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THE EFFECT OF DIHYDROQUERCETIN ON THE GROWTH AND USE OF FEED BY PIGS (*Sus scrofa domesticus* Erxleben, 1777) UNDER MODERATE HEAT STRESS

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Abstract

Crossbred pigs are characterized by nervous instability, limited thermoregulation, and susceptibility to stress. Climate stress causes behavioral, physiological, functional, productive changes in farm animals. The aim of the research was to assess the influence of a moderate climatic stress factor (an increase in ambient temperature) on feeding, the digestibility of nutrients and productivity of intensively growing young pigs fed with dihydroquercetin (DHQ) during different periods of rearing and fattening (the physiological yard of the Ernst Federal Research Center for Animal Husbandry, 2020). For groups of crossbred boars F_2 (Large White × Landrace) × Duroc, $N = 36$) were subjected to moderate heat stress (4-6 °C above the optimum). Control animals (group 1, $n = 9$) fed a basal diet (BD), group 2 ($n = 9$) received BD + adaptogen dihydroquercetin (DHQ) during the rearing period, group 3 ($n = 9$) during the rearing and fattening, and group 4 ($n = 9$) during periods of technological stress (7 days after transportation, after transferring to other feeds, and before slaughter). The adaptogen we used as dietary supplement was Ecostimul-2 (LLC Ametis, Russia; 45 mg/kg of feed, or 32 mg DHQ/kg of feed). Moderate heat stress during feeding period (weeks 12-15 of the experiment) led to a significant increase in air concentration of ammonia up to 16.7 mg/m^3, hydrogen sulfide up to 1.67 mg/m^3, and carbon dioxide up to 0.14 mg/m^3. The blood cortisol level was 291.60 nmol/l in control group 1 (or 23.0 % above the upper value of reference limits of 41-237 nmol/l), 299.89 nmol/l in group 4, and 210 nmol/l ($p > 0.05$) in groups 2 and 3. At slaughter, the cortisol level was the highest in the control animals (284.77 nmol/l) while feeding DHQ in groups 3 and 4 decrease it to 234-253 nmol/l. Adverse external stimuli increased the mortality in the control to 11 % vs. 0 % in other groups. The animals were weighed weekly, and the average daily weight gain was assessed for each of the periods as compared to control with regard to environmental factors (microclimate parameters) and technology elements (change of feed, vaccination, etc.). During the growing period, the weight gain in all groups with DHQ were 1.5-1.7 % greater than in control group 1 (week 1, group 3, $p < 0.05$) that indicates better adaptation after transportation. Our study showed a significant increase in the average daily weight gain in certain periods of co-action of moderate heat stress and other stress factors, e.g., during vaccination (week 8, vaccination against classic swine fever, group 2 at $0.05 < p \le 0.1$; groups 3 and 4 at $p < 0.05$). Over the experiment (growing and fattening periods), the largest average daily weight gain was in group 4 which received 32 mg/kg DHQ during technological stress, the difference with the control was 13.6 % ($p > 0.05$). In group 3 (32 mg/kg DHQ during the final fattening), there was a trend towards an increase in gross growth (by 6.2 %, $0.05 < p \le 0.1$) compared to control. The balance test during the final fattening revealed a tendency to higher digestibility of dry matter in groups 3 and 4 (by 1.31 and 0.93 %, respectively; $0.05 < p \le 0.1$). In the groups received DHQ, the nitrogen excretion with urine was lower (by 21.20, 14.47, and 21.91 g in groups 2, 3, and 4, respectively) compared to control group 1 ($p = 0.18$-0.37). Thus, dietary DHQ contributed to the retention and more efficient use of nitrogen by growing young pigs. With DHQ, excretion of calcium in the feces was also lower (by 3.48 g, $p < 0.05$; 1.68 g, $p > 0.05$; 2.87 g, $p = 0.06$) while its deposition in the body of growing young pigs was higher (by 3.52 g, $p < 0.05$; 1.62 g, $p > 0.05$; 2.85 g, $p = 0.06$) in groups 2, 3, and 4, respectively. Calcium utilization was 9.82 % higher ($p < 0.05$) in the animals of groups 2, 3, and 4. Thus, the control animals were more susceptible to the heat stress and had worse growth parameters, nutrient utilization, and higher mortality. Dietary DHQ applied during pig growing and fattening improves adaptive abilities of animals resulting in their better growth and productive performance.

Keywords: adaptogen, dihydroquercetin, stress, young pigs, productivity, average daily live weight gain, digestibility

To increase the efficiency of pig raising, it is necessary to ensure the optimal microclimate in the premises (temperature and humidity, the concentration of harmful gases, air exchange). A proper microclimate positively influences the physiological state of animals. Conversely, an uncontrolled microclimate or underestimation of the effect of stressors of various strengths and degrees of impact on the animals weakens their resistance, which leads to the emergence and development of diseases of various etiologies, impairs productivity [1], reproductive ability, and causes a number of other undesirable consequences [2], including the decline in pork quality [3].

The physical state and chemical composition of the air environment are fickle factors and are subject to large fluctuations. The animal organism can adapt to these changes, but only up to certain limits. In particular, in order to maintain normal vitality, animals must expend a certain amount of nutrients to generate heat, which is necessary for metabolism [4]. The more the body spends energy materials to adapt to environmental conditions, the less nutrients will be used to ensure productivity [5].

The air environment, which determines the state of the microclimate of closed livestock buildings, affects heat exchange, gas exchange, physical and chemical properties of blood, body and skin temperature, and other indicators [6]. The body reacts to any impact of the environment with a multilevel physiological and biochemical reaction, which causes the development of stress and then, as a consequence, adaptation. The damaging effect of the consequences of stress is due to an excessive increase in the adaptive lipotropic effect, which increases the activity of phospholipases and the intensity of free-radical lipid oxidation through catecholamines and protein kinases. Stress effects lead to the restructuring of metabolism and some physiological functions, which initially increases the stability of the animal organism [7]. However, prolonged exposure to stress depletes the internal defense systems, which ultimately affects the health of animals, their resistance to diseases, productivity and safety [8]. The ambient temperature has a significant impact on the physiological changes in the body and the productivity of pigs, while the effect of air humidity on these indicators is less pronounced. In studies on animals kept under conditions of complex exposure to environmental factors, significant violations of immune reactivity were revealed. It was established [9] that in 60-kilogram pigs the upper critical temperatures for such important physiological reactions as respiratory rate, heat production, rectal temperature were within the range of 21.3-22.4 °C, 22.9-25.5 °C and 24.6-27.1 °C, respectively, depending on the change in relative air humidity from 50 to 80%.

Physiological balance under microclimatic stressors is maintained as long as the action of external stimuli does not exceed the adaptive capacity of the body [10, 11]. The consequences of the manifestation of climatic stress and its duration depend largely on the composition of the diet, the system of housing and watering, the density of animals in the pig breeding complex, microclimate conditions - relative humidity, air velocity and its composition [12].

The trend towards intensification of animal husbandry is likely to continue, and the problem of heat stress is likely to be exacerbated by global warming and climate change. The development of methods for preventing and eliminating the negative consequences of stress, in particular heat stress, is undoubtedly an important tool for increasing the productivity of animal husbandry [1, 13]. During the construction of large pig-breeding complexes, there will be a need for more precise control of all factors affecting production efficiency. It is necessary to identify and study the possibilities to level the consequences of abiotic stresses, including through the use of feed adaptogens. In this regard, a promising solution may be the use of natural and synthetic bioactive substances with antioxidant properties, which reduce the effect of stressors on homeostasis by stabilizing free radical oxidation and increasing the adaptive properties of the body [14, 15]. It has been reported that the use of dihydroquercetin (DHQ) helps to reduce lipid peroxidation, reducing the negative effects on the body of pigs of the effects of transport and feed stress [16, 17].

In the present work, we have shown that the feeding of natural bioflavonoid dihydroquercetin as an additional prophylactic feed component can favorably affect the safety and productivity of intensively growing pigs, contributing to their better adaptation to feeding and housing conditions.

The purpose of this study was to assess the effect of the adaptogen dihydroquercetin, fed to pigs at different periods of growing and fattening, on productivity and nutrient use under conditions of moderately pronounced heat stress with accompanying technological stresses (transportation, switching to another feed recipe, vaccination and slaughter).

Materials and methods. Physiological studies were carried out on 36 hybrid boletus (*Sus scrofa domesticus*) F_2 [(large white × Landrace) × Duroc] with a live weight at the beginning of the experiment of 17.20-17.43 kg at the age of 58 days (physiological yard of the Ernst FRC VIZh, 2020). The duration of the experiment was 120 days.

Young hogs purchased from Verkhnyaya Khava (Voronezh Province) were delivered by special vehicles for animal transport (transportation at a distance of 500 km) in compliance with transportation standards and the necessary veterinary and sanitary control. After delivery, the animals were divided into four groups, which were kept under the same conditions.

In a comparative study, groups 2, 3 and 4 (n = 9 each) received DHQ in addition to the diet (Ecostimul-2 preparation, Ametis JSC, Russia; dosage 45 mg/kg of feed, or 32 mg DHQ/kg feed as per active substance), in group 2 (n = 9) only during the growing period (DHQd), in group 3 (n = 9) during growing and fattening (DHQd+o), in group 4 (n = 9) during feed and technological stresses (DHQtech) (7 days after transportation, 7 days before and 7 days after switching to another type of compound feed, and also not less than 7 days before slaughter). Group 1 (n = 9) fed the basal diet without DHQ was used as a control.

The fattening pigsty for 48 posts complied with the standards for keeping animals (GOST 12.1.005 and MR for technological design) during growing and fattening. Feeding occurred 2 times a day from group feeders with dividers for individual feeding (1.5×2 m pens, 1×1.5 m rubber mat; 3 pigs per pen during growing to slaughter, 0.8 m^2 per head with an actual area of 3 m^2). Teat drinkers were located in the corner of the machine directly in front of the manure removal channel, the animals had constant access to water; dry compound feeds were moistened directly during distribution. The basal components of the diet were SK-4 (during growing), SK-5 (during the 1st fattening period) and SK-6 (during the final fattening) (the manufacturer of the compound feed is Agrovitex LLC, Russia). The feeds were balanced in terms of nutritional substances and energy according to modern norms and the recommended feeding regimen [18].

In the premises, as per veterinary and sanitary requirements, cleaning with the removal of manure was performed twice a day. To control the mode of the simulated environment, the temperature and relative humidity of the air in the pigsty were measured (at 16:00) using a stationary electronic weather station. Using infrared lamps in each machine, in the warm period when the outdoor air temperature was above 10 °C, the indoor temperature was increased by 5 °C relative to the calculated summer outdoor temperature (up to 26-28 °C the most) to simulate stress conditions.

The temperature-humidity index (THI) was calculated for the entire period of the experiment based on the records of the electronic weather station [19]:

$$THI = (0.8 \times t) + [(\varphi/100) \times (t - 14.4)] + 46.4,$$

where t is the dry bulb temperature, °C; φ is relative air humidity, %.

The volume fraction of methane (%vol.f.), mass concentration (mg/m^3) of carbon dioxide, ammonia, hydrogen sulfide and methane in the air of the working area was measured (a multicomponent gas analyzer MAG-6, MAG-6 P-K, EXIS JSC, Russia).

Animals were individually weighed (a REUS-300 electronic balance, OOO Tenzosila, Russia) before the start of the experiment and every 7 days until its completion. Based on the weighing and assessment of feed consumption, gross, average daily weight gains and feed costs per unit of gain were calculated.

In animals from all groups (N = 20, n = 5), blood was taken from the jugular vein at the end of rearing, in the middle of fattening period and before the end of the experiment. Blood concentration of cortisol was measured by the enzyme immunoassay method (an automatic microplate photometer Immunochem-2100, High Technology, Inc., USA; reagent kits X-3964 Cortisol-IFA-BEST, Vector-Best, Russia; sensitivity 5 nmol/l, measurement range 0-1200 nmol/l).

To determine the digestibility of nutrients in the diet and to study the metabolism of nitrogen and minerals in intensively growing young pigs at the end of the final fattening period, a balance experiment was carried out (N = 12, groups of n = 3) as per common standard methods [20, 21]. All animals (N = 12) during the balance experiment were kept in special individual cages to record feed consumption and the amount of excrements. Recording was carried out for 5 days, after which average samples were taken for chemical analysis using standard methods.

Statistical analysis was performed using the STATISTICA package, version 10.0 (StatSoft, Inc., USA). Quantitative data are presented as the arithmetic mean (M) and standard error of the mean (±SEM). Identification of the relationship of the studied factor with the indicators of nutrient digestibility, nitrogen retention, blood parameters was performed on a sample of animals using one-way analysis of variance (ANOVA) with Dunnett's test. Differences from control were statistically significant at $p < 0.05$ and were considered a trend at $p \geq 0.05$ and $p \leq 0.1$.

Results. Table 1 shows the experiment scheme. Table 2 shows the composition of diets over the experiment.

The environmental conditions directly affect the vital activity, substrate and energy metabolism of animals. The temperature is one of the main influencing factors. The optimum temperature for pigs of different sex and age groups is not the same, 27 °C for sucking pigs, 25 °C for piglets with live weight from 15 to 25 kg, 22 °C from 25 to 45 kg, 20 °C from 45 to 85 kg, and 17 °C from 85 to 120 kg. Any deviation from the optimal parameters activates the thermoregulation system, and the greater the deviation, the more the animal is exposed to stress with high energy costs to maintain a constant body temperature [18].

1. Experiment scheme to assess the effect of dihydroquercetin (DHQ) **on pigs (*Sus scrofa domesticus*) F2 [(Large White × Landrace) × Duroc] adaptation to simulated heat stress** (physiological yard of Ernst FRC VIZH, Moscow Province, May-September 2020)

Group	*n*	Diet	DHQ feeding scheme
1(control)	9	BD	
2	9	BD + DHQ	During growing (DHQd)
3	9	BD + DHQ	During growing and fattening (DHQd+o)
4	9	OBD + DHQ	During feed and technological stresses (ДКВtech)

N o t e. BD — a basal diet (Table 2), DHQ (Ecostimul-2 preparation, Ametis JSC, Russia) was fed at a dosage of 45 mg/kg feed (32 mg DHQ active substance/kg feed). The dosage of the supplements was preliminarily estimated [2, 5, 12, 30].

2. The nutritional value of compound feed (at natural humidity) **in assessment of the dihydroquercetin** (DHQ) **effects on pigs (*Sus scrofa domesticus*) F2 [(large white × Landrace) × Duroc] adaptation to simulated heat stress** (physiological yard of Ernst FRC VIZH, Moscow Province, May-September 2020)

Parameter	Units	Compound feed (OOO Agrovitex, Russia)		
		SK-4	SK-5	SK-6
Exchange energy	MJ/kg feed	11.85	11.65	11.47
Moisture	%	12.00	13.50	14.00
Crude protein	%	18.50	17.20	12.20
Crude fat	%	1.86	2.35	2.60
Crude fiber	%	4.24	5.72	5.04
Lysine	%	1.13	1.00	0.75
Methionine + cystine	%	0.65	0.62	0.46
Threonine	%	0.70	0.64	0.50
Tryptophan	%	0.22	0.20	0.16
Calcium	%	0.85	0.75	0.60
Phosphorus	%	0.56	0.55	0.48
Salt (NaCl)	%	0.54	0.53	0.50

In our research, the temperature regime varied from 22.1 to 29.6 °C (or from 71.8 to 85.3 °F). Thus, there was an excess of the temperature optimum of 18-20 °C by an average of 4-6 °C during most of the experiment. Relative air humidity varied on average from 65 to 85% and generally corresponded to zoo-hygienic standards (60-85%) [22] (Fig. 1).

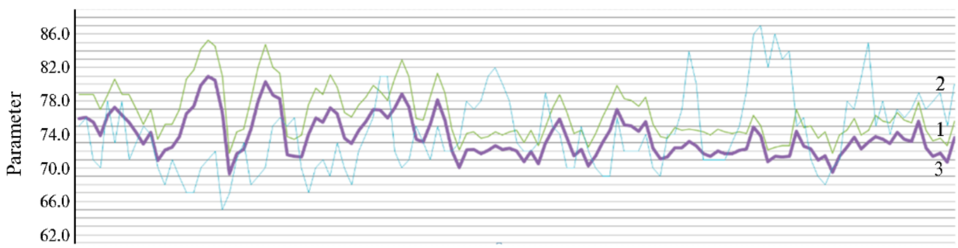

Рис. 1. Temperature (°F, 1)**, relative air humidity** (%, 3) **and temperature-humidity index** (THI, 2) **in the experiment on assessing the dihydroquercetin effects on pigs (*Sus scrofa domesticus*) F2 [(Large White × Landrace) × Duroc] adaptation to simulated thermal stress** (physiological yard of Ernst FRC VIZH, Moscow Province, May-September 2020).

Based on measurements of the relative humidity and temperature of the room where the animals were kept, a temperature-humidity index (THI) was calculated, confirming that the animals were under moderate stress. The THI values were 72.0-77.3 units (Table 3). Along with a moderate increase in temperature, the room air was saturated with CO_2 to 0.14 mg/m^3, remaining within the permissible concentration (< 0.2 mg/m^3). During the experiment, the content of ammonia and hydrogen sulfide also remained within the normal range (up to 20 and 10 mg/m^3, respectively).

The dynamics of blood cortisol level (Table 4) showed that the animals were exposed to stress factors during the experiment. In some periods, the level of cortisol often exceeded the physiological norm for pigs (41-237 nmol/l) [23].

3. Microclimate parameters during assessing the dihydroquercetin effect on pigs (*Sus scrofa domesticus*) F$_2$ [(Large White × Landrace) × Duroc] adaptation to simulated thermal stress (n = 6, M±SEM, physiological yard of Ernst FRC VIZH, Moscow Province, May-September 2020**)**

Week	Parameter							
	φ, %	t, °C	t, °F	THI	NH_3, mg/m^3	H_2S, mg/m^3	CH_4, %	CO_2, %
1	73.2±1.37	21.8±0.28	78.8±0.51	75.8±0.50	4.3±1.1	0	0	0.06±0.01
2	71.6±1.37	22.2±0.45	76.5±0.82	73.7±0.77	3.8±0.8	0	0	0.06±0.01
3	68.1±0.81	25.2±0.88	81.2±1.59	77.3±1.36	5.8±1.1	0.05±0.01	0	0.07±0.01
4	66.7±1.49	24.1±0.84	78.1±1.51	74.7±1.32	5.5±1.0	0.06±0.01	0	0.06±0.01
5	67.5±0.88	24.3±0.40	77.4±0.72	74.5±0.63	6.8±1.2	0.08±0.01	0	0.11±0.02
6	66.0±1.85	25.2±0.51	78.3±0.91	75.1±0.67	6.5±1.2	0	0	0.09±0.02
7	73.5±0.97	25.9±0.73	79.6±1.32	76.7±1.12	5.8±1.1	0.05±0.01	0	0.06±0.01
8	70.5±1.22	24.2±0.08	76.5±0.14	73.8±0.12	6.6±1.2	0.08±0.01	0	0.07±0.01
9	77.3±1.18	22.7±0.51	74.1±0.92	72.2±0.80	5.5±1.1	0	0	0.08±0.01
10	72.7±1.18	23.3±0.58	75.4±1.04	72.9±0.93	4.9±1.3	0	0	0.08±0.01
11	69.8±2.09	23.6±0.07	75.6±0.12	72.8±0.25	7.0±1.3	0	0	0.07±0.01
12	70.9±0.76	24.2±0.49	76.4±0.88	73.5±0.77	16.7±2.0	0	0	0.13±0.04
13	74.4±2.09	22.5±0.07	74.5±0.12	72.3±0.25	12.8±1.8	0.38±0.05	0.01±0.005	0.09±0.02
14	77.6±2.57	22.9±0.28	74.3±0.50	72.5±0.57	15.8±2.2	1.67±0.21	0.02±0.005	0.11±0.02
15	77.4±2.73	22.6±0.38	74.0±0.68	72.0±0.49	13.3±1.8	1.42±0.15	0.02±0.005	0.14±0.03
16	73.1±2.56	22.3±0.28	74.1±0. 50	72.0±0.56	9.6±1.5	0	0	0.11±0.02
17	76.2±0.70	21.5±0.21	76.1±0.37	73.8±0.37	5.5±1.0	0	0	0.11±0.02

4. Blood cortisol levels in pigs (*Sus scrofa domesticus*) F$_2$ [(Large White × Landrace) × Duroc] under simulated heat stress, as influenced by the dihydroquercetin (DHQ) **additive** (physiological yard of Ernst FRC VIZH, Moscow Province, May-September 2020)

Time frames	Group			
	1 (control)	2 (DHQ$_d$)	3 (DHQ$_d$ +o)	4 (DHQ$_{tech}$)
Final growing period	291.60±42.68	210.81±18.46	210.26±33.65	299.89±52.35
Transition to final fattening	147.58±27.50	140.83±13.16	93.53±16.19*	133.74±16.64
Before slaughter	284.77±86.81	275.59±86.41	234.36±61.23	253.20±46.39

N o t e. For a description of the groups, see the Materials and methods section. DHQ was fed during the growing period (DHQd), during growing and fattening (DHQd+o), and during feed and technological stresses (DHQtech).
* Differences from control are statistically significant at $p < 0.05$

5. Weight gain and feed consumption in pigs (*Sus scrofa domesticus*) F$_2$ [(Large White × Landrace) × Duroc] under simulated heat stress, as influenced by the dihydroquercetin (DHQ) **additive** (n = 9, M±SEM, physiological yard of Ernst FRC VIZH, Moscow Province, May-September 2020)

Parameter	Group			
	1 (control)	2 (DHQ$_d$)	3 (DHQ$_d$ +o)	4 (DHQ$_{tech}$)
	O v e r g r o w i n g p e r i o d			
n	9	9	9	9
Days	34	34	34	34
Live weight at the beginning of the experiment kg	17.20±0.63	17.40±0.51	17.31±0.52	17.43±0.65
Live weight at the end of the period, kg	38.83±0.89	39.34±1.09	39.31±0.81	39.39±1.26
Gross gain, kg	21.63±0.46	21.94±0.82	22.00±0.63	21.96±0.80
Daily gain, g	636.18±13.39	645.42±24.01	647.06±18.57	645.75±23.39
	O v e r t h e 1st f a t t e n i n g p e r i o d			
n	8	9	9	9
Days	49	49	49	49
Live weight at the end of 1st fattening period, kg	84.32±1.67^a	84.47±1.93	84.56±1.26	84.38±2.75
Gross gain, kg	45.09±0.99^a	45.12±1.30	45.24±1.18	44.99±1.85
Daily gain, g	920.18±20.11^a	920.86±26.54	923.36±24.08	918.14±37.73
	O v e r t h e 2nd f a t t e n i n g p e r i o d a			
n	8	9	9	9

Continued Table 5

Days on average	37	37	37	37
Live weight at the end of fattening:				
total, kg	121.33±1.59	122.50±1.90	123.86±1.48	123.22±2.28
to control, %	100.0	101.0	102.1	101.6
Gross gain, kg	37.01±0.92	38.03±0.69	39.30±0.93^{+}	38.84±0.74
Daily gain, g	1000.30±24.93	1027.93±18.58	1062.16±25.01^{+}	1049.85±19.94
Over the whole experiment				
n	8	9	9	9
Days	120	120	120	120
Gross gain, kg	103.82±1.44	105.10±1.76	106.54±1.59	105.79±1.90
Daily gain, g	865.19±12.02	875.83±14.63	887.87±13.23	881.57±15.81
Feed consumption over the whole experiment				
Total, kg	320.8	312.1	312.1	312.1
Feed per 1 kg weigh gain:	3.09	2.97	2.93	2.95
total;, kg				
o control, %	100	96.1	94.8	95.5

N o t e. For a description of the groups, see the Materials and methods section. DHQ was fed during the growing period (DHQd), during growing and fattening (DHQd+o), and during feed and technological stresses (DHQtech); [a] — the value is calculated without estimates for one pig died in the middle of the period, 07/28/2020); + means trend of differences from control at $0.05 < p \leq 0.1$.

We revealed differences in the dynamics of live weight in animals during the experiment (Table 5, Fig. 2).

Thus, growing animals fed DHQ differed from the control in the average daily weight gain by 1.5-1.7% ($p > 0.05$). According to the results of the 1st fattening period, the animals showed identical parameters of live weight gain, but one animal dropped out of the control group (because of paralysis of the heart muscle due to a moderately pronounced heat stress), for the rest ($n = 8$) growth parameters were the same as in animals of the experimental groups. In the 2nd fattening period, the animals showed similar growth parameters ($p < 0.05$), but one more animal dropped out of the control group a few days before slaughter (the consequences of stress, the hind limbs were stretched, forced slaughter was carried out). In group 3, there was a tendency ($p = 0.01$) to better weight gains (1062.2 vs. 1000.3 g in the control group), which indicated a positive effect of feeding DHQ during the final fattening period. We have established the fact of greater susceptibility to stress among the animals of the control group compared to those treated with DHQ. Losses due to the disposal of animals in the control group influenced the cost of feed for the 1st fattening period (2.9-3.5% less in the experimental groups), for the 2nd fattening period (2.7-5% and 8% less in the experimental groups) and in general for the experiment (3.9-5.2% less in groups 2-4).

When compared, there was a tendency (0.05 < p 0.1) to increase the digestibility of dry matter by 1.31% in animals treated with DHQ during the fattening period, by 0.93% in those receiving DHQ during technological stress (Table 6). The digestibility of crude fiber increased in group 3 (DHQd+o) by 3.23%. In groups 3 and 4, there was a tendency ($0.05 < p \leq 0.1$) to increase the digestibility of the feed dry matter. Changes in feed digestibility were accompanied by a reduced excretion of nitrogen in the urine (in group 2 by 21.20 g, in group 3 by 14.47 g, and in group 4 by 21.91 g) compared to control group ($p > 0.05$) (Table 7).

DHQ in the diet of pigs from groups 2, 3 and 4 contributed to less calcium excretion with faeces (by 3.48 at $p < 0.05$; 1.68 at $p > 0.05$; 2.87 g at $p = 0.06$. respectively) and its increased deposition in the body (by 3.52 at $p < 0.05$, 1.62 at $p > 0.05$, and 2.85 g at $p = 0.06$) compared to control. The proportion of used Ca from that received with food in groups fed DHQ was higher by 9.82% ($p < 0.05$), 4.52 and 7.94% ($0.05 < p \leq 0.1$), respectively. In animals from groups 3 and 4, the deposition of phosphorus was somewhat lower than in the control, but the decrease was not statistically significant.

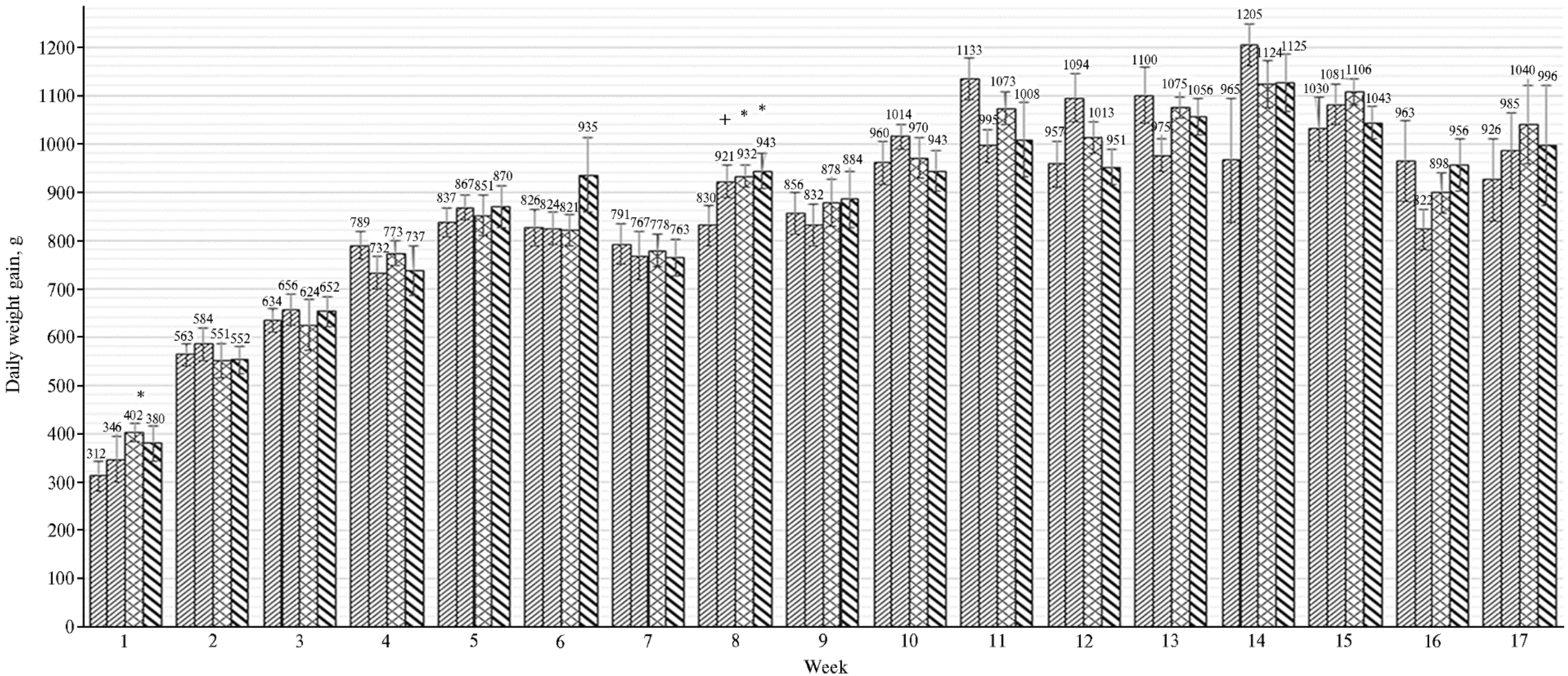

Fig. 2. Dynamics of average daily weight gain (AGA) **in pigs (*Sus scrofa domesticus*) F_2 [(Large White × Landrace) × Duroc] under simulated heat stress, as influenced by the dihydroquercetin** (DHQ) **additive:** weekly from left to right) group 1 (control), groups 2, 3, and 4. For a description of the groups, see the Materials and methods section ($n = 9$, M±SEM, physiological yard of Ernst FRC VIZH, Moscow Province, May-September 2020)).

* Differences from control are statistically significant at $p < 0.05$; + means trend at $0.05 < p \leq 0.1$.

6. Digestibility of nutrients in pigs (*Sus scrofa domesticus*) F2 [(Large White × Landrace) × Duroc] under simulated heat stress, as influenced by the dihydroquercetin (DHQ) **additive** (n = 3, M±SEM, physiological yard of Ernst FRC VIZH, Moscow Province, May-September 2020)

Nutrient	Group			
	1 (control)	2 (DHQd)	3 (DHQd +o)	4 (DHQtech)
Dry matter	73.89±0.36	75.66±0.89	75.20±0.42$^+$	74.82±0.20$^+$
Organic matter Органическое вещество	76.71±0.59	77.51±0.77	77.13±0.39	76.71±0.24
Crude protein	76.45±1.08	77.36±1.83	76.53±1.03	75.61±0.54
Crude fat	59.32±6.63	65.25±0.26	66.81±3.94	60.88±7.42
Crude fiber	40.26±2.47	40.44±1.47	43.49±2.88	40.89±2.10
Nitrogen-free extractives	81.63±0.13	82.24±0.49	81.53±0.37	81.76±0.30

N o t e. For a description of the groups, see the Materials and methods section. DHQ was fed during the growing period (DHQd), during growing and fattening (DHQd+o), and during feed and technological stresses (DHQtech); $^+$ means trend at $0.05 < p \le 0.1$.

7. Nitrogen, calcium, and phosphorus utilization in pigs (*Sus scrofa domesticus*) F2 [(Large White × Landrace) × Duroc] under simulated heat stress, as influenced by the dihydroquercetin (DHQ) **additive** (n = 3, M±SEM, physiological yard of Ernst FRC VIZH, Moscow Province, May-September 2020)

Parameter	Group			
	1 (control)	2 (DHQd)	3 (DHQd +o)	4 (DHQtech)
Nitrogen balance				
Input with feed, g	112.82	112.82	112.82	112.82
Output with faeces, g	27.79±0.38	25.54±2.06	26.48±1.17	27.52±0.61
Digested, g	85.03±0.31	87.28±1.68	86.35±0.95	85.30±0.50
Output with urine, g	60.37±11.48	39.17±8.78	45.90±8.51	38.46±7.32
Deposited in the body:				
total, g	24.66±11.77	48.11±7.23	40.45±9.64	46.84±7.28
of the input, %	21.86±10.44	42.64±6.41	35.85±8.54	41.51±6.45
of digested, %	28.91±13.69	55.46±9.20	46.57±10.60	54.92±8.61
Calcium balance				
Input with feed, g	35.83	35.83	35.83	35.83
Output with faeces, g	20.31±0.77	16.83±0.45*	18.63±1.33	17.44±0.77$^+$
Output with urine, g	0.31±0.04	0.27±0.06	0.38±0.04	0.34±0.04
Deposited in the body	15.20±0.78	18.72±0.45*	16.82±1.34	18.05±0.77$^+$
Utilized, % of the input	42.43±2.19	52.25±1.26*	46.95±3.73	50.37±2.16$^+$
Phosphorus balance				
Input with feed, g	22.53	22.53	22.53	22.53
Output with faeces, g	10.23±0.09	9.48±0.57	9.85±0.18	10.10±0.15
Output with urine, g	3.12±0.39	2.89±0.65	4.40±0.37$^+$	4.01±0.70
Deposited in the body	9.17±0.40	10.16±0.54	8.28±0.54	8.42±0.73
Utilized, % of the input	40.70±1.79	45.09±2.38	36.74±2.39	37.38±3.25

N o t e. For a description of the groups, see the Materials and methods section. DHQ was fed during the growing period (DHQd), during growing and fattening (DHQd+o), and during feed and technological stresses (DHQtech).
* Различия с контролем статистически значимы при $p < 0{,}05$; $^+$ means тенденция при $0{,}05 < p \le 0{,}1$.

Thus, pigs normally performed physiological functions when being in a neutral thermal zone. It also depends on animal age, body weight and the effective perceived temperature, which, in turn, is influenced by air movement, bedding, humidity and temperature of the walls and floor. Pigs do not sweat and have a relatively small lung capacity. Because of these physiological limitations and the relatively thick layer of subcutaneous fat, pigs are more susceptible to heat stress. Pigs with a live weight of 25, 50 and 75 kg respond differently to an increase in ambient temperature from 14 to 35 °C. The average daily live weight gain of 75 kg pigs begins to decrease at the temperature exceeding 23 °C, of 25 kg pigs at the temperature above 27°°C [1].

The concentration of ammonia, hydrogen sulfide and carbon dioxide varied during the experiment depending on the temperature and humidity conditions of the premice. When modeling moderately pronounced heat stress during the fattening period (12-15 weeks of the experiment), with an increase in the animal live weight, the concentration of ammonia increased to 16.7 mg/m^3, hydrogen sulfide up to 1.67 mg/m^3, and carbon dioxide up to 0.14 mg/m^3 (see Table 3). We believe that these values indicate the complex negative impact of the simulated heat stress on animals. The change in the microclimate of the premise together with current technological manipulations to a certain extent influence the physiological processes, reducing the adaptive capabilities of some individuals. As a result, one animal from the control group failed to acclimatize and died. Thus, the mortality of livestock was 11% in the control group vs. 0% in the rest animals.

In growing, individuals fed DHQ had greater average daily weight gains compared to control, especially during the 1st week ($p < 0.05$, group 3). In our opinion, it indicates better adaptation after distant (500 km) transportation together with simulated moderate heat stress (see Table 5, Fig. 2). This followed from the blood cortisol level which in the control exceeded the upper limit of the norm. In animals that received DHQ only during technological and feed stresses (group 4), the cortisol concentration was comparable to control while in groups 2 and 3 it corresponded to the norm. This indicates a positive role of DHQ additives. The effect of DHQ similar to that found during growing was noted in group 3 during the 1st fattening period when cortisol levels decreased to 93.5 vs. 147.6 nmol/l in the control ($p < 0.05$). Dietary DHQ led to an increase in the average daily weight gain in groups 2, 3 and 4 groups during technological stress, together with moderate simulated thermal stress. It occurs during growing (1st week, group 3, $p < 0.05$) and at vaccination against classical swine fever in the beginning of fattening (8th week, group 2, $0.05 < p \leq 0.1$; groups 3 and 4, $p < 0.05$) (see Fig. 2). Note that constantly fed dietary DHQ (group 3) ensured the live weight gain which did not decrease (as compared to the control) throughout the entire experiment. When the supplement was stopped (group 2) or periodically fed (group 4, 11th and 16th weeks of the experiment), the gains were lower than in the control.

The blood cortisol level before slaughter was the highest in the control animals (20.2% above the physiological norm). In animals that received DHQ only during growing, it also exceeded the norm. Feeding with DHQ during the entire growth and at some subsequent periods stabilized this parameter (234-253 nmol/l), indicating a positive effect of DHQ on stress resistance of animals. The best result was provided by the constant DHQ input during growth and fattening.

As is known, in response to heat stress, an animal tries to lower its body temperature by increasing sweating, respiration rate and salivation [18]. These reactions energy-consuming and, accordingly, part of the feed energy used under optimal conditions to ensure live weight gain is redirected to thermoregulation. Digestion also transforms chemical energy in feeds into thermal energy in the body, and animals change their feeding behavior [24]. Some researchers believe [25] that the primary response to heat stress in different animal species is to reduce food intake (metabolic heat reduction strategy).

It is difficult to quantify the effects of climatic stress on animal productivity compared to a normal temperature regime [1, 26, 27]. Stress-induced reduction in feed intake creates the prerequisites for a decrease in the productivity of growing animals. In recent years, the negative consequences of thermal stress in pigs have become more obvious, probably due to increased susceptibility of these animals to heat as a result of genetic selection for heat-producing traits [24]. The negative effect of heat stress on productivity is primarily explained by a decrease in feed intake, although the experimental results of recent years contradict this conclusion. In our experiments, no suppression of feed intake occurred with an increase in ambient temperature. As long as the animal consumes enough food (including dry matter and metabolizable energy) to provide growth, development and physiological response to heat stress, stress does not lead to negative consequences, but at certain periods, the body needs additional support (increased feed energy, administration of adaptogenes, etc.). It is assumed that heat stress directly and indirectly affects the physiological processes that determine the health and productivity of animals. In our experiment, we found a trend towards an increase in dry matter digestibility, by 1.31% in animals fed DHQ during fattening and by 0.93% when subjected to technological stress ($p < 0.1$). In our opinion, it is explained by DHQ-mediated activation of metabolism due to the antioxidant properties of the adaptogene, by optimization of energy consumption with a moderate increase in ambient temperature, and alteration of metabolic priorities through direct or indirect mechanisms. At large pig breeding complexes, it was found [28] that about 5% of the nitrogen used as feed is emitted as NH_3 and another 1% with wastewater. Our data, including those obtained previously [5, 12], suggest that in growing pigs, feeding dietary DHQ may promote better utilization and deposition of nitrogen due to less excretion in the urine. Further studies of the influence of DHQ on the nitrogen compound redistribution in heat-stressed pigs will confirm or refute our assumption. In intensive pig breeding, the DHQ used constantly or in courses under stresses can increase pig adaptability and prevent the undesirable effects of technological, feed, transport and heat stresses. Improving the health of animals increases their livability and stabilizes daily weight gain under stress.

Suray and Fisinina [13] showed that immediately after the temperature impact on chickens, their growth slows down but then compensatory growth follows which contributes to a higher final live weight in broilers compared to birds not subjected to thermal training. That is, short-term stressful situations train animals, causing them to have a physiological response to stress. Long-term chronic stress, even of a moderate strength, worsens the growth performance and leads to premature retirement of the livestock, which was also confirmed by our findings. Dietary adaptogenes can neutralize the negative effects of stress and improves adaptive reactivity of animals [29, 30]. Previously, we have shown that dietary DHQ prevents negative effect of simulated technological stress on metabolism, including lipid peroxidation, stimulates anabolic processes, positively affectes the clinical health and nonspecific resistance of animals. In pigs fed DHQ, the level of cortisol, a hormone involved in the development of stress reactions was the lowest (134 nmol/l during growth and 215 nmol/l at the final fattening) [30], which additionally confirms our data of 2020. There are several aspects of the leveling effect of dietary adaptogens under various stresses. Phytogenic feed additives with a high content of antioxidants may reduce oxidative stress in pigs caused, among other reasons, by thermal exposure of pigs [31]. Feeding gamma-aminobutyric acid provides better performance due to improved functions of the nervous system and increased stress resistance of piglets [32]. Research has shown that targeted bioactives reduce adrenaline production and increase growth hormone production in piglets [32], resulting in consuming more feed and wasting less time and energy on aggressive behavior and associated stress. Immunoprophylaxis of various stresses with immunotropic drugs can reduce the age of the first insemination in gilts and increase their fertility [33, 34]. Normotimics and adaptogens may accelerate animal growth and improve the quality of meat products [35, 36].

Thus, a reduced heat generation and proper intake of dietary nutrients help to mitigate the effects of various stresses in pigs. We suggest an integrated approach to prevent negative effect of heat stress in pigs. It includes breeding animals for heat tolerance; the use of special anti-stress feed additives (with thermal exposure, changes in the feeding regime, transportation and other stress factors); proper ventilation and air cooling regime in the premise. Timely forecasting of heat and other stresses and adequate preventive measures will help to avoid negative consequences of stresses for young animals and economic losses under intensive pig farming.

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