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BIOCHEMICAL STATUS AND PRODUCTIVE PARAMETERS OF PIGS (*Sus scrofa domesticus*) IN MODELING STRESS AND ITS CORRECTION

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Abstract

A peculiarity of living organisms is the internal constancy maintained by self-regulation mechanisms. In higher animals, the functions of control and regulation of biochemical reactions are performed by the neuro-endocrine system. With its help the organism perceives various influences of external and internal environment and reacts to them by means of hormones. In this regard, biomarkers of stress level are primarily the content of hormones, as well as blood concentrations of metabolites and their correlation. The use of dihydroquercetin, vitamins C and E in nutrition can help to reduce the negative effects of stress. In the present work we have established for the first time the positive influence of additional feeding of antioxidant complex on the adaptation of pigs under stress by hormonal regulation and strengthening of antioxidant status of the organism. The aim of the work was to evaluate the effect of feeding a complex of adaptogens DHQEC (dihydroquercetin and vitamins E, C) on the biochemical status and productive qualities of pigs under stress modelling. Fattening experiments were performed on 34 pigs (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] (Ernst Federal Research Center for Animal Husbandry, 2022-2023). Body weight (BW) of piglets aged 99 days at the beginning of the experiment was 40.7-41.0 kg. The duration of the fattening period was 90 days. During the preliminary period, piglets were distributed into four groups by the paired-analogues method: I control (C–, without dietary DHQEC) (9 animals), II control (C+, with dietary DHQEC) (9 animals), III experimental (E–, without dietary DHQEC) (8 animals), IV experimental (E+, with dietary DHQEC) (8 animals). Each stall sized 2.4×2.25 m (5.4 m^2) with 1.05 m feeding front. That is, with four pigs per stall (groups E– and E+) instead of 3 pigs per stall (groups C– and C+), there was a decrease in the stall area per pig from 1.8 to 1.35 m^2, and the feeding front from 0.35 to 0.26 m (according to GOST 28839-2017, the norm is at least 0.3 m per pig). DHQEC contained DHQ (Ecostimul-2, AO Ametis, Russia; DHQ 72-73 %, 32 mg/kg of feed), vitamin E (INNOVIT E60, MEGAMIX, Russia, 10 mg/kg of feed), and vitamin C (Tiger C 35, Anhui Tiger Biotech Co., Ltd., China, 35 mg/kg of feed). Animals from groups C+ and E+ received dietary DHQEC (0.025 % by weight of mixed fodder) throughout the whole period of the tests. Young animals were weighed individually every decade. To assess the clinical, physiological and metabolic status of the organism at the end of the growing period, as well as during transfer to final fattening and before slaughter, blood samples were taken from the jugular vein in the morning before feeding. Calcium, phosphorus, magnesium, aspartate aminotransferase, alanine aminotransferase activity, alkaline phosphatase, total bilirubin, creatinine, cholesterol, glucose, total protein, albumin, chloride and urea were determined in blood serum. To assess antioxidant status, the total amount of free water-soluble antioxidants was determined amperometrically in serum samples. The serum concentrations of total and free thyroxine

(T_{4t} and T_{4f}) and triiodothyronine (T_{3t} and T_{3f}), as well as thyroid hormone, cortisol, adrenaline, insulin-like growth factor-1, and melatonin were also determined by solid-phase enzyme-linked immunosorbent assay. BW of pigs after starvation was evaluated immediately before slaughter. After slaughter, the carcass was weighed, the slaughter yield, thickness of the skin, muscle eye area, and pH were determined 45 min after slaughter and after 24 h of storage. It was found that with increased competition for feed table, DHQEC provides a significant decrease in cortisol ($p = 0.014$) and adrenaline ($p = 0.09$) in piglets during the final fattening. Due to competition for feed, the melatonin concentration decreased ($p = 0.01$), while DHQEC in E+ group normalized the melatonin level to the values for the 1st and 2nd blood draws. Stress had a negative effect on some metabolic processes indicators of which are biochemical blood parameters (blood concentration of triglycerides, cholesterol, bilirubin, AsAT). At final fattening, there were significant shifts in the animal hormonal status. In piglets in groups C compared to E, the concentration of T_{4t} ($p = 0.02$), T_{3t} ($p = 0.05$), T_{3f} ($p = 0.004$) decreased together with an increase in the thyroid hormone (TTG) production ($p = 0.05$). Dietary DHQEC somewhat smoothed the negative influence of the modelled factor. The lowest values of T_{4t}, T_{4f}, T_{3t}, T_{3f} were rebealed in the E+ group. It should be noted that the TTG content and integral thyroid index (ITI) in the E+ group decreased to 0.46 mME/l and 69.7 units vs. 0.51 mME/l and 263.8 units in the E– group, while the conversion of T_{4f} to T_{3f} decreased 1.73 times. With increasing BW of animals (70 kg and more), the effect of limitation of machine area and feeding front was stronger, which was manifested in the decrease of ADG in the E– group in the last fattening period ($p < 0.05$). Under the influence of DHQEC during the final fattening, there was a tendency to increase the amount of insulin-like growth factor-1 (IGF-1) in groups C+ and E+ compared to groups C– and E– (163.7 and 162.8 vs. 141.0 and 142.1 ng/ml, respectively, $p = 0.14$), which correlates with higher ADG. The obtained results indicate that DHQEC supplementation, having antioxidant activity, can improve growth parameters and, apparently, exhibit tissue-specific regulation of IGF-IR mRNA transcription. At the end of the experiment there was an increase in the blood melatonin (MT) concentration in animals from groups C– and C+ ($p < 0.05$), and dietary DHQEC did not affect the change of this index. A statistically significant decrease in MT content in E– and E+ groups compared to the control groups (292.2 and 179.8 pg/ml vs. 457.6 and 458.7 pg/ml, $p = 0.01$) should be noted. Therefore, competition for feed led to a decrease in this index, and feeding of DHQEC in E+ group normalised its values to those at the 1st and 2nd blood draws. Stress had a significant effect on the quality of production. The adaptogen-antioxidant complex DHQEC significantly improves the adaptive abilities of pigs and reduces the influence of stress factors on production performance.

Keywords: adaptogen, dihydroquercetin, vitamin, stress, young pigs, hormones, blood biochemistry, slaughter performance

A characteristic feature of a living organism is its ability to maintain a constant internal environment through self-regulation mechanisms. In higher animals, the functions of controlling and regulating biochemical reactions are performed by a complexly organized neuroendocrine system. Due to it, the body perceives the influences of the external and internal environment and reacts to them through biochemical signals, the hormones [1].

Stress is one of the key factors affecting the body of pigs under modern intensive fattening technologies. Physiological stress increases an individual's vigilance and provides the effort necessary to exhibit behavioral responses. They are associated with increased heart rate and the secretion of stress hormones such as cortisol and catecholamines (adrenaline and norepinephrine). An animal's premortem physiological and behavioral responses can significantly alter meat quality by influencing muscle energy metabolism, including metabolite and glycogen content [2]. In pigs, biomarkers of chronic and acute stress are numerous and include content of hormones [3], antioxidants [4], some metabolites [5] and their ratio [6, 7].

Under stress, the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis are mainly activated due to the production of catecholamines and glucocorticoids [8]. E. Petrosus et al. [9] identified a pattern of changes in the intestinal microbiome and susceptibility to disease due to increased levels of cortisol and catecholamines in piglets during weaning. C.-H. Yu et al. [10] found that before weaning stress in hybrid piglets was modulated, there were no differences in plasma cortisol levels between groups. After weaning, piglets increased the plasma cortisol concentration ($p < 0.05$) compared to animals not exposed to

stress (230±50 vs. 120±50 nmol/l).

Thyroid hormones affect the functions of almost all organs and systems (including the heart, central nervous system, autonomic nervous system, gastrointestinal tract), bone health, metabolism, and activate genes that accelerate metabolism and thermogenesis. Increased metabolic rate is associated with increased oxygen and energy consumption. Thyroid hormones also play a key role in fertility, development, tissue differentiation, and fetal growth, and they regulate cellular metabolism and calcium levels [11-13].

At large pig farms, even relatively comfortable conditions very often adversely affect the physiological and biochemical processes in animals. The thyroid gland, as the most important regulatory element of homeostasis, quickly responds to the influence of endogenous and exogenous factors by changing secretory activity [14].

The stress response is closely related to meat quality. The intravital mechanisms of its deterioration under stress and the long-term effects of stress causing spoilage of meat obtained from animals are still not well understood, although the problem itself is obvious [15]. Before slaughter, animals are subjected to potentially stressful procedures that may include food deprivation, collection and mixing of animals, transport to the slaughterhouse, and waiting before slaughter. Some of these factors are physical or physiological (food deprivation, fatigue or pain), others are psychological (presence of strangers, separation from members of the rearing group, encounter with individuals with whom the animal has not previously been exposed) [2].

Meat characteristics such as PSE (pale, soft, exudative) and DFD (dark, firm, dry) are closely related to glycolysis and oxidative stability in stress-induced tissues. Oxidative stress, which is accompanied by a decrease in intracellular antioxidant capacity and an increase in the production of reactive oxygen species (including free radicals), leads to changes in the metabolism of glycogen, glucose, structural modifications of cell membranes and a decrease in meat quality. Acute stress can cause PSE; with chronic stress, meat acquires signs of DFD which are formed before slaughter, which ultimately causes huge economic losses in pig production [1].

Oxidative stress is a consequence of an imbalance of pro-oxidants and antioxidants, leading to cell and tissue damage. Depletion of antioxidant systems becomes one of the causes of oxidative stress, which causes an avalanche of production of reactive oxygen species (ROS) or free radicals [16]. Recently, there has been increased interest in studying the role of free radicals, the reactive oxygen and nitrogen species (ROS and RNS). On the one hand, ROS and RNS are formed as a result of natural physiological processes and are necessary to maintain the functions of the immune system, cell signal transmission and hormone synthesis, on the other hand, oxidative stress caused by high concentrations of free radicals may lead to damage to DNA, proteins, and membrane lipids. The required amount of ROS in the body is maintained by the antioxidant system [17]. The production of ROS in mammals is caused by the activity of endogenous prooxidant enzymes NADPH oxidase, xanthine oxidase, peroxisomes and cytochrome P450. Their production is balanced by endogenous antioxidant enzymes, including superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione, and heme oxygenase 1 (HO-1) [18]. These antioxidant defense systems are directly regulated by nuclear factor erythroid 2-related factor 2 (Nrf2). Excessive oxidative damage can be controlled with exogenous antioxidants such as vitamins C and E, polyphenols, carotenes, flavonoids, omega-3 fatty acids, and N-acetylcysteine (NAC) [19, 20].

Supplementation with dihydroquercetin and vitamins C and E may reduce the negative effects of stress [21, 22]. However, their effect on the synthesis of hormones and the oxidative stability of meat in fattened young pigs exposed to stress has been practically not studied. It is also of interest to study the relationships between biochemical blood markers (including hormones), growth performance of animals during the fattening period, slaughter performance and meat quality when modeling unfavorable environmental conditions.

This work was the first to establish the positive effect of feeding a complex of antioxidants (dihydroquercetin and vitamins E, C) on pig adaptation to stress conditions due to the optimization of metabolic processes and hormonal status via enhancing antioxidant protection which contributed to the production of higher quality meat.

The purpose of the work is to assess the effect of feeding the adaptogen complex DHQEC (dihydroquercetin and vitamins E, C) on the biochemical status and productive qualities of pigs when modeling stress.

Materials and methods. The experiments were carried out in 2022-2023 (the Ernst Federal Research Center for Animal Husbandry − VIZh). Thirty-four piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] of 40.7-41.0 kg weight and 99-day age, were subjected to 90-day fattening, 40 days for the 1st fattening period and 50 days for the 2nd fattening period.

Experiments were performed in accordance with the fundamentals and principles of proper care and maintenance of laboratory animals [23-26]. All piglets were kept in the same conditions that met zoohygienic requirements (with the exception of simulated factors during the test periods). Feeding was carried out according to modern standards [27] using group self-feeders.

During the preliminary growing period, piglets were assigned to four groups according to age, bodyweight and average daily gain, group I (control, $n = 9$, C–, DHQEC–), II (control, $n = 9$, C+, DHQEC+), III experimental ($n = 8$, E–, DHQEC–), IV ($n = 8$, E+, feeding with DHQEC+).

Each pen was 2.4×2.25 m (5.4 m^2) with 1.05 m feeding front. That is, when placing 4 pigs per pen (groups E– and E+) instead of 3 pigs per pen (groups C– and C+) the pen area per pig decreased from 1.80 to 1.35 m^2, the feeding front from 0.35 m to 0.26 m (with a norm of at least 0.3 m/animal according to GOST 28839-2017). This causes additional competition for feed during fattening and was a technological and feed stress factor.

The DHQEC composition corresponds to new information on action and feeding standards of dihydroquercetin [22, 28], the standards for the use of vitamins in pig feeding [27], the synergistic effect of antioxidants, activation of antioxidant defense, immunostimulation, our previous data on DHQEC (2019 -2022) [21, 29] and other reports [30, 31].

Dietary DHQEC was DHQ (Ekostimul-2, JSC Ametis, Russia, No. PVR-2-9.9/02502; DHQ 72-73%), vitamin E (INNOVIT E60, MEGAMIX, Russia), and vitamin C (Tiger C 35, PVI-2-2.15/04504, Anhui Tiger Biotech Co., Ltd., China) added to feed at 32 mg/kg, 10 mg/kg, and 35 mg/kg, respectively [32]. In lab conditions, the ingredients were mixed with crushed wheat grain and added to the feed (paddle mixer type SV-2.2; AgroPostavka, Russia).

Animals from groups C+ and E+, in addition to the basal diet, were fed DHQEC (0.025% of the feed weight) hroughout the test. The DHQEC added to feed did not change the amount of consumed energy and essential nutrients in animals [27].

The young animals were weighed individually every ten days (a REUS-A-U scales, Tenzosila LLC, Russia). The absolute and average daily weight gain was

calculated for each group during the fattening periods and for the entire experiment in general.

To assess the clinical, physiological and metabolic status, blood was taken from the jugular vein in the morning before feeding three times. The first blood sampling was before the beginning of the experiment (n = 15, animals were randomly selected from a set further used to form groups), the second sampling was when transferred to final fattening (n = 20, 5 animals from each group), and the third was before slaughter (n = 34, all animals). Each blood sample was divided into four vacuum tubes (10 ml EDTA and 5 ml serum) for biochemical and hematological analysis, antioxidant status (AOS) assessment and measurement of hormone levels.

The number of erythrocytes, leukocytes, hemoglobin content, hematocrit were determined (a hematological analyzer ABC VET, Horiba ABZ, France) with Unigem reagents (Reamed, Russia). Blood serum and plasma were separated by centrifugation (3000 rpm for 15 min, lab centrifuge UC-1412A, ULAB, China) and stored at –20 °C. Serum aspartate aminotransferase (AsAT), alanine aminotransferase (AlAT), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), creatinine (CREA), urea (URO), total bilirubin (TOBIL), calcium (Ca), phosphorus (P), magnesium (Mg), cholesterol (CHOL), glucose (GLU), chlorides (CL) were measured (an automatic biochemical analyzer Erba Mannheim automatic XL-640, Erba systemic reagents; Erba Lachema s.r.o., Czech Republic). To assess the antioxidant status (AOS), the total amount of free water-soluble antioxidants (FWSA) was measured in blood serum amperometrically (a chromatograph Tsvet-Yauza 01-AA, NPO Khimavtomatika, Russia).

To measure hormone concentrations, blood samples were centrifuged (a Hettich ROTOFIX 32 device, ANDREAS HETTICH GmbH, Germany) for 10 min at 4500 rpm. Blood serum was collected with an automatic pipette Techno F1 (Lenpipet, Russia) and transferred into 1.5 ml Eppendorf tubes. Tubes with serum were placed on a Multi Bio RS-24 multirotator (BioSan, Latvia) for 10-15 min. The concentration of total and free thyroxine (T_{4t} and T_{4f}) and triiodothyronine (T_{3t} and T_{3f}), thyroid-stimulating hormone (TSH), cortisol (CORT), adrenaline (ADR), insulin-like growth factor 1 (IGF-1), melatonin (MT) was determined by enzyme-linked immunosorbent assay in accordance with the manufacturer's recommendations (an automatic microplate photometer Immunochem-2100, High Technology, Inc., USA).

The integral thyroid index (ITI, the ratio of the amount of thyroid hormones to their pituitary regulator) and the peripheral conversion index (PCI, characterizes the tissue conversion of thyroxine into its biologically more active metabolite triiodothyronine) were calculated: $ITI = (T_{3f} + T_{4f})/TSH$; $PCI = T_{4f}/T_{3f}$.

The control slaughter was performed in accordance with the Rules for veterinary inspection of slaughter animals and veterinary and sanitary examination of meat and meat products (approved by the Main Directorate of Veterinary Medicine of the USSR Ministry of Agriculture on December 27, 1983). Immediately before slaughter, the live weight (LW) of pigs after fasting was determined. After slaughter, the fresh carcass weight (without head, legs, tail, internal organs and internal fat according to GOST 31476-2012) and the slaughter yield (the ratio of the fresh carcass weight to the live weight before slaughter) were determined. The thickness of the backfat was measured with a metal ruler above the spinous processes between the 6th and 7th thoracic vertebrae, not counting the skin thickness. The area of the transverse section of the *longissimus dorsi* muscle at the

10-11th ribs was determined by making an imprint on paper, followed by measurement with a planimeter as per methodological recommendations [33]. pH was measured (Testo 205 device, Testo SE & Co. KGaA, Germany) 45 min after slaughter and after 24 h of storage.

The experimental data were processed biometrically by one- and two-way analysis of variance (ANOVA) using Student's, Dunnett's and Tukey's tests in the STATISTICA 13RU program (StatSoft, Inc., USA). Arithmetic mean values (*M*), standard errors of the means (±SEM) and significance level (p) were calculated. The differences were considered statistically significant and the existence of a relationship between the parameters was accepted at a significance level not exceeding 0.05.

Results. Based on the weighing and feed consumption, we determined the absolute and average daily weight gain in piglets (ADG) and feed costs per unit of gain (Table 1, Fig. 1).

1. Bodyweight of crossbred piglets (*Sus scrofa domesticus*) F_2 [(large white × Landrace) × Duroc] and feed costs when fed dietary complex of adaptogens DHQEC (dihydroquercetin and vitamins E, C) with modeling stress (*M*±SEM, physiological yard of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2022-2023)

Parameter	Group			
	C– (*n* = 9)	C+ (*n* = 9)	E– (*n* = 8)	E+ (*n* = 8)
Growing period (preliminary period)				
Length of period, days	30	30	30	30
Average daily gain, g	735.56±20.67	735.93±11.42	730.0±20.03	737.92±22.36
1st fattening period				
Length of period, days	40	40	40	40
Live weight at the beginning of fattening, kg	40.97±0.77	40.74±0.39	40.80±0.64	40.91±0.72
Live weight at the end of the 1st fattening period, kg	78.99±1.64	78.83±0.45	78.16±1.06	79.03±1.78
Gross gain, kg	38.02±1.16	38.09±0.26	37.36±0.72	38.11±1.40
Average daily gain:				
total, g	950.56±29.05	952.22±6.49	934.06±18.06	952.81±34.93
to control (C–), %	100.0	100.2	98.3	100.2
to control (C+), %	99.8	100.0	98.1	100.0
2nd fattening period				
Length of period, days	50	50	50	50
Live weight at the end of fattening, kg	126.83±1.98	128.13±1.14	125.99±1.29	128.41±2.83
Gross gain, kg	47.84±1.15	49.30±1.19	47.83±1.15	49.39±1.58
Average daily increase:				
total, g	984.38±20.33	1014.54±22.30	958.19±17.41*	991.71±38.97
to control (C–), %	100.0	103.1	97.3	100.7
to control (C+), %	97.0	100.0	94.4	97.7
For the entire period				
Length of period, days	90	90	90	90
Gross gain, kg	85.87±1.72	87.39±1.26	85.19±1.26	87.50±2.59
Average daily increase:				
total, g	968.84±18.58	986.08±13.40	946.99±10.10	973.36±30.95
to control (C–), %	100.0	101.8	97.7	100.5
to control (C+), %				
Feed costs for the entire period				
Total, kg	283.0	283.0	283.0	283.0
Compound feed:				
total, kg/kg gain	3.31±0.07	3.24±0.05	3.33±0.05	3.26±0.10
to control (C–), %	100.0	97.9	100.6	98.5
to control (C+), %	102.2	100.0	102.8	100.6

Note. For a description of the groups, see the Materials and methods section.
* Differences from the control (group C+) are statistically significant by Student's *t*-test at $p < 0.05$.

During the growing period, ADG was 730.0-737.9 g. For the 1st fattening period, animals from the test group E– under simulated external conditions and with a decrease in the feeding front showed a tendency to decrease the ADG value, 934.1 g vs. 950.6-952.8 g in the control and test group E+, or by 1.7-1.9%. It should be noted that the effect of DHQEC was not noticeable under standard

housing in group C+, but in group E+, ADG was comparable to the control, which indicates a positive effect of DHQEC when the feeding front was limited (see Table 1).

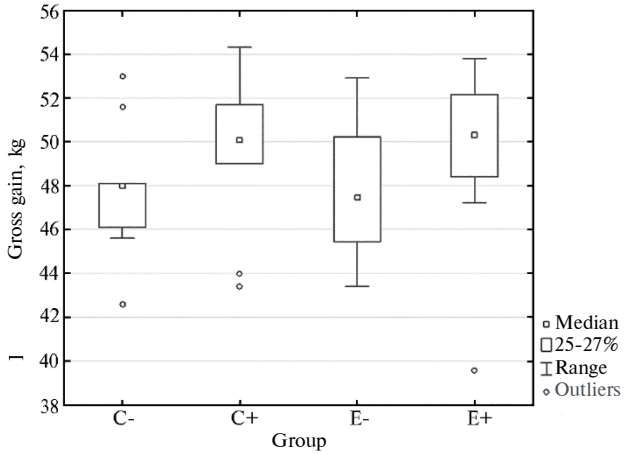

Fig. 1. Absolute bodyweight gain in crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] during the 2nd fattening period when fed dietary complex of adaptogens DHQEC (dihydroquercetin and vitamins E, C) with modeling stress (physiological yard of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2022-2023). For a description of the groups, see the Materials and methods section.

During the 2nd period of fattening, ADG differed slightly from that during the 1st period and amounted to 958.2-1014.5 g, which may indicate both a greater influence of environmental factors and increased competition. The standard keeping of animals from group C– provided ADG = 984.4 g ($p = 0.37$ vs. the 1st period), and dietary DHQEC (group C+) provided better adaptation ($p = 0.02$ vs. the 1st period) and a 3.1% increase in ADG vs. group C–.

With an increase in LW (70 kg or more), the effect of limiting the area of the pen and the feeding front became more pronounced ($p = 0.87$ vs. the 1st period), causing competition for food in groups E– and E+ (see Fig. 1). ADG in group E– was 958.2 g, or 2.7% less compared to group C– and 5.6% ($p < 0.05$) less than in group C+. Dietary DHQEC under a limited feeding front (group E+) contributed to the ADG preservation ($p = 0.82$ vs. the 1st period).

Thus, the main effect of DHQEC as part of compound feeds was a stable ADG at 3.31-3.33 kg feed per 1 kg ADG in amimals not fed DHQEC vs. 3.24-3.26 kg feed per 1 kg ADG in thosed fed with DHQEC.

Biochemical analysis showed (Table 2) that during the 1st period of fattening, the blood TP content in E– pigs compared to other groups was higher (72.1 vs. 68.1-68.5 mmol/l, $p > 0.05$), the urea content was lower (4.5 vs. 4.6-5.3 mmol/l, $p > 0.05$), and the A/G ratio was the lowest (1.13 vs. 1.16-1.25, $p < 0.05$). This may indirectly indicate worse nitrogen utilization under stress. Feeding DHQEC contributed to improved parameters in the E+ pigs. A similar pattern occurred during the final fattening.

The TOBIL in pigs under feeding stress (E–, E+) was 1.15 and 1.20 μmol/l, respectively, or 33.7 and 39.5% higher ($p < 0.05$) than in group C– which could be due to increased oxidative stress.

The blood concentrations of chlorides in groups E– and E+ compared to C– and C+ was 108.8 and 109.6 mmol/l vs. 107.2 and 108.3 mmol/l, or 1. 5 and 1.2% more ($p > 0.05$). In group E–, there was a tendency to increase the content of cholesterol and triglycerides compared to the control ($p < 0.10$). In this group, animals had the highest AlAT concentration (69.4 IU/l) compared to the rest (58.80-59.5 IU/l, $p > 0.05$).

2. Blood morphological and biochemical parameters of crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] when fed dietary complex of adaptogens DHQEC (dihydroquercetin and vitamins E, C) with modeling stress (M±SEM, physiological yard of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2022-2023)

Parameter	Beginning of the test (n = 15)	1st fattening period				2nd fattening period			
		group							
		C– (n = 5)	C+ (n = 5)	E– (n = 5)	E+ (n = 5)	C– (n = 9)	C+ (n = 9)	E- (n = 8)	E+ (n = 8)
Total protein, g/l	65.20±0.77	68.14±2.63	68.52±1.17	71.24±2.20	68.40±2.00	79.60±1.87	78.57±1.20	81.99±1.57	79.56±1.47
Albumin (A), g/l	35.10±0.50	37.78±1.44	37.28±0.71	37.64±0.95	36.74±2.05	45.02±0.92	45.47±0.52	45.50±0.83	44.28±1.04
Globulin (G), g/l	30.20±0.51	30.36±1.25	31.24±0.91	33.60±1.49	31.66±0.75	34.58±1.36	33.10±1.02	36.49±1.31	35.29±0.92
Creatinine, μmol/l	114.80±1.74	134.38±1.82	134.18±3.94	139.16±12.94	133.14±5.88	168.08±5.46	168.20±5.83	169.98±7.80	164.73±4.25
Urea, mmol/l	5.08±0.29	5.27±0.64	4.64±0.31	4.54±0.29	4.83±0.12	7.26±0.38	6.95±0.25	6.71±0.58	6.41±0.53
AsAT, IU/l	37.10±1.98	40.24±7.09	36.18±3.12	37.74±4.08	33.84±3.71	75.76±9.03	60.71±5.26	78.30±16.69	76.51±11.77
AlAT, IU/l	57.10±2.33	58.80±4.95	58.92±2.56	69.36±7.01	59.54±5.80	82.30±4.76	80.34±4.48	75.23±5.14	85.64±5.24
Alkaline phosphatase, IU/l	259.50±18.71	249.80±29.06	221.40±11.17	248.60±18.95	250.60±22.11	241.44±10.94	233.11±11.59	220.50±8.68	272.25±22.56
Total bilirubin, μmol/l	1.05±0.04	0.86±0.07	0.90±0.06	1.15±0.07**	1.20±0.10	3.79±0.40	4.15±1.00	3.43±0.90	4.35±1.00
Total cholesterol, mmol/l	2.50±0.11	2.67±0.14	2.52±0.10	2.97±0.13†	2.67±0.12	2.97±0.11	2.80±0.11	3.06±0.12	3.11±0.12
Triglycerides, mmol/l	0.37±0.02	0.39±0.04	0.39±0.06	0.44±0.03†	0.34±0.05	0.40±0.03	0.41±0.03	0.57±0.09	0.47±0.04
Glucose, mmol/l	5.80±0.13	6.18±0.30	6.53±0.49	6.38±0.66	6.06±0.56	5.59±0.39	5.56±0.28	5.57±0.18	5.72±0.25
Calcium, mmol/l	3.04±0.02	3.05±0.03	3.15±0.04†	3.05±0.06	3.05±0.09	3.10±0.04	3.10±0.05	3.14±0.03	3.13±0.08
Phosphorus, mmol/l	3.41±0.07	3.09±0.03	3.03±0.11	3.15±0.08	3.30±0.15	3.09±0.07	2.97±0.13	3.12±0.06	2.99±0.09
Magnesium, mmol/l	0.96±0.07	1.27±0.08	1.17±0.06	1.20±0.07	1.29±0.06	1.05±0.03	1.00±0.06	1.02±0.05	1.04±0.02
Iron, rmol/l	35.80±1.75	29.11±3.22	24.34±2.59	29.13±1.67	26.69±0.98	21.70±1.90	23.50±2.21	21.87±1.78	24.15±3.30
Chlorides, mmol/l	108.30±0.40	107.16±0.91	108.34±0.47	108.84±0.58	109.58±1.19	109.74±0.48	109.31±1.35	112.21±0.76**	111.86±1.11†
Leukocytes, $\times 10^9$/l	24.20±0.89	32.21±1.73	29.17±1.99	31.54±1.71	29.71±2.59	23.67±1.71	23.45±1.07	23.61±1.46	23.63±2.04
Red blood cells, $\times 10^{12}$/l	11.00±0.27	5.62±0.26	5.55±0.11	5.60±0.20	5.36±0.15	12.03±0.12	11.40±0.36	12.16±0.38	11.59±0.32
Hemoglobin, g/l	106.20±2.48	124.42±2.22	122.92±2.15	129.20±2.35	120.74±6.23	151.53±0.89	141.34±3.64**	145.54±4.76	139.76±2.84***
Hematocrit, %	59.4±1.48	29.91±0.71	29.60±0.44	31.28±0.81	29.79±1.47	74.34±1.01	68.93±1.60**	72.49±1.78	69.00±1.35**
A/G	1.17±0.02	1.25±0.02	1.20±0.04	1.13±0.04*	1.16±0.08	1.31±0.05	1.38±0.04	1.26±0.05	1.26±0.04
Ca/P	1.16±0.03	1.27±0.01	1.35±0.06	1.25±0.02	1.20±0.07	1.30±0.03	1.37±0.07	1.30±0.03	1.36±0.03
TWSA, mg/l	10.76±0.26	13.79±1.34	17.0±1.63	12.04±0.80*	9.60±0.54**	10.93±1.21	9.54±1.61	11.23±1.30	10.98±1.17

N o t e. AsAT — aspartate aminotransferase, AlAT — alanine aminotransferase, TWSA — total amount of water-soluble antioxidants. For a description of the groups, see the Materials and methods section.

*, **, *** Differences to control (C–) are statistically significant by Student's t-test, respectively, at $p < 0.05$, $p < 0.01$, $p < 0.001$; † — tendency towards reliability ($p < 0.1$).

3. The content of various hormones in the blood serum of crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] when fed dietary complex of adaptogens DHQEC (dihydroquercetin and vitamins E, C) with modeling stress (*M*±SEM, physiological yard of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2022-2023)

Group	Parameter			
	CORT, nmol/l	ADR, ng/ml	IGF-1, ng/ml	MT, pg/ml
Beginning of the test				
General sample (n = 8)	66.62±16.24	2.59±0.56	178.20±3.74	201.42±34.46
1st fattening period				
C– (n = 5)	69.02±24.72	2.98±0.39	161.84±14.41	209.88±83.26
C+ (n = 5)	66.33±12.86	1.84±0.50	156.53±5.99	221.18±69.98
E– (n = 5)	47.73±5.11	3.05±0.80	174.52±4.96	326.73±75.46
E+ (n = 5)	44.14±11.40	3.48±0.75	165.56±11.52	246.88±60.82
Average over groups (n = 20)	56.80±8.92	2.85±0.30	164.61±4.83†	251.17±35.02
p-value, group factor 1 (C, E)	0.085	0.197	0.294	0.342
p-value, group factor 2 («–»,«+»)	0.794	0.580	0.486	0.644
p-value, group factor 1 × фактор группы 2	0.970	0.236	0.858	0.540
2nd fattening period				
C– (n = 5)	98.49±23.70	34.21±27.36	140.99±12.54	457.62±102.96
C+ (n = 5)	75.23±22.44	13.80±9.97	163.68±7.46	458.67±100.00
E– (n = 5)	149.81±20.79	66.44±0.59	142.05±11.48	292.15±72.75
E+ (n = 5)	68.88±29.16	2.10±0.68	162.81±20.77	179.78±17.21
Average over groups (n = 20)	98.10±16.83*	25.29±10.23†	152.38±6.86	347.06±45.95†
p-value, group factor 1 (C, E)	0.248	0.655	0.995	0.014
p-value, group factor 2 («–»,«+»)	0.014	0.085	0.138	0.501
p-value, group factor 1 × фактор группы 2	0.144	0.375	0.946	0.493

Note. For a description of the groups, see the Materials and methods section. CORT — cortisol, ADR — adrenaline, IGF-1 — insulin-like growth factor 1, MT — melatonin.
* Differences vs. the previous period are statistically significant by Studen's t-test at $p < 0.01$; †— tendency towards reliability ($p < 0.1$).

4. Content of thyroid hormones and thyroid-stimulating hormone (TSH) **in blood serum of crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] when fed dietary complex of adaptogens DHQEC (dihydroquercetin and vitamins E, C) with modeling stress** (*M*±SEM, physiological yard of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2022-2023)

Group	Parameter					Index	
	T_{4t}, nmol/l	T_{4f}, pmol/l	T_{3t}, nmol/l	T_{3f}, pmol/l	TSH, mIU/l	ITI	PCI
			В начале опыта				
General sample (n = 8)	51.44±4.19	19.86±1.64	2.30±0.10	5.25±0.49	0.32±0.12	127.70±48.15	3.92±0.55
			1-й период откорма				
C– (n = 5)	55.06±4.48	20.89±1.13	2.38±0.13	5.48±0.48	0.23±0.06	139.76±36.24	3.86±0.29
C+ (n = 5)	52.45±5.58	21.79±1.51	2.36±0.19	5.26±0.72	0.23±0.11	296.23±197.73	4.32±0.60
E– (n = 5)	59.10±4.32	20.91±1.32	2.11±0.18	5.49±0.79	0.25±0.10	168.62±69.17	3.93±0.38
E+ (n = 5)	51.16±2.02	20.03±1.17	2.36±0.16	5.49±0.65	0.21±0.08	199.05±87.53	3.74±0.38
Average over groups (n = 20)	54.44±1.72	20.91±0.49	2.30±0.07	5.43±0.24	0.23±0.03	200.91±44.15	3.96±0.16
p-value, group factor 1 (C, E)	0.69	0.38	0.32	0.80	0.94	0.70	0.44
p-value, group factor 2 («–»,«+»)	0.11	0.99	0.40	0.82	0.77	0.29	0.67
p-value, group factor 1 × фактор группы 2	0.38	0.68	0.44	0.99	0.98	0.63	0.64
			2-й период откорма				
C– (n = 5)	41.90±3.00**	19.07±1.52	2.23±0.10	4.91±0.09	0.21±0.12	206.61±80.00	3.88±0.27
C+ (n = 5)	38.64±4.02**	17.64±1.89†	2.13±0.24	3.66±0.93	0.22±0.07	144.69±70.27	6.09±2.13
E– (n = 5)	36.84±4.57***	18.34±1.57	2.08±0.26	3.10±0.99**	0.51±0.26	263.78±257.37	7.42±2.10††
E+ (n = 5)	27.97±3.37***	16.15±2.05†	1.43±0.28**	1.38±0.32***	0.46±0.19*	69.68±39.09†	12.85±2.30***
Average over groups (n = 20)	36.34±1.84	17.80±0.69	1.97±0.11	3.26±0.39	0.35±0.07	171.19±54.02	7.56±1.04
p-value, group factor 1 (C, E)	0.02	0.42	0.05	0.004	0.05	0.93	0.007
p-value, group factor 2 («–»,«+»)	0.09	0.19	0.09	0.050	0.90	0.23	0.060
p-value, group factor 1 × фактор группы 2	0.02	0.50	0.03	0.003	0.30	0.63	0.004

N o t e. For a description of the groups, see the Materials and methods section. T_{4t} — total thyroxine, T_{4f} — free thyroxine, T_{3total} — total triiodothyronine, T_{4cfree} — free triiodothyronine, ITI — integral thyroid index, PCI — peripheral conversion index.

*, **, *** Differences vs. the previous period are statistically significant by Studen's t-test at $p < 0.05$, $p < 0.01$, $p < 0{,}001$, respetively; †— tendency towards reliability ($p < 0.1$).

5. Pre-slaughter, slaughter and meat quality parameters in crossbred piglets (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] when fed dietary complex of adaptogens DHQEC (dihydroquercetin and vitamins E, C) with modeling stress (*M*±SEM, physiological yard of the Ernst Federal Research Center for Animal Husbandry — VIZh, 2022-2023)

Parameter	Group				p-value
	C–	C+	E–	E+	
	n = 9	*n* = 9	*n* = 8	*n* = 8	
Live weight after fasting, kg	124.06±1.95	125.13±1.14	124.99±1.48	126.16±2.31	0.87
Carcass length, cm	117.22±1.59	114.78±1.95	115.00±1.65	114.63±2.18	0.72
Weight of steamed carcass, kg	83.01±0.44	82.45±0.26	82.93±0.42	83.22±0.28	0.80
Slaughter yield, %	74.97±0.46	73.95±0.29†	75.01±0.36	75.01±0.32	0.12
Head weight (with tongue), kg	8.06±0.21	8.77±0.38	8.03±0.20	8.40±0.30	0.22
Leg weight, kg	1.92±0.06	1.86±0.04	1.88±0.03	1.96±0.08	0.56
Liver weight, kg	1.59±0.03	1.66±0.03	1.70±0.03*	1.70±0.05†	0.15
Kidney weight, kg	0.40±0.01	0.41±0.02	0.41±0.02	0.41±0.02	0.97
Heart weight, kg	0.46±0.02	0.50±0.02	0.49±0.02	0.49±0.01	0.25
Spleen weight, kg	0.22±0.01	0.24±0.02	0.21±0.01	0.19±0.01*	0.06
Lung weight, kg	0.81±0.04	0.81±0.08	0.88±0.08	0.77±0.04	0.65
Total mass of offal, kg	13.46±0.23	14.24±0.34	13.60±0.17	13.91±0.33	0.21
Yield of by-products, % by weight of the steamed carcass	14.48±0.22	15.41±0.44†	14.51±0.17	14.71±0.26	0.11
Thickness of the backfat between the 6th and 7th thoracic vertebrae (without skin thickness), mm	28.67±1.67	27.56±1.91	28.38±1.99	24.50±1.46†	0.36
Thickness of backfat on the loin (without skin thickness), mm	16.56±0.80	16.89±2.31	19.50±1.10*	15.50±2.00	0.42
pH of the longissimus dorsi muscle 45 min after slaughter, units	5.88±0.11	6.11±0.10	5.92±0.12	6.05±0.11	0.37
pH of the longissimus dorsi muscle after 24 h storage, units	5.67±0.02	5.72±0.02*	5.73±0.03†	5.70±0.03	0.21
Carcass category according to GOST R 31476-2012	2nd	2nd	2nd	2nd	
The loin eye size, cm^2	58.75±3.02	64.70±2.69	58.68±2.62	64.80±4.23	0.32
WHC, %	70.96±1.81	67.07±0.89†	68.48±1.20	67.42±1.32	0.18
TWSA, мг/г	0.11±0.01	0.12±0.01	0.11±0.01	0.10±0.00	0.45

N o t e. For a description of the groups, see the Materials and methods section. WHC — water holding capacity. TWSA — total amount of water-soluble antioxidants.

* Differences from control (C–) are statistically significant by Studen's *t*-test at $p < 0.05$, † — tendency towards reliability ($p < 0.1$).

In pigs fed DHQEC, the blood concentration was 24.3 and 26.7 μmol/l vs. 29.1 μmol/l in the control, or 16.5 and 8.2% less ($p > 0.05$) and the hemoglobin concentration was 122.9 and 120.7 g/l vs. 124.4 and 129.2 g/l, or 1.2 and 6.6% less ($p > 0.05$). This is obviously due to more intense oxidation and reduction processes in the body. Exposure to a stress factor in the 1st period of fattening led to greater consumption of water-soluble antioxidants (TWSA), and feeding DHQEC (C+ group) probably ensured their accumulation.

In the 2nd period of fattening (before slaughter), animals from groups E– and E+ had a lower blood content of urea (by 7.6 and 7.8%), a higher blood content of TP (by 3.0 and 1.3%) and lower A/G values (by 3.8 and 8.7%). In group E–, ALP and AlAT were 220.5 U/l vs. 233.1-272.3 U/l and 75.2 IU/l vs. 80.3-85.6 IU/l ($p > 0.05$). Despite the fact that in groups C– and E– the iron concentration was lower, in groups C+ and E+, the hemoglobin decreased statistically significantly (141.3 and 139.8 g/l vs. 151.5 and 145.5 g/l, respectively, or by 6.7 and 3.9%, $p < 0.01$). Hematocrit also fecreased (68.9 and 69.0% vs. 74.3 and 72.5%, or by 7.2 and 4.8%, $p < 0.01$). The concentration of chlorides was 2.3% higher ($p < 0.01$), and there was a tendency to increase (by 42.5%) in blood triglycerides ($p = 0.08$) in group E–, which indicated that E– pigs were under stronger stress compared to other groups. In the E+ group fed DHQEC, these indicators improved and were 0.3 and 17.5% lower than the values in the E– group ($p > 0.05$).

Calculation of the total values by growth periods indicated a trend towards an increase in cortisol content in group C– compared to previous periods, 98.1

nmol/l vs. 66.6 and 56.8 nmol/l, or by 47.3 and 72.7 % ($p < 0.01$). At the end of fattening, the amount of cortisol increased in group E– compared to the C– control, 149.81 vs. 98.49 nmol/l, or by 52.1% ($p < 0.05$) (Table 3, data are also presented in graphical form in Fig. 2, see http://www .agrobiology.ru).

To assess the functional state of the thyroid gland, the integral thyroid index and the peripheral conversion index are of interest, characterizing the quantitative ratio of thyroid hormones and their pituitary regulator and the conversion of thyroxine to triiodothyronine. In the 1st period of fattening, no significant changes were found in the blood concentration of thyroid hormones of animals from groups C+, E–, E+ compared to group C– (Table 4, see Fig. 2, http://www.agrobiology.ru). The T_{4t} indicator showed a slight downward trend ($p = 0.11$) in pigs fed DHQEC (groups C+ and E+), which may indicate that the body retained the energy necessary for intensive growth. Maintaining lower thyroid hormone levels may be a mechanism for reducing metabolic demands. Under the influence of increasing stress in groups E– and E+ at the end of fattening, compared to groups C– and C+, the blood concentration of T_{4t} ($p = 0.02$), T_{3t} ($p = 0.05$), T_{3f} ($p = 0.004$) decreased while TSH production ($p = 0.05$) and PCI ($p = 0.007$) were higher.

Weighing the animals before slaughter (after fasting) showed that in pigs in groups C+ and E+ LW was 125.1 and 126.1 kg vs. 124.1 and 125.0 kg in groups C– and E– (a decrease by 0.8 and 0.9%, respectively, $p > 0.05$) (Table 5). The weight of the fresh carcass had no intergroup differences ($p = 0.80$). The slaughter yield was higher in groups C–, E–, E+ compared to C+by 1.0; 1.1 and 1.1%, respectively ($p = 0.12$). In the C+ group, the specific yield of offal was 14.2% vs. 13.5-13.9% ($p = 0.21$).

The influence of stress factors in groups E was expressed in an increase in LW compared to C– and C+ (1.70 kg vs. 1.59 and 1.66 kg, or 6.9 and 2.4% more, $p < 0 .05$) and a tendency towards a decrease in spleen weight (0.21 and 0.19 kg in groups E– and E+ vs. 0.22 and 0.24 kg in groups C– and C+, or by 4.5 and 20.8% less, $p = 0.06$). In group E–, the stress led to an increase in fat depot: the thickness of the back fat on the lower back significantly increased compared to C– (by 17.8%, $p < 0.05$). DHQEC provided a tendency to a decrease in the thickness of the backfat both between the 6th and 7th thoracic vertebrae, and on the lower back. It should be noted that feeding DHQEC did not significantly affect the increase in pH_{45} (6.11 and 6.05 in groups C+ and E+ vs. 5.88 and 5.92 in groups C– and E–, or 3.9 and 2. 2% more, $p = 0.37$). A significant increase in the pH of the *longissimus dorsi* muscle occurred after 24 h in C+ pigds compared to C– pigs, by 0.9% ($p < 0.05$). An increase in pH occurred with a greater water-holding capacity of meat from animals of C– and E– groups compared to C+ and E+ ($p = 0.18$), while the pH value was the highest in group C–. The quality of carcasses is characterized by the area of the muscle eye, which was 67.07 and 64.80 cm^2 in groups C+ and E+, being larger than in C– and E– by 10.1 and 10.4%, respectively.

In modern intensive livestock farming, the use of bioactive substances of both natural and synthetic origin to reduce the impact of stress factors is becoming increasingly important [31, 34-36]. A study performed on newborn buffaloes treated and not treated with vitamin E (DL-α-tocopherol acetate) confirmed that vitamin E supplementation resulted in improved growth, metabolic and endocrine profiles [37].

Cortisol is a steroid hormone, the main representative of glucocorticoids, which is produced in the zona fasciculata of the adrenal cortex under the control of adrenocorticotropic hormone (ACTH) of the pituitary gland. Its production depends on the combination of incoming neuronal and humoral stimuli, as well

as the blood cortisol content (given the negative feedback) [9]. Cortisol regulates the metabolism of proteins, fats, carbohydrates, water and electrolytes, is involved in the regulation of inflammatory reactions and the response to stress factors, and is involved in the development of adaptation syndrome [38, 39]. By affecting protein metabolism, cortisol increases protein synthesis in the liver and inhibits it in muscle, bone and lymphoid tissues. The catabolic and antianabolic effects release amino acids, which are taken up by the liver, deaminated, and converted into carbohydrates [40, 41].

Under stress, glucocorticoids help increase the amount of glucose in the blood. In our studies, the glucose content in animals of the experimental groups remained within the normative values and had no intergroup differences (see Table 2). The creatinine content that characterizes the activity of the creatine phosphokinase and the rate of muscle mass gain was also normal in all animals. This corresponded to high increases in the livestock's fat mass.

In E– animals, there was a tendency to increase the amount of triglicerides during the 1st fattening period (0.44 mmol/l vs. 0.34-0.39 mmol/l, $p < 0.10$), which indicates their stress. AsAT activity (a marker of damage to the liver and cardiovascular system) was normal in all pigs throughout the experiment, but the highest values were characteristic of group E– (78.3 IU/l vs. 60.7-76.5 IU/l at the end of fattening, $p > 0.05$). Dietary DHQEC had a clear effect on the AsAT (E+ vs. E– and C+ vs. C– by 10.0 and 10.3%, respectively) (see Table 2).

It is important that at the 1st stage of fattening, feed stress led to a slight decrease in the blood cortisol content (47.7 and 44.1 nmol/l in E– and E+ vs. 69.0 and 66.33 nmol/l in C– and C+, or by 30.9 and 33.5%) (see Table 3, Fig. 2). We suggest that the simulated stress caused by competition for food could provoke additional physical activity, which provided some exercise for the animals. However, with increased food competition, feeding DHQEC led to a significant decrease in the blood cortisol level during the final fattening ($p = 0.014$).

Adrenaline is synthesized in the adrenal medulla, is a derivative of the amino acid tyrosine and belongs to the group of catecholamines. Adrenaline enhances the response of the nervous system and hormones. In addition, ADR reduces digestive-related responses that are not directly associated with stress [42]. At the beginning of the experiment and in the 1st period of fattening the blood adrenaline concentration was low (2.59 and 2.85 ng/ml, respectively), and at slaughter it tended to change ($p = 0.07$). Importantly, we did not identify a relationship between the blood adrenaline concentration between for a certain group, but there was a clear tendency towards a decrease in the parameter in animals fed DHQEC in groups C+ and E+ compared to groups C– and E– (8.60±5.14 vs. 50.32±20.91, or 5.9-fold, $p = 0.09$). Feeding DHQEC significantly contributed to the stress resistance, as it is evidenced by the levels of adrenaline and cortisol before slaughter (see Table 3, Fig. 2).

Supplementation of diets with bioactive ingredients of various origins can promote the secretion of insulin-like growth factor 1 (IGF-1) which affects animal and poultry the productivity performance [43]. Feeding plant extracts can improve growth performance and nutrient utilizaion in growing pigs, reduce fecal gas emissions, and increase blood components associated with the immune response such as leukocytes and lymphocytes, as well as serum IGF-1 concentrations [44].

G. Liu et al. [45] reported that supplementation of plant extracts resulted in an increase in average daily weight gain, serum IGF-1 content and IGF-1 receptor mRNA content in tissues (stomach, duodenum, muscle) in pigs. In our study, the amount of IGF-1 in pig blood serum at the beginning of the experiment was 178.2 ng/ml (see Table 3, Fig. 2). At the age of 140 days, we did not note

intergroup differences, but there was a general trend towards a decrease in IGF-1 content ($p = 0.10$). On day 180 it remained ($p = 0.03$). However, under the influence of DHQEC, the IGF-1 content had a slight tendency to increase during the final fattening period, in the C+ and E+ groups compared to the C– and E– groups (163.7 and 162.8 vs. 141.0 and 142.1 ng/ml, or by 16.1 and 14.6%, $p = 0.14$), which correlated with more intense increases in body weight. The results we obtained indicated that DHQEC, having antioxidant properties, improved growth performance and, apparently, tissue-specifically regulated the amount of IGF-IR mRNA. In addition, these data may be important for determining the physiological role of the IGF-1 system in marker control of pig growth.

Melatonin is the most effective antioxidant in the body. It has both direct antioxidant activity and indirect activity, stimulating other antioxidant systems. MT directly binds hydroxyl, superoxide anion, hydrogen peroxide, singlet oxygen, perioxynitrite and nitric oxide. MT also stimulates the activity of a cascade of enzymes, most notably intracellular superoxide dismutase, glutathione peroxidase, and catalase [46]. Endogenous MT is capable of modulating the amount of NO in mitochondria and, as a consequence, the circadian rhythm of oxidative phosphorylation and glycolysis in vivo. We did not find statistically significant differences in the content of melatonin in the blood serum between the first and second sampling (see Table 3), that is, stress and handling of the animals during blood sampling did not affect the change in MT concentration. However, in group E–, the MT value was the highest in the 1st fattening period (326.7 pg/ml) compared to the control groups (209.9 and 221.2 pg/ml, $p > 0.05$). Feeding DHQEC in the E+ group led to a decrease in MT to 246.9 pg/ml, or by 24.4% compared to E–. It is interesting that at the end of the experiment, the blood MT concentration in groups C– and C+ increased compared to the 1st fattening period by 218.2 and 207.4% ($p < 0.05$), but feeding DHQEC did not affect this parameter. It should be noted that there was a statistically significant decrease in MT in the E– and E+ groups compared to the controls by 36.2 and 60.8%, respectively ($p = 0.01$), that is, increased feed competition led to a decrease but not to an increase in the MT content. Moreover, feeding DHQEC in the E+ group normalized the indicator to the values of the 1st and 2nd blood samplings.

Consumer preferences for meat are generally determined by its physical characteristics, while nutritional value is related to its chemical composition. Exogenous melatonin negatively affects muscle pH and rate of water loss in finishing pigs, but this may be somewhat dependent on melatonin dose [47]. Melatonin injections also affect pork quality [48].

In the work of Y. Zhou et al. [30], the use of DHQ (15 mg/kg LW) increased the blood concentrations of testosterone derivatives, antioxidants (melatonin and betaine), unsaturated fatty acids (docosahexaenoic acid, DHA) and beneficial amino acids (proline). Thus, the increase in melatonin levels in piglets in our experiment was obviously a necessary response to stress and pre-slaughter holding, while in pigs from the E+ group the melatonin concentration did not change which indicates a significant role of the DHQEC complex in the pre-slaughter period.

Thyroid function and thyroid hormone activity are considered critical for maintaining performance in companion animals [49]. Increased secretion of thyroid hormone activates metabolism and therefore heat production. S. Zhang et al. [50] showed that the blood concentrations of cortisol, T_3, T_4 in growing pigs with 25 kg LW decreased as the ambient temperature increased from 18 to 32 °C, and the amount of T_4 at 18 °C exceeded the values at 27, 29 and 32 °C ($p < 0.05$).

Knowledge of the mechanisms underlying fluctuations in TSH and thyroid

hormone levels is important for understanding animal adaptation processes [51]. Genetic factors account for up to 65% of interindividual variation in TSH and thyroid hormone levels [52, 53], but other factors may also influence thyroid function. These are age and sex [54, 55], internal factors (microbiota) [56], stress [57], drug use [20] and environment [58, 59].

In our experiment, during final fattening, significant changes occurred in the hormonal status of the animals (Table 4). Under the influence of stress (groups C vs. groups E), T_{4t} ($p = 0.02$), T_{3t} ($p = 0.05$), T_{3f} ($p = 0.004$) concentrations decreased, which was accompanied by increased TSH production ($p = 0.05$). The functions of the thyroid gland, pituitary gland and hypothalamus are coordinated, and this ensures control of the content of thyroid hormones. If there is not enough thyroid hormone in the blood, the pituitary gland increases the production of thyroid-stimulating hormone (TSH), which stimulates the production of hormones by the gland. As soon as the level of thyroid hormones is restored, TSH synthesis slows down and its amount approaches normal. At that, PCI (an indicator of tissue conversion of thyroxine) increased several times ($p = 0.007$), which additionally indicates a strong influence of stress on the animal's body. Feeding DHQEC during nutritional stress somewhat smoothed out the negative impact of the modeled factor, leading to additional regulation and reduction of the body's metabolic losses. The lowest T_{4t}, T_{4f}, T_{3t}, T_{3f} values were identified in the E+ group. It should be noted that in the E+ group, TSH and ITI indicators decreased to 0.46 mIU/l and 69.7 units vs. 0.51 mIU/l and 263.8 units, or by 9.8 and 73.6%, in group E– with a 1.73-fold decrease in the T_{4f} to T_{3f} conversion (see Table 4, Fig. 2).

Under simulated stress, the DHQEC supplement provided the best physiological response during the test period (see Table 2). The combination of DHQ with vitamins C and E enhanced mechanisms of antioxidant protection. During the 1st period of fattening, under the influence of stress, water-soluble antioxidants (WSA) were consumed. According to a two-factor analysis, $p < 0.001$ for group E vs. group C, $p = 0.74$ for groups "–" vs. groups "+", and $p = 0.03$ for two factors (see Table 2). The effect of stress in groups E was clear (a decrease in the amount of water-soluble antioxidants compared to group C+). During final fattening, both factors (stress and feeding DHQEC) did not affect the blood WSA level ($p > 0.05$).

As already noted, the quality of meat depends on the physiological and behavioral reactions of the animal before slaughter [39, 60], including changes in the content of metabolites and glycogen [61, 62]. We assessed the quality of pork according to GOST 7269-2015 for most important prameters, the meat water holding capacity (WHC), color, pH. In samples of muscle tissue from groups C+, E–, E+, there was a slight decrease in WHC ($p = 0.18$) compared to group C– (see Table 5). The pH value of muscle tissue samples from group C+ (5.72 ± 0.02) was better ($p < 0.05$) than that of group C– (5.67 ± 0.02). This may resulted from the use of DHQEC which prevented the decrease in pH after slaughter due to improving animal stress resistance and antioxidant effects ex vivo. Under simulated stress, the pH value of muscle tissue samples from group E– also tended to improve compared to the control group C– ($p < 0.10$). Previously, we showed [29, 32] that modulation of social and technological stresses is accompanied by animal body training which apparently we also noted in this experiiment.

In 24 h after slaughter, the pH values of the muscle tissue samples in E+ group were comparable to that for C+ group, 5.70 ± 0.03 and 5.72 ± 0.02, respectively ($p > 0.05$) which indicates action of DHQEC. In terms of pH, meat samples in group E+ were not inferior to those of group C+.

Nowadays, the use of bioactive anti-stress drugs of natural origin is more

preferable [63]. Studies on several biochemical parameters in pigs of different genotypes indicate that local animals are more adapted to changing environmental conditions [64]. The use of feed products, in particular antioxidant additives, can significantly increase the body's resistance and reduce stress load [65] which is achieved through hormonal regulation of functions and an improvement in the body's antioxidant status.

Thus, simulated stress (additional feed competition during fattening) led to production of stress hormones. The antioxidant defense was significantly reduced, as evidenced by a decrease in the blood content of melatonin and free antioxidants of F_2 piglets [(Large White × Landrace) × Duroc]. Stress had a negative impact on metabolism, in particular, on blood biochemical parameters, e.g., the concentrations of total protein, triglycerides, cholesterol, chlorides, total bilirubin, and aspartate aminotransferase. Under stress, the concentration of thyroid hormones also decreased while the production of thyroid-stimulating hormone increased and the peripheral conversion index (PCI, characterizes the tissue conversion of thyroxine to triiodothyronine) increased. Stress significantly affected meat quality. A complex of antioxidants (dihydroquercetin and vitamins E, C), used as a feed additive, improved metabolic processes, including protein and mineral metabolism, increased the body's protective functions, nutrient utilization, and animal adaptability. The effect of the antioxidant feed additive was evident throughout the entire fattening period. As a result, the characteristics of the resulting products (slaughter performance, meat quality) were improved.

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