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THE EMBRYONIC METABOLISM OF NITRIC OXIDE AND ITS INTERRELATION WITH POSTEMBRYONIC DEVELOPMENT IN CHICKEN (*Gallus gallus domesticus* L.) AND QUAILS (*Coturnix coturnix* L.)

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Abstract

The embryonic development is accompanied by the intense synthesis of nitric oxide (NO). Many processes of the embryogenesis (e.g. tissue differentiation, apoptosis) were found to be NO-dependent. However, due to the difficulties related to the control of NO metabolites in living tissues the physiological effects of NO have been studied by the indirect methods exclusively, via the effects of the inhibitors of NO synthesis or the effects of NO donor compounds and arginine as the precursor in the NO biosynthesis. But this does not allow us to establish the mechanism of the relationship between the observed effect and the metabolism of nitric oxide. Myogenesis is also considered NO-dependent since arginine, NO-synthase inhibitors, and NO donors were reported to affect the development of muscles. However, these effects are quite contradictory. The lack of data on the relationship between nitric oxide metabolism and these effects does not allow us to suggest in detail the role of NO in myogenesis and the mechanism of its influence on muscle development. And the lack of understanding of this mechanism does not allow the use of nitric oxide to correct the animal development. In this study we are presenting a pioneer view on the interrelationships of embryonic NO metabolism with the features of the postembryonic body development in different poultry species determined with the use of highly sensitive and highly specific enzymatic sensor for determination of the NO metabolites. The study was aimed at the determination of interrelationships between the intensities of embryonic NO synthesis and its oxidation and embryonic and postembryonic body growth in poultry and at the evaluation of possible application of these interrelationships for the enhancement of meat productivity. The experiments were performed in 2017–2019 on different breeds of chickens and quails. It was found that the intensity of embryonic NO synthesis is similar within any given poultry species. In most cases no significant differences between the breeds ($p > 0,05$). This was determined by the total concentration of all NO metabolites in the embryo. However, the intensity of embryonic NO oxidation to nitrate can vary drastically. Differences between embryos of egg and meat breeds, lines and crosses on this indicator reach several orders of magnitude. In the embryos of egg breeds, there is mainly an accumulation of nitric oxide in the so-called donor compounds. By the end of embryogenesis, their concentration reaches several hundred of micromoles. In meat breed embryos NO is mainly oxidized to nitrate. The variance of the intensity of embryonic NO oxidation within a given breed does not exceed 10–15 %. This oxidation was found to occur predominantly in the embryonic muscle tissues. The intensity of NO oxidation is similar for endogenous (synthesized by embryos) and exogenous (injected in ovo) NO donors. The injections of inhibitors of NO synthesis decreased the embryonic concentration of total NO metabolites while the NO donors to nitrate ratio was not affected. These effects suggest that the intensity of NO oxidation to nitrate is directly correlated with certain features of embryonic tissues. Therefore, it can be considered as a biochemical marker of these features. It correlates with meat productivity and is an indicator inherent to a given breed. It is not sex-linked and

does not depend on the layer age, nutrition, etc. Thus, it can be regarded as a highly sensitive and highly specific genetically preconditioned marker. The 2-fold increase or decrease of embryonic concentration of oxidized NO (by the intraembryonic injections of NO donors or inhibitors of NO synthesis, respectively) did not significantly affect the postnatal body growth rate. The exact mechanism of the embryonic NO oxidation and the interrelationships of the latter with the development of muscular tissues are still to be elucidated.

Keywords: poultry, nitric oxide, NO donors, nitrate, embryogenesis, post-embryonic growth

Embryogenesis is known to be accompanied by the synthesis of NO [1-4]. It is believed that many processes occurring in the developing embryo, for example, implantation [5], cell differentiation and apoptosis [6, 7], adaptation to hypoxia [8], are NO-dependent. Nitric oxide has been reported to affect muscle development [9, 10]. This is shown both at cellular [11-14] and at body [13, 15, 16] levels, including the embryonic level [9, 17]. The conclusions were made on the basis of experiments with NO donor compounds [9, 11, 18], studies of the consequences of blocking NO synthesis [9, 10, 12, 13], and the use of arginine as a source of NO [9, 12, 17]. The observed effects are rather contradictory and do not allow researchers to suggest in detail any mechanism of NO influence on myogenesis. The drawback of all previous studies, from our point of view, is the lack of control estimates of the drug metabolism and the initial level of NO in the tissue. Until now, such measurements have been methodologically problematic [19, 20], but these estimates are obligatory to clarify the mechanism of NO participation in physiological processes.

Using a highly sensitive enzymatic sensor, which allows quantification of all physiologically significant nitro- and nitroso compounds [20], we have previously identified the main regularities of the synthesis and metabolism of nitric oxide in an avian embryo [21]. It was found that in the embryos of breeds, lines and crosses resulted from breeding for improved meat productivity, there is an intense oxidation of NO synthesized in the embryo to nitrate. In embryos of the initial and egg poultry, NO accumulates mainly in so-called NO donor compounds: the nitrosothiols (RSNO), dinitrosyl iron complexes (DNIC), high molecular weight nitro compounds (RNO₂) that are capable of transforming into DNIC. The difference in the intensity of NO oxidation between meat and egg forms can be up to two orders of magnitude [21].

This paper presents findings on the relationship between the rate of synthesis and metabolism of nitric oxide (NO) in the embryo, on the one hand, and the embryonic and postembryonic development of birds, on the other hand. It was shown for the first time that the oxidation of NO to nitrate occurs predominantly in the muscles. It is the intensity of this oxidation that correlates with meat productivity. It is conditioned by genetically determined characteristics of embryonic tissues, and these features are associated with the growth rate of muscle tissue.

The work was aimed to quantify the intensity of synthesis and oxidation of nitric oxide in the embryo and to collate these processes with embryonic and postembryonic growth rate of poultry in order to reveal regulatory potential of NO in increasing meat productivity.

Materials and methods. The experiment involved chicken (*Gallus gallus domesticus* L.) breeds Andalusian blue, Yurlovskaya golosistaya, Orlovskaya sittsevaya, Cornish (line B56), Plymouth Rock (line B79), Malay and kulangi fighting breeds, and crosses Hisex White, Smena 8, Cobb 500, Ross 308, as well as quail (*Coturnix coturnix* L.) breeds Estonian meat-egg, Japanese gray, Manchurian golden, Pharaoh and heavy white. Seventy fertilized eggs from each breed and cross provided by Genofond LLC (Russia) were placed in incubators (Stimul-Ink-1000, Stimul Group LLC, Russia) at 37.6 °C during incubation period, and 37.2

°C during hatching (a vivarium of Breeding Genetic Center Zagorskoe, VNITIP, Sergiev Posad, Moscow Province, 2017-2019). Each egg was weighted before incubation.

Four eggs of each breed, line and cross were taken before incubation and during incubation, on days 7 and 17 for chickens, and every day from day 1 to day 13 for quails, to remove embryos for quantification of NO metabolites (analysis was performed no later than 30 min after sampling). The embryos were mechanically cut into small pieces and poured with a 158 mM sodium chloride solution containing 10 mM potassium phosphate, pH 7.4, 20 ml solution per 1 g tissue, and homogenized (a glass homogenizer, 8 min, 40 frictions per min, 6 °C). De-shelled eggs without embryos were subjected to a similar procedure.

The dinitrosyl iron complex with two glutathione molecules (DNIC/GSH), prepared according to the method described earlier [19, 20], was used as an exogenous NO donor compound. L-nitroarginine (NA) (Sigma-Aldrich, USA) was used to block the NO synthesis. Donor and blocker solutions were prepared in sterile physiological saline and injected in ovo into the air chamber before setting eggs in incubation according to the experimental scheme: 0.3 ml of saline (control), 0.3 ml of 13.0 mM DNIC/GSH (donor), 0.3 ml 6.0 mM NA (blocker).

An enzymatic sensor developed by us was used to quantify NO metabolites. The sensor is based on the ability of nitrite, nitrosamines (RNNO), nitrosothiols (RSNO), dinitrosyl iron complexes (DNIC), and nitro derivatives of high molecular weight compounds (RNO₂) to inhibit catalase in the presence of halogen ions and on the loss of this property under the influence of factors specific to each group of compounds. Nitrates were reduced to nitrites with vanadium trichloride and quantified [20]. The method allows quantification of NO derivatives with a sensitivity of up to 50 nM without sample preparation [20] using facility based on a Dithermanal device (Vaskut-EMG, Hungary).

Classical Griess test was also used to measure nitrite (NO₂⁻) ions [22].

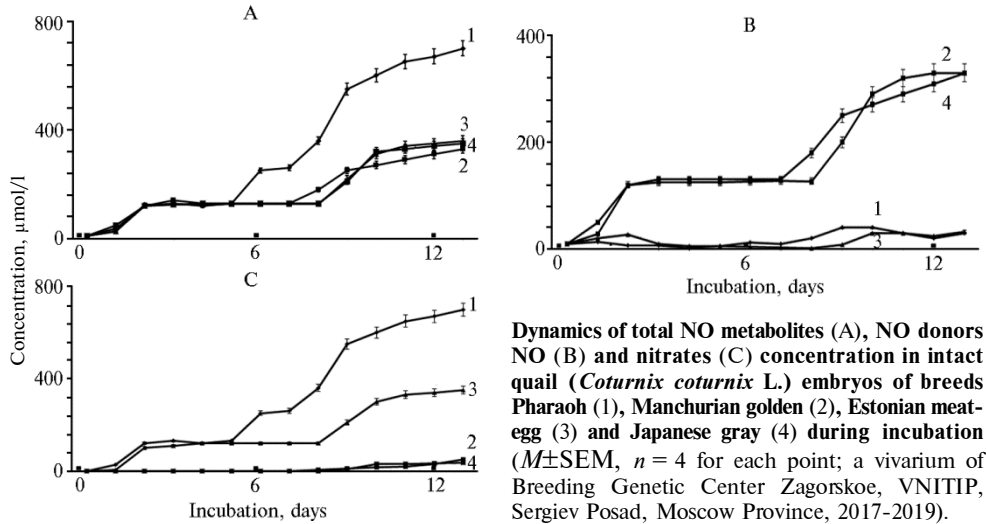
The hatched young birds were grown in KBN-1640 cages, 20 birds each up to 10 days of life and 8-10 birds each from day 10 to day 28 of life. Feeding corresponded to the recommendations of VNITIP for the age and the breed (cross) (Moscow, 2014). Growth was estimated by individual weighing on days 1, 14 and 28.

Results are shown as means (*M*) and standard errors of means (\pm SEM). The significance of the differences between the compared indicators was assessed by the Student's *t*-test. Differences were deemed statistically significant at $p < 0.05$.

Results. Synthesis and oxidation of NO in avian embryos. The development of the avian embryo is accompanied by intense production of nitric oxide. Synthesized NO, according to recent concepts, is involved in formation of NO-derived complexes, the so-called NO donors. These compounds are assumed to directly interact with the physiological target [19, 23-25]. As can be seen from the data presented in the figure, by day 3, the total concentration of NO metabolites, i.e. the NO donors and nitrates in the homogenous content of a quail egg reaches 150 μ mol/l. We have found a similar relationship in chickens [21]. The NO donor compounds accumulate in the amnion, while nitrate ions concentrate outside the amniotic bladder. Nitrite and nitrosamines appeared in trace amounts in all tissues of the embryo [25]. From day 3 to day 6 in quails (see Fig.) and to day 11 in chickens [21], the total concentration of nitro and nitroso compounds in the embryo homogenate is practically unchanged. Further, the indicator sharply increases (see Fig.), which is associated with the onset of intense synthesis of NO in embryonic tissues [21].

Until this time, the total concentration of NO donors and nitrates derived from NO oxidation, in embryos of all breeds of one species is approximately the

same (see Fig., Table 1). However, embryos in different breeds, lines, and crosses differed in the proportion of nitrates and NO donors. Within one species, this proportion can vary by several orders of magnitude (see Table 1). As we previously found, this is due to the fact that in some embryos NO is intensively oxidized to nitrate, while in others it is predominantly accumulated in NO donors [21]. This oxidation begins on days 2-3 and continues throughout embryogenesis (see Fig.).



Dynamics of total NO metabolites (A), NO donors NO (B) and nitrates (C) concentration in intact quail (*Coturnix coturnix* L.) embryos of breeds Pharaoh (1), Manchurian golden (2), Estonian meat-egg (3) and Japanese gray (4) during incubation ($M \pm \text{SEM}$, $n = 4$ for each point; a vivarium of Breeding Genetic Center Zagorskoe, VNITIP, Sergiev Posad, Moscow Province, 2017-2019).

1. Relation of NO metabolites (nitrates and NO donors, $\mu\text{mol/l}$) in homogenates of intact 7-day-old embryos of chickens and quails of different breeds, lines and crosses in collation with the dynamics of live weight ($M \pm \text{SEM}$, $n = 50$ for each breed, line and cross; a vivarium of Breeding Genetic Center Zagorskoe, VNITIP, Sergiev Posad, Moscow Province, 2017-2019)

Breeds, lines and crosses	Egg weight, g	Chick weight, g				Proportion of NO metabolites, day 7
		day 1	day 14	day 28		
				♀	♂	
<i>Chicken (Gallus gallus domesticus L.)</i>						
Hisex White	64.2 \pm 0.8	42.4 \pm 3.1	79.8 \pm 3.9	222.4 \pm 5.2		≤ 0.1 138.9 \pm 8.9
Smena 8	64.8 \pm 0.6	47.5 \pm 0.7	311.9 \pm 19.2	1157.0 \pm 50.0		<u>145.4\pm9.8</u> 3.3 \pm 2.1
Cobb 500	62.7 \pm 0.7	48.5 \pm 1.9	276.5 \pm 17.5	1244.5 \pm 38.4		<u>151.3\pm10.1</u> 2.3 \pm 1.5
Yurlovskaya golosistaya	60.4 \pm 0.7	39.1 \pm 0.8	107.5 \pm 4.5	241.7 \pm 8.9		≤ 0.1 149.6 \pm 9.1
Malay fighting breed	54.5 \pm 0.5	37.9 \pm 0.8	98.8 \pm 4.4	214.8 \pm 9.1		<u>148.4\pm9.3</u> 5.8 \pm 1.9
Orlovskaya sittsevaya	51.7 \pm 0.5	35.5 \pm 0.9	93.1 \pm 5.8	167.8 \pm 8.7		≤ 0.1 131.5 \pm 6.9
Andalusian blue	48.5 \pm 0.6	37.2 \pm 0.97	85.4 \pm 3.1	170.6 \pm 5.4		≤ 0.1 141.8 \pm 8.4
Kulangi	55.7 \pm 0.6	41.3 \pm 0.9	92.4 \pm 3.8	223.7 \pm 8.3		<u>143.4\pm7.6</u> 8.8 \pm 1.6
Cornish (line B56)	65.8 \pm 0.6	49.3 \pm 0.7	291.7 \pm 9.4	1287.5 \pm 49.1		<u>152.2\pm9.9</u> 9.5 \pm 1.7
Plymouth Rock (line B79)	64.3 \pm 0.8	44.9 \pm 0.7	265.2 \pm 9.7	1058.4 \pm 32.6		≤ 0.1 141.8 \pm 8.4
Ross 308	65.6 \pm 0.7	43.4 \pm 0.5	341.7 \pm 10.1	1146.4 \pm 30.5		<u>138.5\pm8.6</u> 4.4 \pm 1.9
<i>Quails (Coturnix coturnix L.)</i>						
Manchurian golden	12.7 \pm 0.2	11.7 \pm 0.2	95.8 \pm 1.1	178.2 \pm 3.7	163.6 \pm 1.7	≤ 0.1 131.8 \pm 4.3
Estonian	13.0 \pm 0.1	12.1 \pm 0.2	111.0 \pm 1.1	194.5 \pm 3.8	181.8 \pm 2.3	<u>139.4\pm11.2</u> 3.1 \pm 1.5
♀ Manchurian golden \times ♂ Estonian	13.6 \pm 0.2	12.6 \pm 0.2	109.3 \pm 1.4	184.6 \pm 4.1	176.9 \pm 3.8	<u>168.5\pm10.9</u> 1.5 \pm 0.8

Japanese gray	12.2±0.2	10.6±0.3	95.6±1.2	163.5±2.7	158.1±3.3	≤ 0.1 134.3±2.7
♀ Japanese gray × ♂ Estonian	12.1±0.1	10.4±0.3	98.7±1.4	171.3±2.4	167.5±2.1	<u>168.5±10.9</u> 1.5±0.8
Pharaoh	13.4±0.1	11.9±0.2	105.2±1.6	205.1±3.4	198.6±6.0	<u>235.3±12.8</u> 12.8±1.9
Heavy white	13.5±0.2	10.9±0.2	105.0±2.6	283.3±3.1	277.4±6.1	<u>157.0±6.7</u> 3.1±1.0
♀ Manchurian golden × ♂ Japanese gray	12.6±0.1	10.2±0.1	96.3±0.8	179.4±2.9	165.2±2.3	≤ 0.1 131.0±4.8

Note. Concentration of nitrites and nitrosamines in all specimens is $< 0.1 \mu\text{mol/l}$. The numbers above and below the line indicate the concentration of nitrates and NO donors.

High intensity of oxidation is characteristic of the meat poultry. It is observed in broiler embryos (Cobb 500, Smena 8, Ross 308), their paternal line Cornish B56, and also in embryos of fighting breeds (Malay and kulangi). Their embryos are characterized by low levels of NO donors (from units to tens of micromoles per liter) with a high content of nitrate (hundreds of micromoles per liter) (see Table 1). In embryos of egg breeds (Hisex, Orlovskaya sinttsevaya, Yurlovskaya golosistaya, Andalusian blue), as well as in the maternal line of Plymouth Rock B79 of the cross Smena 8, the intensity of NO oxidation is minimal, which shows the relation of nitrate to NO donors (see Fig., Table 1). By analogy, for the embryos of heavy quail breeds (Pharaoh, heavy white, Estonian), there is a high intensity of nitric oxide oxidation in the embryo, while in the light breed (Japanese gray, Manchurian golden) oxidation is negligible (see Table 1).

Relationship between embryonic NO oxidation and bird growth rate. Analysis of the content of nitro- and nitroso compounds in the tissues of 17-day-old chicken embryos, characterized by both high and low rates of NO oxidation, shows in all cases the highest level of NO donor compounds in the liver. The liver, as well as the gastrointestinal tract, contains mainly nitric oxide donors, and the amount of nitrate is insignificant. A significant accumulation of nitrate, many times higher than that of NO donors, occurred in muscles of the Cornish B56 embryo. In the Hisex White cross line, the muscles predominantly contained NO donors (Table 2). Consequently, nitrate accumulates mainly in muscle tissue. It is in it, apparently, that NO oxidation to nitrate occurs. This gives one more reason to assume that NO oxidation in the embryo is mainly associated with myogenesis, namely with myogenesis of skeletal muscles (see Table 2).

2. Concentration (nmol/mg) of nitro and nitroso compounds in tissues of 17-day-old intact embryos of chicken (*Gallus gallus domesticus* L.) egg cross Hisex White and meat line Cornish B56 ($M \pm \text{SEM}$, $n = 8$; a vivarium of Breeding Genetic Center Zagorskoe, VNITIP, Sergiev Posad, Moscow Province, 2017-2019)

Cross	Liver		Gastrointestinal tract		Muscle	
	NO donors	NO ₃ ⁻	NO donors	NO ₃ ⁻	NO donors	NO ₃ ⁻
Hisex White	11.5±0.9	< 0.1	1.1±0.1	< 0.1	3.2±0.3	0.3±0.1*
Cornish B56	33.5±0.3	0.2±0.1	4.3±0.3	< 0.1	0.2±0.1	4.2±0.3*

Note. Concentration of nitrites and nitrosamines in all specimens is $< 0.1 \mu\text{mol/l}$.

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

Our previous works show that there is no qualitative difference in the concentration of NO donors and nitrates between the pectoral and leg muscles of chickens and quails. In meat birds, nitrate predominates, in egg birds, NO donors. Quantitative differences in the concentration of NO metabolites between these muscle groups of one individual are no more than 10-12% [21, 26], which is incomparable with the difference between egg and meat forms (see Table 2). That is, NO oxidation is not directly related to the biochemical characteristics of these muscle groups. Since muscle tissue dominates in weight over others, nitrate predominates in homogenates of embryos of meat forms (see Fig.).

We found that 8-15 days after hatching, the concentration of NO donors and nitrate in chick tissues sharply decreases and becomes approximately the same in all breeds, lines and crosses [21]. That is, a high content of nitro- and nitroso compounds and intensive oxidation of NO donors to nitrates in muscle tissue are embryonic phenomena. It was also shown that the development of muscle tissue in embryos with high and low rates of NO oxidation proceeds at the same rate and does not have a qualitative difference in any parameters [26]. The difference may be in the intensity of muscle growth. But how can you evaluate it? Relation of gutted carcasses weight of broilers to their body weight is no than 5% more than that index of egg breeds [21]. It was the live weight that we decided to use as a criterion for the growth of muscle and bone tissue.

On day 1 after hatching, chickens of different breeds differ slightly in live weight (see Table 1). The increase in weight in embryos with high and low rates of embryonic NO oxidation, based on the data in Table 3, also occurs without significant differences. However, after hatching, the chickens show significant differences in growth (see Table 1).

3. Weigh (g) of intact quail (*Coturnix coturnix* L.) embryos during incubation
($M \pm SEM$, $n = 8$; a vivarium of Breeding Genetic Center Zagorskoe, VNITIP, Sergiev Posad, Moscow Province, 2017-2019)

Breed	Days on incubation				
	8	10	12	15	day 1 after hatching
Manchurian golden	0.53±0.02	1.23±0.08	2.63±2.1	5.32±3.1	11.7±0.2
Pharaoh	0.57±0.02	1.20±0.10	2.75±2.1	5.34±3.4	11.9±0.2

The difference in live weight and its gain between broilers and egg chickens, as well as between heavy and light quail breeds, is obvious (see Table 1). But Cornish and Plymouth Rock, the paternal and maternal lines of the Smena 8 cross with high and low rates of embryonic NO oxidation, respectively, differed insignificantly in terms of live weight. A significant difference ($p < 0.05$) occurred only on week 3 after hatching. There was no significant difference in live weight between fighting breeds and egg breeds Yurlovskaya golosistaya and Hisex White (see Table 1).

Quails of heavy (Pharaoh, white heavy, Estonian) and light (Japanese gray, Manchurian golden) breeds clearly differed in live weight ($p < 0.05$). In the embryos of hybrids of Japanese gray and Estonian, as well as Manchurian golden and Estonian breeds, as follows from the proportion of nitrate and NO donors on day 7, embryonic oxidation of nitric oxide occurs with a high intensity (it is not lower than in embryos of the Estonian breed). In terms of live weight, these hybrids occupied an intermediate position between the parental breeds, while the hybrids of the Manchurian golden and Japanese gray breeds were similar in live weight and the rate of NO oxidation to their parents (see Table 1).

Thence two questions arise. What is the mechanism of the relationship between NO oxidation and the rate of weight gain resulted primarily from the development of bone and muscle tissue? And can this process be controlled to increase meat productivity?

Exogenous NO donors, introduced into eggs before incubation, underwent oxidation to nitrate in those embryos where there was intense oxidation of endogenously synthesized NO (Ross 308), and practically did not oxidize where the latter was not oxidized (Hisex White) (Table 4).

It means that the intensity of oxidation is determined by certain features of the embryo tissues. The data in Table 2 allow us to assume that oxidation occurs in muscle tissue. These features are determined genetically, since they are inherent in specific breeds, lines and crosses regardless of the age of the laying hen, the

conditions of its keeping and the conditions of eggs incubation [21], and can also be fully or partially inherited (see Table 1). The intensity of post-embryonic growth is somehow connected with these features. In this case, as an explanation, it can be suggested that nitric oxide is intensively oxidized in the embryo, and phenotypic manifestations associated with this process are observed after hatching (see Table 1).

4. Concentration ($\mu\text{mol/l}$) of NO donors and NO_3^- in homogenates of 7-day-old embryos in chicken (*Gallus gallus domesticus* L.) crosses, as influenced by an exogenous blocker of NO synthesis and DNIC/GSH applied prior to incubation ($M \pm \text{SEM}$, $n = 25$ for each group; a vivarium of Breeding Genetic Center Zagorskoe, VNITIP, Sergiev Posad, Moscow Province, 2017-2019)

Treatment	NO donors	NO_3^-
Cross Hisex White:		
+ 0.3 ml saline (control)	138.3 \pm 7.8	< 0.1
+ 0.3 ml 13.0 mM DNIC/GSH	210.9 \pm 9.4*	< 0.1
+ 0.3 ml 6.0 mM NA	39.8 \pm 3.1*	< 0.1
Cross Ross 308:		
+ 0.3 ml saline (control)	3.7 \pm 1.2	131.2 \pm 7.5
+ 0.3 ml 5.0 mM DNIC/GSH	3.9 \pm 1.3	165.0 \pm 8.3*
+ 0.3 ml 13.0 mM DNIC/GSH	34.1 \pm 2.9	179.6 \pm 8.1*
+ 0.3 ml 6.0 mM NA	3.6 \pm 1.2	46.7 \pm 4.2*

Note. NA — N ω -nitro-L-arginine, DNIC/GSH — dinitrosyl-iron-di-glutathione. Concentration of nitrites and nitrosamines in all specimens is < 0.1 $\mu\text{mol/l}$.

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

Thus, NO oxidation serves as a marker of the presence of such features, which, apparently, can manifest themselves not only in the embryonic, but also in the postembryonic period. This marker can be used both to determine phenotypically unexpressed forms [27] and, possibly, to control the breeding process.

Consequently, if the intensity of endogenous NO oxidation is low, then the exogenous NO donor compounds, most likely, should not have any effect. Or, if a similar effect does occur, then it is not associated with NO. According to our data, exogenous NO donors, when injected into the embryo of both egg and meat chickens at the concentrations used in Table 4, did not have a significant effect on the live weight of chickens during the first 3 weeks after hatching ($p > 0.05$).

The use of NO synthase blockers reduced the amount of nitro and nitroso compounds in the embryo without changing the ratio between NO donors and nitrate (see Table 4). A 70% decrease in the total concentration of NO donors and nitrates did not significantly affect the increase in live weight in the first three weeks after hatching ($p > 0.05$). That is, we cannot yet give an unambiguous answer whether the oxidation of NO itself affects myogenesis, or whether this oxidation is a side effect of specific processes in the developing muscles of embryos of meat poultry.

So, we revealed that the rate of nitric oxide synthesis in the embryos of birds of the same species is approximately the same, while the rate of NO oxidation to nitrate varies up to several orders of magnitude when comparing egg and meat breeds, lines and crosses. In egg poultry, nitric oxide in embryos accumulates in amounts of up to several hundred micromoles, mainly as NO donor compounds, while in meat poultry, oxidation of NO to nitrate predominates. Within the breed, line and cross, the rate of NO oxidation varies by no more than 10-15%. Oxidation occurs mainly in muscle tissue. Exogenous NO donors are oxidized in the embryo with the same intensity as endogenous ones, and the blocker of NO synthesis, decreasing the total concentration of its metabolites, does not affect the quantitative ratio between NO donors and nitrate. Consequently, the intensity of embryonic oxidation is a biochemical marker of gene expression that determines the rate of muscle growth. The identification of these genes is an important scientific and

practical task. The possibility of regulation of meat productivity at the embryonic level is seen primarily in the regulation of the expression of these genes.

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