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FEATURES OF NITRIC OXIDE METABOLISM IN EMBRYOS OF DIFFERENT BIRD SPECIES AS GENETICALLY DETERMINED SIGN ASSOCIATED WITH MEAT PRODUCTIVITY

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Abstract

At present, the role of nitric oxide (NO) in embryogenesis, in particular in myogenesis, is widely discussed. Earlier we noted that the main part of nitric oxide synthesized in the avian embryo can accumulate in tissues as part of the so-called NO donor compounds or be oxidized to nitrate. The degree of this oxidation correlates with the meat productivity of adults. This report shows that in broiler embryos NO is oxidized to nitrate by 90% or more, while in embryos of egg poultry NO oxidation is negligible. That is, the degree of NO oxidation is determined by some features of the embryo tissues rather than NO itself determines these features. Consequently, the degree of NO oxidation in bird embryogenesis is an indicator associated with tissue properties correlating with meat productivity. Since this sign is inherited, it is assumed to be genetically determined. The purpose of this work is to characterize the manifestation and inheritance of the intensity of nitric oxide oxidation and the associated physiological characteristics of embryos in birds of different species. The experiments were carried out in a vivarium (Zagorskoye, Sergiev Posad, Moscow Province, 2015-2021). It was shown that in poultry of different breeds characterized by the same degree of NO oxidation the live weight can vary significantly. This is especially evident in hens. The proportion of oxidized NO in the embryo was higher in lines, breeds and crosses obtained as a result of breeding to increase meat productivity. Thus, in the embryos of broilers and meat quails, by the day 7th, more than 90% of embryonic NO is oxidized, in egg forms oxidation was insignificant (several percent), most meat-egg forms occupied an intermediate position according to this index. The analysis of inheritance of the index in the F_1 generation in several bird species suggests that this trait is formed due to the expression of various genes that can both promote and counteract its manifestation. Oxidation of NO to nitrate in embryos of both meat and egg forms can be induced by light at the beginning of incubation. In embryos of egg forms, the proportion of oxidized NO can increase up to 60 % under the action of light. Consequently, there is a possibility of oxidation of NO in embryos of both meat and egg forms. Apparently, the mechanism of activation of this process is inherited, which can also be partially induced by light. Further analysis of the inheritance of the intensity of oxidation of embryonic NO in a number of generations will show which genes are associated with the intensity of oxidation of NO. This will allow using this indicator as a highly sensitive marker for the corresponding genes.

Keywords: nitric oxide, NO, NO donors, NO oxidation, trait inheritance, nitrate, myogenesis, *Gallus gallus domesticus* L., chickens, *Coturnix coturnix* L., quail, *Numida meleagris* L., guinea fowl, *Struthio camelus* L., ostriches

At present, the role of nitric oxide (NO) in embryogenesis, in particular in myogenesis, is widely discussed. It is believed that NO mediates proliferation of myocytes [1-5], formation of muscle fibers [3, 4], and proliferation of satellite

cells [6]. According to modern concepts, the physiological effect of NO is due to the nitrosation of enzymes guanylate cyclase [7, 8], caspase]9-11] and the structures that determine gene expression [12, 13].

It is believed that the synthesized nitric oxide is incorporated in the NO donor molecules — S-nitrosothiols (RSNO), dinitrosyl iron complexes (DNIC), and high molecular weight nitro compounds (RNO_2). These compounds play the role of a nitric oxide depot, prolonging its physiological lifetime [7, 14, 15]. Their concentration in cells can reach tens of micromoles [16, 17], being comparable with the concentration of nitrate, the end product of NO metabolism. Therefore, to determine the role of NO in a particular process, it is necessary to control the content of deposited NO and the products of its metabolism.

But until now, the quantifiction of all NO metabolites in living tissues is difficult due to the lack of methods that allow one to quickly analyze the entire spectrum of nitro and nitroso compounds and its changes during physiological processes.

The enzyme biosensor we have developed jointly with the Federal Research Center for Chemical Physics RAS is based on the reversible inhibition of catalase by all nitroso compounds that initially have an NO^+ group or acquire it when influenced by sertain factors. Halide ions increase the inhibition efficiency by two orders of magnitude. Nitroso compounds lose their inhibitory properties when interact with substances specific to each of their groups. This allows the concentration of S-nitrosothiols (RSNO), DNIC, nitrite, and nitrosamines to be measured with a 50 nM accuracy [17].

Using the developed sensor, it was shown that embryogenesis in birds, as in other animals, is associated with increased production of NO which either accumulates in the embryo as part of donor compounds or is oxidized to nitrate. Within a species, the NO synthesis is approximately of the same intensity while the intensity of nitric oxide oxidation to nitrate varies. The latter indicator in meat poultry is many times higher than in egg poultry [17, 18].

Earlier we evaluated the embryonic NO oxidation level in 42 breeds, lines and crosses of 5 poultry species [18-20]. All 25 egg breeds, lines and crosses were low in intensity of embryonic NO oxidation, no more than 5% of the total synthesized NO on day 14. In 19 breeds, crosses and lines designated as meat poultry, the level of NO oxidation was high (90% or more), and in meat-and-egg breeds, the indicator showed intermediate values [18-20]. Such data suggest that the intensity of NO oxidation is somehow related to meat productivity, especially since NO oxidation occurs predominantly in muscle tissues [20]. Since the intensity of NO oxidation is a hereditary trait with a deviation of less than 10% within the line and cross [18-20], it is reasonable to assume that the property is genetically determined.

The hypothesis of using the NO oxidation intensity as a selection marker could be confirmed or refuted by data on this trait manifestation in the embryos of birds of different species and different types of productivity. We did not find such data in the available scientific publications.

In this work, we have studied for the first time the relationship between the intensity of NO oxidation to nitrate in the embryo and the rate of postembryonic growth in quails, chickens, and ostriches. Also, the mechanism of heritability of this trait was analyzed for the first time in F_1 from crosses of different lines and breeds were crossed. The proposed methodology allowed us to estimate a highly sensitive parameter associated with meat productivity. Its successful use in breeding programs requires an in-depth study of the mechanism of such a relationship.

Our goal was to reveal the features of the inheritance of nitric oxide oxidation intensity and related physiological traits in the embryos of birds of different species.

Materials and methods. Experiments were carried out in a vivarium of the Genetic and Breeding Center Zagorskoye (VNITIP, Sergiev Posad, Moscow Province, 2015-2021) using fertile eggs of chickens (*Gallus gallus domrticus* L., the White Cochinchina, Fawn Brama, Andalusian Blue, Cornish lines B5, B6, B56, Plymouth Rock lines B7, B9, B79, Kulangi breeds; cross Hisex White and its lines X1, X2, X12, X3, X34; crosses Hisex Brown, Smena 8, Cobb 500, Ross 308, mini-hens of lines B77 and A77 groups No. 1 and No. 2), quails (*Coturnix coturnix* L., Estonian meat-egg, Japanese Gray, Manchurian Golden, Pharaoh, White Heavy breeds), and guinea fowl (*Numida meleagris* L., lines ZB1 and ZB2 of the Zagorskaya white-breasted breed, obtained from Genofond LLC, Russia). The eggs of ostriche (*Struthio camelus* L.) black-necked and blue-necked subspecies were obtained from the Vorob'i bird park (Kaluga Province). F_1 hybrids of the Japanese Grey, Estonian Meat-Egg, and Manchurian Golden breeds derived from various crossing combinations.

The temperature during incubation was 37.6 °C, during the hatching period 37.2 °C in accordance with the recommendations of VNITIP (Moscow, 2014) (incubators Stimul Ink-1000, Russia),.

With the use of green light during incubation, the control group was kept in the dark. The experimental group was kept under around-the-clock lighting (15 W Navigator NCL-SH10 energy-saving lamp with a green filter, light flux of 975 lm). The illumination period continued in course of 4 or 14 days from the beginning of incubation. The experiment was repeated 4 times, repetitions were performed with a change of incubators at the incubation temperature of 37.6 °C and hatching temperature of 37.2 °C as described (Recommendations of VNITIP. Moscow, 2014).

To determine the proportion of oxidized NO on days 2, 3 (formation of the amniotic membrane, the embryo is separated from the egg contents), 7, 8, 10 and 15 (after the complete formation of allantois), for ostriches on day 24, 20-40 eggs of each breed, line and cross were selected from the incubated batches. The concetration of NO metabolites in the samples (egg content homogenate, amnion content, allantois content) was measured no later than 30 min after sampling.

Shell-free eggs were homogenized (8 min, 6 °C, 40 frictions/min, a glass homogenizer, DWK Life Sciences GmbH, Germany); after 11-day incubation, a chopper (Oster, Mexico) was used.

The concentration of nitro and nitroso compounds was measured by using the highly sensitive sensor based on Dithermanal calorimeter (Hungary) [21]. The total concentration of NO donor compounds (depot), the S-nitrosothiols (RSNO), dinitrosyl iron complexes (DNIC), high-molecular-weight nitro compounds capable of transforming into DNIC (RNO_2), as well as the concentration of nitrite and nitrosamines, nitrate (NO_3^-) were assessed {17]. The nitrate content was evaluated after reduction with vanadium trichloride to nitrite, followed by quantitative determination [21, 22]. The proportion of NO oxidized to nitrate was determined from the concentration ratio nitrate/(NO donors + nitrate) × 100%.

Meat productivity was assessed by the live weight of birds in samples of 20-40 individuals after hatching and at the age of 28 days [23]. Young birds were kept in cages, feeding and rearing conditions, sex ratio during rearing were as commonly used (Recommendations of VNITIP. Moscow, 2014).

The BioStat software package (https://www.softsalad.ru/software/znaniya/matematika-i-nauka/biostat-2008) was used for statistical processing. Mean values (M) and standard errors of the mean (±SEM) were calculated with a 95%

confidence interval ($t_{0.05}$ × SEM). Differences between the variants were assessed by parametric statistics (Student's *t*-test) and were considered statistically significant at $p < 0.05$.

Results. To assess the amount of deposited NO and its oxidation product, nitrate, we used an enzymatic sensor developed by us based on the property of nitrite, nitrosamines (RNNO), nitrosothiols (RSNO), dinitrosyl iron complexes (DNIC) and nitro derivatives of macromolecular compounds (RNO_2) to inhibit catalase in the presence of halide ions and the loss of this ability under the influence of factors different for each group of compounds. Since the catalase reaction is highly exothermic (47.2 kcal/mol of released oxygen), its kinetics can be analyzed based on the kinetics of heat production accompanying the process [17, 21]. The method makes it possible to estimate the content of NO derivatives without preliminary sample preparation, since there is no need to remove colored impurities and turbidity, and is characterized by a sensitivity of 50 nM [17, 21].

1. The concentration of NO donors and nitrate (μmol/l) **in the egg content homogenate, in the amnion and allantois of embryos of different breeds, lines and crosses of chickens** (*Gallus gallus domesticus* L.) **during incubation** ($n = 40$, vivarium, Genetic and Breeding Center Zagorskoye, VNITIP, Sergiev Posad, Moscow Province)

Sample	Day 2		Day 3		Day 10		Day 15	
	NO donors	nitrate	NO donors	nitrate	NO donors	nitrate	NO donors	nitrate
Cornish line B56, $M \pm (t_{0.05} \times SEM)$								
Homogenate	37.1±2.9	< 0.1	134.5±8.9	8.1±3.2	5.7±1.6	146.6±8.8	18.7±2.5	465.5±19.9
Amnion			19500.3±45.5	< 0.1	23.7±7.6	< 0.1	19.8±7.4	< 0.1
Allantois			6.1±3.5	12.3±3.4	5.9±1.4	138.4±8.8	4.2±1.7	448.7±20.5
Plymouth Rock line B79, $M \pm (t_{0.05} \times SEM)$								
Homogenate	45.8±3.1	< 0.1	148.4±9.9	< 0.1	158.5±10.1	< 0.1	437.3±19.4	16.4±3.5
Amnion			12125.0±169.5	< 0.1	5500.0±150.0	< 0.1	5340.0±180.0	< 0.1
Allantois			10.5±3.3	< 0.1	10.2±3.9	< 0.1	8.9±2.9	13.9±4.1
Cross Smena 8, $M \pm (t_{0.05} \times SEM)$								
Homogenate	25.8±4.4	4.1±3.2	7.6±1.8	133.6±10.1	5.8±1.8	153.6±8.2	12.8±4.8	569.8±16.4
Cross Hisex White, $M \pm (t_{0.05} \times SEM)$								
Homogenate	40.4±6.2	< 0.1	142.4±8.1	< 0.1	160.4±8.7	< 0.1	466.4±15.4	12.5±2.8

Note. On day 3, given the small size of the amnion, homogenates of three embryos covered with an amniotic membrane were used in 1.5 ml of 40 mM K-phosphate buffer, pH 7.4. Up to day 15 (until the allantois was completely closed on days 12-13), the concentration in the liquid medium outside the amnion was measured. The concentration of nitrite and nitrosamines in all samples was < 0.1 μmol/l.

Table 1 shows the data on the content of deposited NO and its oxidation product, nitrate, in the homogenates of avian embryos of the Hisex White egg cross, Smena 8 broiler, its paternal form Cornish B56, and maternal Plymouth Rock B79. NO donor compounds are accumulated from day 1 to day 3 in the amniotic fluid. At the age of 2-3 days, their concentration decreases in the embryos of the Smena 8 cross and the Cornish B56 line. On the example of the Cornish B56 line, we see that there is a decrease in the concentration of NO donors in the amniotic fluid and a simultaneous increase in the concentration of nitrate outside the amnion. In the amnion, nitrate and nitrite are present in trace concentrations. The total content of nitrate and NO donors in the homogenates of all embryos differed insignificantly (see Table 1), 140-160 μmol/l on day 10 and 450-570 μmol/l on day 15. However, the proportion of oxidized NO deposited in donors to nitrate varied tremendously: from more than 90% in embryos of the Cornish B56 line and the Smena 8 cross to less than 5% in the embryos of the Highsex White cross and the Plymouth Rock B79 line (see Table 1). Consequently, the intensity of embryonal NO oxidation is due to definite features of the embryo tissues. It is not NO donors that spontaneously dissociate with the release of NO, but physiological targets in tissues are responsible for degradation of donor compounds and binding NO which is rapidly oxidized to nitrate and moved out of the amnion. This is confirmed by data on the oxidation of exogenous NO

donors introduced into the egg. They were completely oxidized in broiler embryos and almost not oxidized in egg poultry embryos [18, 20].

NO oxidation occurs during the entire embryonic period. Before hetching, the total concentration of nitrate and NO donors can reach several hundred micromoles (see Table 1).

2. Oxidation of NO to nitrate in egg content homogenates on day 7 in chickens, quails and guinea fowls vs the live weight gain after hatching (vivarium, Genetic and Breeding Center Zagorskoye, VNITIP, Sergiev Posad, Moscow Province)

Breed, line, cross	Purpose of use	Egg weight, g	Chick weight, g			NO oxidized to nitrate, %
			day 1	day 28		
				♂	♀	
Chickens (*Gallus gallus domesticus* L.), $M \pm (t_{0.05} \times SEM)$						
Highsex White (n = 40)	Eggs	63.8±0.8	41.8±2.1	230.1±4.5		2.4±1.3
Oryol calico (n = 20)	Eggs	51.7±0.5	35.5±0.9	167.8±8.7		2.1±1.3
Yurlovskaya golosistaya (n = 20)	Meat-and-egg	63.8±0.7	40.6±0.9	253.9±7.7		3.8±1.8
Plymouth Rock B79 (n = 40)	Meat (bred for egg production)	63.9±0.8	47.1±0.7	1044.6±34.3		2.6±1.5
Andalusian Blue (n = 30)	Eggs	48.5±0.6	38.2±1.0	218.6±5.6		2.1±1.3
Blue Meat-Egg (n = 30)	Meat-and-egg	49.9±0.6	40.1±1.0	231.8±5.5		61.8±2.9
Brama Fawn (n = 20)	Meat-and-egg	52.1±0.5	38.2±0.3	254.8±4.4		82.2±3.1
Smena 8 (n = 40)	Meat	64.9±0.6	48.1±0.7	1188.4±53.2		98.1 ±2.5
Cornish B56 (n = 40)	Meat	65.4±0.6	47.9±0.7	1299.7±54.6		96.9±3.1
Kulangi (n = 20)	Fighting	57.1±0.6	39.9±0.9	234.4±9.1		96.6±2.9
Cobb 500 (n=40)	Meat	63.1±0.7	48.3±0.7	1214.2±44.2		97.8±2.6
Ross 308 (n = 40)	Meat	63.8±0.7	45.8±0.5	1177.5±31.6		97.4±2.5
Guinea fowl (*Numida meleagris* L.), $M \pm (t_{0.05} \times SEM)$						
ZB2 (n = 20)	Eggs	46.8±0.5	31.4±0.6	376.4±5.4		2.2±1.4
ZB1 (n = 20)	Meat	6.7±0.5	31.3±0.5	395.5±8.5		97.8±2.6
Quail (*Coturnix coturnix* L.), $M \pm (t_{0.05} \times SEM)$						
Manchurian Golden (n = 40)	Eggs	12.9±0.2	10.0±0.2	181.1±3.6	163.4±1.9	2.3±1.3
Japanese Gray (n = 40)	Eggs	12.7±0.2	10.8±0.2	173.4±3.0	161.2±3.1	2.4±1.4
Estonian Meat-and-Egg (n = 40)	Meat-and-egg	13.1±0.2	11.2±0.2	198.4±3.9	182.1±2.4	95.5±3.6
♂Japanese Gray × ♀Estonian Meat-and-Egg (n = 20)		12.2±0.2	10.3±0.2	178.3±2.5	171.6±2.3	98.8±3.4
♂Manchurian Golden × ♀Japanese Gray (n = 20)		12.4±0.2	10.2±0.2	176.9±2.9	163.7±2.5	2.2±1.4
Pharaoh (n = 30)	Meat	13.3±0.2	11.0±0.2	208.8±3.5	196.5±4.1	98.4±3.6
White Heavy (n = 20)	Meat	13.9±0.2	11.5±0.2	289.1±3.5	274.0±3.9	97.1±3.3

Poultry with a high intensity of NO oxidation (Table 2) turned out to be meat or fighting birds. In these breeds, lines, and crosses, chicks on day 28 mostly exceeded in weight the birds with low NO oxidation, which are considered egg or initial forms.

But, as follows from the data of Table 2, in birds of different breeds, despite the same intensity of NO oxidation, the live weight differs significantly, especially in chickens. Therefore, it makes sense to determine not the correlation between the proportion of oxidized embryonic NO and body weight, but to assess how the proportion of oxidized embryonic NO changes within the same breed during selection for meat productivity.

The available data suggest that selection for increasing meat productivity within the same breed results in an increase in the intensity of oxidation of NO synthesized during embryogenesis. The Blue Meat-Egg breed is a product of the Andalusian Blue chicken breeding for meat productivity. In the embryos of Blue Meat-Egg chickens, the level of oxidized NO is appr. 60%, while in the Andalusian Blue breed, as in all egg-type chickens, this figure is insignificant (see Tables 2, 3). The growth rate of the Blue Meat-Egg breed is significantly ($p < 0.05$) higher than that of the Andalusian Blue, so, the difference in live weight reaches 10% on day 14 and 7% on day 21 (see Table 2).

3. The proportion of NO oxidized to nitrate in egg content homogenates in different breeds, lines and crosses of chickens (*Gallus gallus domesticus* L.) on day 10 of incubation and its inheritance in F_1 (vivarium, Genetic and Breeding Center Zagorskoye, VNITIP, Sergiev Posad, Moscow Province)

Breed, line, cross	Description	Purpose of use	NO oxidized to nitrate, %, $M \pm (t_{0,05} \times \text{SEM})$
X_1 ($n = 20$)	Paternal line of the paternal form of the Hisex White cross		44.2±3.9
X_2 ($n = 20$)	Maternal line of the paternal form of the Hisex White cross		1.9±1.3
X_{12} ($n = 20$)	The paternal form of the Hisex White cross ♂X_1 × ♀X_2		2.1±1.4
X_3 ($n = 20$)	The paternal line of the maternal form of the Hisex White cross		2.2±1.4
X_{34} ($n = 20$)	Maternal form ♂X_3 × ♀X_4		2.4±1.4
Highsex White ($n = 30$)	Final hybrid ♂X_{12} × ♀X_{34}	Egg	2.3±1.4
Andalusian Blue ($n = 30$)		Egg	2.3±1.6
Blue Meat-Egg ($n = 30$)	Derived from selection of Andalusian Blue breed for live weight gain	Meat-and-egg	59.9±2.7
Cornish B5 ($n = 30$)	Line of paternal form of Smena 8 cross		98.2±2.7
Cornish B6 ($n = 30$)	Line of paternal form of Smena 8 cross		97.9±2.8
Cornish B56 ($n = 30$)	Parenal form of cross Smena 8 ♂B5 × ♀B6		96.9±3.1
Plymouth Rock B7 ($n = 30$)	Line of maternal form of cross Smena 8		3.3±1.4
Plymouth Rock B9 ($n = 30$)	Line of maternal form of cross Smena 8		2.9±1.5
Plymouth Rock B79 ($n = 30$)	Maternal form of cross Smena 8 ♂B7 × ♀B9		2.6±1.5
Smena 8 ($n = 30$)	Final cross, ♂B56 × ♀B78	Meat	98.4±2.4
B77(1) ($n = 20$)	Mini-hen line B77 of group 1	Mini-egg	81.4±3.1
B77(2) ($n = 20$)	Mini-hen line B77 of group 2	Mini-egg	90.7±3.5
A77(1) ($n = 20$)	Mini-hen line A77 of group 1	Mini-meat	98.8±3.4
A77(2) ($n = 20$)	Mini-hen line A77 of group 2	Mini-meat	99.3±3.5

N o t e. The proportion of NO oxidized to nitrate is calculated as the concentration ratio nitrate/(NO donors + nitrate) × 100%. Groups 1 and 2 are derived from selection of the corresponding lines for an increase in live weight.

Mini-hens are characterized by an increased NO oxidation compared to ordunary chickens. However, for mini-hens, selection for higer growth rate leads to intensification of nitric oxide oxidation (Table 3). Thus, 8 weeks after hatching, the live weight in line B77 group 2 is 17% higher compared to group 1 [23] with 81.4 and 90.7% ($p < 0.05$) NO oxidation on day 10 of incubation (see Table 3). A77 line group 2 on week 8 also exceeded group 1 in live weight 17% [23]. Since the eviscerated carcass yield of broilers and egg hens differs by 5%, we considered live weight as an indicator of meat productivity. It is a known that the higher the live weight, the higher the eviscerated carcass yield [23].

Lines ZB1 and ZB2 derived from Zagorskaya white-breasted (ZB) guinea fowl after beedng for meat (ZB1) and egg (ZB2) productivity. In ZB1, almost complete oxidation of NO synthesized in the embryo occurs, while in ZB2, only a few percent are oxidized, although the growth rate of ZB1 slightly exceeds that of ZB2 (see Table 2). Therefore, the growth rate is determined by many factors, and not all of them are associated with the activation of NO oxidation. Nevertheless, selection for higher growth rate leads to intensification of nitric oxide oxidation.

It can be assumed that there is a gene that is either not present in the original forms (and the selection process captures some mutant forms), or this gene or genes are present everywhere, but its (their) expression can be suppressed by other genes. In this regard, it was of interest to assess the inheritance of this trait when crossing different breeds. The Smena 8 cross broilers were obtained by crossing a maternal and paternal forms, which, in turn, also result from crossing certain lines of the Cornish and Plymouth Rock breeds. The data of Table 3 indicate that the Smena 8 cross and its paternal lines and forms (Cornish B5, B6, and B56) show almost complete oxidation of NO in the embryo. On the contrary, in the maternal lines and forms (Plymouth Rock B7, B9, and B79), only a few

percent of NO is oxidized. The growth rate of the Smena 8 cross is somewhat lower than that of the Cornish B56 line, but higher than that of the Plymouthrock B79 line (see Table 3).

4. The proportion of NO oxidized to nitrate in egg content homogenates in different breeds of quails (*Coturnix coturnix* L.) on day 8 of incubation and its inheritance in F_1 (vivarium, Genetic and Breeding Center Zagorskoye, VNITIP, Sergiev Posad, Moscow Province)

Breed	Purpose of use	NO oxidized to nitrate, %, $M\pm(t_{0.05} \times \text{SEM})$
Manchurian Golden (M) ($n = 40$)	Egg	2.6±1.4
Japanese Gray (J) ($n = 40$)	Egg	2.5±1.5
Estonian Meat-and-Egg (E) ($n = 40$)	Meat-and-egg	93.8±4.1
Hybrid (♂J × ♀E) ($n = 20$)		97.9±3.5
Hybrid (♂E × ♀J) ($n = 20$)		99.1±3.1
Hybrid (♂J × ♀M) ($n = 20$)		2.4±1.5

N o t e. The proportion of NO oxidized to nitrate is calculated as the concentration ratio nitrate/(NO donors + nitrate) × 100%.

In quails, heavy breeds (Pharaoh, White Heavy, and Estonian) also show almost complete oxidation of embryonic NO, while it is insignificant in egg breeds Golden Manchurian and Japanese Gray. In hybrids of the Estonian Meat-and-Egg and Japanese Gray breeds, embryonic NO is almost completely oxidized (Table 4). The growth rate of the hybrids turned out to be intermediate between that of the Estonian Meat-Egg and Japanese Gray breeds, regardless of which breed were males and which were females (see Table 2). In the embryos of hybrids of the Japanese Gray and Manchurian Golden breeds, NO oxidation is insignificant, as it is in the embryos of the parent breeds (see Table 4). In terms of growth rate, these hybrids also do not differ from their parents (see Table 2).

In addition to cases when it is possible to assume the presence of a gene or its dominant allele associated with intense NO oxidation, insignificant ($\sim$ 2%) oxidation of embryonic NO can occur in the offspring from crossing a line with significant (44.2±3.9%) NO oxidation (e.g., X_1, the paternal line of the Hisex White paternal form) with a line in which this oxidation is insignificant ($\sim$ 2%, $p < 0.05$) (e.g., X_2, the maternal line of the Hisex White paternal form (see Table 3, X_1, X_2, X_{12} lines and the final hybrid). The X_1 line used as the paternal line of the paternal cross is the heaviest with the strongest skeleton. On day 28, it exceeds the final hybrid and all other lines in live weight by 11-12% [23]. X_1 is also the only line of the Hisex White cross with a significant (44.2%) oxidation of embryonic NO (see Table 3). However, this property was not inherited in further crosses. Therefore, our hypothesis about the dominant allele of the gene that determines the intense oxidation of NO is not confirmed.

5. The proportion of NO oxidized to nitrate in egg content homogenates in different subspecies of ostriches (*Struthio camelus* L.) on day 24 of incubation and its inheritance in F_1 ($n = 20$, vivarium, Genetic and Breeding Center Zagorskoye, VNITIP, Sergiev Posad, Moscow Province)

Breed, subspecies	Описание Description	NO oxidized to nitrate, %, $M\pm(t_{0.05} \times \text{SEM})$
Black-necked (B)		1.6±0.8
Blue-necked (Bl)		1.5±0.8
Hybrid (♂B × ♀ Bl)	A significantly higher rate of postembryonic growth than parental forms.	90.7±3.8
Hybrid (♂Bl × ♀ B)	A significantly higher rate of postembryonic growth than parental forms.	91.2±3.8

N o t e. The proportion of NO oxidized to nitrate is calculated as the concentration ratio nitrate/(NO donors + nitrate) × 100%.

Crossing the black-necked and blue-necked subspecies of ostriches gives us another example that refutes the hypothesis about the dominant allele of the gene for NO oxidation intensity. The offspring of ostriches is characterized by 90%

oxidation of NO in the embryo to nitrate (Table 5) and a significantly higher growth rate than parental forms [19, 24]. NO oxidation in embryos of parental forms is negligible (see Table 5).

All the data obtained, on the one hand, indicate that the intensity of embryonic NO oxidation is genetically determined and inherited within the line, cross, and breed with a variation of the indicator of no more than 10% [18-20]. On the other hand, the considered examples allow us to assume that the intensity of NO oxidation is determined not by single specific gene, but, apparently, by the combined action of different genes. Perhaps they can both provide and reduce the manifestation of the trait in question.

Previously, we showed that the intensity of embryonic NO oxidation does not depend on the age of laying hens, keeping conditions, and the sex of the embryo [18, 19]. However, the use of green light during incubation is known to stimulate post-embryonic growth [25, 26]. We have shown that green light promotes the intensification of embryonic NO oxidation. In this, no activation of NO synthesis occurs, since the total concentration of nitrate and NO donors did not change, remaining within 150-160 μmol/l on day 10 and 470-490 μmol/l on the day 15, whereas NO oxidation intensifies, since the concentration of nitrate increased, and the concentration of NO donors decreased (Table 6). The following regularities turned out to be characteristic. According to our data for 7, 10 and 15 days, illumination of the Hisex White cross eggs from day 1 to day 6 of incubation and from day 1 to day 15 of incubation induces oxidation of up to 60% of the synthesized NO to nitrate (see Table 6). A further increase in light intensity did not increase the proportion of oxidized NO. This percentage is maintained throughout the embryogenesis, even when green illumination is canceled from day 6. Moreover, nitrate was accumulated outside the amnion, as in embryos with an initially high NO oxidation (see Tables 1, 6). Resumed illumination on day 6 no longer led to the intensification of nitric oxide oxidation, although donor compounds were also present in the embryo. Thereofore, the light acts on the embryonic tissues that cause oxidation but does not affect the NO donor compounds itsef (see Table 6).

6. Influence of green light regimes on the composition of nitro- and nitroso compounds in the egg content homogenate, amnion and allantois of Hisex White (*Gallus gallus domesticus* L.) embryos on day 15 of incubation ($N = 4$, $n = 20$, vivarium, Genetic and Breeding Center Zagorskoye, VNITIP, Sergiev Posad, Moscow Province)

Sample	Day 5		Day 7		Day 10		Day 15	
	NO donors	nitrate	NO donors	nitrate	NO donors	nitrate	NO donors	nitrate
Control (darkness), $M \pm (t_{0.05} \times SEM)$								
Homogenate	139.8±7.7	< 0.1	148.5±8.2	< 0,1	155,8±8,9	< 0,1	465,5±19,9	18,6±1,9
Amnion							5230.0±170.0	< 0.1
Allantois							11.2±2.2	12.7±2.9
Lighting from day 1 to day 15, $M \pm (t_{0.05} \times SEM)$								
Homogenate	141.1±7.5	< 0.1	59.8±3.6	90,3±4,4	64,1±10,1	96,1±4,5	164,3±10,2	316,4±15,5
Amnion							1870.0±90.0	< 0.1
Allantois							12.6±2.4	278.6±12.1
Lighting from day 1 to day 4, $M \pm (t_{0.05} \times SEM)$								
Homogenate	147.8±7.4	< 0.1	151.4±7.8	< 0.1	158.8±8.1	< 0.1	478.3±17.9	22.3±1.9
Lighting from day 1 to day 6, $M \pm (t_{0.05} \times SEM)$								
Homogenate	146.6±7.9	< 0.1	79.4±8.6	74.6±7.9	82.7±8.2	81.9±7.9	217.4±10.4	261.5±12.2
Lighting from day 6 to day 14, $M \pm (t_{0.05} \times SEM)$								
Homogenate	145.5±7.2	< 0.1	150.2±7.6	< 0.1	154.9±7.8	< 0.1	469.7±18.1	20.8±2.0

Note. Until day 15, the concentration was measured in a liquid medium outside the amnion. The concentration of nitrite and nitrosamines in all samples was < 0.1 μmol/L.

Based on the data obtained, we can conclude that nitric oxide is involved in a specific process in avian embryogenesis, in which it does not participate (or participates, but not so actively) after hatching. The fact that most of the deposited

NO (90%) in broiler embryos is oxidized to nitrate indicates that the high concentrations of deposited NO (over 90%) that we observe in the amnion of egg forms (see Table 1) are not vital necessary (at least in order to provide vital NO-dependent processes). As shown earlier, the oxidation of NO to nitrate occurs in the tissues of the embryo, and mainly in the muscle tissue. There is virtually no oxidation in the liver and intestines [18, 20]. Therefore, it is reasonable to believe that this oxidation is somehow related to the development of muscle tissue.

To assess the role of NO in increasing the rate of body weight growth, two approaches are possible. The first is to try to artificially minimize NO oxidation in the embryo, for example, by using NO synthase blockers. The second is to use exogenous NO donors. According to our data, a decrease in the intensity of NO synthesis even by 80% from the initial one at the beginning of incubation did not significantly affect the rate of postembryonic growth [18, 20]. In addition, NO donor compounds in an amount equal to that occurred on day 3 had no reliable effect when introduced into the egg [18, 20].

It is possible that NO is synthesized in excess in the embryo to ensure any physiological processes. That is, it is not the amount of oxidized NO that is of interest, but the features of the tissues of the embryo that induce this oxidation. From the data presented in Table 1, it follows that NO donor compounds are initially accumulated in the embryo. Starting from a certain time (from days 2-3), these compounds begin to oxidize to nitrate. In embryos of egg poultry, oxidation is practically absent (see Tables 1-5). What is this period and what process is it associated with? Myotome formation is intiated in the embryo at the age of 2-3 days. Proliferation of myoblasts lasts up to 14 days. But NO oxidation in embryos of meat forms occurs throughout the embryogenesis. Histological studies did not reveal any qualitative differences in the development of muscle tissue in embryos of broilers and egg forms of chickens, as well as in embryos of egg and meat forms of quails, characterized by high and low intensity of embryonic NO oxidation [18]. It can be assumed that some structures appear on days 2-5, the further development of which is associated with NO oxidation. The formation of these structures, apparently, is genetically determined, since the oxidation intensity index is inherited within lines, crosses [18]. Perhaps, under the influence of genetically determined factors, a population of some cells is formed, the growth of which is associated with oxidation processes.

But what is the nature of these genetically determined factors and what genes are associated with their appearance? D. Cazzato et al. [27] studied the expression of seven genes that determine the course of myogenesis at the earliest stages of embryogenesis. The expression was influenced by the NO synthase inhibitor and NO donors. Without denying these data, we note that they refer to the beginning of embryogenesis. The studied factors did not affect the features of postembryonic development [18, 20].

Embryonic oxidation of NO can also be partly due to external factors such as light (see Table 6). This effect was also shown by us in a number of other works [20, 28]. It has been shown that light induced NO oxidation in embryos of both meat and egg chickens, followed with a slight (by a few percent) increase in the rate of postembryonic growth [20]. The latter is consistent with other reportes [26, 29].

Thus, the analysis of the embryonic NO oxidation intensity inheritance in F_1 of several bird species suggests that this trait is the result of the expression of various genes that can both promote and suppress the NO oxidation. The oxidation of NO to nitrate in all avian embryos can be induced by light at the beginning of incubation, after which the process proceeds even in the dark. In embryos of egg forms, under the action of light, the proportion of oxidized NO can increase up to 60%. Therefore, embryos of both meat and egg forms have mechanisms that

ensure the oxidation of NO. Apparently, the ability to activate this process is inherited, which can also be partially induced by external factors (light). To find out for which genes the embryonic intensity of NO oxidation can serve as a marker and how it can be used in the theory and practice of breeding, we will study the inheritance of this trait in several generations. The mechanism of NO oxidation in the embryo and the specific physiological role of this process are still not clear.

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