (see Table 1).

Green light is known to increase the growth rate and meat production of poultry [28-30]. We have previously shown that green light enhances NO oxidation in the embryo [15]. In the present study, green light used during incubation induces the oxidation of embryonic NO throughout embryogenesis (see Table 1).

Effect of NO synthesis blockers and green light on the expression of some genes. Amplification conditions for assessing gene expression are presented in Table 2.

2. Primers and amplification modes used in assessing the expression of genes involved in or influencing myogenesis

Gene (protein)	Annealing temperature, °C	Primer pair (5′ → 3′)
TBP (TATA box binding protein)	64	F: AGCTCTGGGATAGTGCCACAG
		R: ATAATAACAGCAGCAAAACGCTTG
Myh 1 (myosin)	64	F:AGACAAAAACACCTGGTGCC
		R: CCTCCTCTCCACCATTCTTG
MyoD1 (myogenic differentiation 1 protein)	64	F: CGTGAGCAGGAGGATGCATA
		R:GGGACATGTGGAGTTGTCTG
Myf 5 (myogenic factor 5)	64	F: TGCCCTGAGGAAGAGGAACAC
		R: ACGATGCTGGAGAGGCAGTC
Mef 2c (myocyte enhancer factor 2C)	58	F: AGCAGCTCAGCCACTTTCTC
		R:AATATTCACCACCCGGTTCA
Myog (myogenin)	58	F: AGCCTCAACCAGCAGGAG
		R: TGCGCCAGCTCAGTTTTGGA
MSTN (myostatin)	64	F: TTTAGAGGTCAGAGTTACAGACAC
		R: TTTAGGTGCTATAATCCAGTCCCA
GHR (growth hormone receptor)	54	F: CAGATACTGACAGGCTCCTGAGT
		R: GAGATGGCATCACATGTGTCGCT

Note. Amplification protocol: 95 °C, 600 s; 40 cycles of denaturation at 95 °C, 15 s, primer annealing at the temperature indicated in the Table, 30 s, elongation at 72 °C, 30 s.

3. Expression of genes involved in or influencing myogenesis in 6-day-old embryos of meat- and egg-type chicken (*Gallus gallus domesticus*) exposed to the NO synthesis blocker nitroarginine and green light (*M*±SEM, в vivarium of the SGC Zagorskoe, EPH VNITIP, Sergiev Posad, Moscow Province)

Treatment	NO_3^-/NO, %	Donors NO + NO_3^-, μmol/l	Gene rxxpression (ΔCt = Ct – Ct *TBP*)						
			MSTN	*GHR*	*MyoD1*	*Myf 5*	*Myog*	*Mef 2c*	*Myh 1*
Nitroarginine									
Egg cross Hisex White (*n* = 8)									
Control	1.9±1.1	144.1±8.2	1.2±0.1	–0.4±0.2	1.7±0.3	13.7±0.8	4.2±0.4	2.7±0.2	20.7±0.3
Test	2.2±1.3	40.1±5.7*	1.7±0.1	–0.2±0.1	–0.1±0.1*	11.8±1.1	2.4±0.5*	0.9±0.1*	21.1±0.2
Mini-mear breed (line A77, group 2) (*n* = 8)									
Control	97.8±2.5	131.4±7.8	0.9±0.2	–0.1±0.1	0.5±0.1	11.7±0.5	1.2±0.2	0.46±0.2	–3.4±0.3
Test	97.1±2.2	39.7±5.8*	1.5±0.5	0.9±0.4*	–0.9±0.3*	12.3±1.1	0.9±0.6	0.98±0.5	–3.0±0.4
Green light									
Egg cross Hisex White (*n* = 10)									
Control	1.2±0.7	139.2±8.8	1.8±0.2	0.6±0.1	–0.2±0.2	8.0±1.1	0.2±0.5	1.2±0.4	–1.1±0.3
Test	63.7±2.1*	135.4±8.1	1.9±0.4	0.5±0.2	–0.7±0.4*	8.8±1.3	–0.6±0.2*	0.4±0.2*	–0.5±0.8

Note. NO/NO_3^- — the proportion of embryonic NO oxidized to NO_3^-. For the experimental design, see the Materials and methods section. The concentration of NO and nitrate donors and the degree of NO oxidation to nitrate were determined in the embryo homogenate. *MSTN* — myostatin, *GHR* — growth hormone receptor, *MyoD1* — myogenic differentiation 1, *Myf 5* — myogenic factor 5, *Myog* — myogenin, *Mef 2c* — myocyte enhancer factor 2C, *Myh 1* — myosin. The housekeeping gene *TBP* (TATA box binding protein) was used as a reference gene.

* Differences from control are statistically significant at $p < 0.05$.

4. Expression of genes involved in or influencing myogenesis in breast, thigh and shin muscles of 14-day old emvryos of Hisex White egg-type chicken (*Gallus gallus domesticus*) as influences by green light (*M*±SEM, в vivarium of the SGC Zagorskoe, EPH VNITIP, Sergiev Posad, Moscow Province)

Treatment	NO_3^-/NO, %	Donors NO + NO_3^-, μmol/l	Gene rxxpression (ΔCt = Ct – Ct *TBP*)						
			MSTN	*GHR*	*MyoD1*	*Myf 5*	*Myog*	*Mef 2c*	*Myh 1*
			Breast muscles (n = 10)						
Control	2.4±0.6	618.8±15.1	–0.9±0.2	–1.3±0.1	–4.5±0.5	0.3±2.5	–0.8±0.9	–0.7±0.9	–2.6±0.6
Test	61.3±2.8	621.4±16.6	–0.2±0.4	–1.0±0.1	–2.6±0.6*	6.6±0.5*	–2.3±0.4*	–1.6±0.4	–2.6±0.3
			Thigh and shin muscles (n = 10)						
Control	2.4±0.6	618.8±15.1	–0.8±0.2	–1.1±0.2	–4.3±0.5	1.1±2.4	–1.6±0.6	–1.5±0.3	–2.8±0.3
Test	61.3±2.8	621.4±16.6	–1.1±0.2	–1.2±0.1	–3.6±0.2	6.2±1.0*	–2.8±0.5*	–1.6±0.2	–3.0±0.2

Note. NO/NO_3^- — the proportion of embryonic NO oxidized to NO_3^-. For the experimental design, see the Materials and methods section. The concentration of NO and nitrate donors and the degree of NO oxidation to nitrate were determined in the embryo homogenate. *MSTN* — myostatin, *GHR* — growth hormone receptor, *MyoD1* — myogenic differentiation 1, *Myf 5* — myogenic factor 5, *Myog* — myogenin, *Mef 2c* — myocyte enhancer factor 2C, *Myh 1* — myosin. The housekeeping gene *TBP* (TATA box binding protein) was used as a reference gene.

* Differences from control are statistically significant at $p < 0.05$.

The relative expression of genes of interest was assessed by the $2^{-\Delta\Delta CT}$ method, where Ct is the PCR cycle in which the fluorescent signal from the dye crosses the established threshold level. Thus, the lower the Ct value, the more intense the amplification and, consequently, the higher the gene expression.

The introduction of NA before incubation reduced the concentration of NO metabolites in the embryo body, recorded on day 6, by 70%, but did not affect the degree of their oxidation to nitrate (Table 3).

This decrease was accompanied by an increase in the expression of myogenic differentiation 1 (*MyoD1*), myogenin (*Myog*), and myocyte proliferation factor 2c (*Mef 2c*) genes. In the Hisex White egg cross, the expression of these genes was significantly increased in embryonic tissues; in the mini-meat chicken breed, only the expression of the *MyoD1* gene was significantly increased, but the same trend was observed as in the Hisex White cross (see Table 3).

The expression of the same genes (*MyoD1*, *Myog* and *Mef 2c*) significantly increased under the influence of green light (see Table 3). Green light does not block NO synthesis, but intensifies the oxidation of NO to nitrate [15, 16]. Therefore, a blocker of NO synthesis and an activator of its oxidation have a similar effect on the expression of the genes under study.

The oxidation level of embryonic NO under the effect influence of green light does not change until the final stage of embryogenesis (see Table 1). However, on day 14, in embryo muscle homogenates the expression of most of the studied genes decreased compared to the control, while in the pectoral muscles a significant decrease was noted for *MyoD1* and *Myf 5*, while in the muscles of the thigh and lower leg only for *Myf 5* (Table 4). In all samples of muscle tissue of embryos from the test group, there was a higher expression of the myogenin gene *Myog*.

Note that a high degree of oxidation of embryonic NO is characteristic of meat forms of poultry. This trait is inherited and varies by no more than 10-15% within the line and cross. In the postembryonic period, the content of nitro- and nitroso compounds levels out [15, 16]. Therefore, the analyzed trait is associated with processes during embryonic development and is inherited genetically. The question arises as to how NO oxidation may affect the rate of muscle tissue growth. It can be assumed that NO binds to some physiological target, which determines processes that promote the activation of postembryonic growth, while NO, after binding to the target, is ultimately oxidized to nitrate. It is also possible that the oxidation of NO produces some intermediate compounds (NO_2, NO^+), which serve as stimulators of muscle tissue growth.

According to our data, in different breeds and crosses, characterized by the same degree of oxidation of embryonic NO, the expression of genes responsible for myogenesis can differ several times. In this regard, in the control and test groups we used eggs from the same laying hen. The data obtained show (see Tables 1, 3) that gene expression in our experiment depends on the concentration of NO donor compounds. Its decrease promotes an increase in the expression of *MyoD1*, *Myog* and *Mef 2c* at the initial stage of embryogenesis. Such a reduction can be achieved either by reducing NO production or by intensifying the oxidation of synthesized NO to nitrate (see Table 3).

Thus, the results obtained in this work indicate that accumulated NO affects the expression of these genes and it is NO, and not its oxidation products. It can be assumed that NO oxidation in embryonic tissues is a method (or one of the methods) for regulating gene expression. The influence of light on the process of NO oxidation indicates that structures that ensure oxidation are present in all embryos, but their activation is either genetically programmed or can partially occur under the influence of external factors (in particular, light). The mechanisms

that ensure this oxidation are inherited. The inheritance of this trait in hybrids shows that it is not caused by one gene. The low concentration of NO donors in meat embryos (at several μmol/l) indicates that the amount of NO donors, measured in hundreds of μmol/l, is not vital. Artificial regulation of the content of NO donors in embryonic tissues is problematic, since in the avian embryo arginine is in a saturation concentration for NO synthase, and the NO synthesis blocker nitroarginine effectively blocks NO synthesis only during the first 7 days of incubation. The degree of NO oxidation in the embryo is a highly sensitive parameter for selection, since the difference between egg and meat forms in this indicator reaches two orders of magnitude. The degree of NO oxidation is inherited; therefore, it is determined genetically. Determining the mechanism of inheritance of these genes in different bird species is the task of our future research.

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