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BREED-SPECIFIC PROOXIDANT-ANTIOXIDANT BALANCE OF GEESE MUSCLE TISSUE IN ONTOGENESIS

Maximum content of lipid peroxidation end-products in the striated muscles of Legart geese was found at the end of embryonic ontogenesis. The content increased by 1.88 times compared to the input values. An antioxidant activity of the tissue reduced by 3.00 times during the ontogenesis. However, the increasing of antioxidant enzymes activity provided the maintenance of prooxidant-antioxidant balance in this period. There were no significant changes of the total unsaturated fatty acid content and the total tissue lipid unsaturation during the last week of embryogenesis in Legart breed. The content of lipid breakdown end-products in the original homogenate and after induction of peroxidation by Fe2+ ions in the skeletal muscles of Kharkiv breed had no weighty changes during the embryonic period. The highest values were on the 1st day of postnatal ontogenesis. There was a minimal value of antioxidant activity with its subsequent increase during the same period. Due to the activity of antioxidant protection enzymes, prooxidant-antioxidant balance in the skeletal muscles of Kharkiv breed was maintained. The activity is at a consistently high level during 22nd-28th days of embryogenesis. The average level of superoxide dismutase activity in the skeletal muscles of Kharkiv breed exceeded the value in Legart breed by 2.09 times, while glutathione peroxidase and catalase activity were at the same level. A prevalence of the superoxide dismutase in the antioxidant activity system indicated on higher adaptive breed potential – an average antioxidant activity was 1.5 times higher for the skeletal muscles of Kharkiv breed. Breed specificity is aimed to adapt goose organism to hyperoxia of atmospheric respiration in the skeletal muscles. Legart breed geese use the activation of antioxidant enzymes, whereas Kharkiv breed geese involve much more antioxidant enzymes – superoxide dismutase, probably, alternative mechanisms and low molecular weight antioxidants. It is established that the reduction of the total content of unsaturated fatty acids and unsaturation for this type of tissue and these breeds is not typical.

Keywords: skeletal muscle, superoxide dismutase, glutathione peroxidase, catalase, fatty acids.

The antioxidant defense system (ADS) plays an important role in the body's prooxidant-antioxidant homeostasis maintaining at all stages of ontogenesis. The system neutralizes free radicals and prevents the accumulation of lipid peroxidation products [11, 21]. According to the physiological norm, these processes are an integral part of metabolism, because a number of substances involved in the processes of intracellular signalling and functions regulation are produced in the course of oxidative processes [16, 19]. However, the normal functioning of the organism is possible only in the case of maintaining balance between the production and inactivation of reactive oxygen forms. Their excessive formation

activates the processes of lipid peroxidation and disruption of various cell functions [21]. The maintenance mechanisms of redox homeostasis in different types of tissues differ due to the intensity of metabolism and the degree of tissue oxygen consumption [11]. In addition, species traits and breed features determine these mechanisms. This issue is especially relevant in poultry farming. There is much data in the papers about the particular biochemical features in various poultry species, including geese [3, 5, 14, 18]. It is known that the violation of prooxidant-antioxidant balance in the mismatch of the keeping and feeding technology leads to the decrease of productivity and interrupts realization of the bird's genetic potential. A number of recent studies have investigated the particularities of prooxidant-antioxidant balance in different geese breeds all over the world [5, 13, 14, 18]. However, the breed and tissue specificity of the redox balance requires in-depth study, in particular, for the most common breeds: Kharkiv (Great Gray) and Legart. Therefore, the goal of this study was the assessment of the ontogenetic peculiarities of prooxidant-antioxidant balance in the tissues of these goose breeds during the embryonic and early postnatal ontogenesis.

Materials and methods

The research followed the principles of bioethics, legislation and requirements in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes (Strasbourg, 1986), the General Ethical Principles for Animal Experiments (Ukraine, 2001) and Commission on Bioethics of the Bogdan Khmelnitsky Melitopol State Pedagogical University (№1, 15.09.2015).

Eggs of breeds Legart (average weight = 150.52 ± 7.53 *g*) and Kharkiv (average weight = 145.7 ± 2.6 *g*) were used for incubation. Studies of the ADS system in embryogenesis were performed in physiologically justified terms: 15th day – closure of the allantois, the presence of a formed liver, 22nd day – transition from protein to yolk nutrition, 28th day – embryos transfer to excretion. In the postnatal period, studies were limited by the 14th day of age [2]. The activity of antioxidant enzymes was determined by commonly used methods: superoxide dismutase activity (SOD; EC 1.15.1.1.) [6], catalase activity (CAT; EC 1.11.1.6) [7], glutathione peroxidase activity (GPO; EC 1.11.1.9 [1].

The intensity of lipids peroxidation (LPO) in the tissues was assessed by the level of TBA-active products (TBCA) [8]. Determination of the concentration of these products was performed in tissue homogenates ($TBCA_v$) and after LPO initiated by Fe^{2+} ($TBCA_i$). The coefficient of antioxidant activity (K_{AOA}), which is calculated as the ratio of $TBCA_v$ to $TBCA_i$, was used to carry out the integrated assessment of the ADS state [2].

The FA content was determined by gas-liquid chromatography. Lipids were extracted using Bligh and Dyer method with modification [17, 20]. Preparation of samples, hydrolysis of esters and methylation of FA were performed by the method [15]. The FA composition of lipids was determined on a Carlo Erba chromatograph. Chromosorb W/DP with phase of Silar 5CP («Serva», Germany) was used (concentration: 10 %, temperature: 140-250 ºC, growth rate: 2 ºC/min, injector temperature: 210 ºC, detector temperature: 240 ºC). In addition to the total content of unsaturated FAs (UFA) (Σ_C), the total equivalent concentration of UFA relative to multiple bonds (unsaturation, Σ_N) $\mu mol*g^{-1}$ was calculated [2].

Statistical processing of the results was performed using analysis of variance. Assessing the reliability of the difference between the control and experimental groups of geese was determined using the ANOVA test [12]. The difference was considered as significant at $p \leq 0.05$, using the software package SPSS v.23, and MS Excel 2019.

Results and discussion

In the muscle tissues of Legart geese, a maximum content of LPO final products ($TBCA_i$) was detected at the end of their embryonic development. Their content increased 1.88 times compared to 22-day-old embryos value (Table).

The activity of antioxidant system decreased (the decrease of K_{AOA} by 3.00 times was observed) corresponding to the activation of peroxidation (Fig. 1). However, the maintenance of prooxidant-antioxidant balance in this period was provided by increasing the activity of all enzymes of antioxidant defence (SOD, CAT, GPO) at the 1st day of postnatal ontogenesis. A significant 13.5-time increase of the antioxidant activity of the tissue confirmed this fact.

The total content of unsaturated fatty acids (UFA) and the total unsaturation of tissue lipids did not change significantly in the tissue of Legart breed during the last week of embryogenesis (Fig. 2). The results of our previous work showed a significant decrease in the content of docosapentaenoic (1.59 times) and arachidonic (1.40 times) acids [10]. These changes can be regarded as the one of the mechanisms of LPO inhibition in the content of polyunsaturated fatty acids during the hyperoxia in the beginning of atmospheric respiration [11].

Table

Biochemical parameters of skeletal muscle tissue in geese (X ± E, n = 6; B - breed; $\sum_N$ - total lipid unsaturation; L – Legart breed; K – Kharkiv breed)

Parameter	B	Age, days				
		22-day-old embryos	28-day old embryos	1st postnatal day	7th postnatal day	14th postnatal day
Σ_C, % of the total FA	L	50,83±0,77	51,51±0,64	53,98±0,57	57,83±0,80	52,98±0,43
	K	51,17±0,56	55,21±0,76	52,08±0,60	52,55±0,63	51,60±0,58
$\sum_N$, *μmol/g FW*	L	0,29±0,01	0,27±0,01	0,33±0,00*	0,30±0,00	0,36±0,01*
	K	0,32±0,02#	0,35±0,02*#	0,31±0,02	0,29±0,01	0,31±0,01#
$TBCA_v$ content, *nmol/g FW*	L	6,50±0,50	3,65±086*	13,40±0,86*	38,57±2,49*	6,09±0,01*
	K	34,26±1,60#	31,83±1,30#	44,50±1,90*#	41,57±0,40	18,35±1,00*#
$TBCA_i$ content, *nmol/g FW*	L	54,81±0,01	103,12±0,50*	24,76±0,50*	58,87±2,49*	35,73±3,48*
	K	84,61±0,40#	83,47±1,60#	135,27±2,10*#	56,03±3,20*	43,03±0,50*#
K_{AOA}, *conditional units*	L	0,12±0,01	0,04±0,01*	0,54±0,03*	0,66±0,04*	0,17±0,02*
	K	0,40±0,02#	0,38±0,02#	0,33±0,02*#	0,74±0,04*#	0,43±0,02*#
CAT, *нKat/(min×g FW)*	L	37,43±3,59	65,98±6,17*	24,78±2,05*	30,48±2,07*	47,03±4,47*
	K	33,00±1,50	27,00±0,80*#	29,50±0,90	31,00±0,90	29,00±1,70#
SOD, *conditional units/(min×g FW)*	L	9,37±0,36	11,75±0,87	19,05±1,67*	6,16±0,30*	4,99±0,23*
	K	33,30±2,40#	26,20±2,10*#	21,40±1,20	9,50±0,60*#	15,10±1,40*#
GPO, *mmol/(min×g FW)*	L	9,56±0,75	15,61±0,61*	2,26±0,18*	45,22±0,01*	4,51±0,16*
	K	6,53±0,80#	21,15±1,90*#	20,67±0,60#	8,25±0,60*#	13,33±1,40*#

Note: FW, fresh weight; ***,** the difference is significant relative to the previous value, *p* - ≤0.05; #, the difference is significant relative to group 1, *p*-≤0.05

Thus, genetically programmed transition from hypoxia at the end of the embryonic period to hyperoxia of atmospheric respiration of 28-day-old embryos involves activation of the antioxidant system. There was a decrease in the content of individual PUFAs during the fourth week of embryogenesis. It caused the increase of tissues resistance to the disturbance of prooxidant-antioxidant balance and the start of LPO processes. There was no significant decrease in unsaturation and in the content of unsaturated fatty acids. Therefore, the low molecular weight antioxidants (vitamin A, E, β-carotene and others) can be very important in this period [9].

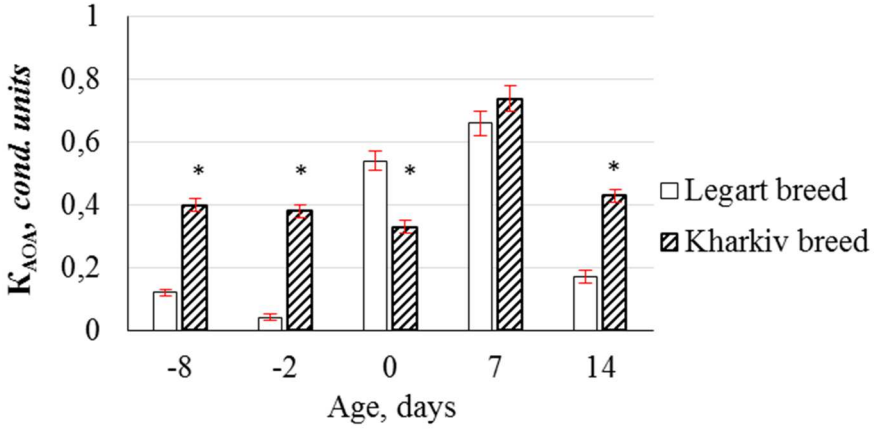

Fig.1. Antioxidant activities of skeletal muscle tissue in geese (M ± m, n = 6).

Note. *, the difference is significant between the groups, $p \leq 0.05$; -8, -2 – 22nd and 28th days of embryogenesis; 0, 7, and 14 – 1, 7th and 14th days of postnatal ontogenesis respectively.

The content of $TBCA_v$ and $TBCA_i$ in the skeletal muscles of Kharkiv breed had no significant changes during the embryonic period. The highest value of both indicators acquired on the 1st day of postnatal ontogenesis – the content of $TBCA_v$ and $TBCA_i$ increased by 1.40 and 1.62 times compared to the previous value. The minimum value of K_{AOA} (decreasing by 1.15 times relative to the previous value) was in the same period. Further, the increase in K_{AOA} was registered – it reached a maximum level on the 7th day of postnatal ontogenesis. The obtained results coincide with the data for a similar tissue of Legart geese and for the myocardium of Kharkiv breed [18]. Nevertheless, we found the contradiction in the data for skeletal muscles of Italian breed, which have the maximum value of K_{AOA} on the 14th day of embryogenesis [5]. Probably the low molecular weight antioxidants, which come with food, causes such increase in tissue antioxidant status on the 7th day of postnatal ontogenesis. The activity of antioxidant enzymes in this period was significantly reduced (GPO in 2.51 and SOD in 2.25 times, respectively). Analysis of changes in lipid FAC indicated a stable level of total UFA content and lipids unsaturation during the experiment. Thus, the maintenance of prooxidant-antioxidant balance in the skeletal muscles of Kharkiv breed geese was realized mainly due to the antioxidant protection enzymes – their activity was at a consistently high level during the 22-28th days of embryogenesis. Probably, alternative mechanisms, such as low molecular weight antioxidants can be additionally involved.

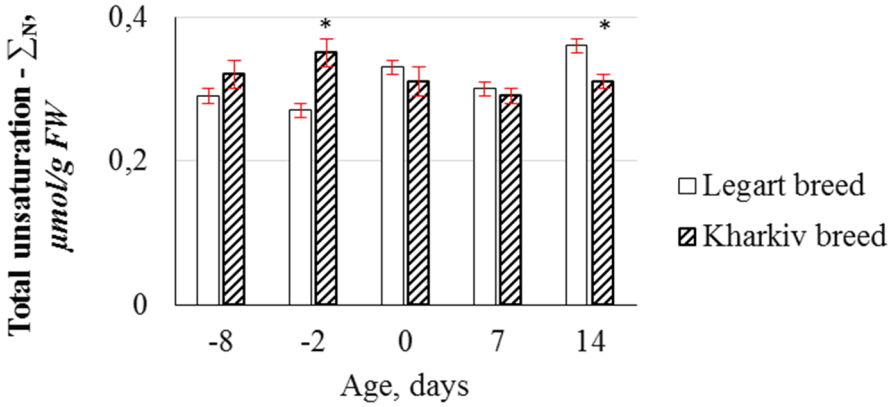

Fig. 2. Total unsaturation of fatty acids of skeletal muscle tissue in geese (M ± m, n = 6).

The skeletal muscles of Kharkiv breed geese exceed the corresponding Legart's SOD-activity index by 2.09 times, while GPO and CAT activities were at the same level compared to the average level of SOD activity. Since SOD is the main enzyme of the ADS, it may indicate a higher adaptive potential of the breed. The average value of K_{AOA}, which is 1.5 times higher for the skeletal muscles of Kharkiv breed geese, points to it. However, the skeletal muscle tissue of Legart breed has a lower average content of $TBCA_v$ and $TBCA_i$ in 2.63 and 1.54 times.

Conclusions

Breed specificity is aimed to adapt the geese functionality to hyperoxia of atmospheric respiration in the skeletal muscles. Legart breed use the activation of the antioxidant enzymes, and Kharkiv breed geese utilized also superoxide dismutase activation, accompanied, probably, with some other defense mechanisms, including low molecular weight antioxidants. The reduction strategy of the total content of unsaturated fatty acids and the rate of their unsaturation for this type of tissue and goose breeds is not typical.

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ПОРОДНА СПЕЦИФІЧНІСТЬ ПІДТРИМКИ ПРООКСИДАНТНО-АНТИОКСИДАНТНОЇ РІВНОВАГИ М'ЯЗОВОЇ ТКАНИНИ ГУСЕЙ В ОНТОГЕНЕЗІ

Встановлено максимальний вміст кінцевих продуктів пероксидного окиснення ліпідів у посмугованих м'язах гусей породи Легарт у кінці ембріонального онтогенезу – він підвищувався у 1,88 разів порівняно з попереднім значенням. На тлі активації пероксидного окиснення знижується антиоксидантна активність тканини у 3,00 рази. Однак підтримка прооксидантно-антиоксидантної рівноваги в даний період забезпечується завдяки підвищенню активності ензимів антиоксидантного захисту. Для породи Легарт, впродовж останнього тижня ембріогенезу сумарний вміст ненасичених жирних кислот та загальна ненасиченість ліпідів тканини достовірно не змінювалися. Для скелетних м'язів гусей харківської породи впродовж ембріонального періоду достовірних змін вмісту кінцевих продуктів розпаду ліпідів як у вихідному гомогенаті так і після індукування пероксидного окиснення йонами Fe^{2+} не встановлено, найвищого значення обидва показники набували на 1-у добу постнатального онтогенезу. У цей період відзначалося також мінімальне значення антиоксидантної активності із подальшим його підвищенням. Підтримка прооксидантно-антиоксидантної рівноваги у скелетних м'язах гусей харківської породи реалізується, головним чином, за рахунок ензимів антиоксидантного захисту, активність яких знаходиться на стабільно високому рівні впродовж 22–28-ї діб ембріогенезу. За середнім рівнем супероксиддисмутазної активності скелетні м'язи гусей харківської породи перевищують відповідний показник легартів у 2,09 разів, в той час як глутатіонпероксидазна таі каталазна активності знаходяться на однаковому рівні. Оскільки супероксиддисмутаза є основним ензимом системи антиоксидантного захисту, це може вказувати на більш високий адаптаційний потенціал породи, яка демонструє вище у 1,5 разів середнє значення антиоксидантної активності у скелетних м'язах гусей у харківської породи. Породна специфічність спрямована на адаптацію організму гусей до гіпероксії атмосферного дихання у скелетних м'язах гусей харківської породи та породи легард визначається у легардів - шляхом активізації антиоксидантних ензимів, а у гусей харківської породи за рахунок антиоксидантних ензимів, переважно супероксиддисмутази, та, ймовірно, залучення альтернативних механізмів, та низькомолекулярних антиоксидантів. Встановлено, що механізми зниження загального вмісту ненасичених жирних кислот та ненасиченості для даного типу тканини та порід гусей не характерні.

Ключові слова. скелетні м'язи, супероксиддисмутаза, глутатіонпероксидаза, каталаза, жирні кислоти.

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