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## ROLE OF FEED ADAPTOGENS IN FORMING THE CONCEPT OF ORGANIC PIG PRODUCTION

Roman V. Nekrasov<sup>1,\*</sup>, Nadezhda V. Bogolyubova<sup>1</sup>, Magomed G. Chabaev<sup>1</sup>, Konstantin S. Ostrenko<sup>1</sup>, Roman A. Rykov<sup>1</sup>, Anastasia A. Semenova<sup>2</sup>, Viktoria V. Nasonova<sup>2</sup>

<sup>1</sup> L.K. Ernst Federal Research Center for Animal Husbandry, Podolsk, Moscow Region, Russia

<sup>2</sup> V.M. Gorbatov Federal Research Center for Food Systems, Moscow, Russia

### KEY WORDS:

*taxifolin, vitamins, stress, feeding method, animal adaptation, productivity, meat quality*

### ABSTRACT

One of the priorities of the Strategy of scientific and technological development of the Russian Federation (approved by Presidential Decree № 642 of December 1, 2016) is the transition to a highly productive and environmentally friendly agriculture. Organic agriculture can help to ensure sufficient food for the population by 2050 and simultaneously reduce the impact of agriculture on the environment only if it is implemented as part of a system of agricultural production, which should provide the same volume and composition of products as in the baseline scenario. An important aspect of organic livestock production is to reduce the impact of stress factors on animals. A promising solution can be the use of natural biologically active substances with pronounced antioxidant properties. The action of natural bioflavonoids (Taxifolin) and also in a complex with vitamins on the organism of fattening pigs during the periods of stress of various etiology (climatic, fodder, social etc.) has been studied. A complex including vitamin C, vitamin E and Taxifolin has been developed. The provisions of the method of feeding pigs as a way to reduce the negative impact of stress factors on meat quality by means of the studied nutritional factor have been studied experimentally.

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## РОЛЬ КОРМОВЫХ АДАПТОГЕНОВ ПРИ ФОРМИРОВАНИИ КОНЦЕПЦИИ ОРГАНИЧЕСКОГО ПРОИЗВОДСТВА ПРОДУКЦИИ СВИНОВОДСТВА

Некрасов Р. В.<sup>1</sup>, Боголюбова Н. В.<sup>1</sup>, Чабаев М. Г.<sup>1</sup>, Остренко К. С.<sup>1</sup>, Рыков Р. А.<sup>1</sup>, Семенова А. А.<sup>2</sup>, Насонова В. В.<sup>2</sup>

<sup>1</sup> Федеральное исследовательское учреждение животноводства — ВИЖ имени Академика Л. К. Эрнста, Подольск, Московская область, Россия

<sup>2</sup> Федеральное научное учреждение пищевых систем им. В. М. Горбатова, Москва, Россия

### КЛЮЧЕВЫЕ СЛОВА:

*таксифолин, витамины, стресс, способ кормления, адаптация животных, продуктивность, качество мяса*

### АННОТАЦИЯ

Одним из приоритетов Стратегии научно-технологического развития Российской Федерации (утверждена Указом Президента Российской Федерации от 1 декабря 2016 г. № 642) является переход к высокопродуктивному и экологически чистому агрохозяйству. Органическое сельское хозяйство может способствовать обеспечению достаточного количества продовольствия для населения к 2050 году и одновременному снижению воздействия сельского хозяйства на окружающую среду только в том случае, если оно будет реализовано в рамках системы сельскохозяйственного производства, которая должна обеспечивать те же объемы и состав продукции, что и при базовом сценарии. Важным аспектом получения органической животноводческой продукции является снижение воздействия факторов стресса на животных. Поэтому существует острая необходимость в разработке эффективных и устойчивых подходов к управлению для смягчения негативных последствий стрессов и повышения эффективности конверсии корма при получении свинины более высокого качества. Перспективным решением может быть применение природных биологически активных веществ с выраженными антиоксидантными свойствами, которые могут посредством стабилизации свободнорадикального окисления повышать адаптационные свойства организма к воздействию стресс-факторов среды. Изучено действие природных биофлавоноидов (таксифолин), а также в комплексе с витаминами на организм откармливаемых свиней в периоды стрессов различной этиологии (климатического, кормового, транспортного, социального и т. д.). Разработан комплекс, включающий витамин С, витамин Е, таксифолин. Экспериментальным путем изучены положения способа кормления свиней, как способа снижения негативного влияния стресс-факторов на качество мяса посредством изученного фактора питания.

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## 1. Introduction

In the practice of animal husbandry and, in particular, pig breeding, there are stresses of different etiology associated with feeding and housing conditions, climatic conditions, physiological characteristics of the organism. Under chronic exposure to various stress situations there is an activation of free radical oxidation with simultaneous depletion of antioxidant protection. The body forms the syndrome of stress maladaptation, ketosis, hepatodystopia, autoimmune processes [1].

There are known studies on the use of various biologically active substances — adaptogens, in particular, antioxidants, trace elements, bioflavonoids, vitamins and other substances, in pig feeding. Vitamins are organic compounds necessary for normal life activity of organisms, some of them are simultaneously antioxidants.

It is known that antioxidants are added to feed or feed ingredients to inhibit lipid oxidation processes, the development of which can lead to changes in the chemical composition, nutritional and energy value of feed, the appearance of foreign flavors (rancidity, sedimentation), loss of important substances in the diet of animals, especially unsaturated fatty acids and several vitamins [2]. Vitamin E, Vitamin C and Se are effective antioxidants that help increase the antioxidant status of the body [3,4].

Fat-soluble vitamin E (D-alpha-tocopherol) is widely used in premixes for animals, but often its insufficient content in feed and stress loading of animals is not taken into account. Vitamin E is a universal protector of cell membranes and an effective immunomodulator that activates the immune system. Vitamin E is also important for the formation of catalase and peroxidase, which neutralize peroxides, which is necessary for adequate immune response of the body. The vitamin E action develops gradually over several weeks of intake, therefore vitamin E must be given as long term doses [5].

The oxidized form of vitamin E is reduced by Vitamin C, and Vitamin E can again perform the function of an antioxidant [6].

Water-soluble vitamin C (L-ascorbic acid), entering the animal's body with food, does not accumulate and is quickly eliminated from the organism. For this reason, its content in the body must be constantly replenished. As an antioxidant, ascorbic acid performs the biological functions of reducing agent and coenzyme of a number of metabolic processes. It is an important substance in the diet of animals, necessary for normal development and functioning of all body systems, including bone and connective tissue [7].

Vitamin C participates in biological redox reactions of the body, has antiradical properties, which causes inhibition of the process of peroxidation of proteins, lipids and other components of cells and their protection from damage, has membrane stabilizing and immunomodulatory effects [8].

It is known that polyphenols (flavonoids) have pronounced antioxidant properties, similar to vitamin E, such as protection from reactive oxygen species, chelation of metal ions and induction of antioxidant enzymes [9]. Our 2019–2021 studies showed that the use of adaptogens-bioflavonoids (in particular, Taxifolin) during periods of stress of various etiologies is reasonable [10,11,12,13].

However, the complete replacement of tocopherols in the diet with polyphenols is questionable. Polyphenols cannot replace the unique antioxidant function of vitamin E, which, due to its lipophilic structure, is embedded in biological membranes and effectively neutralizes fatty acid radicals and AFC [14].

Based on the analysis of information obtained from public sources, as well as the norms of using vitamins in feeding pigs [15], taking into account their synergistic effect on activation of antioxidant protection of the body, immunostimulation; as well as taking into account the previously obtained results of works,

a complex of biologically active substances including Taxifolin, Vitamin E and Vitamin C (TaxEC) was developed. The aim of the research was to study the effectiveness of using a new complex of biologically active substances to provide an antioxidant effect on pigs during the fattening period against the background of environmental stressors.

## 2. Materials and Methods

For the scientific experimentation 30 clinically healthy cross-bred castrated young boars (F-2:(LW×L)×D) were used. The age of piglets at the beginning of the experiment was 60 days. According to the principle analogue scale, the animals are divided into two groups, based on the body weight (Table 1).

Table 1. Experimental design

Таблица 1. Дизайн эксперимента

Groups	Number of animals	Feeding characteristics
1 Control (C)	15	Basic diet (BD)
2 Experimental (E)	15	BD + Feeding complex (Taxifolin with vitamins C and E, TaxEC)*

\* The composition of the additive is currently under registration. The experimental party contained Tax ("Ekostimul-2", JSC Ametis, Russia), vitamin E (INNOVIT E60, GC "MEGAMIKS", Russia), vitamin C (Tiger C35, "Anhui Tiger Biotech Co. Ltd.", China). The claim of the invention is stated in the application for the invention "Method of feeding young pigs during the feeding period" No. 2022129798/20(065177), filing date 17.11.2022.

The basis of diet was mixed fodder SK-4 (during pig growing), SK-5 (during 1st period of fattening) and SK-6 (during final fattening) (Table 2), balanced by nutrients and energy according to modern standards and recommended feeding regime [15]. TaxEC was fed with mixed fodder to pigs of the experimental group (0.25% of the feed composition).

The research was conducted in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123, Strasbourg, 1986). The research was approved by the bioethical commission of the L. K. Ernst Federal Research Center for Animal Husbandry (Protocol No. 6, dated May 27, 2022).

The production area where the experiment was conducted had all necessary equipment, tools, and feed distribution system. All animals were kept under the same conditions and had free access to feed and water during the whole period of the experiment. The conditions of housing and care of the animals were identical.

When simulating environmental conditions, temperature ranged from 14.0 to 32.0 °C (in sections up to 33.0 °C), and relative humidity from 60 to 85%.

Growth rate of the experimental animals was monitored by individual weighing on electronic scales REUS-300 (Tenzosila LLC, Russia) before the experiment and then every 7 days until its end. Gross and average daily gains, as well as feed expenses per growth unit were calculated based on the results of weighing and feed consumption.

At the beginning (n=5), in the middle (n=5) of fattening and before slaughter (n=5) blood from the jugular veins was sampled in vacuum tubes. Serum was separated by centrifugation (3000 rpm for 15 min) and stored at -20 °C until analysis. Stress markers (glucose (GLU), triglycerides (TG), lactate dehydrogenase (LDG), creatine phosphokinase (CPK), aspartate aminotransferase (AST), were examined on a BioChemFC-360 automated analyzer (HTI, USA) according to the procedures enclosed with the reagents (HTI, USA). The antioxidant status of the blood was determined on the basis of the following parameters:

total antioxidant status (TAS) and total amount of water-soluble antioxidants (TAWSA) in blood by amperometric method on a Color-Yauza-01-AA device (NPO Khimavtomatika, Russia).

Control slaughter of pigs with determination of: body weight before slaughter, carcass weight, fat thickness (over spinous processes between 6th and 7th thoracic vertebrae, excluding the thickness of the skin, mm); slaughter yield (ratio of carcass weight to body weight before slaughter) was conducted in accordance with the “Rules of veterinary inspection of slaughter animals and veterinary and sanitary examination of meat and meat products”. Meat quality indicators: determination of moisture-holding capacity (MHC) by Grau and Hamm pressing method with Volovinskaya modification; total amount of water-soluble antioxidants (TAWSA) — as per Tsvet-Yauza-01-AA (NPO Khimavtomatika, Russia) device by amperometric method.

Data were analyzed using a one-way ANOVA analysis of variance using STATISTICA statistical software (Version 13RU, StatSoft Inc., USA). Differences of  $p < 0.05$  were considered statistically significant, whereas a trend was considered when  $0.05 \leq p < 0.1$ .

### 3. Results

Against the background of feeding the TaxEC during the period of rearing, the ADG in group 2 were higher than in group 1 by 2.5% ( $p > 0.05$ ). At the same time, in the first week there was a tendency to better growth of animals in the experimental group ( $p < 0.10$ ). By simulating the conditions, the temperature optimum (18–20 °C) was exceeded to 32–33 °C during the first fattening period. On this background, the animals of

Table 2. Live weight of pigs (kg) and feed consumption for the whole period of the experiment ( $M \pm m$ ,  $n = 15$ )

Таблица 2. Живая масса свиней (кг) и потребление корма за весь период эксперимента ( $M \pm m$ ,  $n = 15$ )

Indicator	Groups		p-value
	C	E	
Growing period			
Duration of period, days	35	35	—
BW at staging, kg	15.87 ± 0.42	15.92 ± 0.25	0.92
BW at the end of the period, kg	36.37 ± 1.02	36.93 ± 1.04	0.69
Gross gain, kg	20.50 ± 0.91	21.01 ± 0.98	0.69
ADG, g	585.71 ± 25.87	600.38 ± 28.0	0.69
1st fattening period			
Duration of period, days	42	42	—
BW at the end of the period, kg	77.69 ± 1.48	80.16 ± 1.39	0.22
Gross gain, kg	41.32 ± 0.98	43.23 ± 0.56	0.09
ADG, g	983.81 ± 23.26	1029.21 ± 13.39	0.09
2nd fattening period			
Duration of period, days	37	37	—
BW at the end of the period, kg	125.27 ± 1.89	127.21 ± 1.34	0.39
Gross gain, kg	46.70 ± 1.27	46.35 ± 0.83	0.81
ADG, g	953.06 ± 25.85	945.85 ± 16.98	0.58
For the whole period of experiment			
Duration of period, days	126	126	—
Gross gain, kg	108.52 ± 1.91	110.59 ± 1.29	0.36
ADG, g	861.27 ± 15.17	877.67 ± 10.26	0.36
Feed expenses for the whole period of experiment			
Fodder, kg/kg gain	3.14	3.08	—
% to control	100	98.1	—
Safety, %			
For the whole period of experiment	100.0	100.0	—

Compiled by the authors

experimental group against the background of simulated high ambient temperature also showed the tendency ( $p = 0.09$ ) to better growth — 1029,21 vs. 983,81 g in the control, or by 4,6%. During the 8th week of experiment, the growth of experimental group animals was reliably higher than the control values ( $p = 0.03$ ), indicating the effectiveness of feeding TaxEC during the temperature stress. In the 2nd period of fattening, the animals showed similar growth parameters ( $p > 0.05$ ) — 945,85 g vs. 953,06 g in control, or lower by 0,8%. On the whole the gains during the experiment were 877,67 vs. 861,27 in the control, the animals of the control group were more exposed to the environment stress-factors during the fattening period, but later on they slightly improved their results.

Additional feeding of pigs with TaxEC resulted in the improvement of metabolic processes, first of all, it was reflected in the increase of antioxidant protection, better adaptability of animals to the conditions of temperature stress, which was characterized by the level of stress markers (Table 3).

Feeding TaxEC was effective already in the early periods of rearing and fattening, and further feeding led to the preservation of general antioxidant status and improved the formation of product characteristics (slaughter performance, meat quality) (Table 4).

Table 3. Blood serum indices of experimental animals ( $M \pm m$ )

Таблица 3. Показатели сыворотки крови экспериментальных животных ( $M \pm m$ )

Indicator	Groups		p-value
	C	E	
The rearing period ( $n = 5$ )			
GLU, mmol/l	5,22 ± 0,24	5,40 ± 0,23	0,75
TG, mmol/l	0,17 ± 0,02	0,21 ± 0,02	0,43
LDH, U/l	207,21 ± 29,00	201,05 ± 27,48	0,93
CPK, U/l	815,18 ± 203,89	786,58 ± 98,89	0,94
AST, U/l	29,73 ± 0,66	28,67 ± 1,28	0,66
CREA, μMol/L	102,26 ± 1,99	110,04 ± 1,50†	0,09
TAS, mmol/l	1,55 ± 0,05	1,39 ± 0,04*	<0.05
TAWSA, mg/l	19,01 ± 1,53	14,17 ± 1,05*	<0.05
The 1 <sup>st</sup> period of fattening ( $n = 5$ )			
GLU, mmol/l	5,67 ± 0,45	5,57 ± 1,02	0,96
TG, mmol/l	0,22 ± 0,02	0,22 ± 0,02	0,96
LDH, U/l	325,65 ± 27,39	187,82 ± 29,83†	0,07
CPK, U/l	1845,67 ± 503,94	1573,54 ± 336,29	0,79
AST, U/l	35,94 ± 2,08	32,46 ± 3,08	0,58
CREA, μMol/L	129,19 ± 2,45	133,53 ± 2,22	0,44
TAS, mmol/l	1,47 ± 0,04	1,65 ± 0,08*	<0.05
TAWSA, mg/l	11,69 ± 0,93	12,99 ± 0,52	>0.05
The 2 <sup>nd</sup> period of fattening ( $n = 5$ )			
GLU, mmol/l	6,15 ± 0,14	5,95 ± 0,05	0,44
TG, mmol/l	0,21 ± 0,03	0,15 ± 0,02	0,39
LDH, U/l	374,51 ± 3,36	343,96 ± 26,94	0,50
CPK, U/l	2617,40 ± 344,39	1290,00 ± 144,62†	0,06
AST, U/l	59,16 ± 4,69	39,14 ± 1,15	0,03
CREA, μMol/L	145,16 ± 2,50	148,27 ± 2,54	0,60
TAS, mmol/l	1,47 ± 0,06	1,60 ± 0,02†	0,10
TAWSA, mg/l	11,31 ± 0,67	11,61 ± 1,01	>0.05

GLU, glucose; TG, triglycerides; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; AST, aspartate aminotransferase; CREA, creatinine; TAS, total antioxidant status; TAWSA, total amount of water-soluble antioxidants. Significantly at \* —  $p < 0.05$  compared to the control; † — tendency at  $p < 0.10$ .  
Compiled by the authors



Table 4. Pre-slaughter and slaughter performance of pigs (M ± m, n = 15)

Таблица 4. Предубойные и убойные показатели свиней (M ± m, n = 15)

Indicator	Groups		p-value
	C	E	
Weight of a fresh carcass, kg	93,30 ± 1,58	94,50 ± 1,43	0,49
Slaughter yield, %	74,43 ± 0,45	74,38 ± 0,38	0,85
Fat thickness between 6th and 7th thoracic vertebrae, mm	23,53 ± 0,98	23,50 ± 2,03	0,78
Fat thickness on loin, mm	16,80 ± 0,77	15,29 ± 1,36	0,33
pH of the longest muscle of back, 45 min, units	5,79 ± 0,08	5,84 ± 0,11	0,57
pH of the longest muscle of the back, 24 hours, units	5,44 ± 0,01	5,52 ± 0,03**	0,01
Area of muscle eye, cm <sup>2</sup>	46,52 ± 1,62	49,26 ± 2,35	0,22
MHC, %	81,62 ± 1,09	82,54 ± 1,41	0,87
TAWSA, mg/g	0,081 ± 0,002	0,0083 ± 0,002	0,60

MHC, moisture-holding capacity; TAWSA, total amount of water-soluble antioxidants. Significantly at \*\* – p < 0.01 compared to the control. Compiled by the authors

#### 4. Discussion

The use of combinations of various antioxidant-adaptogens has been extensively studied in the poultry industry [16]. Regarding applications in swine production, it has been reported that studies on the effects of Vitamins E and C in stressed pigs are necessary elements that can form part of a strategy to improve animal health and productivity [17].

Positive effects of polyphenol supplementation have been observed in studies with sick or stressed animals and have been attributed to their systemic anti-inflammatory effects, improved gut health and reduced translocation of pro-inflammatory and pro-oxidant stimuli into the bloodstream. The health effects of bioflavonoids likely result not only from direct antioxidant activity, but also include inhibition of radical-forming enzymes such as xanthine oxidase, NOX and lipoxigenase, in addition to effects on platelet aggregation, leukocyte adhesion and vasodilatory properties. Flavonoids have different biological activities in different cells, tissues and disease states [18].

The antioxidant properties of flavonoids are convincing and in a number of cases they were even more effective than traditional antioxidants such as vitamins E and C [14]. Attempts to completely replace vitamins (particularly E) in animal diets with various plant extracts containing flavonoids were found to be unsuccessful. At the same time, the need to clarify the effective doses of polyphenols in studies on farm animals was noted [19]. Therefore, we chose the strategy of using a combination of the action of polyphenols with vitamins E and C.

Additional feeding Tax pigs in combination with vitamins (C and E) led to improvement of metabolic processes, first of all, it was reflected in the increase of antioxidant protection, better adaptability of animals to the conditions of temperature stress.

Stress metabolites such as lactate and glucose in blood can be useful indicators to assess pre-slaughter stress, and their high levels in blood indicate an accelerated rate and high degree of postmortem muscle metabolism, resulting in low muscle pH, while carcass temperature remains high, which causes a higher incidence of pale, soft and exudative (PSE) pork [20]. Glucose levels in animals of control and experimental groups were within normal values. Feeding TaxEC had no effect on this parameter in this case. At the same time, the level of triglycerides in animals of both groups was ≤ 0.22 mmol/l, which confirms the high level of animal stress throughout the experiment.

Evaluation of the potential use of various biomarkers of physiological stress in slaughter pigs is important in terms of predicting carcass and meat quality parameters. According to [21] LDH

can help pork producers predict changes in pork quality, while cortisol, alanine aminotransferase and albumin can be useful in predicting carcass quality. In our experiment, LDH activity was normal in animals of groups C and E throughout the experiment, but during the temperature stress (mid-fattening), there was a tendency (p = 0.07) to decrease this index in animals of group E. Elevated CPK content was observed in all animals throughout the whole experiment, but the highest level was in the middle and end of fattening. In group E, this index was insignificantly lower in the beginning and middle of fattening, and at the end of the experiment there was a tendency to lower values (p = 0.06), which indicates a favorable protective role of antioxidants.

AST is a marker of liver and cardiovascular damage, and CREA characterizes the rate of creatine phosphokinase reaction and the rate of muscle mass gain. AST and CREA values were normal in all animals throughout the experiment. At the end of the experiment in Group E AST was reliably lower (p < 0.01) and there was a tendency (p = 0.09) to a higher CREA level, indicating a positive effect of TaxEC on the animals' condition against the background of better growth.

It was found that combining Tax with vitamins C and E enhanced the mechanism of antioxidant protection. For instance, at the beginning of the experiment, there was a loss of water-soluble forms of antioxidants (TAWSA) (p < 0.01) (Table 3), but later, their level in the blood of experimental animals exceeded the control level, indicating a better protective mechanism against environmental factors (including temperature stress during the period of fattening).

The effect of TaxEC was clearly manifested at the end of the experiment. In pigs of Group E, the level of TAS in blood serum became higher (p < 0.05) during basic fattening and before slaughter (p = 0.1) as compared to the control under the influence of TaxEC feeding.

The slaughter yield in Group E was 74.38% vs. 74.43% (p > 0.05) in Group C (Table 4). Slaughter weight correlated with paired carcass weight without head and legs (r = 0.34 and r = 0.46, respectively groups), whose mean values were 1.29% higher in Group E (p = 0.49). The thickness of the backbone, both at the level between the 6th and 7th thoracic vertebrae, and in the lumbar part of the carcass were: 23.50 vs. 23.53 mm (p = 0.78) and 15.29 vs. 16.80 mm (p = 0.33), respectively. Muscle eye area was higher in animals of the experimental group by 2.74 cm<sup>2</sup> (p = 0.22).

The pH value of the longest muscle back 45 minutes after slaughter was relatively the same for carcasses of groups C and E, 5.79 and 5.84 (p = 0.57), respectively. Anaerobic glycolysis produces lactate, which accumulates and lowers intracellular pH, so that by 24 hours after death, pH can fall to about 5.4–5.7 [22]. The use of TaxEC resulted in an improvement of this index. 24 h after slaughter, the pH in the experimental group was higher by 0.07 units (p < 0.01).

Prolonged chronic stress, even at a moderate level of exposure, can worsen the growth of animals and lead to a decrease in the quality of production. Tax in combination with vitamins can regulate in animals through suppression of excessive activation of the RAAS system, improve water and sodium retention, reduce inflammatory response [23]. Tax inhibits free-radical oxidation of both water-soluble and fat-soluble substrates and can function as a trap for reactive oxygen species and as a chelator of variable valence metals [24]. Complex feeding of Tax with vitamins E and C inhibited oxidation reactions, which resulted in the accumulation of water-soluble antioxidant reserves in the animals over time. The results of control slaughter testified to obtaining the carcasses from the Group E with higher values of the following indices: slaughter yield, muscle eye area, pH of the longest muscle of the back 24 hours after slaughter demonstrating the correctness of the chosen strategy.

Thus, the study and application of poorly studied feedstuffs, as well as combining them with well-studied ones, is important to reduce the exposure of animals to stress without significantly affecting the characteristics of organic production.

## 5. Conclusions

One of the priorities of the Strategy of scientific and technological development of the Russian Federation is the transition to a highly productive and environmentally friendly agriculture. Organic agriculture can help to ensure sufficient food for the population by 2050 and simultaneously reduce the impact of agriculture on the environment only if it is implemented within the system of agricultural production, which

should provide the same volume and composition of products, as in the baseline scenario. The use of targeted feed additives to reduce the effects of stressors is the most convenient and cost-effective method of reducing their impact on the animal body. In the given research we considered feeding young pigs including feeding them with complete fodder a micro additive consisting of vitamins complex (C and E) and Taxifolin during the period of fattening against the background of stresses of different etiology. The implementation of this strategy contributes to a better adaptation of animals to environmental conditions and can contribute to obtaining products of higher quality when implementing the direction of development of pig breeding.

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AUTHOR INFORMATION	СВЕДЕНИЯ ОБ АВТОРАХ
Affiliation	Принадлежность к организации
<p><b>Roman V. Nekrasov</b>, Doctor of Agricultural Sciences, Professor of RAS, Chief Researcher, Department of Feeding of Farm Animals, L. K. Ernst Federal Research Center for Animal Husbandry Dubrovitsy 60, Podolsk Municipal District, Moscow Region, 142132 Russia Tel.: +7-496-765-12-77 E-mail: nek_roman@mail.ru ORCID: <a href="https://orcid.org/0000-0003-4242-2239">https://orcid.org/0000-0003-4242-2239</a> * corresponding author</p>	<p><b>Некрасов Роман Владимирович</b> — доктор сельскохозяйственных наук, профессор РАН, главный научный сотрудник, отдел кормления сельскохозяйственных животных, Федеральный исследовательский центр животноводства — ВИЖ имени академика Л. К. Эрнста 142132, Московская область, Городской округ Подольск, поселок Дубровицы, 60 Тел.: +7-496-765-12-77 E-mail: nek_roman@mail.ru ORCID: <a href="https://orcid.org/0000-0003-4242-2239">https://orcid.org/0000-0003-4242-2239</a> * автор для переписки</p>
<p><b>Nadezhda V. Bogolubova</b>, Doctor of Biological Sciences, Leading Researcher, Department of Physiology and Biochemistry of Farm Animals, L. K. Ernst Federal Research Center for Animal Husbandry Dubrovitsy 60, Podolsk Municipal District, Moscow Region, 142132 Russia Tel.: +7-496-765-11-69 E-mail: 652202@mail.ru ORCID: <a href="https://orcid.org/0000-0002-0520-7022">https://orcid.org/0000-0002-0520-7022</a></p>	<p><b>Боголюбова Надежда Владимировна</b> — доктор биологических наук, ведущий научный сотрудник, отдел физиологии и биохимии сельскохозяйственных животных, Федеральный исследовательский центр животноводства — ВИЖ имени академика Л. К. Эрнста 142132, Московская область, Городской округ Подольск, поселок Дубровицы, 60 Тел.: +7-496-765-11-69 E-mail: 652202@mail.ru ORCID: <a href="https://orcid.org/0000-0002-0520-7022">https://orcid.org/0000-0002-0520-7022</a></p>
<p><b>Magomed G. Chabaev</b>, Doctor of Agricultural Sciences, Professor, Leading Researcher, Department of Feeding of Farm Animals, L. K. Ernst Federal Research Center for Animal Husbandry, Dubrovitsy 60, Podolsk Municipal District, Moscow Region, 142132 Russia Tel.: +7-496-765-12-90 E-mail: chabaev.m.g-1@mail.ru ORCID: <a href="https://orcid.org/0000-0003-1889-6063">https://orcid.org/0000-0003-1889-6063</a></p>	<p><b>Чабаев Магомед Газиевич</b>, доктор сельскохозяйственных наук, профессор, ведущий научный сотрудник, отдел кормления сельскохозяйственных животных, Федеральный исследовательский центр животноводства — ВИЖ имени академика Л. К. Эрнста, 142132, Московская область, Городской округ Подольск, поселок Дубровицы, 60 Тел.: +7-496-765-12-90 E-mail: chabaev.m.g-1@mail.ru ORCID: <a href="https://orcid.org/0000-0003-1889-6063">https://orcid.org/0000-0003-1889-6063</a></p>
<p><b>Konstantin S. Ostrenko</b>, Doctor of Biological Sciences, Leading Researcher, Laboratory of Immunobiotechnology and Microbiology, L. K. Ernst Federal Research Center for Animal Husbandry, Dubrovitsy 60, Podolsk Municipal District, Moscow Region, 142132 Russia Tel.: +7-496-765-12-90 E-mail: ostrenkoks@gmail.com ORCID: <a href="https://orcid.org/0000-0001-8862-3648">https://orcid.org/0000-0001-8862-3648</a></p>	<p><b>Остренко Константин Сергеевич</b> — доктор биологических наук, ведущий научный сотрудник, лаборатория иммунобиотехнологии и микробиологии, Федеральный исследовательский центр животноводства — ВИЖ имени Академика Л. К. Эрнста 142132, Московская область, Городской округ Подольск, поселок Дубровицы, 60 Тел.: +7-496-765-12-90 E-mail: ostrenkoks@gmail.com ORCID: <a href="https://orcid.org/0000-0001-8862-3648">https://orcid.org/0000-0001-8862-3648</a></p>
<p><b>Roman A. Rykov</b>, Senior Researcher, L. K. Ernst Federal Research Center for Animal Husbandry Dubrovitsy 60, Podolsk Municipal District, Moscow Region, 142132 Russia Tel.: +7-496-765-11-69 E-mail: brukw@bk.ru ORCID: <a href="https://orcid.org/0000-0003-0228-8901">https://orcid.org/0000-0003-0228-8901</a></p>	<p><b>Рыков Роман Анатольевич</b>, старший научный сотрудник, отдел физиологии и биохимии сельскохозяйственных животных, Федеральный исследовательский центр животноводства — ВИЖ имени академика Л. К. Эрнста 142132, Московская область, Городской округ Подольск, поселок Дубровицы, 60 Тел.: +7-496-765-11-69 E-mail: brukw@bk.ru ORCID: <a href="https://orcid.org/0000-0003-0228-8901">https://orcid.org/0000-0003-0228-8901</a></p>
<p><b>Anastasia A. Semenova</b>, Doctor of Technical Sciences, Professor, Deputy Director, V. M. Gorbатов Federal Research Center for Food Systems 26, Talalikhina str., 109316, Moscow, Russia Tel.: +7-495-676-95-11 (105) E-mail: a.semenova@fncps.ru ORCID: <a href="https://orcid.org/0000-0002-4372-6448">https://orcid.org/0000-0002-4372-6448</a></p>	<p><b>Семенова Анастасия Артуровна</b>, доктор технических наук, профессор, заместитель директора, Федеральный научный центр пищевых систем им. В. М. Горбатова 109316, г. Москва, ул. Талалихина, 26 Тел.: +7-495-676-95-11 (105) E-mail: a.semenova@fncps.ru ORCID: <a href="https://orcid.org/0000-0002-4372-6448">https://orcid.org/0000-0002-4372-6448</a></p>
<p><b>Victoria V. Nasonova</b>, Doctor of Technical Sciences, Head of Department of Applied Scientific and Technological Development, V. M. Gorbатов Federal Research Center for Food Systems 26, Talalikhina str., 109316, Moscow, Russia Tel.: +7-495-676-95-11 (307) E-mail: v.nasonova@fncps.ru ORCID: <a href="https://orcid.org/0000-0001-7625-3838">https://orcid.org/0000-0001-7625-3838</a></p>	<p><b>Насонова Виктория Викторовна</b>, доктор технических наук, руководитель отдела научно-прикладных и технологических разработок, Федеральный научный центр пищевых систем им. В. М. Горбатова 109316, г. Москва, ул. Талалихина, 26 Тел.: +7-495-676-95-11 (307) E-mail: v.nasonova@fncps.ru ORCID: <a href="https://orcid.org/0000-0001-7625-3838">https://orcid.org/0000-0001-7625-3838</a></p>
<p><b>Contribution</b></p>	<p><b>Критерии авторства</b></p>
<p>Authors are equally relevant to the writing of the manuscript, and equally responsible for plagiarism.</p>	<p>Авторы в равных долях имеют отношение к написанию рукописи и одинаково несут ответственность за плагиат.</p>
<p><b>Conflict of interest</b></p>	<p><b>Конфликт интересов</b></p>
<p>The authors declare no conflict of interest.</p>	<p>Авторы заявляют об отсутствии конфликта интересов.</p>