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СИБИРСКИЙ ВЕСТНИК СЕЛЬСКОХОЗЯЙСТВЕННОЙ НАУКИ

SIBIRSKII VESTNIK SEL'SKOKHOZYAISTVENNOI NAUKI

УЧРЕДИТЕЛИ: СИБИРСКИЙ ФЕДЕРАЛЬНЫЙ НАУЧНЫЙ ЦЕНТР АГРОБИОТЕХНОЛОГИЙ РОССИЙСКОЙ АКАДЕМИИ НАУК
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ВЛИЯНИЕ АММОНИЙНОЙ И КАЛИЙНОЙ СЕЛИТРЫ НА ЗИМОСТОЙКОСТЬ И УРОЖАЙНОСТЬ РЖИ ОЗИМОЙ (*SECALE CEREALE* L.)

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Представлены результаты исследований за 3 года (2019–2021) на посевах ржи озимой (*Secale cereale* L.) в Северо-Западном регионе Российской Федерации (Ленинградская область). Изучалось влияние различных доз и сроков внесения аммонийной и калийной селитры на зимостойкость и продуктивность растений озимой ржи на примере сортов Волхова и Эврика. Почва на опытных полях – дерново-подзолистая суглинистая с глубиной пахотного горизонта 20–25 см. Содержание гумуса – 2,0–2,3%, pH солевой вытяжки – 5,2–5,5. Удобрения вносили в сухом виде осенью через неделю после появления всходов, весной – при возобновлении вегетации. Изучаемые дозы удобрений в пересчете на действующее вещество по азоту составляли 0 (контроль), 30, 60 и 90 кг/га. Показано, что зимостойкость обоих сортов практически не зависела от доз и сроков внесения, а также от вида удобрений. Максимальную урожайность зерна сорт Волхова показал при дозе азота по действующему веществу 60 кг/га, сорт Эврика – 90 кг/га, при этом максимальная за все годы урожайность сорта Эврика превысила таковую сорта Волхова на 24,5%. В пределах указанных доз азота для обоих сортов наилучшим вариантом оказалось единовременное внесение весной смеси аммонийной и калийной селитры, наихудшим – единовременное внесение весной одной аммонийной селитры. В среднем по обоим сортам внесение калийной селитры весной увеличивало урожайность зерна на 16% по сравнению с вариантами, где калийную селитру не использовали.

Ключевые слова: нитрат, аммиачная селитра, азотнокислый калий, сложное удобрение, подкормка по всходам, весенняя подкормка

EFFECT OF AMMONIUM AND POTASSIUM NITRATE ON WINTER HARDINESS AND YIELD OF WINTER RYE (*SECALE CEREALE* L.)

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The results of research for 3 years (2019–2021) on winter rye (*Secale cereale* L.) crops in the North-West region of the Russian Federation (Leningrad region) are presented. The effect of different doses and timing of ammonium and potassium nitrate application on winter hardiness and productivity of winter rye plants on the example of Volkhova and Eureka varieties was studied. The soil in the experimental fields was sod-podzolic loamy with the depth of the arable horizon of 20–25 cm. Humus content – 2.0–2.3%, pH of the salt extract – 5.2–5.5. Fertilizers were applied in dry form in the autumn one week after the emergence of the seedlings, and in the spring – at the resumption of vegetation. The studied fertilizer doses in terms of active substance for nitrogen were 0 (control), 30, 60 and 90 kg/ha. It is shown that winter hardiness of both varieties practically did not depend on the doses and timing

of the application, as well as on the type of fertilizers. The maximum grain yield was shown by the Volkhova variety at a dose of nitrogen of 60 kg/ha, the Eureka variety – 90 kg/ha, with the maximum yield of the Eureka variety for all the years exceeding that of the Volkhova variety by 24.5%. Within the specified doses of nitrogen for both varieties, the best option was a single spring application of a mixture of ammonium and potassium nitrate, the worst - a single spring application of ammonium nitrate alone. On average for both varieties, potassium nitrate application in spring increased the grain yield by 16% compared to the variants where potassium nitrate was not used.

Keywords: nitrate, ammonium nitrate, potassium nitrate, complex fertilizer, seedling feeding, spring feeding

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INTRODUCTION

Winter rye is one of the most important grain crops not only in Russia, but also in the neighboring countries [1, 2], so the issues of increasing the yield and quality of its grain do not lose their importance, especially with noticeable climatic changes. It is known that the average temperature on the Earth's surface has increased by almost 1 °C over the century. In this case, the issues of changing the conditions of overwintering of winter crops become relevant [3–6].

Formation of winter rye productivity largely depends on the conditions of mineral nutrition of plants in critical periods of vegetation – from sprouting to going into winter and at the resumption of spring growth. If there is a lack of nitrogen in the autumn period, the development of winter cereals slows down, as a result of which their winter hardiness may decrease. It is possible to solve the problem of nitrogen compensation in this period through autumn application of nitrogen fertilizers. However, their use in the autumn period may increase the sensitivity of plants to winter frosts, especially in case of prolonged warm autumn, when there is a risk of overgrowth of plants. It is well known that for good overwintering, plants must accumulate a sufficient amount of sugars and potassium is one of the most important elements stimulating plastic metabolism. From this comes the known positive effect of potassium on winter hardiness of plants. At the same time, there are studies showing that the concentration of potassium in winter tillering nodes is determined to a greater extent by the content of easily available potassium in the soil at sowing and it does not depend much on the pre-sowing application of potassium fertilizers[1]. For a successful uptake of potassium by seedlings, it is important that the soil contains exchangeable and readily available potassium; with traditional pre-sowing application, a significant part of potassium can be fixed in non-exchangeable form [7-9]. There is also data that winter rye most intensively absorbs nitrogen and potassium in the phase of spring tillering, so the question of optimal timing and methods of fertilizer application for this crop remains open [10].

Numerous studies have established that winter rye has a very high biological potassium removal among grain crops [11, 12]. At the same time, the widely used liming of sod-podzolic soils leads to an even greater increase in the need of plants in potassium.

[1]*Akimova O.I.* Formation of winter rye winter hardiness elements during the period of autumn growth and development // Siberian Herald of Agricultural Science, 2007, N 4 (172), pp. 23-31.

This is associated with the violation of the normal ratio of K: Ca in plants due to an increase in the intake of calcium, which competes with potassium ions in this regard [13]. Also, ammonium ions close to its ionic radius[2] can compete with potassium, so when using ammonium sources of nitrogen nutrition, it is important to provide plants with a sufficient amount of available potassium.

The purpose of the study was to investigate the effect of autumn and spring application of increasing doses of ammonium and potassium nitrate on winter hardiness and yields of winter rye.

MATERIAL AND METHODS

The research was conducted during 3 years (2019-2021) in the Gatchina district of the Leningrad region of the North-West region of the Russian Federation on the experimental fields of the Leningrad Research Institute of Agriculture "Belogorka". The experiment was conducted on winter rye plants of two varieties zoned in the North-Western region of the Russian Federation, Volkhova (old variety 1988) and Eureka (new variety 2021). The soil in the experimental fields was sod-podzolic loamy medium-cultivated with the depth of the arable horizon of 20-25 cm. Humus content – 2,0–2,3%, pH of the salt extract – 5,2–5,5, mineral nitrogen content – 1,2–1,5 mg/100 g of soil, mobile potassium compounds – 8–10 mg/100 g of soil. Sowing was carried out in the first ten-day period of September, the seeding rate – 4.5 million germinated seeds/ha. The total area of the experiment – 0.1 ha, the experiment was laid by the method of randomized repetitions, the size of the record plot – 4 m^2 (the area of 0.5 m^2 was allocated within its limits for counting the number of overwintered plants), repetition – 3-fold. Sowing was carried out with a "twirler" seeder, fertilizer application in autumn and spring – manually scattered with subsequent embedding with rakes-markers to a depth of 2.0–2.5 cm. Grain harvesting was carried out by the Hege combine harvester.

Experiments were laid out and the results of the experiments were statistically processed according to the generally accepted methods (according to Dospekhov).

In the experiments, no other chemical means other than the studied fertilizers were used. The influence of three factors was studied: with and without potassium (*A*), single and fractional application of fertilizers (*B*), fertilizer dose by active ingredient (a.i.) nitrogen (*C*). Fertilizers in the form of NH_4NO_3 and KNO_3 were applied in dry form uniformly over the whole plot area, in autumn - one week after the emergence of the seedlings, in spring - at the resumption of vegetation. The total fertilizer dose applied during the vegetation period was 30, 60 or 90 kg/ha of nitrogen active substance; in case of split application, the autumn dose was 1/3 and the spring dose was 2/3 of the total dose.

Scheme of the experiment:

1. Without fertilizers (control).

2. With potassium: 1) split application - 30 kg a.i./ha for nitrogen (KNO_3 in autumn (10 kg a.i./ha for nitrogen) + NH_4NO_3 in spring (20 kg a.i./ha)); 60 kg a.i./ha for nitrogen (KNO_3 in autumn (20 kg a.i./ha for nitrogen) + NH_4NO_3 in spring (40 kg a.i./ha)); 90 kg a.i./ha for nitrogen (30 kg a.i./ha for nitrogen) + NH_4NO_3 in spring (60 kg a.i. /ha)); 2) one-time application - 30 kg a.i./ha for nitrogen (KNO_3 in spring (10 kg a.i./ha for nitrogen) + NH_4NO_3 in spring (20 kg a.i./ha)); 60 kg a.i./ha for nitrogen (KNO_3 in spring (20 kg a.i./ha for nitrogen) + NH_4NO_3 in spring (40 kg a.i./ha)); 90 kg a.i./ha for nitrogen (KNO_3 in spring (30 kg a.i./ha for nitrogen) + NH_4NO_3 in spring (60 kg a.i./ha)).

3. Without potassium: 1) split application - 30 kg a.i./ha for nitrogen (NH_4NO_3 in autumn (10 kg a.i./ha) + NH_4NO_3 in spring (20 kg a.i./ha)); 60 kg a.i./ha for nitrogen (NH_4NO_3 in autumn (20 kg a.i./ha) + NH_4NO_3 in spring (40 kg a.i./ha)); 90 kg a.i./ha for nitrogen (NH_4NO_3 in autumn (30 kg a.i./ha) + NH_4NO_3 in spring (60 kg a.i./ha)); 2) one-time application - 30 kg a.i./ha for nitrogen (NH_4NO_3 in

[2]*Yakimenko V.N.* Effect of fertilizer application in agrocenoses on soil fixation of potassium and ammonium // Problems of agrochemistry and ecology, 2010, N 4, pp. 9-12.

spring (30 kg a.i./ha)); 60 kg a.i./ha for nitrogen (NH_4NO_3 in spring (60 kg a.i./ha)); 90 kg a.i./ha for nitrogen (NH_4NO_3 in spring (90 kg a.i./ha)).

Characteristics of weather conditions. *2019.* The autumn-winter period of 2018/19 was characterized by a warm and prolonged autumn, which is usual for the Leningrad region in recent times, snow cover was established only in late November with a slight freezing of the soil. In mid-February, the soil under the snow completely thawed, which contributed to the death of some plants from desiccation. Weather conditions in spring and early summer were standard for the region, but July was cold and excessively rainy, which resulted in lodging of some plants.

2020. The autumn-winter period of 2019/20 was characterized by an early onset of cold weather, with average daily temperatures not exceeding 9.5 °C after September 15. However, the winter was very warm, with a lot of precipitation, but without a stable snow cover, which contributed to the leaching of nutrients from the soil and the death of some plants from soaking. Weather conditions in spring and summer were generally typical for the Leningrad region.

2021. The autumn-winter period of 2020/21 again saw a warm and prolonged autumn, with snow cover established in mid-December. The summer period was characterized by hot and very dry weather, the HTC for the period from flowering to harvesting was only 0.3.

RESULTS AND DISCUSSION

As the studies have shown, under the conditions of weather in the autumn-winter period - prolonged warm autumn and moderately cold winter - usual for the North-Western region of the Russian Federation at present, winter hardiness of winter rye plants, regardless of the variety, almost did not change with autumn fertilization, remaining at the level of the control (see Tables 1, 2). A weak effect was observed only in 2020, which was characterized by an abnormally warm winter. The variety Volkhova reliable difference from the control was observed only in the variant with the maximum dose of ammonium nitrate and was only +7.3%. Winter hardiness of plants of the variety Eureka slightly increased already at N_{20}, but in general the effect was also little reliable – the maximum increase in plant survival was 7.4% at N_{30}. At the same time, the introduction of potassium did not give the expected protector effect, which may be due to the fact that in modern realities, the safety of winter rye plants in winter is determined mainly

Табл. 1. Зимостойкость ржи озимой сорта Волхова при осеннем внесении разных доз аммонийной и калийной селитры, 2019–2021 гг., %

Table 1. Winter hardiness of the winter rye variety Volkhova at autumn application of different doses of ammonium and potassium nitrate, 2019–2021, %

Type of fertilizer applied in autumn	Winter hardiness			
	N0	N10	N20	N30
	2019			
KNO_3	–	63,3	64,1	62,6
NH_4NO_3	–	65,8	64,6	63,4
Control	63,1	–	–	–
$LSD_{0,05}$	7,8			
$F_{theor.}$ *A/C*	4,5/3,2			
$F_{fact.}$ *A/C*	0,3/0,2			
	2020			
KNO_3	–	78,1	70,5	73,9
NH_4NO_3	–	76,9	79,4	83,1
Control	75,8	–	–	–
$LSD_{0,05}$	6,2			
$F_{theor.}$ *A/C*	4,5/3,2			
F_{fact} *A/C*	4,5/0,7			
	2021			
KNO_3	–	76,5	74,6	75,2
NH_4NO_3	–	80,2	73,1	76,2
Control	78,4	–	–	–
$LSD_{0,05}$	16,4			
$F_{theor.}$ *A/C*	4,49/3,23			
F_{fact} *A/C*	0,04/0,32			

Note. Here and in Table 2. N0, N10, N20, N30 – fertilizer dose 0, 10, 20, 30 kg/ha by active substance nitrogen, respectively. Factor *A* – type of fertilizer, factor *C* – fertilizer dose.

Табл. 2. Зимостойкость ржи озимой сорта Эврика при осеннем внесении разных доз аммонийной и калийной селитры, 2019–2021 гг., %

Table 2. Winter hardiness of the winter rye variety Eureka at autumn application of different doses of ammonium and potassium nitrate, 2019–2021, %

Type of fertilizer applied in autumn	Winter hardiness			
	N0	N10	N20	N30
	2019			
KNO_3	–	74,0	75,0	78,8
NH_4NO_3	–	77,2	74,0	75,0
Control	74,9	–	–	–
$LSD_{0,05}$	12,2			
$F_{theor.}$ *A/C*	4,5/3,2			
F_{fact} *A/C*	0,01/0,15			
2020				
KNO_3	–	76,8	75,7	79,4
NH_4NO_3	–	82,4	84,2	85,3
Control	77,9	–	–	–
$LSD_{0,05}$	8,0			
F_{theor} *A/C*	4,5/3,2			
F_{fact} *A/C*	7,0/1,0			
2021				
KNO_3	–	83,4	78	83,6
NH_4NO_3	–	86,3	80,9	76,8
Control	84,1	–	–	–
$LSD_{0,05}$	11,1			
$F_{theor.}$ *A/C*	4,5/3,2			
F_{fact} *A/C*	0,01/1,04			

not by frost resistance, and resistance to soaking and uprooting, potassium, as is well known, contributes to an increase in frost resistance of plants.

Tables 3 and 4 present data on the yield of winter rye varieties Volkhova and Eureka at different variants of ammonium and potassium nitrate application.

In 2019, Volkhova variety showed the highest yield at N_{60} in all the variants. With abundant nitrogen nutrition (N_{90}) in conditions of rainy July,

Табл. 3. Урожайность ржи озимой сорта Волхова при разных сроках и дозах внесения аммонийной и калийной селитры, 2019–2021 гг., т/га

Table 3. The yield of winter rye variety Volkhova at different dates and doses of ammonium and potassium nitrate application, 2019–2021, t/ha

Option	Crop yield			
	N0	N30	N60	N90
2019				
KNO_3 autumn + NH_4NO_3 spring	–	2,46	2,99	1,95
NH_4NO_3 autumn + NH_4NO_3 spring	–	2,57	3,11	1,98
KNO_3 spring + NH_4NO_3 spring	–	2,51	3,48	2,06
NH_4NO_3 spring	–	2,23	2,76	1,98
Control	2,05	–	–	–
$LSD_{0,05}$	0,16			
$F_{theor.}$ *A/B/C*	2,90/2,90/2,82			
$F_{fact.}$ *A/B/C*	4,30/87,16/62,68			
2020				
KNO_3 autumn + NH_4NO_3 spring	–	2,11	3,18	3,05
NH_4NO_3 autumn + NH_4NO_3 spring	–	2,41	3,29	3,49
KNO_3 spring + NH_4NO_3 spring	–	3,80	4,15	3,60
NH_4NO_3 spring	–	2,93	3,29	3,97
Control	2,16	–	–	–
$LSD_{0,05}$	0,44			
$F_{theor.}$ *A/B/C*	2,90/2,90/2,82			
$F_{fact.}$ *A/B/C*	4,48/17,23/12,32			
2021				
KNO_3 autumn + NH_4NO_3 spring	–	2,38	2,63	2,17
NH_4NO_3 autumn + NH_4NO_3 spring	–	2,88	2,81	1,72
KNO_3 spring + NH_4NO_3 spring	–	1,32	2,69	2,19
NH_4NO_3 spring	–	1,75	2,25	1,62
Control	1,39	–	–	–
$LSD_{0,05}$	0,55			
$F_{theor.}$ *A/B/C*	2,90/2,90/2,82			
$F_{fact.}$ *A/B/C*	2,96/16,10/11,35			
Average for 3 years				
KNO_3 autumn + NH_4NO_3 spring	–	2,32	2,93	2,39
NH_4NO_3 autumn + NH_4NO_3 spring	–	2,62	3,07	2,40
KNO_3 spring + NH_4NO_3 spring	–	2,54	3,44	2,64
NH_4NO_3 spring	–	2,30	2,77	2,52
Control	1,78	–	–	–

Note. Here and in Table 4. N0, N30, N60, N90 – dosage of fertilizer 0, 30, 60, 90 kg/ha by active substance and nitrogen, respectively. Factor A – type of fertilizer, factor B – timing of fertilizer application, factor C – fertilizer dose.

Табл. 4. Урожайность ржи озимой сорта Эврика при разных сроках и дозах внесения аммонийной и калийной селитры, 2019–2021 гг., т/га

Table 4. The yield of winter rye variety Eureka at different timing and doses of ammonium and potassium nitrate application, 2019–2021, t/ha

Option	Crop yield			
	N0	N30	N60	N90
2019				
KNO_3 autumn + NH_4NO_3 spring	–	2,38	2,55	3,40
NH_4NO_3 autumn + NH_4NO_3 spring	–	2,43	2,70	2,69
KNO_3 spring + NH_4NO_3 spring	–	2,45	2,86	3,19
NH_4NO_3 spring	–	2,27	2,63	2,60
Control	2,21	–	–	–
$LSD_{0,05}$	0,35			
$F_{theor.}$ *A/B/C*	2,90/2,90/2,84			
$F_{fact.}$ *A/B/C*	2,90/7,34/7,49			
2020				
KNO_3 autumn + NH_4NO_3 spring	–	3,03	4,08	3,92
NH_4NO_3 autumn + NH_4NO_3 spring	–	3,51	4,17	4,78
KNO_3 spring + NH_4NO_3 spring	–	3,35	4,11	5,17
NH_4NO_3 spring	–	3,52	4,37	4,40
Control	2,75	–	–	–
$LSD_{0,05}$	0,48			
$F_{theor.}$ *A/B/C*	2,90/2,90/2,84			
$F_{fact.}$ *A/B/C*	2,90/50,55/35,62			
2021				
KNO_3 autumn + NH_4NO_3 spring	–	2,06	2,38	2,13
NH_4NO_3 autumn + NH_4NO_3 spring	–	1,69	2,13	1,90
KNO_3 spring + NH_4NO_3 spring	–	1,44	1,63	1,75
NH_4NO_3 spring	–	1,70	1,73	1,76
Control	1,28	–	–	–
$LSD_{0,05}$	0,28			
$F_{theor.}$ *A/B/C*	2,90/2,90/2,84			
$F_{fact.}$ *A/B/C*	15,2/38,9/31,1			
Average for 3 years				
KNO_3 autumn + NH_4NO_3 spring	–	2,49	3,00	3,15
NH_4NO_3 autumn + NH_4NO_3 spring	–	2,54	3,00	3,12
KNO_3 spring + NH_4NO_3 spring	–	2,41	2,86	3,37
NH_4NO_3 spring	–	2,50	2,91	2,92
Control	2,08	–	–	–

this variety showed strong regrowth of podding, which caused a significant decrease in the yield. According to the timing and type of fertilizer at the optimum dose of nitrogen (N_{60}) the most effective was a simultaneous spring application of a mixture of ammonium and potassium nitrate, which may indicate the positive effect of an additional source of potassium ions for plant development in the spring and summer period. Eureka variety showed maximum yield at N_{90} in the variants with potassium nitrate. This can be explained by the fact that the less developed root system of Eureka, as a shorter-stemmed variety, in conditions of cold rainy weather during the grain filling phase less effectively used nutrients from the soil. At the same time, a good supply of potassium, which stimulates, as is known, plastic metabolism, somewhat corrected the situation at high doses of fertilizers. Significant differences between the variants with different timing of fertilizer application were not revealed.

In 2020, the situation was comparable to 2019 – the yield of the Volkhova variety reliably increased to N_{60}, the Eureka variety – to N_{90}. The determining role in 2020 was played by the resistance of plants to lodging, as weather conditions were favorable for intensive grain filling, but at the same time contributed to increased lodging. At N_{90} the degree of lodging of the Volkhova variety reached 60–80% in all variants, while the lodging resistance of the Eureka variety did not exceed 20%[3]. As a result, the maximum yield of the Eureka variety exceeded the maximum yield of the Volkhova variety by 24.5%. In terms of timing and type of fertilizer application, the most effective within the optimal nitrogen doses for each variety, as well as in 2019, were the variants of a single fertilizer application with the presence of potassium.

In 2021, fractional fertilizer application was more effective – Volkhova plants reached maximum yield at N_{30} and Eureka at N_{60}. This is probably due to the prolonged dry weather that occurred during the entire period from flowering to harvesting, when the large doses of fertilizers made at once in the spring could not be ful-

[3]*Petrovtseva N.A.* New variety of winter rye Eureka and evaluation of its resistance to lodging under different schemes of nitrogen fertilizer application // Agrobiotechnology - 2021: collection of articles of the international scientific conference. (Moscow, November 24-25, 2021). Moscow, 2021, pp. 601-605.

ly assimilated by plants in conditions of severe moisture deficit. Also, in contrast to the previous 2 years, the positive effect of potassium application was not manifested. This can be explained by the fact that, as the results of 2019 and 2020 showed, potassium application influenced mainly the spring-summer vegetation. In conditions of severe summer drought, potassium supply to plants was limited not by the amount of the element in the soil, but by the ability of plants to extract it.

CONCLUSIONS

1. Winter hardiness of winter rye plants, regardless of the variety, was not affected by the autumn application of neither ammonium nor potassium nitrate.

2. The effectiveness of different doses of fertilizers was determined by the variety and weather conditions: the Volkhova variety achieved the maximum yield at low and medium doses of fertilizers (30 kg a.i./ha for nitrogen in 2021 and 60 kg a.i./ha for nitrogen in 2019 and 2020), the Eureka variety - at medium and high (60 kg a.i./ha for nitrogen in 2021 and 90 kg a.i./ha for nitrogen in 2019 and 2020). At the same time, under favorable conditions in 2020 the highest yield shown by the variety Eureka exceeded that of the variety Volkhova by 24.5%.

3. Within the limits of the same nitrogen doses, a positive effect on yield of potassium nitrate application at a single spring application of fertilizers was observed - for the Volkhova variety at N_{30} and N_{60}, for the Eureka variety - at N_{90} (except for extremely dry 2021, when the effect of potassium nitrate application was not manifested). With fractional application of fertilizers, the effect of potassium nitrate application is noted only on the variety Eureka at N_{90}.

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ЭФФЕКТИВНОСТЬ ИСПОЛЬЗОВАНИЯ КАС-32 И БИОПРЕПАРАТА СТЕРНИФАГ НА ЯРОВОЙ ПШЕНИЦЕ, ВЫРАЩИВАЕМОЙ С ПРИМЕНЕНИЕМ ТЕХНОЛОГИИ NO-TILL

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Изложены результаты совместного применения биопрепарата Стернифаг, СП на основе гриба *Trichoderma harzianum* и карбамидно-аммиачной смеси (КАС-32) на яровой пшенице, возделываемой по технологии No-till в северной лесостепи Приобья. Данная зона отличается коротким вегетационным периодом и среднемноголетним коэффициентом увлажнения 1,04–1,08. В таких условиях технология No-till, или прямой посев, позволяет минимизировать затраты на производство зерна. При выращивании культуры по указанной технологии в условиях северной лесостепи отодвигаются сроки посева, что актуализирует использование биопрепарата для ускорения минерализации соломистых остатков. В производственном и стационарном опытах на выщелоченном черноземе совместное применение Стернифага и азотного удобрения осенью предыдущего года на 33,0–49,0% активизировало разложение растительных остатков на второй пшенице. Внесение биопрепарата совместно с КАС-32 позволило получить в годы исследования дополнительно с 1 га по 1,5 и 0,96 т зерна яровой пшеницы (43,7–44,4%) за счет увеличения массы 1000 зерен, озерненности колоса и лучшей сохранности растений к уборке. Применение биопрепарата Стернифаг и КАС-32 на яровой пшенице, выращиваемой на основе технологии No-till в северной лесостепи Приобья, экономически выгодно. Оно способствует повышению прибыли в 2,0–2,4 раза и росту уровня рентабельности производства зерна на 27,0–36,0%. Результативность возделывания культуры на фоне жидкого азотного удобрения КАС-32 ниже. В этом варианте зерновая продуктивность яровой пшеницы по отношению к контролю возрастала в 2018 г., отличавшемся повышенной влажностью, на 1,1 т/га, в 2020 г., характеризуемом июньской засухой, на 0,74 т/га. Уровень рентабельности производства зерна при применении КАС-32 выше, чем в контроле, на 15,0–17,2%.

Ключевые слова: яровая пшеница, биопрепарат Стернифаг, КАС-32, урожайность, экономическая эффективность

EFFICIENCY OF USING UAN-32 AND STERNIFAG BIOPREPARATION ON SPRING WHEAT GROWN WITH NO-TILL TECHNOLOGY

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The results of joint application of the biopreparation Sternifag, WP based on the fungus *Trichoderma harzianum* and urea ammonium nitrate (UAN-32) on spring wheat cultivated using the No-till technology in the northern forest-steppe of the Priobie region are presented. This zone is characterized by a short growing season and a mean annual moisture coefficient of 1.04–1.08. Under such conditions, No-till, or direct seeding, technology can minimize the cost of grain production. When growing crops according to this technology in the conditions of the northern forest-steppe, the sowing dates are postponed, which actualizes the use of biopreparation to accelerate the mineralization of straw residues. In production and stationary experiments on leached chernozem, the joint application of Sternifag and nitrogen fertilizer in autumn of the previous year activated decomposition of plant residues on second

wheat by 33.0–49.0%. Application of the biopreparation together with UAN-32 allowed to obtain in the years of research additional 1 ha 1.5 and 0.96 tons of spring wheat grain (43.7–44.4%) due to an increase in the weight of 1000 grains, ear fineness and better preservation of plants for harvesting. Application of biopreparation Sternifag and UAN-32 on spring wheat grown on the basis of No-till technology in the northern forest-steppe of Priobie is economically beneficial. It is accompanied by an increase in profit by 2.0–2.4 times and an increase in the level of profitability of grain production by 27.0–36.0%. Effectiveness of cultivation of the crop on the background of liquid nitrogen fertilizer UAN-32 is lower. In this variant, grain productivity of spring wheat relative to the control increased in 2018, characterized by high humidity, by 1.1 t/ha, in 2020, characterized by June drought, by 0.74 t/ha. The level of profitability of grain production at application of UAN-32 is higher than in the control by 15.0–17.2%.

Keywords: spring wheat, biopreparation Sternifag, UAN-32, productivity, economic efficiency

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The authors declare no conflict of interest.

INTRODUCTION

The zone of the northern forest-steppe of Priobie is characterized by a short growing season and average annual moisture coefficient of 1.04-1.08 (according to the data of the agrometeorological station "Ogurtsovo"), which is not typical for the application of No till or direct seeding technology. However, even in such conditions, the use of the specified farming system allows minimizing the costs of spring wheat grain production[1]. When cultivating according to the above technology in the northern forest-steppe, the sowing dates are postponed, which makes the use of biopreparations to accelerate the mineralization of straw residues relevant [1–3].

In the No-till system the plant residues left after harvesting grain crops are 80.0% represented by straw, which is the main mass of organic matter entering the soil. Organic matter accumulates reserves of carbon, nitrogen, phosphorus, potassium, thus acting as an additional source of nutrients. Up to 12-15 kg/ha of nitrogen, 7-8 kg/ha of phosphorus, 20-25 kg/ha of potassium[2] go back to the soil together with straw [4]. When using resource-saving technologies and crop rotations dominated by grain crops, due to slow stubble decomposition (3-5 years on average), large amounts of crop residues accumulate on the soil surface, which leads to an increase in the population density of phytopathogenic microorganisms[3]. In this regard, it is important to select biological means that will promote accelerated decomposition of plant residues and simultaneously reduce the stock of soil infection[4]. Inoculation of soil with *Trichoderma* micromycetes helps to solve these problems. This fungus is a

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recognized biocontrol agent and has the ability to degrade complex polymers such as cellulose and lignin. As a result, *Trichoderma* successfully returns nutrients to the soil and enhances its microbial activity in the long term [5–10].

Since the rate of decomposition of plant residues depends on the optimal ratio of C: N (about 20-30: 1), for better work of the fungus-destructor and regulation of such ratio it is necessary to additionally apply a compensating dose of nitrogen fertilizers [11, 12].

The purpose of the study was to investigate the effect of combined application of urea ammonium nitrate (UAN 32) and biopreparation Sternifag, WP (wettable powder) on the process of decomposition of plant residues, economic and economic efficiency of spring wheat grain production in No till technology in the conditions of the northern forest-steppe of Priobie.

MATERIAL AND METHODS

Field studies were conducted at the experimental field and at the station of the Novosibirsk State Agrarian University in the left-bank suburbs of Novosibirsk in 2018 and 2020. The designated area belongs to the north forest-steppe Priobsky agrolandscape region. It is believed that up to 80.0% of grain yield variability in this zone is determined by the moisture conditions of the first half of the growing season [13]. According to hydro-thermal conditions, 2018 was favorable for the formation of good grain productivity. In this period, the moisture factor was 1.44 (according to Selyaninov). At the same time, May was excessively cold (temperature deficit of -4°C was recorded) and strongly overwatered. As a result, sowing dates had to be postponed by two weeks. In terms of hydrothermal characteristics, 2020 was sufficiently moist and warm. In June 2020, a typical spring-summer drought was observed in the Priobsky agro-landscape region (56.0% of the monthly precipitation shortfall). All this reduced potential grain yields.

The experiments were laid on leached chernozem of medium loamy granulometric composition with neutral reaction of medium on stubble background. The humus content was 6.4 and 6.7%, mobile P_2O_5 – 230 and 213 mg/kg of soil (according to Chirikov), exchangeable K_2O – 213 and 186 mg/kg of soil, respectively. Novosibirskaya 31 medium early spring wheat variety (seeding rate 5.5 million seeds/ha) was sown. Spring wheat was used as a forecrop.

The biopreparation Sternifag, WP and nitrogen fertilizer UAN 32 were applied by spraying in the first ten-day period of October of the previous year. In 2018, plots of 15 ha were laid as part of the production experiment. In 2020, the experiment was a field experiment. The area of plots of each variant of the experiment was 1 m^2 (this is due to the fact that the experiments were conducted as part of a stationary experiment, in which zero-tillage technology was used since 2013). Repetition is eightfold, the arrangement is randomized. The following experiment variants are presented:

1) control (without fertilizer application);
2) UAN-32 (application rate 60 kg a.i./ha);
3) UAN-32 (application rate 60 kg a.i./ha) + Sternifag, WP (application rate 80 g/ha).

The selected dose of nitrogen is recommended for grain forecrops at intensive technology of wheat cultivation in the subzone of the northern forest-steppe[5].

Urea-ammonia mixture is a liquid nitrogen fertilizer containing three forms of nitrogen: nitrate, ammonium (8.0% each) and amide (16.0%).

Sternifag, WP is a biopreparation of the group of biofungicides based on the fungus Trichoderma harzianum (strain VKM F-4099D, titer 10^{10} CFU/g), produced by ZAO "Agrobiotechnology" (Moscow). It is recommended for use at a rate of 80 g/ha.

In the production experiment 2018, in which all technological operations were carried out by machines, and in the experiment 2020, which is a model of the production experiment, the same dependencies were determined in field conditions. The degree of decomposition of

[5]*Kiryushin V.I., Vlasenko A.N., Kalichkin V.K., Vlasenko N.G., Filimonov Y.P., Iodko L.N., Sharkov I.N., Tarasov A.S., Ponko V.A., Yuzhakov A.I., Khmelev V.A., Semendyaeva N.V., Kincht A.V., Sineschekov V.E., Novikov V.M., Shoba V.N., Kozhevnikov A.I., Usolkin V.T., Dobrotvorskaya N.I., Kim S.A., Solosich N.A., Filimonova L.N., Konyaeva N.M., Polukhin N.I.* Adaptive-landscape farming systems of the Novosibirsk region. Novosibirsk, 2000, p. 388.

crop residues was established using the weight method[6] twice during the growing season: after sowing the crop and one month later. The biological grain yield was determined according to the method of field experiment[7]. In each variant, sheaves were sampled from 1 m^2 in five and six repetitions and structural yield analysis was carried out. The following indicators were determined: number of plants, productive bushiness, grain weight, number of grains in the ear, weight of 1000 grains (the last two indicators were taken into account in 25 repetitions to improve the accuracy of statistical processing). Microsoft Excel and Snedecor programs were used for statistical data processing.

RESULTS AND DISCUSSION

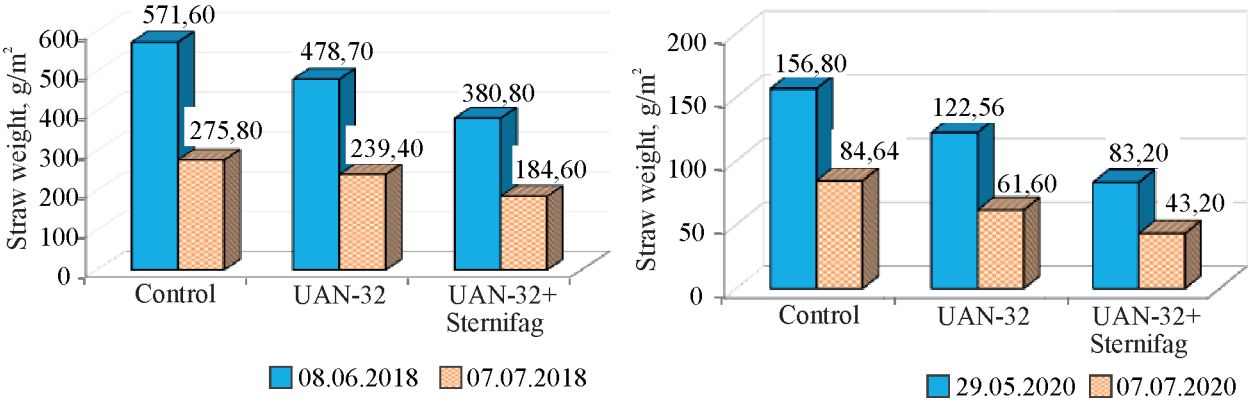
On the experimental variants in both years of research, more intensive decomposition of crop residues was observed. In early June 2018. as a result of a significant shortage of May temperatures, the activity of natural microbiological decomposition of crop residues in general was strongly reduced. In this case, in the variant with the application of UAN 32 straw residues decomposed more than in the control by 16.3%, in the variant with the joint application of UAN 32 and Sternifag the increase in the indicator amounted to 33.4% (see the figure). A month after sowing on the background of joint application of Sternifag and UAN 32 straw remained 3 times less than it was initially, on the background of UAN 32 – 2.4 times less. In the control, the initial mass of straw decreased by 2 times.

In 2020, the joint application of UAN 32 and fungal inoculant under conditions of optimal thermal regime in spring further reduced the period of decomposition of wheat straw residues. In May, their amount decreased by 47.0% in comparison with the control, and by 22.0% in comparison with the variant that implied application of UAN 32 alone. In the following month, the volume of plant residues on the soil surface at joint application decreased to 49.0%.

On the background of UAN 32 their number decreased by 1.4 times compared to the control.

Additional release of nutrient elements from plant residues in the experiment variants had a positive effect on plant productivity, contributing to the formation of larger and more accomplished grain in spring wheat (see Table 1). In 2018, characterized by excessive moisture and lack of heat in May, the combined use of biopreparation and fertilizer led to an increase in the weight of 1000 grains by 4.7 g, in 2020, which turned out to be wet and warm by 3.6 g. The use of liquid fertilizer alone increased the grain plumpness by 3.1 and 3.3 g, respectively.

In 2020, rainy weather in July and August contributed to the decrease in the difference be-



Влияние КАС-32 и биопрепарата Стернифаг на разложение стерни яровой пшеницы
Influence of UAN-32 and the Sternifag biopreparation on the decomposition of spring wheat stubble

[6]Photosynthesis and bioproductivity: methods of determination / edited by A.T. Mokronosov. Moscow: Agropromizdat, 1989, p. 460.

[7]*Dospekhov B.A.* Methodology of field experiment with the basics of statistical processing of research results. Moscow: Kolos, 1985, p. 351.

tween the control and the variant with UAN 32 and Sternifag in terms of 1000 grain weight. The excess of precipitation over the average annual values amounted to 41.0 and 24.0%, respectively. This provoked the development of root rots (at this time they affect yield through 1000 grain weight) and possibly enzyme-mycosis depletion of grain. In 2018, grain filling took place in dry August.

Structural analysis showed that the second element that made a significant contribution to the change in the yield of the experimental variants was the density of productive stalks by the time of harvesting. In 2018, the addition of Sternifag to UAN 32 increased plant safety by 31.0% (+ 84.6 pcs./m^2), in 2020 – by 14.8% (+ 38 pcs./m^2). In the variant with UAN 32 the plant stand density by years exceeded the control by 29.0 (+ 78.6 pcs./m^2) and 8.7% (+ 22.4 pcs./m^2). It should be noted that the small safety of plants to harvesting in the experiment as a whole is most likely associated with the development of root rot. This is a common phenomenon for No till wheat cultivation technology characterized by an increased background of soil phytopathogens[8].

In 2020, the joint application of Sternifag and UAN 32 and liquid fertilizer separately also contributed to the establishment of more grains in the ear. The value of the index relative to the control in the variants amounted to 4.2 and 2.0 pieces, respectively.

Changes in the yield structure under the influence of the joint use of Sternifag and UAN 32 led to a significant increase in crop productivity (see Table 2). The highest grain increment in this variant was obtained in 2018 – 1.50 t/ha. In 2020, the economic efficiency was at the level of 0.96 t/ha. Relative to the control, grain productivity in 2018 increased by 43.7%, in 2020 – by 44.4%.

Application of UAN-32 in 2018 increased wheat yield by 1.10 t/ha (32.0%), in 2020 – by 0.74 t/ha (34.3%). In 2018 in control and variants with fertilizer and biopreparation application, grain of different quality and class was obtained. In the control the content of gluten was 28.8%, protein – 13.5%, in the fertilized variants – respectively 29.2–33.0 and 15.0–16.5%, i.e. the class of grain was higher, due to which the selling price increased.

Calculation of economic efficiency showed that the increase in grain yield after application of UAN 32 together with Sternifag and UAN 32 separately at the stated rates of consumption provided an increase in profit (see Table 3). The effect from the use of fertilizer and biopreparation exceeded all the costs associated with their purchase, application and harvesting of additional yield. The level of profitability of grain production in the experimental variants was the highest in 2018: from 153.0% in the control to 189.0% in the UAN 32 + Sternifag variant (an increase of 36.0%, or 1.24 times). When applying only

Табл. 1. Структура урожайности яровой пшеницы на фоне применения Стернифага и КАС-32
Table 1. Yield structure of spring wheat against the background of the use of Sternifag and UAN-32

Option	Number of plants, pcs./m^2	Productive bushiness	Number of grains per ear, pcs.	Weight of 1000 grains, g
2018				
Control (without fertilizers)	270,00 ± 2,70	1,17	33,70 ± 0,50	32,20 ± 0,80
UAN-32 (60 kg a.i./ha)	348,60 ± 7,20	1,12	33,20 ± 0,04	35,30 ± 0,60
UAN-32 (60 kg a.i./ha) + Sternifag (80 g/ha)	354,60 ± 5,70	1,16	32,40 ± 0,50	36,90 ± 1,00
2020				
Control (without fertilizers)	256,40 ± 3,19	1,11	28,80 ± 0,45	26,40 ± 0,78
UAN-32 (60 kg a.i./ha)	278,80 ± 5,86	1,14	30,80 ± 0,84	29,70 ± 0,50
UAN-32 (60 kg a.i./ha) + Sternifag (80 g/ha)	294,40 ± 1,92	1,07	33,00 ± 0,71	30,00 ± 0,88

[8]*Korobova L.N., Marmulev A.N., Lyakh A.A.* Effect of soil treatment on the development of root rot of spring wheat in Priobie // Plant Protection and Quarantine, 2017, N 10, pp. 45-46.

Табл. 2. Различия в урожайности яровой пшеницы на фоне внесения КАС-32 и биопрепарата Стернифаг

Table 2. Differences in the yield of spring wheat against the background of the introduction of UAN-32 and the biological product Sternifag

Option	2018			2020		
	Crop yield		Increment to the control, t/ha	Crop yield		Increment to the control, t/ha
	g/m^2	t/ha		g/m^2	t/ha	
Control (without fertilizers)	343,20	3,43	–	216,30	2,16	–
UAN-32 (60 kg a.i./ha)	449,20	4,49	+ 1,10	290,10	2,90	+ 0,74
UAN-32 (60 kg a.i./ha) + Sternifag (80 g/ha)	493,40	4,93	+ 1,50	311,90	3,12	+ 0,96
LSD_{05}	72,90	0,73		37,80	0,38	

urea-ammonia mixture, profitability increased to 168.0% (an increase of 15.0%, or 1.1 times).

In 2020, application of liquid fertilizer UAN 32 on spring wheat grown with No till technology increased profit by 4300 rubles/ha, profitability level - 1.6 times. In the control these indicators amounted to 4694 rubles / ha and 27.7%, respectively. In the variant with the application of UAN 32 and Sternifag economic effect was naturally higher. Profit amounted to 11044 rubles / ha; the level of profitability increased by 2.0 times.

The level of payback of 1 rubles of costs for spring wheat grain production under No till technology with the joint use of UAN 32 and Sternifag in the experiment 2018 amounted to 2.91, in the experiment 2020 - 1.54. In the variant with UAN 32 it was less - 2.68 and 1.44. In the control, the level of payback of 1 rubles of costs amounted to 2.47 and 1.28, which reflects the increase in economic efficiency of growing spring wheat in the northern forest-steppe of Priobie at the application of UAN 32 and, especially, its tank mixture with the biological preparation Sternifag, WP.

CONCLUSIONS

1. Preparation Sternifag, WP on the basis of fungus *Trichoderma harzianum* when used together with UAN 32 within the No till technology in the conditions of the northern forest-steppe of Priobie contributed to the acceleration of mineralization of plant residues in the soil and on its surface by 33.0-49.0%. The use of UAN 32 alone accelerated the decomposition of straw

Табл. 3. Экономические показатели использования КАС-32 и биопрепарата Стернифаг на яровой пшенице, выращиваемой с применением технологии No-till

Table 3. Economic indicators of the use of UAN-32 and the biological product Sternifag on spring wheat with No-till technology

Option	Crop yield, t/ha	Production cost, rubles/ha	Costs, rubles/ha	Profit, rubles/ha	Profitability, %
2018					
Control (without fertilizers)	3,40	23 800	9628,30	14171,70	153,00
UAN-32 (60 kg a.i./ha)	4,50	40 500	15095,40	25404,50	168,00
UAN-32 (60 kg a.i./ha) + Sternifag (80 g/ha)	4,90	44 100	15215,40	28884,60	189,00
2020					
Control (without fertilizers)	2,16	21 600	16 906	4694	27,70
UAN-32 (60 kg a.i./ha)	2,90	29 000	20 006	8994	44,90
UAN-32 (60 kg a.i./ha) + Sternifag (80 g/ha)	3,12	31 200	20 156	11 044	54,70

residues by 16.0-27.0%. This improved the nutrition of spring wheat plants and stimulated the grain yield.

2. The maximum increase in wheat grain at the use of zero technology was provided by the joint application of liquid fertilizer UAN 32 at a dose of 60 kg a.i.d./ha and biopreparation Sternifag, WP at a concentration of 80 g/ha. The yield of spring wheat in this variant in the years of the study exceeded the control by 43.7 and 44.4%, amounting to 4.93 and 3.12 tons of grain per 1 ha. Application of the preparation with a destructor and bioagent (trichoderma) increased the weight of 1000 grains and the safety of plants for harvesting by 31.0 and 14.8%, respectively. In addition, the number of grains in the ear increased.

3. Application of liquid nitrogen fertilizer UAN 32 contributed to better grain plumpness and plant preservation: these indicators increased by 29.0 and 8.7% in relation to the control. This increased grain productivity of spring wheat by 1.10 t/ha in wet 2018 and by 0.74 t/ha in 2020, characterized by June drought.

4. Application of UAN-32 and biopreparation Sternifag, WP on spring wheat within the No till technology in the northern forest-steppe of Priobie is economically profitable. Their joint application increases the profit by 2,00–2,35 times with the growth of profitability of production by 27,0–36,0%. In this variant the economic effect of payback of 1 rubles of costs for grain production corresponds to the level from 1.54 to 2.90.

5. Effectiveness of cultivation of the crop at application of UAN 32 fertilizer is lower. The level of payback of 1 rubles of costs in this variant is from 1.44 to 2.68 and is accompanied by an increase in profitability of spring wheat grain production by 15.0–17.2%.

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ФЛУОРЕСЦЕНТНАЯ РЕАКЦИЯ ПРОРОСТКОВ ПШЕНИЦЫ ПРИ ИНФИЦИРОВАНИИ *BIPOLARIS SOROKINIANA*

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Представлены результаты исследований фотосинтетической активности проростков трех сортов яровой мягкой пшеницы, выращенных в регулируемых климатических условиях, и их реакции на инфицирование возбудителем обыкновенной корневой гнили злаков *Bipolaris sorokiniana* Shoem. по параметрам флуоресценции хлорофилла (ФлХ). ФлХ регистрировали флуориметром Dual-PAM-100/F в режиме записи медленной кинетики темновых индукционных кривых с импульсным анализом насыщения (Slow Kinetics). Исследования проводили в 2021, 2022 гг. Установлена сортовая специфика формирования адаптивных реакций 10–16-суточных проростков яровой пшеницы при инфицировании *B. sorokiniana* (5000 конидий на зерно) по посуточной динамике параметров ФлХ. Подтверждена информативность параметров ФлХ *Y*(II), *ETR, qP*, *Fv/Fm, Fv/F*0*, Y*(*NPQ*)*, qN* и *Y*(*NO*) в качестве биомаркеров оценки устойчивости сортов к патогену на уровне фотосинтетического аппарата. Реакция более устойчивых сортов пшеницы Новосибирская 29 и Сибирская 21 на внедрение патогена оказалась менее выраженной по сравнению с менее устойчивым сортом Новосибирская 41. У более устойчивых сортов установлены наименьшие изменения параметров ФлХ относительно контроля. Наибольшие межсортовые различия при инфицировании *B. sorokiniana* (от 1,9 до 8,6 раза) проявлялись у 16-суточных проростков. Уровень фотосинтетической активности при формировании адаптивных реакций при патогенезе *B. sorokiniana* может служить критерием стрессоустойчивости, что позволит проводить комплексные исследования по моделированию системы растение – хозяин – патоген, оценивать первичные неспецифические реакции и адаптивные компоненты стрессоустойчивости сортов яровой пшеницы.

Ключевые слова: пшеница, сорт, устойчивость, биострессор, фотосинтез, параметры флуоресценции хлорофилла

FLUORESCENT REACTION OF WHEAT SEEDLINGS WHEN INFECTED BY *BIPOLARIS SOROKINIANA*

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Results of the studies of photosynthetic activity of seedlings of three varieties of spring soft wheat grown under controlled climatic conditions and their response to infection with the pathogen of common root rot of cereals *Bipolaris sorokiniana* Shoem. by chlorophyll fluorescence (ChlF) parameters are presented. Chlorophyll fluorescence was recorded with a Dual-PAM-100/F fluorimeter in the mode of recording slow kinetics of dark induction curves with pulse saturation analysis (Slow Kinetics). The studies were conducted in 2021, 2022. The varietal specificity of formation of adaptive reactions of 10–16-day-old spring wheat seedlings under infection with *B. sorokiniana* (5000 conidia per grain) according to the daily dynamics of the ChlF parameters was established. The information content of

the ChlF parameters *Y*(II), *ETR*, *qP*, *Fv*/*Fm*, *Fv*/*F*0, *Y*(*NPQ*), *qN* and *Y*(*NO*) as biomarkers for assessing pathogen resistance of the varieties at the level of photosynthetic apparatus was confirmed. The response of the more resistant wheat varieties Novosibirskaya 29 and Sibirskaya 21 to the pathogen introduction was less pronounced compared to the less resistant variety Novosibirskaya 41. The more resistant varieties exhibited the smallest changes in the ChlF parameters relative to the control. The greatest inter-variety differences in infection with *B. sorokiniana* (from 1.9 to 8.6 times) were manifested in 16-day-old seedlings. The level of the photosynthetic activity in the formation of adaptive reactions during the pathogenesis of *B. sorokiniana* can serve as a criterion of stress resistance, which will make it possible to conduct complex studies on modeling the system plant – host – pathogen, to assess the primary nonspecific reactions and adaptive components of stress resistance of spring wheat varieties.

Keywords: wheat, variety, resistance, biostressor, photosynthesis, chlorophyll fluorescence parameters

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INTRODUCTION

Common root rot of cereals (causative agent - fungus *Bipolaris sorokiniana* Shoem.) is a harmful disease of wheat in major grain-growing areas of the world, including the Siberian region [1, 2]. The average yield loss is 15-25% as a result of the reduction of productive bushiness, ear greenness and grain weight[1].

One of the ways to reduce the negative effect of the disease and obtain high and stable grain yields of spring wheat is the creation of adaptive (resistant) to the disease varieties in breeding, as well as their reasonable choice in agrotechnologies [3]. Stress resistance is a complex trait, which is realized in different genotypes through a variety of adaptive reactions (specific and nonspecific) and can change during ontogenesis. To obtain an objective assessment of genotype resistance, it is necessary to apply a set of methods that assess resistance at the cellular, organismal and population levels[2]. Most often in breeding programs, resistance of varieties to the pathogen is assessed in the field by the development and prevalence of the disease, as well as by yield depression [4]. In addition to the intensity of the disease symptoms, it is proposed to determine the varietal etiology of root infections and the phytosanitary condition of the soil in the rhizosphere of the evaluated plants during the growing season on all infected underground organs [5]. Field methods, being quite accurate, are time-consuming and labor-intensive[3]. In the realization of a comprehensive approach to diagnostics of stress tolerance, there is a need to search for evaluation criteria and development of non-destructive selection methods, allowing to accelerate the screening of resistant genotypes to biotic stress, especially at early stages of plant development. Biophysical methods, including those based on the analysis of photosynthesis,

[1]*Dolzhenko V.I., Vlasenko N.G., Vlasenko A.N. et al.* Zonal systems of spring wheat protection from weeds, diseases and pests in Western Siberia. Novosibirsk: SibNIIZIH, 2014, 124 p.

[2]*Gurova T.A., Denisyuk S.G., Lugovskaya O.S., Svezhintseva E.A., Mineev V.V.* Methodological provisions of early diagnostics of spring wheat and barley resistance to the combined action of stressors. Novosibirsk: SFSCA RAS, 2017, 62 p.

[3]*Goncharova E.A., Eremin G.V., Hasanova T.A.* Express methods for assessing stress tolerance of agricultural crops and their diagnostic strategy for breeding // Reports of the Russian Academy of Agricultural Sciences, 2015, N 5, pp. 21-24.

one of the stress-sensitive processes in plant cells, meet such requirements [6, 7].

During photosynthesis, all light energy absorbed by chlorophyll molecules is expended in photochemical reactions (photochemical quenching), thermal dissipation (non-photochemical quenching), and fluorescence, processes that compete to deactivate excited states of photosystem II (PS II) pigments[4]. Fluorescence values are proportional to changes in the energy used in photochemical reactions and its dissipation into heat. By the method of chlorophyll fluorescence (ChlF) registration it is possible to determine the disturbance of photosynthetic activity of plants, including the share of energy used for photochemical reactions [8]. Changes in the emission of ChlF are directly or indirectly related to all stages of the light phase of the photosynthesis process: water photolysis, electron transfer, generation of a pH gradient on thylakoid membranes, and ATP[5] synthesis [9]. The method of ChlF registration widely used in research is nondestructive, highly sensitive and allows obtaining information on the efficiency of photosynthesis and the integrity of the photosynthetic apparatus of plants at the earliest stages of stress development[6] [10]. In particular, it is used to assess the resistance of wheat and barley to abiotic and biotic stresses: temperature, drought, increased acidity, salinity, herbicides and diseases [6, 11-14]. Most often, a set of characteristics of ChlF kinetics of plant leaves obtained by the Pulse-Amplitude-Modulation method on modern RAM-fluorimeters is used in the assessment [15].

The method of ChlF registration is also used in the study of root rot infection of winter and spring wheat plants. The stages of photosynthesis of the plants most susceptible to *B. sorokiniana* infection are determined [16]. They identify promising parameters for assessing the condition of infected plants before visible changes in shoots appear[7] [17].

There is insufficient research on varietal specificity of formation of adaptive reactions of plants under the action of the stressor to develop a method for assessing stress tolerance of spring wheat varieties to *B. sorokiniana*.

The purpose of the work was to investigate adaptive reactions of spring soft wheat varieties to the action of biostressor *B. sorokiniana* by daily dynamics of ChlF kinetics parameters to assess stress tolerance of the varieties and to establish maximum intervarietal differences depending on the age of seedlings.

MATERIAL AND METHODS

Experimental work was carried out in the laboratory of the study of physical processes in agrophytocenoses of SFSCA RAS.

The research was carried out in vegetation experiments (water crops) in laboratory conditions on seedlings of released medium early varieties of spring soft wheat Novosibirskaya 41, Sibirskaya 21 and Novosibirskaya 29 of SibNIIRS - ICG SB RAS selection.

Experiment options:

– control (no stressor load);

– infection background (infection of seeds with *B. sorokiniana* 5000 conidia per grain).

The level of stress load – conidial suspension of B. sorokiniana 5000 conidia per grain – was determined by us in specially conducted vegetation experiments as allowing to differentiate wheat varieties of Siberian selection when assessing their resistance to this stress factor by biometric indices and permeability of cell membranes of seedlings [18].

Wheat seeds were pre-sterilized with 96% ethyl alcohol for 1 min followed by three times rinsing with distilled water. Then the seeds were placed in Petri dishes with moistened filter pa-

[4]*Baker N.R.* Chlorophyll fluorescence: a probe of photosynthesis in vivo // Annual review of plant biology, 2008, vol. 59, pp. 89– 113. DOI: 10.1146/annurev.arplant.59.032607.092759.

[5]*Goltsev V.N., Kalaji H.M., Paunov M., Baba V., Horacek T., Mojski J., Kotsel H., Allakhverdiev S.I.* Use of variable chlorophyll fluorescence to assess the physiological state of the photosynthetic apparatus of plants // Plant Physiology, 2016, vol. 63, N 6, pp. 881-907.

[6]*Lysenko V.S., Varduni T.V., Sawyer V.G., Krasnov V.P.* Plant chlorophyll fluorescence as an indicator of environmental stress: theoretical basis of the method // Fundamental Research, 2013, N 4, pp. 112-121.

[7]*Gurova T.A., Osipova G.M.* Instrumental methods and hardware-software tools in solving the problem of stress resistance in crop production // Computational Technologies, 2016, vol. 21, Special issue 1, pp. 65-74.

per and germinated in the thermostat at 22 ºC for three days.

The seeds were infected in the germination phase (on the 3rd day of cultivation) with a conidial suspension of a mixture of medium pathogenic isolates of *B. sorokiniana* prepared on 0.1% water agar (one drop per grain). Then, the seedlings of control and experimental variants were grown in a climacamera "Biotron-7" in roll culture on tap water under day-night photoperiod of 16 and 8 h, respectively, illumination 20 000 and 0 lx (day–night), temperature 22 and 18 ºC (day–night), humidity 60%.

The daily kinetics and parameters of ChlF were recorded using a Dual-PAM-100/F fluorimeter (Heinz Walz, Germany) using the amplitude-pulse modulation method in the mode of recording slow kinetics of dark induction curves with pulse saturation analysis (Slow Kinetics)[8]. The delay time for recording induction curves after determining the minimum and maximum ChlF α is 40 s, which is sufficient for complete re-oxidation of acceptors ("opening" of reaction centers). The interval between saturation pulses when recording induction curves is 20 s, and the data recording time is 4 min.

Excitation of chlorophyll α molecules was performed by a blue LED with a wavelength of 460 nm, and detection of ChlF was performed by a red photodiode with a wavelength of 680 nm. The operation of the fluorimeter was controlled using specialized software. Before measuring the ChlF, 10-16-day-old wheat seedlings were adapted to darkness in the sample chamber for 30 min to reach the fully oxidized state of PS II acceptors (all reaction centers of PS II are "open"). To record the ChlF parameters, a seedling leaf was fixed on a tripod with an optical holder and the program for recording the ChlF induction curves was started. The following fluorescence parameters were obtained: *F*0, *Fm*', minimum and maximum ChlF levels induced by the light pulse after adaptation of leaves to darkness; *F*0', *Fm*', minimum and maximum ChlF levels induced by the light pulse after adaptation of leaves to light; *Fv*/*Fm*, maximum photochemical quantum yield of PS II; Y(II), effective photochemical quantum yield of PS II after adaptation of leaves to light; *Y*(*NPQ*), quantum yield of regulated non-photochemical quenching of ChlF; *Y*(*NO*), quantum yield of unregulated non-photochemical quenching of ChlF; qP, photochemical quenching coefficient of ChlF; *qN*, non-photochemical quenching coefficient of ChlF; *ETR*, electron transport rate. We calculated the variable (variable) ChlF: *Fv* = *Fm* - *F*0 and the ratio of photochemical to nonphotochemical efficiency *Fv*/*F*0, a value proportional to the activity of the water-splitting complex on the donor side of PS II [19].

Variety response was determined by the relative change in the measured parameters of seedling fluorescence after exposure of the plants to the stressor. The smaller the changes in parameters, the higher the resistance in the studied group of varieties. Repetition of analytical and biological experiments was 6- and 3-fold. Statistical processing of data was carried out in Microsoft Excel 2010 using a standard package of data analysis. The ChlF parameters recorded during 4 min were analyzed. The mean error did not exceed 1.5-2.0%. Three series of experiments were performed. Student's *t*-criterion was used to determine the significance of the differences between the mean values.

RESULTS AND DISCUSSION

Earlier our studies have established the informativeness of ChlF parameters for diagnostics of photosynthetic activity and assessment of stress tolerance of two spring wheat varieties contrasting in resistance to *B. sorokiniana* and chloride salinity [20].

This study was conducted to confirm the possibility of using the method of ChlF registration in assessing stress tolerance of other three varieties of spring soft wheat and selecting the age of seedlings to establish maximum intervarietal differences in the study of adaptation of wheat seedlings to the action of *B. sorokiniana.*

There are two main target sites for biotic and abiotic stress in chloroplasts: the electron transport chain (ETC) and chlorophyll and carotenoid synthesis. The ETC with its electron transporters

[8] Product catalog of the German company "Heinz Walz GmbH". http://www.heinzwalz.ru/

and enzymes is involved in phosphorylation and photoreduction of NADP, whereas the synthesis of chlorophyll and carotenoids can be associated with the light-gathering complex (LGC) and the antennae of photosynthetic reaction centers (RCs)[9].

The common root rot pathogen of cereals *B. sorokiniana* and its toxins inhibit photosynthesis by blocking electron transport between photosystems, dissociating photophosphorylation, which is expressed in a decrease in the rate of ATP synthesis. Thus, the process of energy storage and, accordingly, the energy status of the organism is disturbed[10, 11]. Inhibition of the main link of photosynthesis - the process of energy storage – is the cause of productivity decrease. It is clearly traced at the early stages of ontogenesis (tillering - booting) of plants[12].

The level of tolerance of varieties to the pathogen is determined by their reaction to stress during growth and development. The study of daily dynamics of the ChlF parameters of seedlings when the seeds were infected with conidial suspension of *B. sorokiniana* isolates showed non-significant changes in the ChlF parameters on the 10th and 12th days of seedling cultivation. Disturbance of photosynthesis intensity associated with the increase of pathological process was registered in 14-day-old seedlings (see the Table).

The observed primary stress reactions are associated with an increase in thermal dissipation of absorbed light energy - the parameters of regulated non-photochemical quenching Y(NPQ) and qN increased significantly ($p \leq 0.05$) in all varieties in the range of 24.9-55.3% compared to the control. The parameter of unregulated non-photochemical quenching *Y*(*NO*), related to the generation of free-radical oxidation, increased significantly ($p \leq 0.05$) in Novosibirskaya 29 and Sibirskaya 21 varieties by 18.6 and 25.5%. Photochemical activity decreased only in the variety Sibirskaya 21 – reliable ($p \leq 0.05$) decrease in the values of parameters *Y*(II) and *ETR* by 18.8 and 18.6%, respectively.

Inhibition of photochemical activity of seedlings by the pathogen *B. sorokiniana* was found on the 16th day of cultivation. A reliable ($p \leq 0.05$) decrease in the effective quantum yield *Y*(II), the rate of electron transport *ETR* in varieties Sibirskaya 21 and Novosibirskaya 41 by 12.6; 13.6; 35.1 and 36.9 %, respectively, compared with the control was found. In the variety Novosibirskaya 29 these parameters changed unreliably. Inhibition of light-dependent reactions was accompanied by a reliable ($p \leq 0.05$) increase in the values of non-photochemical quenching parameters of ChlF – *qN* coefficient, quantum yields *Y*(*NO*) and *Y*(*NPQ*) from 23.0 (Novosibirskaya 29) to 233.3% (Novosibirskaya 41) compared with the control.

Varietal specificity of adaptive reactions formation was established. In the Novosibirskaya 29 variety, thermal dissipation of excited chlorophyll energy by PS II decreased, which led to a decrease in the values of *Y*(*NPQ*), *qN* and *Y*(*NO*) from 1.8 to 4.7 times, while the parameters of photochemical quenching *Y*(II), *ETR* and *qP* did not change significantly from the 14th to 16th days of seedling cultivation. The parameters *Fv*/*F*0 and *Fv*/*Fm* changed unreliably.

Adaptation mechanisms of the variety Sibirskaya 21 were aimed at reducing the suppression of photochemical quenching - the values of the relative change in the parameters *Y*(II), *ETR* decreased up to 1.4 times. In addition, the values of relative change of the parameters F0 and *Fv*/*F*0, as well as *Y*(*NPQ*) and *qN* decreased by 1.5–1.7 times up to 27.5% from the 14th to 16th days of cultivation.

A sharp increase in the values of *qN* and *Y*(*NPQ*) – by 129.2 and 233.0% compared to the control - was observed in the Novosibirskaya 41 variety from the 14th to 16th days of cultivation,

[9] *Dayan F.E., Zaccaro M.L.M.* Chlorophyll fluorescence as a marker for herbicide mechanisms of action // Pesticide Biochemistry and Physiology, 2012, vol. 102, pp. 189–197.

[10] *Tarabrin G.A., Bystrykh E.E.* Activity of primary reactions of photosynthesis as a test for selection of wheat for resistance to helminthosporiosis // Agricultural Biology, 1990, Plant biology series, N 5, pp. 59-69.

[11] *Fadeev Y.N., Bystrykh E.E., Tarabrin G.A.* Effect of toxins of the causative agent of helminthosporiosis root rot on photoreduction of NADP by chloroplasts of spring wheat // Agricultural Biology, 1987, N 12, pp. 29-34.

[12] *Fadeev Y.N., Bystrykh E.E., Tarabrin G.A.* Photochemical activity of wheat chloroplasts in the period of tillering – flowering depending on the lesion of *Bipolaris sorokiniana* // Reports of VASKHNIL, 1989, N 1, pp. 4-7.

Посуточная динамика параметров флуоресценции хлорофилла листьев проростков сортов пшеницы при инфицировании *B. sorokiniana*
Daily dynamics of chlorophyll fluorescence parameters in the leaves of seedlings of wheat cultivars under *B. sorokiniana* infection

Age of seedlings, days	Option	Parameter							
		Y(II)	*Y(NPQ)*	*Y(NO)*	*qP*	*qN*	*ETR*	F_V/Fm	F_V/F_0
Novosibirskaya 41									
14th	Control	5,9 ± 0,3	0,9 ± 0,04	5,9 ± 0,3	3,5 ± 0,1	2,3 ± 0,2	241,3 ± 6,3	0,68 ± 0,01	2,1 ± 0,2
	B. sorokiniana	5,9 ± 0,2	1,2 ± 0,03*	4,9 ± 0,2	3,5 ± 0,2	2,9 ± 0,3*	239,8 ± 6,3	0,67 ± 0,02	2,0 ± 0,1
16th	Control	6,1 ± 0,3	0,9 ± 0,3	4,9 ± 0,2	3,8 ± 0,3	2,4 ± 0,3	248,9 ± 0,3	0,65 ± 0,02	1,8 ± 0,1
	B. sorokiniana	3,9 ± 0,2*	3,0 ± 0,03**	5,0 ± 0,2	3,2±0,2*	5,5 ± 0,4**	157,1 ± 7,2*	0,58 ± 0,01*	1,3 ± 0,1*
Novosibirskaya 29									
14th	Control	5,9 ± 0,2	0,85 ± 0,02	4,3 ± 0,1	3,6 ± 0,2	2,1 ± 0,1	226,6 ± 7,3	0,72 ± 0,02	2,5 ± 0,2
	B. sorokiniana	5,6 ± 0,3	1,32 ± 0,01*	5,1 ± 0,2*	3,2 ± 0,1	3,0 ± 0,3	224,0 ± 6,9	0,71 ± 0,02	2,4 ± 0,2
16th	Control	5,2 ± 0,2	1,19 ± 0,01	5,5 ± 0,2	2,9 ± 0,1	2,6 ± 0,1	210,5 ± 6,1	0,74 ± 0,02	2,7 ± 0,2
	B. sorokiniana	5,1 ± 0,1	1,51 ± 0,02*	5,3 ± 0,1	3,1 ± 0,1	3,2 ± 0,2*	205,7 ± 6,0	0,71 ± 0,02	2,4 ± 0,2
Sibirskaya 21									
14th	Control	6,4 ± 0,3	0,76 ± 0,02	4,7 ± 0,2	3,5 ± 0,2	2,1 ± 0,1	263,5 ± 8,9	0,71 ± 0,02	2,4 ± 0,2
	B. sorokiniana	5,2 ± 0,1*	0,75 ± 0,02	5,9 ± 0,3*	3,2 ± 0,1	1,9 ± 0,1	214,6 ± 6,9*	0,63 ± 0,01	1,7 ± 0,1*
16th	Control	6,3 ± 0,2	1,11 ± 0,1	4,5 ± 0,1	3,6 ± 0,2	2,9 ± 0,2	260,6 ± 8,8	0,70 ± 0,02	2,4 ± 0,2
	B. sorokiniana	5,5 ± 0,2*	0,84 ± 0,02*	5,6 ± 0,2*	3,4 ± 0,1	2,1 ± 0,1*	225,1 ± 7,1*	0,66 ± 0,01	2,0 ± 0,1*

* Differences with the control are reliable at the significance level $p \leq 0,05$.

i.e. the regulated non-photochemical quenching of fluorescence acts as a protective mechanism against excessive excitation energy. However, a significant ($p \leq 0.05$) decrease in the values of parameters *Y*(II), *ETR*, *qP*, *F*0, *Fm*, *Fv*, *Fv*/*Fm*, *Fv*/*F*0 from 15.3 to 45.6% was found, i.e. there was a suppression of photosynthetic activity under pathogen action.

The difference in the change of photosynthetic activity parameters under the same stress load of *B. sorokiniana* between the varieties Novosibirskaya 29, Sibirskaya 21 and Novosibirskaya 41 indicates different tolerance mechanisms and strategies for converting light energy into chemical energy. In general, plants respond to stressors by activating defense and adaptive mechanisms in order to maintain photosynthetic activity to adapt to new environmental conditions. This may include an increase in the ability to dissipate energy, which is detected by an increase in the non-photochemical quenching parameters *Y*(*NPQ*), *qN* without changing the maximum quantum efficiency of *Fv*/*Fm* and *Fv*/*F*0 PS [21]. The dissipation of excess excitation energy into safe heat acts as a protective mechanism to avoid damage to the RC of PS II by light, the intensity of which exceeds the capabilities of electron transport[13]. This process was observed in the seedlings of all varieties on the 14th day of cultivation. Only in the variety Sibirskaya 21 there was a partial photoinhibition, detected by a decrease in the effective photochemical quantum yield of *Y*(II). However, on the 16th day of cultivation in this variety the inhibition of photochemistry parameters *Y*(II) and *ETR* decreased by 1.5 times.

Increasing stress on the 16th day of cultivation of Sibirskaya 21 and Novosibirskaya 41 varieties seedlings led to a decrease in the effective quantum yield Y(II), photochemical quenching coefficient *qP* and electron transport rate *ETR*, which means inhibition of the electron transfer chain and suppression of photosynthetic activity. Inhibition of light-dependent reactions was accompanied by an increase in *Y*(*NPQ*) and *qN* compared to the control in all cultivars. However, no irreversible loss of PS II functionality

[13]*Lichtenthaler H.K., Buschmann C., Knapp M.* How to Correctly Determine the Different Chlorophyll Fluorescence Parameters and the Chlorophyll Fluorescence Decrease Ratio RFd of Leaves with the PAM Fluorometer // Photosynthetica, 2005, vol. 43, pp. 379–393.

was observed under the conditions of our experiment. According to Pérez-Bueno M.-L. et al., only in the case of a serious irreversible loss of PS II functionality can both photochemical and non-photochemical ChlF quenching parameters be reduced [21]. The decrease in the values of the variable ChlF parameter *Fv*, as well as the background *F*0 and maximum *Fm*ChlF, observed only in Novosibirskaya 41, indicate a weakening of photosynthetic activity and damage to thylakoids, as well as a negative effect of the pathogen on the antenna complex of the RC of PS II, leading to energy loss during its migration[14] [10].

The selected stressor load of *B. sorokiniana* (5000 conidia per grain) and the duration of its action in the experimental conditions (16 days) allowed to establish intervarietal differences. As a result of ranking on the 16th day of cultivation of the seedlings (maximum intervarietal differences) resistance of the varieties to *B. sorokiniana* varied in descending order: Novosibirskaya 29 – Sibirskaya 21 – Novosibirskaya 41. The response of more resistant varieties Novosibirskaya 29 and Sibirskaya 21 to the pathogen introduction was less pronounced – the smallest or unreliable changes in the ChlF parameters relative to the control (see Fig. 1–3).

In seedlings of Novosibirskaya 29 variety under the action of the pathogen, a reliable change in only two parameters was observed – an increase in *Y*(*NPQ*) and *qN* by 26.9 and 23.0%, respectively – with an insignificant change in other parameters compared with the control. Similar results of the response of cereal cultivars differing in resistance to biostressors were obtained by other researchers. Rios J.A.et al. found a sharp disruption of photosynthetic activity of *Fv*/*Fm* and *Y*(II) of wheat leaves infected with *B. sorokiniana* in a more susceptible variety compared to a less susceptible one [1]. In a varietal response study of wheat leaves when infected with Blumeria graminis f., the activity of PS II (*Fv*/*Fm* and *ETR*) was strongly decreased in the susceptible variety, whereas the activity of PS II was unchanged in the resistant variety [14]. Katanić Z. et al. [22] also reported an established dependence of photosynthetic changes caused by Fusarium ear blight on winter wheat cultivar.

CONCLUSIONS

1. The conducted studies confirm the informativeness of the parameters *Y*(II), *ETR*, *qP*, *Fv*/*Fm*, *Fv*/*F*0, *Y*(*NPQ*), *qN* and *Y*(*NO*) as biomarkers of photosynthetic activity and assessment of resistance of soft spring wheat varieties to the common root rot pathogen *B. sorokiniana* at the level of photosynthetic apparatus.

2. Increasing stress from the 10th to 16th day of wheat seedlings cultivation under conditions of infection with *B. sorokiniana* resulted in the disruption of photo-synthetic activity in all the studied varieties - an increase in the inhibition of photochemical quenching (*Y*(II), *ETR*, *qP*, and *Fv*/*F*0) from 15.4 to 37.5% and the increase in thermal dissipation of the excited chlorophyll energy of PS II (*Y*(*NPQ*), *qN* and *Y*(*NO*)) from 25.0 to 233.0%. Primary protective and adaptive reactions of the seedlings to the action of the biostressor are associated with an increase in energy dissipation, then there was a decrease in photochemical activity.

3. The adaptive systems of the Novosibirskaya 29 variety were more labile (reliable change of two parameters – increase of *Y*(*NPQ*) and qN by 26.9 and 23.0%, respectively – with non-significant change of other parameters in comparison with the control). The greatest destructive effect of *B. sorokiniana* infection was revealed in Novosibirskaya 41 (decrease of *Y*(II) and *ETR* up to 37.0%, decrease of *Fm*, *Fv*, *Fv*/*F*0 up to 45.6%, increase of *Y*(*NPQ*), *Y*(*NO*) and *qN* up to 233.0% in comparison with the control. The parameter *Fv*/*Fm*, which is most frequently reported in the literature, was found to be less sensitive compared with its counterpart *Fv*/*F*0.

4. The age of seedlings (16 days) and infection load of *B. sorokiniana* (5000 conidia per grain) were determined for differentiation of the varieties by resistance. Significant intervarietal differences (from 1.9 to 8.6 times) were found

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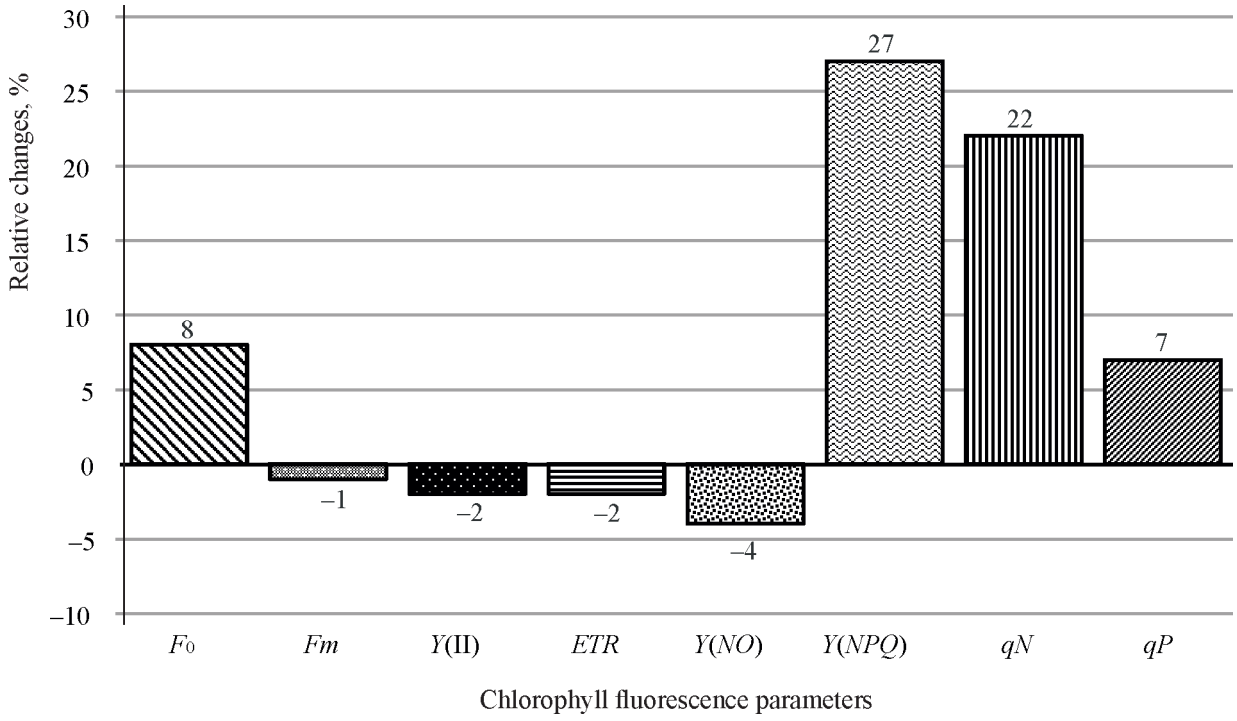

Рис. 1. Параметры флуоресценции хлорофилла листьев 16-суточных проростков сорта Новосибирская 29 при инфицировании *B. sorokiniana* (относительно устойчивый)

Fig. 1. Chlorophyll fluorescence parameters of the leaves of 16-day-old seedlings of Novosibirskaya 29 variety when infected with *B. sorokiniana* (relatively resistant)

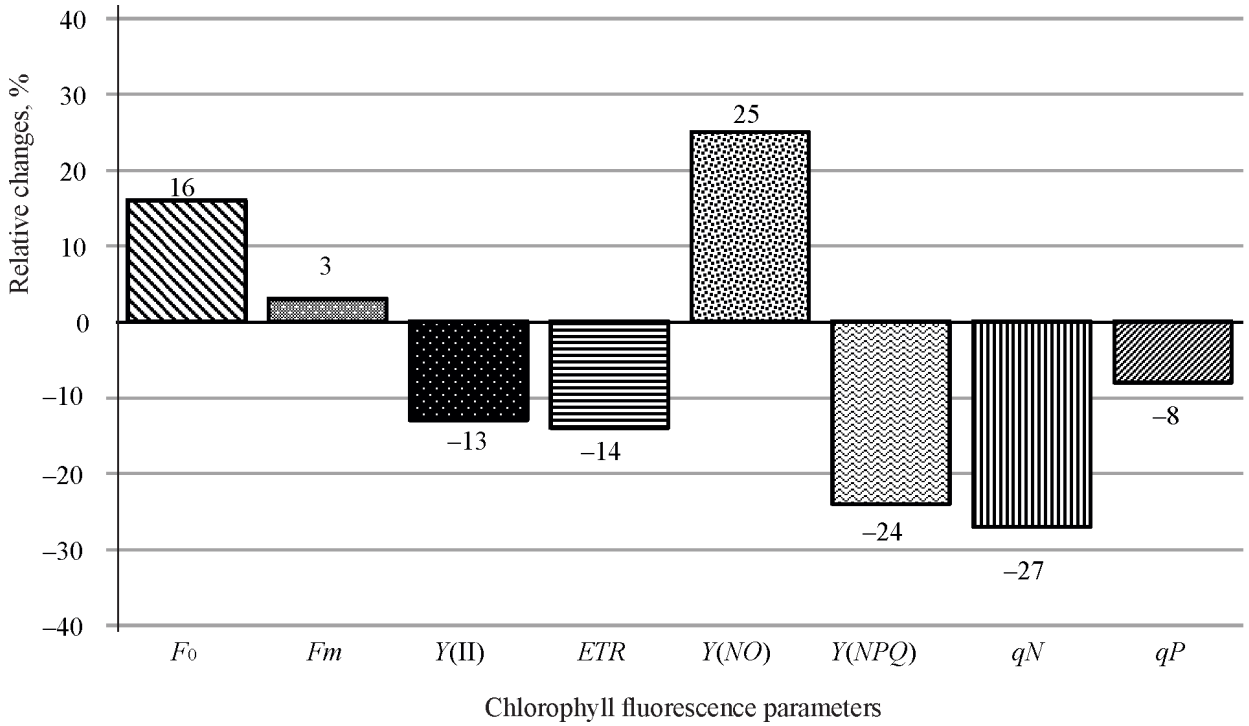

Рис. 2. Параметры флуоресценции хлорофилла листьев 16-суточных проростков сорта Сибирская 21 при инфицировании *B. sorokiniana* (относительно устойчивый)

Fig. 2. Chlorophyll fluorescence parameters of the leaves of 16-day-old seedlings of Sibirskaya 21 variety when infected with *B. sorokiniana* (relatively resistant)

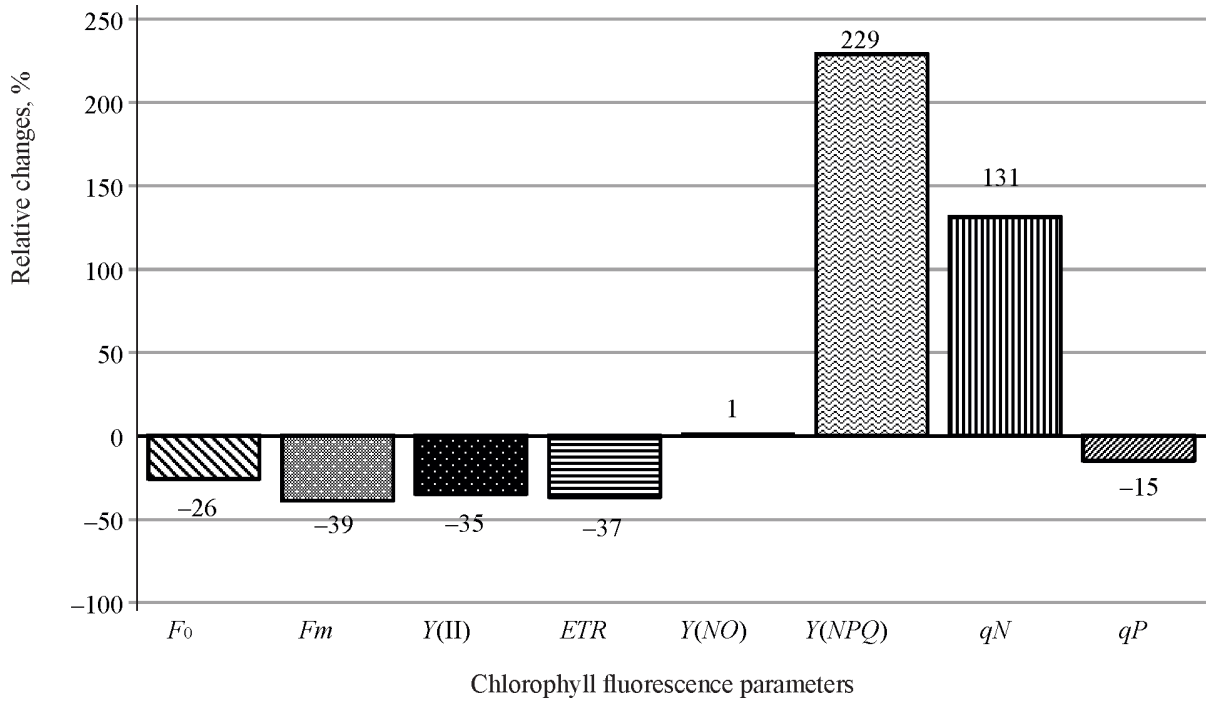

Рис. 3. Параметры флуоресценции хлорофилла листьев 16-суточных проростков сорта Новосибирская 41 при инфицировании *B. sorokiniana* (относительно неустойчивый)

Fig. 3. Chlorophyll fluorescence parameters of the leaves of 16-day-old seedlings of Novosibirskaya 41 variety when infected with *B. sorokiniana* (relatively unstable)

for all parameters in 16-day-old seedlings. Varietal specificity was established – the smallest changes in parameters relative to the control were in more resistant varieties Novosibirskaya 29 and Sibirskaya 21.

The proposed approach will make it possible to develop a nondestructive method for early diagnosis of stress resistance of spring wheat varieties to the pathogen of common cereal root rot *B. sorokiniana* Shoem.

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АКТИВНОСТЬ ОКСИДОРЕДУКТАЗ ПРОРОСТКОВ СОИ НА РАННЕЙ СТАДИИ ОНТОГЕНЕЗА

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Представлены результаты анализа удельной активности и множественных форм ферментов класса оксидоредуктаз: антиоксидантного комплекса (каталазы и пероксидазы) и дегидрогеназ (алкогольдегидрогеназы, НАД$^+$-малатдегидрогеназы, глюкозо-6-фосфатдегидрогеназы). Объектом исследования служили семь сортов сои (*Glycine max* (L.) Merrill). Для анализа использовали покоящиеся семена и 3- и 7-дневные проростки сои. Содержание белка определяли методом Лоури, активность пероксидазы – колориметрическим, каталазы и исследуемых дегидрогеназ – спектрофотометрическим методами, электрофоретические спектры ферментов – методом электрофореза на колонках 7,5%-го полиакриламидного геля. Выявление на геле зон с ферментативной активностью проводили соответствующими гистохимическими методами. Анализ удельной активности антиоксидантных ферментов в покоящихся семенах сои выявил повышенную активность пероксидазы и невысокую активность каталазы. При прорастании семян наблюдается обратная зависимость удельной активности этих ферментов. На 7-е сутки удельная активность каталазы повышается, пероксидазы снижается до минимума. При проращивании сои выявлено 5 форм каталазы, что свидетельствует о невысоком полиморфизме и стабильности фермента, и 18 форм пероксидазы, которые подтверждают высокий полиморфизм и возможность использования этого фермента в качестве маркера биохимических процессов. Установлено, что алкогольдегидрогеназа и глюкозо-6-фосфатдегидрогеназа обладают невысокой гетерогенностью, причем удельная активность этих ферментов при прорастании снижается по сравнению с периодом покоя семян сои. Удельная активность НАД$^+$-малатдегидрогеназы при проращивании незначительно повышается. В исследуемых сортах сои выявлено 8 форм этого фермента, что свидетельствует о повышенном полиморфизме. В покоящихся семенах сои электрофоретические спектры НАД$^+$-малатдегидрогеназы отличались сортовым разнообразием, что позволяет использовать фермент в качестве маркера для дальнейших исследований.

Ключевые слова: *Glycine max*, семена, проростки, каталаза, пероксидаза, дегидрогеназы, множественные формы

OXIDOREDUCTASE ACTIVITY OF SOYBEAN SEEDLINGS AT THE EARLY STAGE OF ONTOGENESIS

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The results of analysis of specific activity and multiple forms of oxidoreductase class enzymes are presented: antioxidant complex (catalase and peroxidase) and dehydrogenases (alcohol dehydrogenase, NAD$^+$-malate dehydrogenase, glucose-6-phosphate dehydrogenase). Seven varieties of soybean (*Glycine max* (L.) Merrill) served as the object of the study. Dormant seeds and 3- and 7-day-old soybean seedlings were used for the analysis. Protein content was determined by the Lowry method, peroxidase activity was determined by colorimetric, catalase and the studied dehydrogenases – by spectrophotometric methods, electrophoretic spectra of enzymes – by electrophoresis on 7.5% polyacrylamide gel columns. Identification of the zones with enzymatic activity on the gel was performed

by appropriate histochemical methods. Analysis of specific activity of antioxidant enzymes in dormant soybean seeds revealed increased activity of peroxidase and low activity of catalase. During seed germination, the inverse relationship of the specific activity of these enzymes is observed. On the 7th day the specific activity of catalase increases, that of peroxidase decreases to a minimum. In soybean germination, 5 forms of catalase were detected, indicating low polymorphism and stability of the enzyme, and 18 forms of peroxidase, which confirm high polymorphism and the possibility of using this enzyme as a marker of biochemical processes. Alcohol dehydrogenase and glucose-6-phosphate dehydrogenase were found to be of low heterogeneity, with the specific activity of these enzymes decreasing during germination compared to the dormant period of soybean seeds. The specific activity of NAD^+-malate dehydrogenase increases slightly during germination. Eight forms of this enzyme were detected in the soybean varieties studied indicating increased polymorphism. In dormant soybean seeds, the electrophoretic spectra of NAD^+-malate dehydrogenase exhibited varietal diversity allowing the enzyme to be used as a marker for further studies.

Keywords: *Glycine max*, soybean seeds, seedlings, catalase, peroxidase, dehydrogenases, multiple forms

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INTRODUCTION

Seed germination is associated with physiological, biochemical and morphological changes, and represents a critical stage in the life cycle of plants and is poorly understood [1]. Plant growth processes are mainly determined by the factors of the internal environment, and the leading role is played by genetic and hormonal regulation [2].

Plants at different stages of ontogenesis are exposed to various unfavorable environmental factors, which leads to increased formation of reactive oxygen species and oxidative stress [3]. The first phases of ontogenesis are impossible without the functioning of key enzyme systems aimed at maintaining a stable level of reactive oxygen intermediates (ROIs) in the cell and more complete utilization of spare substances in metabolic processes. During germination under conditions of saturation with moisture, oxygen and positive temperature in swollen plant seeds, the main metabolic processes are activated, the activity of mitochondrial enzymes of Krebs cycle and oxidative phosphorylation increases, i.e. respiration is activated[1][3].

Recently, the ability of AFCs to act as signaling molecules and regulators of gene expression determining the plant defense response has been actively discussed [4]. ROIs, including hydrogen peroxide, modulate metabolic and hormonal signaling pathways that maintain seed dormancy and germination. Oxidative stress is accompanied by changes in cellular structures, disruption of cytosolic and mitochondrial enzyme activity, membrane integrity, changes in the composition of metabolites, and increased lipid peroxidation[2] [5]. To protect against the negative effects of stressors, plants have an antioxidant system that includes low molecular weight antioxidants and well-studied enzymes of the antioxidant complex class of oxidoreductases, which include catalase (CAT, KE 1.11.1.6), peroxidase (POD,

[1]*Rogozhin V.V., Rogozhina T.V.* Physiological and biochemical mechanisms of germination of wheat grains // Bulletin Altai State Agricultural University, 2011, N 8 (82), pp. 17-21.

[2]*Rogozhin V.V., Kurilyuk T.T.* The role of peroxidase in the mechanisms of dormancy and germination of grains of some cereal crops // Proceedings of TSKhA, 2010, Issue 4, pp. 22-32.

KE 1.11.1.7) and superoxide dismutase (SOD, KE 1.15.1.1) [6]. It has been established that oxidoreductases participate in the defense and adaptive reactions of soybean plants to the effects of hypo- and hyperthermia [7]. Little-studied enzymes of the oxidoreductase class are dehydrogenases, which determine the operation of the main metabolic pathways necessary for the preservation of seed viability and germination. Malate dehydrogenase (NAD+MDH, KE 1.1.1.37) is the key enzyme of the tricarboxylic acid cycle, catalyzing the reversible interconversion of oxaloacetate and malate, associated with the oxidation/reduction of coenzymes. It occupies an important place in catabolic and anabolic processes, participates in adaptive responses to stress factors, which indicates the important role of the enzyme in the regulation of plant metabolism [8]. Alcohol dehydrogenase (ADH, KE 1.1.1.1.1) catalyzes the reduction of ethyl alcohol into acetic aldehyde during alcoholic fermentation. The equilibrium of this reaction is shifted towards the formation of ethanol, the accumulation of which may serve as an adaptive trait of plants and seeds to tolerate a number of external unfavorable conditions[3]. Glucose-6-phosphate dehydrogenase (G6PDH, KE 1.1.1.49) is an important enzyme of the pentose phosphate pathway, modulating glucose metabolism, and is involved in the regulation of NADP content and regulation of seed germination and seedling development [5]. ADH and G6PDH are indicators of anaerobic metabolic processes (see footnote 3). A promising direction is the study of biochemical adaptation at the molecular level with the participation of multiple forms of enzymes that provide plasticity of biochemical processes of the organism to environmental conditions. Moreover, enzymes are used as genetic markers [9].

Soybean is one of the most famous legume crops of multipurpose use in world agriculture. The main soybean-growing region of the Russian Federation is the Amur region, where 30% of sown areas of the crop are concentrated [10]. Most of the varieties grown in the Amur region are created in the Federal Research Center "All-Russian Scientific Research Institute of Soybean" (FRC "VNII of soybean"). Soybean cultivation takes place in different natural and climatic zones of the region, which differ significantly in the amount of precipitation and temperature regime. Therefore, to obtain high yields of soybean varieties adaptive to agro-ecological conditions of cultivation in the region are necessary [11]. In studies on soybean introduction in Russia, breeders focus on the study of economically valuable traits of the crop [12], but not enough on the enzymes involved in increasing soybean adaptation to unfavorable environmental factors [3]. Currently, the specific activity and multiple forms of enzymes of hydrolase class (acid phosphatase, amylase, esterase, ribonuclease) and some antioxidant oxidoreductases (catalase, peroxidase, superoxide dismutase, polyphenol oxidase) have been studied in soybean seeds [3, 13, 14]. Poorly studied enzymes of soybean are dehydrogenases.

The purpose of the study was to investigate the activity of enzymes of the oxidoreductase class during the dormancy period of soybean seeds and at the initial stages of their germination.

MATERIAL AND METHODS

The object of the study was a collection of seeds of seven soybean varieties (Harmony, Lydia, Sonata, MK-100, Nega 1, Grazia, Persona) (*Glycine max* (L.) Merrill) of the selection of FRC "VNII of soybean", which are widely used in production. The seeds were germinated in the thermostat on filter paper in Petri dishes at 25°C for 3 and 7 days. Ungerminated seeds at the dormancy stage served as a control. All studies were carried out in two biological and three analytical repetitions. To obtain protein extracts of soybean seeds and seedlings, a 500 mg weight of material was homogenized with 0.15 M sodium chloride solution for 15 min at a temperature from 0 to +5 °C. The obtained homogenate was centrifuged at 3000 rpm for 15 min. The precip-

[3]*Rogozhin V.V., Rogozhina T.V.* The role of alcohol dehydrogenase in the mechanisms of resting wheat grains // Bulletin Altai State Agricultural University, 2012, N 3 (89), pp. 32-36.

itate was discarded, the supernatant was filtered through mill gas and used for analysis. Protein content was determined by the Lowry method on a photoelectrocolorimeter (KFK-3, Russia) in cells with an optical layer thickness of 1 cm at 750 nm against the control. CAT activity was determined spectrophotometrically[4] at 240 nm by the rate of decomposition of hydrogen peroxide with the formation of water and oxygen against the control in cuvettes with a thickness of the absorbing layer of 1 cm. POD activity was measured by colorimetric method according to A.N. Boyarkin in modification of A.T. Mokronosov on KFK-2 at a wavelength of 670 nm in a cell with an absorbing layer of 2 cm according to the reaction rate of oxidation of benzidine to form benzidine blue in the presence of hydrogen peroxide[5]. The activity of dehydrogenases was determined spectrophotometrically at a wavelength of 340 nm in cells with an optical layer thickness of 1 cm by the increase or decrease in optical density proportional to the concentration of NADH or NADPH. The specific activity was expressed in units per milligram (units/mg) of protein. Electrophoretic spectra of the studied enzymes were revealed by electrophoresis on columns of 7.5% polyacrylamide gel. Identification of zones with enzymatic activity on the gel was performed by appropriate histochemical methods[6]. The values of their relative electrophoretic mobility (Rf) were determined for the identified multiple forms of enzymes and enzymograms were plotted. The numbering of enzyme forms is given from more highly mobile to low-mobile forms. The results of the study were processed using STATISTICA 10. Reliability of changes in the studied parameters was determined by the differences of mean values using Student's criterion. A 5% significance level was used in the calculations.

RESULTS AND DISCUSSION

As a result of the experiment, the activity of enzymes of the class of oxidoreductases of the antioxidant complex (CAT and POD) and dehydrogenases (ADH, NAD^+MDH and G6PDH) in soybean seeds at dormancy and at the early stages of germination was studied. Studies of the specific activity of CAT, one of the main enzymes involved in the utilization of hydrogen peroxide, in dormant seeds of the soybean varieties studied showed low values due to the low affinity of CAT to low concentrations of hydrogen peroxide. The electrophoresis method for CAT in dormant seeds revealed 4 forms of enzyme with the same electrophoretic mobility for each variety (see Fig. 1). The specific activity of POD in the studied resting seeds, in contrast to CAT, was high (with a maximum of 1422.8 units/mg protein for variety MK-100), which corresponded to a high heterogeneity of the enzyme (from 4 to 7 forms). This fact, apparently, indicates that POD actively participates in maintaining the viability of dormant seeds and activates germination processes. At the same time, the product of reactions involving CAT and POD is water, which is necessary for dormant seeds (see footnote 1).

At seed germination within 3 days, the specific activity of CAT and POD sharply decreases (see Fig. 1), which correlates with a decrease in the number of multiple forms of these enzymes. It should be noted that for CAT at this stage, all soybean varieties studied contained only 3 forms of the enzyme detected at the dormancy stage, except for the form (Rf = 0.23). For POD it was found from 1 form (Harmony, Grazia) to 3 forms (MK-100, Lidia). On the 7th day of exposure, the specific activity of POD remains low, but there is an increase in the number of multiple forms of this enzyme for all soybean varieties. At the same time, 3 new highly mobile forms were found in seedlings (Rf = 0.96, Rf = 0.91,

[4]*Sibgatullina G.V., Haertdinova L.R., Gumerova E.A., Akulov A.N., Kostyukova Y.A., Nikonorova N.A., Rumyantseva N.I.* Methods for determining the redox status of cultured plant cells: a textbook. Kazan: Kazan (Volga Region) Federal University, 2011, 61 p.

[5]*Korobko V.V., Kasatkin M.Yu.* Plant Physiology. Large workshop: Textbook for students of the Faculty of Biology. Saratov: Publishinghouse «Saratovsource», 2017, 120 p.

[6]*Ivachenko L.E., Kashina V.A., Maskaltsova E.S., Razantsvei V.I., Stasiuk E.M., Trofimtsova I.A.* Methods of studying polymorphism of soybean enzymes. Blagoveshchensk: BSPU Publishing House, 2008, 138 p.

Rf = 0.87). The CAT activity on the 7th day, on the contrary, sharply increases (on average 20 times), which is probably due to the formation of a form with low electrophoretic mobility (Rf = 0.13).

The specific activity of ADH in the seeds of the soybean varieties under study varied almost 2-fold (from 1.4 $\cdot 10^{-6}$ to 2.7 $\cdot$ 10^{-6} units/mg protein (for varieties Nega 1, Harmonia and Sonata, respectively)) and only 1 form of the enzyme

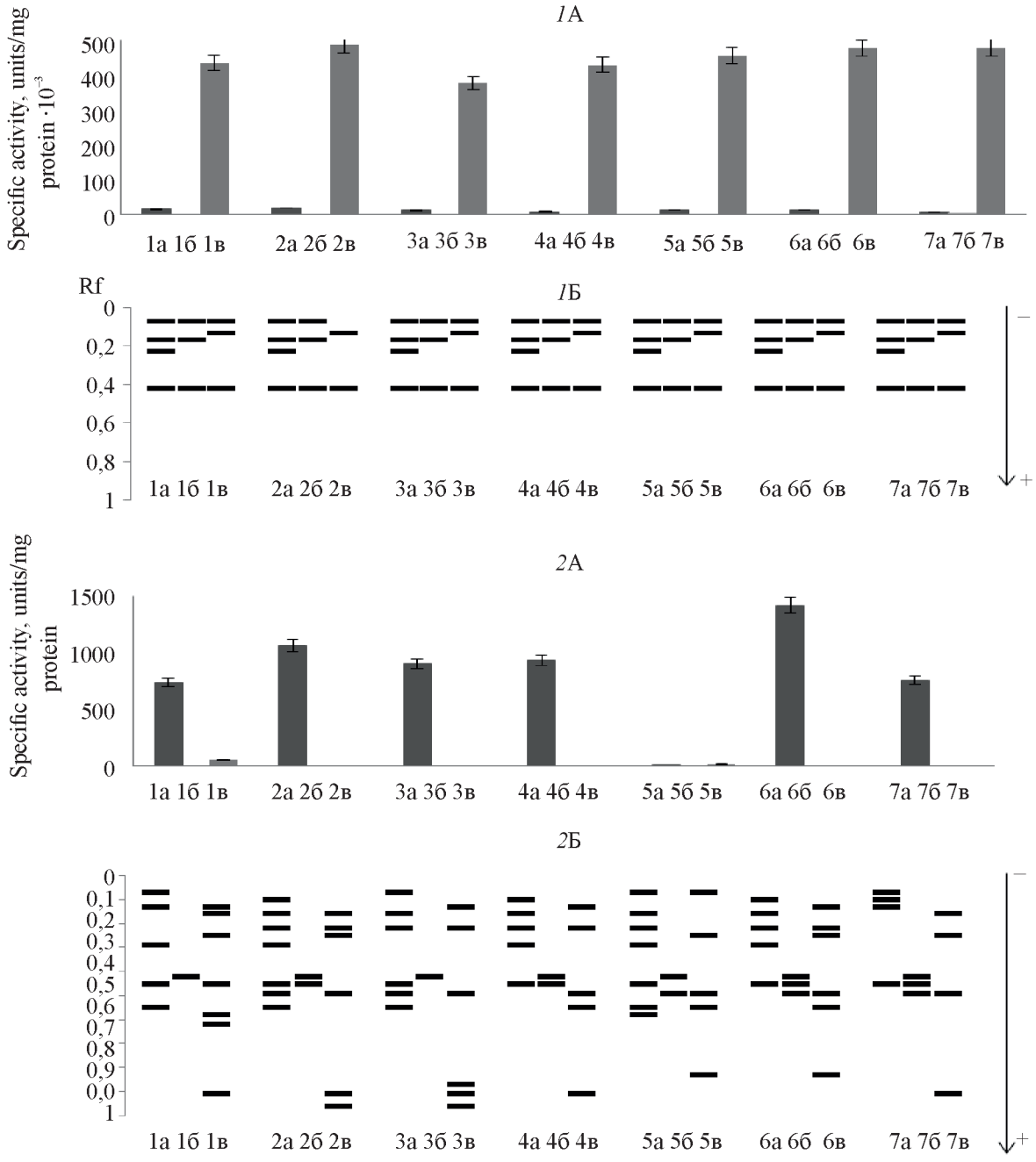

Рис. 1. Удельная активность (А) и схемы энзимограмм (Б) каталазы (*1*) и пероксидазы (*2*) коллекции семян сортов сои:
1 – Гармония; 2 – Соната; 3 – Грация; 4 – Нега 1; 5 – Персона; 6 – МК-100; 7 – Лидия (а – семена, находящиеся в стадии вынужденного покоя, б – 3-дневные проростки семян, в – 7-дневные проростки)

Fig. 1. Specific activity (A) and enzymogram schemes (Б) of catalase (*1*) and peroxidase (*2*) from a collection of seeds of soybean varieties:
1 – Harmony; 2 – Sonata; 3 – Gratsia; 4 – Nega 1; 5 – Persona; 6 – MK-100; 7 – Lydia (a – seeds at the stage of forced dormancy, б – 3-day seedlings, в – 7-day seedlings)

with medium electrophoretic mobility (Rf = 0.40) was detected, indicating a low polymorphism of ADH in seeds of the released soybean varieties (see Fig. 2). When the seeds were germinated for 3 days, the specific activity of ADH decreased significantly, but in all soybean varieties under study 2 forms of ADH with medium electrophoretic mobility were detected. In the varieties Persona, MK-100 and Lydia an additional form of ADH was detected (Rf = 0.40). This is probably due to the fact that the enzyme is necessary, first of all, to preserve the viability of seeds and to ensure the processes associated with their germination, as it maintains the equilibrium in the system ethanol – acetaldehyde in plant cells. On the 7th day of germination, only 1 form of ADH with low electrophoretic mobility (Rf = 0.20) remains, although the specific activity of the enzyme for varieties Harmony, Sonata, Grazia, Nega 1 slightly increases, while in varieties Persona, MK-100 and Lydia the activity is low.

The specific activity of G6PDH in the seeds under forced dormancy varied from $0.235 \cdot 10^{-8}$ units/mg protein for Lidia to $0.44 \cdot 10^{-8}$ units/mg protein for Harmony and Persona (see Fig. 2). At germination on the 3rd day, the enzyme activity decreases significantly, except for the variety Persona, and increases on the 7th day. Probably, the increase in G6PDH activity is associated with high mobilization of nutrients, which leads to intensive growth of seedlings. Only 1 form of G6PDH with low electrophoretic mobility (Rf = 0.24) was detected for all soybean varieties studied, which indicates the stability of this most important enzyme of the pentose phosphate pathway.

The specific activity of NAD^+MDH in seeds in the forced dormancy stage was low and varied insignificantly from $4.1 \cdot 10^{-6}$ to $5.1 \cdot 10^{-6}$ units/mg protein (varieties Nega 1 and Grazia) (see Fig. 2). On the 3rd day of germination, the specific activity of NAD^+MDH increases by 1.5–2.0 times, which is apparently associated with the strengthening of tricarboxylic acid cycle processes. On the 7th day of germination, the enzyme activity for the studied varieties exceeds the level of specific activity detected earlier in seeds in the forced dormancy stage, with the exception of the variety MK-100. Electrophoretic spectra of NAD^+MDH were characterized by significant diversity. High heterogeneity of NAD^+MDH was revealed in soybean seeds in the forced dormancy state. From 2 (Lydia) to 5 (Harmony and Grazia) forms of the enzyme with different electrophoretic mobility were found for each variety. It should be noted that a specific set of MDH forms was detected for each variety at the seed stage. In all the studied varieties a form of the enzyme with Rf = 0.51 with average electrophoretic mobility was detected. After 3 days of exposure, 5 forms of NAD^+MDH were detected. And 2 forms with average (Rf = 0.48 and Rf = 0.40) electrophoretic mobility were revealed, which is characteristic for all varieties. On the 7th day of exposure for all the zoned varieties a molecular form with medium electrophoretic mobility (Rf = 0.48) was found, and it can be called the main form for seedlings, as it is found during seed exposure for 3 and 7 days. A total of 8 multiple forms of NAD^+MDH were detected, indicating an increased polymorphism of the enzyme and the possibility of its use as a marker of biochemical processes in further studies.

CONCLUSIONS

1. The activity of oxidoreductase enzymes of the antioxidant system (CAT and POD) and dehydrogenases (ADH, G6PDH and NAD^+MDH) was found to differ significantly during germination. Increased specific activity of POD (from 737.5 units/mg protein for Harmony variety to 1422.8 units/mg protein for MK-100 variety) and low activity of CAT (from $7.7 \cdot 10^{-3}$ units/mg protein for Lydia variety to $18.9 \cdot 10^{-3}$ units/mg protein for Sonata variety) were detected in dormant soybean seeds. During germination of seeds within 3 days, the specific activity of enzymes sharply decreases - on average by 5 times. On the 7th day, the specific activity of CAT increases (from $377.2 \cdot 10^{-3}$ units/mg protein for the variety Grazia to $488.4 \cdot 10^{-3}$ units/mg protein for the variety Sonata), and POD decreases to a minimum (from 2.7 units/mg protein for the variety Harmony to 55.5 units/mg protein for the variety Nega-1). In soybean germination, 5

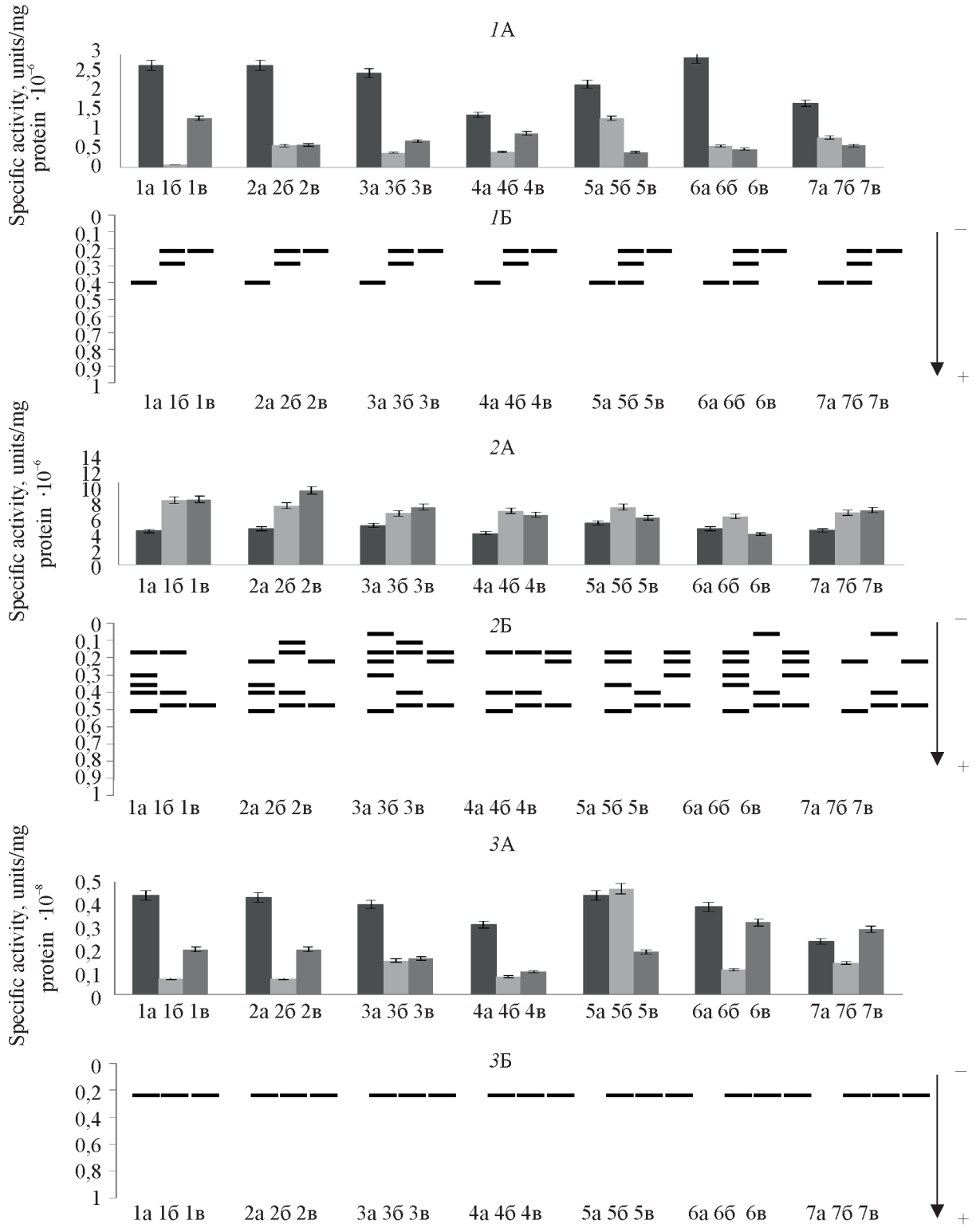

Рис. 2. Удельная активность (А) и схемы энзимограмм (Б) алкогольдегидрогеназы (*1*), малатдегидрогеназы (*2*) и глюкозо-6-фосфатдегидрогеназы (*3*) коллекции семян сортов сои:

1 – Гармония; 2 – Соната; 3 – Грация; 4 – Нега 1; 5 – Персона; 6 – МК-100; 7 – Лидия (а – семена находящиеся в стадии вынужденного покоя, б – 3-дневные проростки семян, в – 7-дневные проростки)

Fig. 2. Specific activity (A) and enzymogram patterns (Б) of alcohol dehydrogenase (*1*), malate dehydrogenase (*2*) and glucose-6-phosphate dehydrogenase (*3*) from a collection of soybean varieties seeds: 1 – Harmony; 2 – Sonata; 3 – Gratsia; 4 – Nega 1; 5 – Persona; 6 – MK-100; 7 – Lydia (a – seeds at the stage of forced dormancy, б – 3-day seedlings, в – 7-day seedlings)

CAT forms were detected, indicating a low polymorphism and stability of the enzyme, and 18 POD forms, confirming a high polymorphism. On the 7th day, 3 new highly mobile forms of POD were detected in the seedlings. The important role of these enzymes in the detoxification of soybean seeds and seedlings from reactive oxygen species and in the formation of metabolic water, contributing to the enhancement of biochemical processes and better seed germination should be noted.

2. The specific activity of dehydrogenases in the studied soybean seeds and seedlings also differed significantly. Thus, in soybean seeds in the dormant stage, an increased activity of ADH (from $1.4 \cdot 10^{-6}$ units/mg protein for Nega-1 to $2.7 \cdot 10^{-6}$ units. /mg protein for Harmony and Sonata varieties) and G6FDG (from $0.235 \cdot 10^{-8}$ units/mg protein for Lydia variety to $0.44 \cdot 10^{-8}$ units/mg protein for Harmony and Persona varieties), which decreased during germination. Moreover, a low heterogeneity of these enzymes in the seeds and seedlings of the studied soybean varieties should be noted, where 3 forms of ADH and 1 form of G6FDH were detected.

3. The specific activity of NAD^+MDH, the most important enzyme of the tricarboxylic acid cycle, increased insignificantly during germination (from $4 \cdot 10^{-6}$ units/mg protein for MK-100 variety to $9.7 \cdot 10^{-6}$ units/mg protein for Sonata variety), but its electrophoretic spectra were characterized by diversity. A total of 8 forms of this enzyme were identified, indicating increased polymorphism and the possibility of using this enzyme as a marker of biochemical processes. The electrophoretic spectra of NAD^+MDH in dormant seeds of the soybean varieties under study deserve special attention, having a specific distribution of forms of this enzyme, differing in electrophoretic mobility, further studies of which will allow a deeper understanding of the physiological and biochemical role of NAD^+MDH in the cellular metabolism of soybeans.

4. The study of specific activity and multiple forms of the most important enzymes of the class of oxidoreductases opens new perspectives for further investigation of biochemical processes at early stages of ontogenesis and has important practical and theoretical significance.

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КОНТАМИНАЦИЯ МИКОТОКСИНАМИ СИЛОСА, ЗАГОТАВЛИВАЕМОГО ЖИВОТНОВОДЧЕСКИМИ ХОЗЯЙСТВАМИ ВОЛОГОДСКОЙ ОБЛАСТИ

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В статье отражены результаты изучения инфицированности микотоксинами силоса, заготовленного в 2021–2023 гг. на территории Вологодской области. Основная задача исследования – оценка степени контаминации микотоксинами различных видов силоса. Анализируемые образцы были представлены силосами различного состава: бобово-злаковый, злаковый, кукурузный, разнотравный и силос из трав с естественных угодий. Всего исследовано свыше 150 образцов силоса из 22 хозяйств региона. Содержание микотоксинов (сумма афлатоксинов, охратоксин А, зеараленон) в кормах определяли методом иммуноферментного анализа с помощью стандартных тест-систем компании «КомПродСервис» (Беларусь). Установлено, что практически во всех кормах, заготавливаемых хозяйствами Вологодской области, присутствуют микотоксины. Так, более 75% образцов содержали афлатоксины в концентрации выше 5 мкг/кг (ПДК), 45% – содержали охратоксин А в концентрации, также превышающей ПДК. Наиболее подверженным заражению афлатоксинами оказался бобово-злаковый и кукурузный силос, доля зараженных образцов в данном случае составила 94 и 85% соответственно. Наличие охратоксина А в высоких концентрациях чаще встречалось в силосе, полученном из разнотравья (91%). Высокая доля контаминированных образцов также обнаружена в силосе из трав, собранных с естественных угодий (73% – афлатоксины, 48% – охратоксин А). Полученные результаты свидетельствуют о необходимости более тщательного контроля за заготовкой и хранением силоса на всех этапах производства, принятия сельхозпроизводителями превентивных мер по снижению негативных последствий использования токсичных кормов.

Ключевые слова: силос, микотоксины, афлатоксины, зеараленон, охратоксин А

MYCOTOXIN CONTAMINATION OF SILAGE HARVESTED BY LIVESTOCK FARMS IN THE VOLOGDA REGION

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The article reflects the results of the study of mycotoxin contamination of silage harvested in 2021–2023 in the Vologda region. The main objective of the study was to assess the degree of mycotoxin contamination of different types of silage. The analyzed samples were represented by silages of different composition: legume-grass, cereal, corn, mixed grass and grass silage from natural lands. In total, more than 150 silage samples from 22 farms in the region were investigated. The content of mycotoxins (aflatoxin sum, ochratoxin A, zearalenone) in feeds was determined by enzyme immunoassay using standard test systems of ComProdService (Belarus). It has been established that almost all fodders harvested by farms in the Vologda region contain mycotoxins. Thus, more than 75% of samples

contained aflatoxins at a concentration above 5 μg/kg (MAC), 45% contained ochratoxin A at a concentration also exceeding MAC. The most susceptible to aflatoxin contamination was legume-grass and corn silage, the proportion of contaminated samples in this case was 94 and 85%, respectively. The presence of ochratoxin A in high concentrations was more frequent in silage obtained from mixed grasses (91%). A high proportion of contaminated samples was also found in grass silage harvested from natural lands (73% aflatoxins, 48% ochratoxin A). The obtained results indicate the need for more careful control of silage preparation and storage at all stages of production, taking preventive measures by agricultural producers to reduce the negative consequences of the use of toxic fodder.

Keywords: silage, mycotoxins, aflatoxins, zearalenone, ochratoxin A

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INTRODUCTION

Nowadays there is a problem of mycotoxins infestation of forages of plant origin – a group of chemical compounds, which are extremely diverse in structure and properties. Mycotoxins pose a certain danger, causing diseases in farm animals, which are commonly called mycotoxicoses, thus causing great economic damage to agricultural producers.

Mycotoxins in the broad sense are considered all metabolites of mold fungi. They also include antibiotics produced by mold fungi, thus mycotoxins provide their producer with a competitive advantage in their natural habitat. Among more than 400 toxins, the most studied classes include zearalenone, ochratoxins, fumonisins, aflatoxins, trichothecene mycotoxins, T-2 toxin, dioxynivalenol, ergotoxins. *Penicillium* mold fungi (*P. roqueforti* and *P. paneum*) are routinely detected in feed samples and produce many toxic substances, including PR toxin, markfortorins A, B and C, penitrem A, roquefortine, andrazines A and B, patulin and microphenolic acid. These toxins have a wide range of effects: roquefortine, penitrem A and markfortorins are neurotoxic, PR toxin and patulin are carcinogenic and genotoxic, etc.[1]

In recent years, a pronounced antibacterial effect of mycotoxins has been found, affecting the composition and number of microflora of the digestive tract of cattle [1]. This results in impaired digestibility of nutrients and decreased protective function of animal microflora.

As toxicants capable of causing a variety of clinical symptoms, mycotoxins often mask their negative effects on living organisms [2]. The presence of mycotoxins in the feed eaten by cattle can have serious adverse effects on their health and productivity. Mycotoxins can affect the condition and operation of various organs and systems: damage to the rumen and

[1] *Sarkisov A.H.* Mycotoxicosis (fungal poisoning). Moscow: Selkhozgiz, 1954, 215 p.

intestine can cause impaired digestion and absorption of nutrients; damage to the liver and kidneys contributes to the disruption of metabolism and purification of the body; when affecting the immune system there is a suppression of its functions, resulting in increased vulnerability to infections and diseases; when affecting the reproductive system increases the number of abortions and reduces fertility; when the nervous system is disturbed, the number of abortions increases and fertility decreases; and with disorders in the nervous system various behavioral changes may be manifested.

It has been previously established that the rumen microflora of a cow of high productivity (from 5 thousand kg/year and above) as a result of dysbiotic disorders loses the ability to natural detoxification of mycotoxins[2]. However, the issues of mutual influence of several toxins and the effect of toxins on the cattle organism at concentrations less than the established norms are poorly studied [3, 4].

Contamination of plant material with mycotoxins is possible both before harvest (during the plant growth period) and after it. *Penicillium* spp. infection occurs, as a rule, after harvesting, *Alternaria* spp. and *Fusarium* spp. fungi affect plants in crops, *Aspergillus* spp. contamination can occur in both ways. Unfortunately, treatment of crops with fungicides does not solve the problem of contamination of plants and feed with mycotoxins [6]. Many species of forage crops are sources of a complex of mycotoxins entering the body of animals. In addition, the ratios of individual components of the complex are subject to changes during vegetation and have their own peculiarities in different crops [7].

The presence of mycotoxins in bulky cattle feed is a global problem. According to some data, in the USA, mycotoxin contamination of feed causes losses at all stages of agricultural production of up to 10 billion dollars, in the European Union - about 5 billion euros (about 2% of the total GDP generated by agriculture in the European Union), and, apparently, both estimates are not accurate enough. The problem has a similar scale in Russia. Reduced productivity and health problems can lead to a loss of up to 250 rubles per animal per day and up to 100 thousand rubles per year, even if the cows have not shown obvious signs of mycotoxicosis [8].

Due to the ongoing globalization of the market, the distribution of contaminated farm animal feed and human food products is increasing, resulting in an increasing number of mycotoxin producers and consequently an increasing number of mycotoxin contaminated samples [7, 9, 10]. Further spread of mycotoxin producers can increase the probability of mass lesions of agricultural crops. This poses a serious threat both to crop yields and to the health of animals and humans who may potentially consume mycotoxin-contaminated products. Therefore, control and prevention of mycotoxins in agriculture is very important to ensure food security and the health of all parties involved. In this regard, proper storage and handling of feed, regular monitoring of mycotoxin levels in food and feed are essential.

A large number of cattle in combination with unfavorable climatic conditions on a large part of the country's territory cause the fact that Russia ranks first in the world in terms of silage production (30-40 million tons/year). The high share of silage in the diet of animals leads to a wide spread of severe toxicoses among cattle. This is primarily due to the ability of micromycetes, such as *Aspergillus fumigatus*, *Byssochlamys nivea*, *Fusarium* sp., *Monascus* sp. and *Penicillium roqueforti*, to actively proliferate in silage ecosystem conditions, where high levels of organic acids and carbon dioxide, but low oxygen levels are maintained. The development of micromycetes in silage can lead to loss of its nutritional value, which reduces feed quality and can negatively affect animal health and performance. Industrial processing

[2] *Yıldırım E.A.* Theoretical and experimental bases of microbiological safety of canned forages for ruminants: Extended abstract of Doctor's thesis in Biology. Dubrovitsy, 2019, 43 p.

is not able to significantly affect the content of mycotoxins in feed, so they are easily transmitted along the production chain, getting into the final products and animal organism [5]. Despite all measures taken to reduce mycotoxin contamination [11], silage forages are universally contaminated with mold fungi [12]. At the same time, even taking into account the significant relevance of the above-mentioned problems, the number of publications on mycotoxin contamination of silage fodder is small relative to other forms of fodder.

Poorly studied distribution of mycotoxins in the Russian Federation in general and in the north-western regions in particular leads to underestimation of risks for farms-producers. With sufficiently warm and humid summer, abundant precipitation and high humidity of autumn and relatively warm winter favorable conditions for the growth of mold fungi in the harvested fodder material are formed. Dairy specialization of cattle breeding in the North-West of Russia requires the harvesting of large volumes of juicy and dry fodder. Many farms do not control the content of mycotoxins in harvested raw materials, and measures to prevent contamination and decontamination of fodder are not implemented.

Taking into account the scientific and economic significance of this problem, the Laboratory of Bioeconomics and Sustainable Development of the Vologda Scientific Center of the Russian Academy of Sciences (VolSC RAS) has started to study the distribution of mycotoxins in forages harvested in the Vologda region. It should be noted that such studies have not been conducted in the region before.

The purpose of the study was to determine the nature and degree of mycotoxin damage of silages prepared in the farms of the Vologda region.

MATERIAL AND METHODS

Sampling of silage harvested in 2021-2023 was carried out by farms of the Vologda region agro-industrial complex in accordance with the recommendations developed by the specialists of the Laboratory of Bioeconomics and Sustainable Development of VolSC RAS on the basis of GOST R ISO 6497-2011. The studied samples are represented by silages of different composition: legume-grass, cereal, corn, mixed grass and silages from natural lands. Samples in which the content of ochratoxin A, zearalenone and the sum of aflatoxins B_1, B_2, G_1, G_2 was below the lower detection limit of the test systems (less than 1 μg/kg) were considered free from mycotoxins. Unfortunately, the MAC levels, which are reflected in the veterinary and sanitary requirements of the Customs Union (approved by the decision of the Customs Union Commission of the Eurasian Economic Community from 18.06.2010 № 317), regulate the content of mycotoxins in such forages as wheat, barley, oats, corn, soybean and others, ignoring silage. The value of MAC for the above-mentioned crops does not have significant differences, so in our work we were guided by the specified norm (5 μg/kg).

The received samples were dried to constant weight and pulverized (particle size not more than 0.2 mm). Then extraction and sample preparation were performed according to the test system manufacturer's protocol. During the period of storage of dry samples and during sample preparation, the exposure of the test material to light was minimized. The prepared extract was transferred into 2 ml cryotubes and stored at -30 °C until analysis (not more than 2-3 days).

Mycotoxins were analyzed according to GOST 31653-2012 "Feed. Method of immunoenzymatic determination of mycotoxins" using standard test systems produced by "ComProdService" (Belarus), using the analyzer of immunoenzymatic reactions AIFR 01 UNIPLAN (ZAO "Picon", Russia) in the Center for Agricultural Research and Biotechnology, VolSC RAS.

RESULTS AND DISCUSSION

Due to the fact that silage is the main type of succulent fodder harvested by farms in the region, the control of the amount of mycotoxins in this type of voluminous fodder is the most important for enterprises engaged in forage

harvesting. In total, more than 150 silage samples from 22 farms of the Vologda region were studied. The obtained data show that almost the whole sample contains mycotoxins (see Fig. 1, 2). Among the analyzed samples, only two samples did not contain the toxins under study.

The total aflatoxin content was determined in 91 samples (see Fig. 1). Of 16 samples of legume-grass silage, 15 were toxic: aflatoxin levels in them exceeded the MAC, and in 12 samples the norm was exceeded more than 2 times. Six out of ten samples of cereal silage also had aflatoxin concentrations above the MAC. Corn silage (seven samples) was toxic in 85% of cases. Meadow grass silage (12 samples) in 66% of cases was characterized by increased aflatoxin content. Among 46 samples of grass silage collected from natural lands, 34 samples had aflatoxin content above MPC, 20 of them exceeded the norm by 2 times.

The content of ochratoxin A was studied on the basis of 49 samples of feed (see Fig. 2). Out of nine samples of legume-grass silage, only one sample had mycotoxin concentration 2 times higher than MAC. In corn silage the ochratoxin content was insignificant, while in grass silage ten samples out of 11 had ochratoxin concentrations exceeding MAC 2 times. Among 28 grass silage samples collected from natural lands, ten samples had ochratoxin concentrations above the MAC.

The content of zearalenone was determined in 14 samples of silage. In all samples the toxin concentration was below the established norms (500 µg/kg).

The results of the study showed that out of 91 analyzed silage samples, more than 75% contained aflatoxins at concentrations above 5 µg/kg. Excess of MAC (5 µg/kg) for ochratoxin A was observed in 45% of samples. As

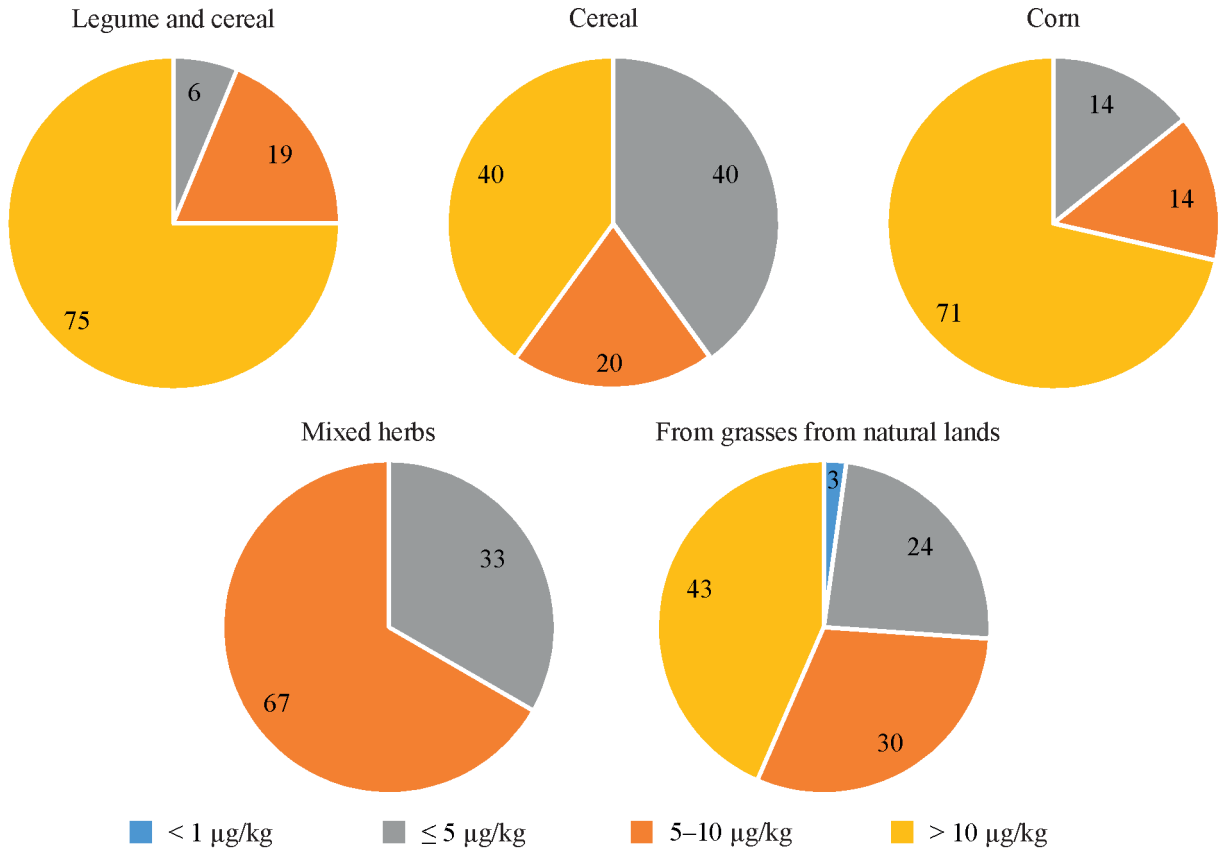

Рис. 1. Содержание афлатоксинов в образцах силосов различного состава (% от общего количества)
Fig. 1. Aflatoxin content in silage samples of different composition (% of total amount)

is known, aflatoxins and ochratoxin A are produced by fungi of the genera *Aspergillum* and *Penicillum*, for the development of which the conditions of ensiling are favorable [13]. Some representatives of these genera successfully develop at pH equal to 2.5 and lactic acid content up to 3.5%. Probably, contamination of the studied samples with mycotoxins occurred as a result of fungi infestation of silage mass. In addition, one cannot exclude the contribution to the contamination of fodder from the plants that served as raw material for silage preparation. Thus, there are works showing the widespread infestation of sown grasses on the root by fungi producing mycotoxins [9].

It should be noted that, according to the study, the most susceptible to aflatoxin contamination is legume-grass and corn silage, where the proportion of affected samples was 94 and 85%, respectively. In the case of ochratoxin A, silage from mixed grasses was the most contaminated (91%). A high proportion of contaminated samples was recorded in the variants from grasses harvested from natural lands (73% for aflatoxins and 48% for ochratoxin A). This is probably due to the longer period of use of natural lands. As a rule, these meadows are not strictly controlled by agricultural producers, agrotechnical practices are minimal, and grass harvesting is carried out in the second turn, while there may still be dried remains of last year's grasses, which may serve as a storage area for fungi. All this leads to the accumulation of various mycotoxins.

CONCLUSION

The results of the conducted research make it clear that practically all types of silage harvested by the farms in the region contain mycotoxins, and in many cases their content either exceeds the norms established for forage products or is in close proximity to such norms.

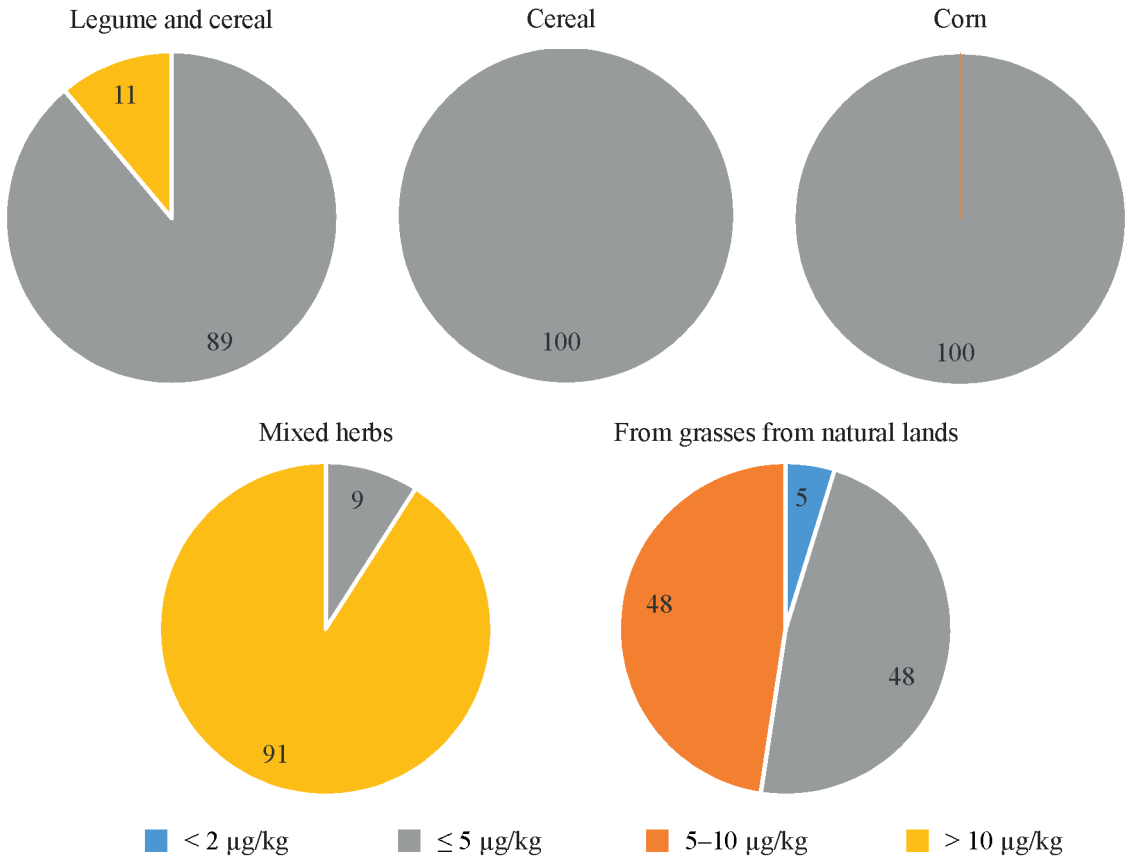

Рис. 2. Содержание охратоксина А в образцах силосов различного состава (% от общего количества)
Fig. 2. Ochratoxin A content in silage samples of different composition (% of total amount)

Firstly, it confirms the thesis that mycotoxins and their producers are everywhere and affect any type of feed, therefore, their toxic effects can affect the condition of animals, including chronic poisoning by small doses.

Secondly, it shows the importance of quality control of harvested forages in terms of mycotoxin content, especially silage, the conditions of harvesting and maturation of which are favorable for the growth of many fungi.

Thirdly, it is already important at this stage to take measures to decontaminate feed on farms (including preventive decontamination prior to or in the absence of sample testing) and measures to reduce toxic effects on animals (e.g. the use of sorbents).

The plans of the Laboratory of Bioeconomics and Sustainable Development of VolSC RAS for the near future include not only a significant expansion of research in terms of the number of samples analyzed, but also the study of the dynamics of accumulation of major toxins in forages differing in composition, time and method of harvesting.

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ИСПОЛЬЗОВАНИЕ ТРИТИКАЛЕ В КОРМОПРОИЗВОДСТВЕ

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Изложены результаты выращивания новых сортов озимой тритикале с целью получения зеленой массы и зерна. Обоснована эффективность использования тритикале в кормопроизводстве, в частности в составе полнорационных комбикормов при выращивании молодняка свиней на откорме и использовании в зеленом конвейере при выращивании крупного рогатого скота (КРС). Исследования проведены в Брянской области в 2021, 2022 гг. Доказано превосходство тритикале по основным параметрам в сравнении с традиционно возделываемыми культурами: рожью и пшеницей. Сочетание высоких показателей урожайности и содержания протеина позволяет увеличить сбор с единицы площади ценного зерна. Содержание протеина в зерне ржи сорта Пуховчанка составило 12,4%, в зерне тритикале сорта Форте – 15,2%, в зерне пшеницы сорта Влади – 14,1%, что указывает на высокую белковость зерна тритикале в сравнении с наиболее распространенными культурами. Лучшие результаты получены на варианте возделывания тритикале сорта Форте при использовании минеральных удобрений в количестве $N_{120}P_{130}K_{130}$, в котором урожайность зерна составила в среднем за два года 91,4 ц/га, превышение урожайности пшеницы сорта Влади – 7,6 ц/га. Увеличение урожайности тритикале произошло за счет сочетания многоколосковости ржи и многоцветковости пшеницы, что обеспечило увеличение массы зерна с колоса. При незначительном уменьшении густоты стеблестоя в посевах тритикале ее урожайность достоверно превышала другие изучаемые культуры. При возделывании тритикале на зеленый корм сорта Слон урожайность составила 407,1 ц/га, что на 106,8 ц/га превышает урожайность сорта Пуховчанка. Срок технологической спелости тритикале наступает на 7-й день позднее ржи, поэтому эта культура хорошо вписывается в зеленый конвейер. Благодаря высокой устойчивости к таким наиболее распространенным и вредоносным болезням, как бурая и стеблевая ржавчина, мучнистая роса, зеленый корм тритикале в период технологической спелости сохраняет потребительские качества на высоком уровне.

Ключевые слова: тритикале, протеин, зеленый корм, комбикорм, молодняк свиней, зеленый конвейер, урожайность

THE USE OF TRITICALE IN FEED PRODUCTION

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The results of growing new varieties of winter triticale in order to obtain herbage and grain are presented. The effectiveness of the use of triticale in fodder production, in particular, as part of complete mixed fodders when growing young fattening pigs on the materials of the Bryansk region and using it in a green forage chain when growing cattle is substantiated. The research was conducted in the Bryansk region in 2021, 2022. The superiority of triticale in terms of the main parameters in comparison with traditionally cultivated crops: rye and wheat has been proven. The combination of high yield and protein content allows increasing the yield per unit area of more valuable grain. The protein content in the grain of the rye variety Pukhovchanka was 12,4%, in the grain of the triticale variety Forte – 15,2%, and in the grain of the wheat variety Vladi – 14,1%, which indicates the highest protein content of triticale grain in comparison with the most common crops. The best results were obtained on the cultivation of the triticale variety Forte using mineral fertilizers in the amount of $N_{120}P_{130}K_{130}$, in which the grain yield averaged 91,4 centners per hectare over two years, the excess yield of wheat variety Vladi was 7,6 centners per hectare. The increase in the yield of triticale was due to the combination of

rye with many ears and wheat with many flowers which ensured an increase in the mass of grain per ear. With a slight decrease in the density of the stem stand in the crops of triticale, its yield significantly exceeded other studied crops of the variety. When cultivating triticale for green fodder of the Elephant variety, the yield was 407,1 c/ha, which is 106,8 c/ha higher than the yield of the Pukhovchanka variety. The technological ripeness of triticale comes 7 days later than that of rye, so this crop fits well into the green forage chain. Due to high resistance to the most common and harmful diseases such as brown and stem rust, powdery mildew, green fodder triticale during the period of technological ripeness retains consumer qualities at a high level.

Keywords: triticale, protein, green feed, compound feed, young pigs, green forage chain, yield

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INTRODUCTION

Triticale as a crop is actively introduced into agricultural production due to the combination of such positive features as high yield potential, favorable biochemical and technological characteristics and other criteria, which has been widely reflected in scientific literature [1-5]. The qualitative characteristic of triticale has ensured the versatile use of this crop: in baking, for ethylene production in the confectionery industry, in beer preparation, etc., as well as in the preparation of beer. Triticale is of special value for fodder production, and triticale is grown to produce green mass and grain. According to T.A. Gorina [6], the green mass of triticale contains more fat, sugar, carotene B compared to wheat and rye. Triticale grain, as studies by many authors show, contains more protein, well balanced in amino acid composition and protein fractions, and has high nutrition and energy balance [7-10]. Consequently, triticale grain is superior to all grain crops in terms of protein content, thus, triticale is undoubtedly an important forage crop. The study of the peculiarities of cultivation and use of triticale will contribute to the implementation of the Resolution of the Government of the Russian Federation from 01.12.2022 № 2201 "On Amendments to some acts of the Government of the Russian Federation on the implementation of the State Program of Agricultural Development and Regulation of Markets of Agricultural Products, Raw Materials and Food".

The purpose of the research is to substantiate the efficiency of triticale utilization in fodder production, in particular, in the composition of complete mixed fodders when growing young pigs for fattening and in the green forage chain when growing cattle.

MATERIAL AND METHODS

The study of the possibility and efficiency of triticale use in fodder production was conducted on new varieties Forte, Slon, included in the State Register of Breeding Achievements, allowed for production use in the Central region in 2022 and in other regions. These varieties are characterized by high yield potential, immunity and intensity. Thus, the Forte variety in 2022 at the Bolsheboldinsky state crop testing site (SCTS) yielded 114.8 c/ha. Triticale Elephant – silage variety, Forte – grain triticale, Vladi – grain wheat.

Sowing of triticale and rye was carried out in optimal for the region terms, from 25.08 to 10.09, with a seed drill SN-16 with a seeding rate of 4.5 million germinated seeds/ha at the

experimental station of the Bryansk State Agricultural University in 2021, 2022. The area of the plot – 35 m^2, threefold repetition, systematic placement of variants. Harvesting of rye and triticale for green fodder was carried out in the phase of earing – flowering manually. Harvesting for grain was carried out by a combine harvester Terion 250 in the phase of full ripeness. Protein content was determined by the Kjeldahl method, the amount of mineral fertilizers according to M.K. Kayumov[1], according to M.A. Albert, P.P. Galeev, E.A. Kovalev [11]. Mineral fertilizers $N_{120}P_{130}K_{130}$ were applied, allowing to obtain triticale grain yields of 100 c/ha and green mass yields of 300 – 400 c/ha. Statistical processing and related observations were carried out according to B.A. Dospekhov[2]. Research on studying the use of triticale grain in the composition of complete mixed fodders was carried out in the conditions of industrial type pig complex. The material of conducted studies were standard complete mixed fodders SPK-7 and SPK-8, designed for fattening young pigs, with the inclusion of different levels of triticale grain by replacing wheat grain. The object of research was a mixed tri-breed (Large White × Landrace × Duroc) young fattening pigs. For the experiment 10 young pigs were selected on the principle of pair-analogs with regard to sex, age, live weight and breed. Duration of the experiment corresponded to the period of intensive fattening and amounted to 90 days. The scheme of the research is presented in Table 1.

In accordance with the scheme of the experiment, the 1st group (control) received the main ration, which included full feed SPK-7 (the first period of fattening) and SPK-8 (the second period) with the content of wheat grain at the level of 45.6%. In the 2nd experimental group, the main ration was used with replacement of wheat grain with triticale grain at the level of 10% in the composition of mixed fodder, in the 3rd group – with replacement by 20%, in the 4th group – by 30% and in the 5th experimental group – with replacement by 40%. The data obtained in the research were processed by the method of variation statistics[3] using the package of applied computer program of statistical analysis Microsoft Excel 2010.

Табл. 1. Схема опыта
Table 1. Experiment scheme

Group	Number of heads	Average live weight at the beginning of the experiment, kg/head
1st (control)	10	44,31 ± 0,52
2nd experimental	10	44,26 ± 0,46
3rd experimental	10	44,30 ± 0,57
4th experimental	10	44,27 ± 0,56
5th experimental	10	44,32 ± 0,48

РЕЗУЛЬТАТЫ И ОБСУЖДЕНИЕ

Rye occupies an important place in the green conveyor belt, but the duration of rye green fodder utilization is limited by the "earing" phase of development. Continued supply of green fodder can be achieved by cultivation of triticale, the earing of which, according to our data, comes 7-8 days later, which fits well into the green conveyor (see Table 2).

Triticale varieties, recommended for obtaining green fodder, have a greater yield potential in comparison with other grain crops. The obtained results show that the yield of specialized triticale variety Slon on average for 2 years significantly exceeds other crops and varieties by this indicator and amounts to 407.1 c/ha (see Table 3).

Taking into account high immunological characteristics in relation to such diseases as brown and stem rust, powdery mildew, the obtained forage was characterized by high consumer properties.

The combination of positive features of rye (multispike spikelets) and wheat multiflowering allows increasing spikelet productivity while maintaining comparable stem density and forming a significantly higher yield among the

[1]*Kayumov M.K.* Programming of yield of agricultural crops. Moscow: Agropromizdat, 1989, 317 p.

[2]*Dospekhov B.A.* Methodology of field experiment (with the basics of statistical processing of research results). Moscow: Alliance, 2011, 352 p.

[3]*Kerdyashov N.N.* Variational statistics Penza. PSAU, 2018, 131 p. URL: https://e.lanbook.com/book/131161.

Табл. 2. Продолжительность вегетационного периода, дни
Table 2. Length of the growing season, days

Culture, variety	Sprouting – earing		Sprouting – full ripeness	
	2021	2022	2021	2022
Pukhovchanka rye	268	270	320	323
Elephant triticale	276	277	328	329
Forte triticale	275	277	329	330
Vladi wheat	271	273	324	326

studied crops. On average for 2 years (see Table 4), the yield of grain triticale of Forte variety amounted to 91.4 c/ha.

Analysis of protein content in the grain of the studied crops, varieties showed that the grain of rye variety Puhovchanka contains 12.4% protein, the grain of triticale variety Elephant – 14.9%, the grain of triticale variety Forte – 15.2%, the grain of wheat variety Vladi – 14.1%. Calculations show that the cultivation of the above varieties allows to receive from one hectare of crops respectively 5.3; 11.6; 13.8; 11.8 c/ha. Thus, triticale grain is the most high-protein among grain crops, which makes it an important component in the preparation of forage. The analysis of the main indicators characterizing the intensity of growth of fattening young pigs and feed costs at inclusion of triticale grain in the composition of complete mixed fodders of different levels was carried out (see Table 5).

The results of the conducted studies showed that at inclusion of triticale grain of different levels in the composition of complete feeds by replacing wheat in all experimental groups there was an increase in live weight of pigs compared to the control group. This, in our opinion, is connected, on the one hand, with a higher level of protein content in triticale grain in comparison with wheat grain, on the other hand, with a higher level of essential amino acid (lysine), the level of which in mixed fodders, the basis of which is wheat grain, is insufficient.

Табл. 3. Урожайность зеленой массы, ц/га
Table 3. Productivity of herbage, c/ha

Culture, variety	2021				2022				Average
	Replication			Average	Replication			Среднее	
	first	second	third		first	second	third		
Pukhovchanka rye	293	287	300	293,3	307	315	301	307,6	300,4
Elephant triticale	407	415	402	408,0	403	416	400	406,3	407,1
Forte triticale	396	394	368	386,6	376	389	387	384,0	385,3
Vladi wheat	210	197	203	202,6	211	207	198	205,3	203,9
LSD	–			19,85	–			11,55	–

Табл. 4. Урожайность зерна, ц/га
Table 4. Grain yield, c/ha

Culture, variety	2021				2022				Average
	Replication			Average	Replication			Average	
	first	second	third		first	second	third		
Pukhovchanka rye	42,3	41,9	43,3	42,5	44,0	43,2	42,6	43,2	42,8
Elephant triticale	79,5	80,2	78,7	79,4	80,1	76,5	74,9	77,1	78,2
Forte triticale	91,7	92,6	93,0	92,4	93,2	94,4	83,8	90,4	91,4
Vladi wheat	82,4	83,8	81,9	82,7	85,5	87,0	82,2	84,9	83,8
HCP	–			1,64	–			4,94	–

Табл. 5. Динамика живой массы и среднесуточных приростов у молодняка свиней на откорме
Table 5. Dynamics of live weight and average daily gains in young fattening pigs

Indicator	Group				
	1st (control)	2nd experimental	3rd experimental	4th experimental	5th experimental
Live weight at the beginning of the period, kg	44,31 ± 0,52	44,28 ± 0,46	44,30 ± 0,57	44,29 ± 0,56	44,30 ± 0,48
Live weight at the end of the period, kg	112,42 ± 1,34	113,27 ± 1,25	114,32 ± 1,41	114,71 ± 1,52	114,25 ± 1,67
Absolute gain, kg	68,11 ± 0,72	68,99 ± 0,69	70,02 ± 0,78	70,42 ± 0,76*	69,95 ± 0,81
Average daily gain, g/head	801,29 ± 8,54	811,65 ± 8,43	823,76 ± 8,76	828,47 ± 9,08*	822,94 ± 10,14
In % of the control	100,00	101,29	102,8	103,39	102,70
Feed costs per 1 kg of gain, kg	3,23	3,179	3,19	3,12	3,15

* $p < 0,05$.

On the basis of the data of the conducted research it is established that with the increase of the level of inclusion of triticale grain in the composition of complete mixed fodders the absolute growth rate of live weight of pigs in all experimental groups increased in comparison with the control group. It should be noted that from all experimental groups the highest level of increase in absolute gain (70.42 ± 0.94 kg) and average daily gain (828.47 ± 12.28 g/head) for the period of research was obtained in the 4th experimental group at the inclusion of triticale grain in the composition of complete mixed fodders at the level of 30%. Further increase in the share of triticale grain does not lead to the growth of weight gain. In our opinion, this is due to a higher content of 5-alkylresorcinols in triticale grain compared to wheat, the average excess is 24 mg/kg.

It should be noted that the use of triticale grain in the composition of diets at fattening of pigs had an impact on the reduction of feed costs for live weight gain, the costs in the 4th experimental group were lower compared to the control group by 3.41%, or by 0.11 kg. Thus, based on the data obtained in the studies on the use of triticale grain in the composition of complete mixed fodders, it can be concluded that triticale can be used at a level of 30%, providing the most intensive growth in pig fattening.

CONCLUSION

Cultivation of new varieties of winter triticale allows obtaining yields significantly higher in comparison with traditional crops. In the present study, the excess in triticale grain yield of the Forte variety amounted to 7.6 c/ha, in green mass yield of the triticale variety Elephant – 106.7 c/ha. Protein content in triticale grain exceeds this indicator in wheat by 1.1%, in rye – by 2.8%. The use of green mass of triticale will stabilize the need of cattle in green fodder in late spring and early summer. Inclusion of triticale grain in the composition of complete mixed fodders at fattening of young pigs instead of wheat at the level of 30% allows to increase the weight gain and reduce the consumption of feed and balance the diet.

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ОСОБЕННОСТИ РАЗВИТИЯ И РАСПРОСТРАНЕННОСТИ БОЛЕЗНЕЙ НА СОЕ В ЛЕСОСТЕПИ ЗАПАДНОЙ СИБИРИ

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В последние годы отмечено значительное ухудшение фитосанитарной ситуации в посевах многих сельскохозяйственных культур, в том числе кормовых. Одной из ценных кормовых культур является соя, получающая все большее распространение за пределами ее природной климатической зоны. В условиях Западной Сибири посевы сои почти ежегодно поражаются комплексом болезней, существенно снижающих урожайность и качество семенного материала, что требует комплексного подхода к обеспечению минимального инфекционного фона в посевах. Развитие эпифитотий на посевах сельскохозяйственных культур подчиняется определенной зависимости. Пик заболеваемости грибных инфекций связан с повышенными температурой и влажностью. Бактериальные инфекции обладают большей толерантностью к низким температурам и могут развиваться в заключительную фазу вегетации сои. Нами проведены исследования корреляционной зависимости между показателями заболеваемости грибными и бактериальными заболеваниями сои и погодно-климатическими условиями. Исследования проведены в 2016–2022 гг. в Новосибирской области. Выбор данных годов обусловлен формированием устоявшихся популяций соевых фитопатогенов, вызывающих ежегодные эпифитотии. В качестве образца для исследования заболеваемости посевов использован сорт сои СибНИИК 315. По результатам многолетних исследований этот сорт является наиболее поражаемым из всех. В качестве учитываемых заболеваний рассматривали листостебельные инфекции пероноспороз и пустульный бактериоз. Применение корреляционного анализа для установления степени взаимосвязи основных показателей зараженности посевов сои и погодно-климатических условий вегетационного периода позволило выявить определенную взаимосвязь, в основном за счет того, что грибные инфекции во все годы исследований проявлялись по типу эпифитотий. Вместе с тем этот способ мало подходит для заболеваний малораспространенных и имеющих характер кратковременных вспышек.

Ключевые слова: соя, сорт СибНИИК 315, пероноспороз, пустульный бактериоз, гидротермический коэффициент, корреляция

FEATURES OF DEVELOPMENT AND PREVALENCE OF DISEASES ON SOYBEAN IN THE FOREST-STEPPE OF WESTERN SIBERIA

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In recent years, there has been a significant deterioration of phytosanitary situation in plantings of many agricultural crops, including fodder crops. One of the valuable forage crops is soybean, which is becoming increasingly widespread outside its natural climatic zone. Under the conditions of Western Siberia, soybean crops are almost annually affected by a complex of diseases that significantly reduce

the yield and quality of the seed material, which requires a comprehensive approach to ensure a minimum infectious background in crops. The development of epiphytotics on crops follows a certain dependence. The peak incidence of fungal infections is associated with increased temperature and humidity. Bacterial infections have a greater tolerance to low temperatures and may develop during the final phase of soybean vegetation. We investigated the correlation between the incidence rates of fungal and bacterial diseases of soybean and weather and climatic conditions of all the years of research. The research was conducted in 2016–2022 in the Novosibirsk region. The choice of the years of research is due to the formation of established populations of soybean phytopathogens that cause annual epiphytotics. SibNIIK 315 soybean variety was used as a sample for the study of the disease incidence in soybean crops. Based on long-term studies, this variety is the most affected of all the varieties. The leaf-stalk infections such as false mildew and pustular bacteriosis were considered as countable diseases. The use of correlation analysis to establish the degree of interrelation between the main indicators of soybean crop infestation and weather and climatic conditions of the growing season allowed to reveal a certain relationship – mainly due to the fact that fungal infections in all the years of research were manifested by the type of the epiphytotics. However, this method is not well suited for low prevalence diseases and diseases with short-term outbreaks.

Keywords: soybean, SibNIIK 315, diseases, false mildew, pustular bacteriosis, hydrothermal coefficient, correlation

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INTRODUCTION

An important factor of sustainable development of agricultural production in Siberia is the prevention of crop losses from a complex of pests. In recent years, a significant deterioration of phytosanitary situation in crops of many agricultural crops, including fodder crops has been noted[1]. The long-term phytosanitary monitoring of seed material, soil and crops of annual and perennial fodder crops, conducted by us, indicates a significant spread and harmfulness of an extensive complex of diseases[2].

Infestation of forage crops by pathogens of various diseases leads to a number of problems [1, 2]:

– reduction of overall plant productivity: plant biomass, seed productivity;

– reduction of fodder quality during vegetation and during storage;

– reduction of sowing qualities of seeds;

– losses of nutrients, as pathogens affecting feedstuffs use them for their growth and reproduction. Mold deterioration of grain leads to a decrease in the content of vitamins by more than 25%, protein – by 20, lysine – by 45%. There is a deterioration of taste qualities, as the infection of feed raw materials by some species of fungi causes the appearance of a repulsive odor and unpleasant taste, reducing the consumption of feeds;

– changes in the physical properties of raw materials, manifested in the formation of dense clumps that hinder their transportation and lead to hanging in silos;

[1] *Ashmarina L.F., Gorobei I.M., Konyaeva N.M., Agarkova Z.V.* Atlas of diseases of forage crops in Western Siberia. Novosibirsk, 2010, 173 p.

[2] *Zhuchenko A.A.* Adaptive system of plant breeding (ecological and genetic bases). Moscow, 2001, vol. 2, 708 p.

– growth and reproduction of fungi and insect parasites, increase in humidity, self-heating of grain, which leads to increased ventilation costs and the need for regular movement during storage;

– formation of mycotoxins, which causes poor health, stunted growth of animals and reduced productivity.

These problems necessitate the development of a set of protective measures to reduce the harmfulness of diseases in forage crops.

Cultivation of high-yielding varieties with complex resistance to abiotic and biotic stressors is one of the most effective and promising directions in modern plant protection, meeting the needs of modern adaptive crop production [3]. Immune varieties are able to provide crop protection from losses, obtain high quality products, and ensure the recovery of ecosystems [4]. Creation of resistant varieties and on their basis optimization of agrotechnology of their cultivation contribute to fundamental changes in the system of plant protection and increase the yield of forage crops and preserve the ecological integrity of the environment [5, 6].

One of the most valuable fodder crops is soybean, which is becoming increasingly widespread outside its natural climatic zone. New promising varieties are regularly emerging, including those aimed at producing highly nutritious fodder for the needs of the livestock industry[3] [7].

Under the conditions of Western Siberia, soybean crops are almost annually affected by a complex of diseases that significantly reduce yield and quality of seed material, which requires a comprehensive approach to ensuring a minimum infectious background in crops [8]. Currently, there is a large number of various techniques and methods to control disease incidence in soybean crops[4] [9]. In addition, one of the achievements of Siberian breeders at the end of the XX century was the creation of the Siberian ecotype varieties and their introduction into culture in the extreme conditions of Siberia [10].

The development of epiphytotics on crops is subject to a certain dependence. It has been established that the peak of fungal diseases is usually associated with high temperature and humidity [11, 12]. Bacterial infections are more tolerant to low temperatures and can develop in the final phase of soybean vegetation.

The purpose of the work is to establish the dependence of the prevalence and development of fungal and bacterial diseases of soybean on weather and climatic conditions of the West Siberian region.

The objectives of the research included evaluation of soybean disease incidence over several years, as well as identification of correlation between the disease incidence rates and weather and climatic conditions of all years of the study.

MATERTIAL AND METHODS

The studies were conducted in 2016-2022 on the basis of experimental plots of the Siberian Research Institute of Fodder Crops (SibNII of fodder crops) SFSCA RAS (Novosibirsk region). The choice of research years was due to the formation of established populations of soybean phytopathogens causing annual epiphytotics.

SibNIIK variety 315 was used as a sample to study the disease incidence in soybean crops. Bred in 1991, it has been used in field conditions as a standard variety for many years. According to the results of long-term studies, this variety is also the most affected of all. The leaf-stalk infections false mildew and pustular bacteriosis were considered as the diseases taken into account.

The main method of recording leaf-stalk infections was visual assessment in accordance

[3]*Toropova E.Yu., Shulga T.V., Seluk M.P.* Effectiveness of soybean seed dressing agent in the protection against diseases // Grain legume crops, an emerging trend in Russia: a collection of articles. Omsk, 2018, pp. 172-175.

[4]*Sidorik I.V., Zinchenko A.V.* Importance of soybean in farming in Kazakhstan // Oilseed crops: scientific and technical bulletin. VNII of oilseed crops. Krasnodar, 2018, N. 2 (174), pp. 75-78.

with the methodological guidelines of VIZR, VIK and VIR[5].

The assessment was carried out on the following scale:

Score	Degree of leaf and stem damage, %
0	No symptoms
2	11–25
3	11–25
4	11–25
5	11–25, plant death.

Counts of soybean leaf-stalk infections were carried out throughout the growing season.

Based on the obtained data, prevalence and disease progression indices were calculated. The index of disease development was determined by the formula

$$R = \frac{\Sigma (a \cdot b) \cdot 100}{N \cdot K},$$

where $\Sigma (a \cdot b)$ – sum of products of the number of diseased plants (a) by their corresponding infestation score (b); N – total number of the counted plants; K – highest score on the scale.

Moisture content of the vegetation period was expressed through the Selyaninov hydrothermal coefficient (HTC), calculated according to the formula[6]

$$K = \frac{R \cdot 10}{\Sigma t},$$

where R – sum of precipitation in millimeters for the period with the temperatures higher than 10 °C; Σt – sum of the temperatures (°C) over the same time.

The dependence of soybean plant disease incidence on HTC was determined through the correlation coefficient with the construction of a scatter diagram using the program Microsoft Office Excel 2021.

RESULTS AND DISCUSSION

Climatic conditions of all the years of research differed significantly in terms of average temperatures and moisture content. 2016 was characterized by almost complete absence of precipitation in the first half of the growing season. In the following months (July – August) precipitation was scarce, which made the conditions of soybean cultivation in this year close to the conditions of dry farming zones. In 2017, a different situation was observed: periodic heavy precipitation from June through August against the background of a relatively dry May. Conditions for soybean cultivation in this year were optimal. The wettest months of the 2018 growing season were June and July, with several excessively wet days (25-30 mm of precipitation). In general, the year was as favorable for soybean as the previous year. Nearly identical conditions were observed in 2020. In 2019, the only wetted day of the growing season was one day in July with 37 mm of precipitation. Otherwise, moisture content was at a medium-low level, which allows us to classify this year as a dry year. In 2021, there was a kind of a gap between two average moist months: June and August. Against the background of weak moisture content of May, this year can also be called dry. In 2022, the lowest amount of precipitation was detected against the background of increased average temperatures of the growing season: by its characteristics, it is closest to 2016.

Based on the data of average temperatures and precipitation for two months of the growing season (July-August), the hydrothermal coefficient of Selyaninov was calculated (see Fig. 1). These months of the growing season in Western Siberia are the months with the highest amount of precipitation. These data are necessary to identify the relationship between the conditions of the growing season and the development of the most typical soybean diseases.

The prevalence and development of soybean diseases by years of research were uneven. The prevalence and development of false mildew in 2017 were very low, in 2022 the disease was completely absent (see Fig. 2).

Pustular bacteriosis on soybean developed as an epiphytotic disease only in some years: this year was 2022. In other years, it manifested it-

[5]Methodological guidelines for the study of resistance of grain legumes to diseases. Leningrad: VIR, 1976, 74 p.

[6]*Selyaninov G.T.* World agroclimatic reference book. Leningrad; Moscow, 1937.

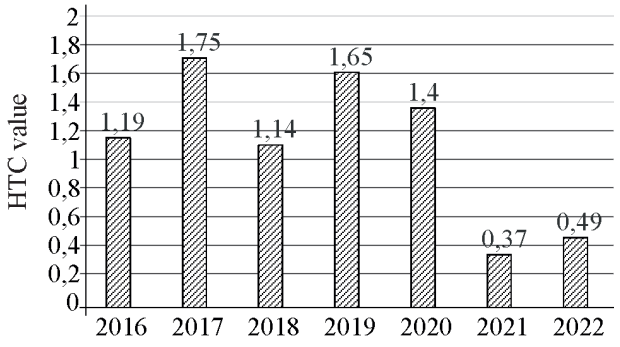

Рис. 1. Гидротермический коэффициент Селянинова по всем годам исследований

Fig 1. Selyaninov's hydrothermal coefficient for all the years of research

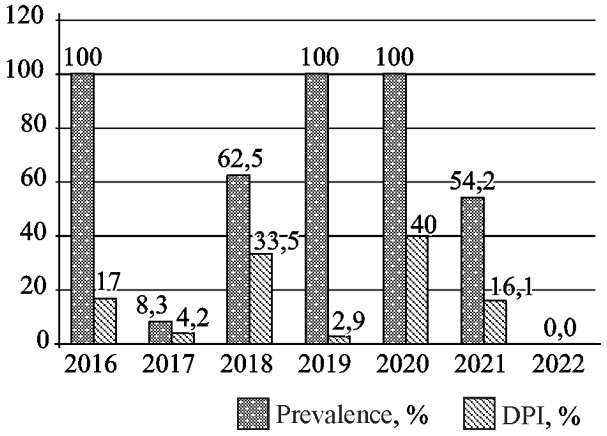

Рис. 2. Распространенность и развитие пероноспороза в 2016–2022 гг.

Fig 2. Prevalence and development of false mildew in 2016–2022

self as a concomitant disease, in most years it was absent (see Fig. 3).

Correlation analysis of prevalence and disease progression with HTC was performed in 2023. The results are presented in Figs. 4 и 5.

According to the results, the most pronounced correlation between false mildew development index and weather and climatic conditions of growing seasons was observed in 2016, 2018, 2019 and 2021. In case of false mildew prevalence, such correlation was observed in 2018 and 2019. The negative correlation between HTC, prevalence and development of bacteriosis is mostly due to the uneven occurrence of these pathogens, which may require further research using artificial infection backgrounds.

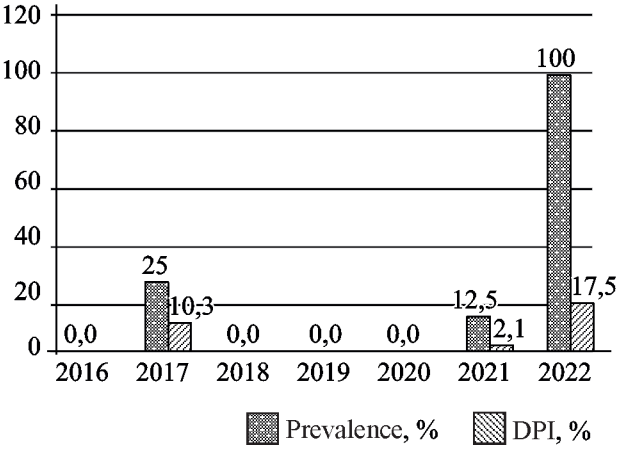

Рис. 3. Распространенность и развитие пустульного бактериоза в 2016–2022 гг.

Fig. 3. Prevalence and development of pustular bacteriosis in 2016–2022

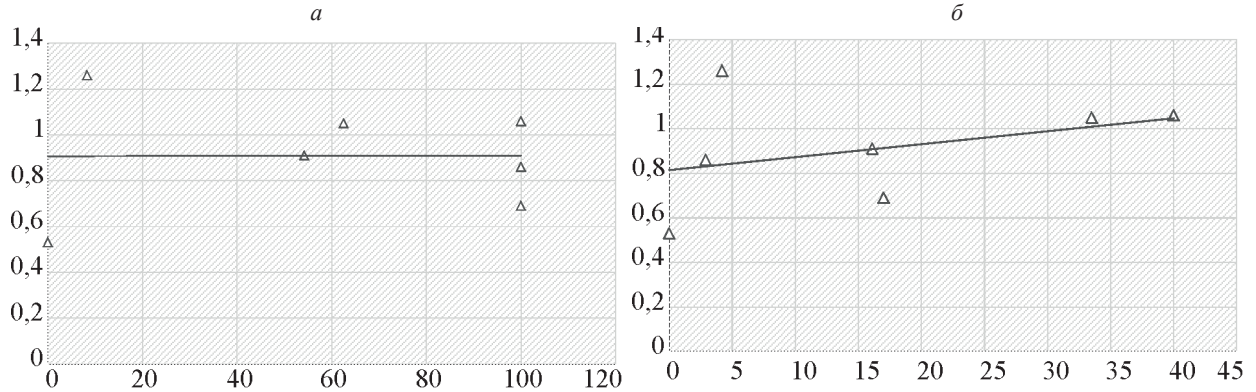

Рис. 4. Корреляция распространенности и развития пероноспороза с ГТК:
а – $r = 0{,}14$; *б* – $r = 0{,}29$

Fig. 4. Correlation of the prevalence and development of false mildew with HTC:
а –$r = 0{,}14$; *б* – $r = 0{,}29$

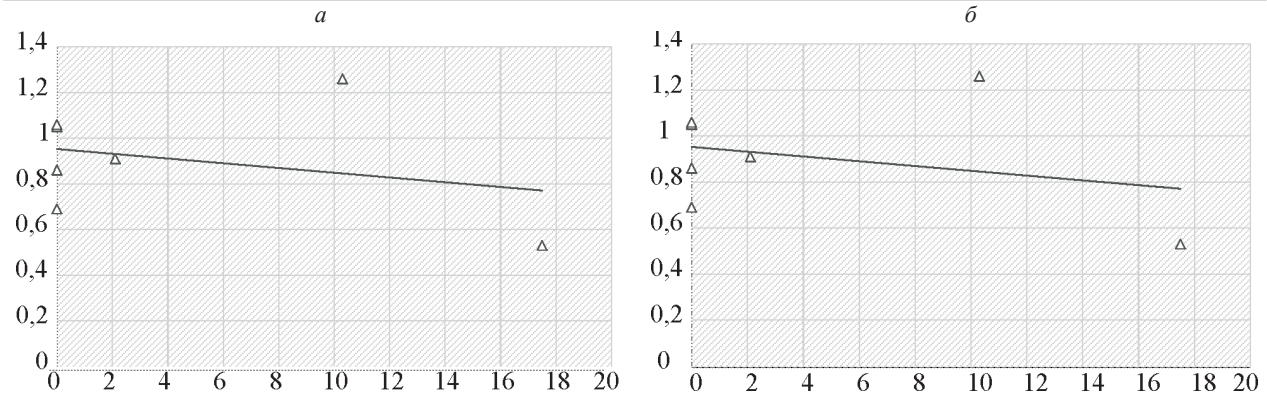

Рис. 5. Корреляция распространенности и развития пустульного бактериоза с ГТК:
а – *r* = –0,49; *б* – *r* = –0,24

Fig. 5. Correlation of the prevalence and development of pustular bacteriosis with HTC:
a – r = –0.49; б – r = –0.24

CONCLUSION

The use of correlation analysis to establish the degree of relationship between the main indicators of infection of soybean crops and weather and climatic conditions of the growing season allowed us to identify a certain relationship between the main indicators of plant infestation by fungal diseases and HTC, mainly due to the fact that false mildew in all years of research (excluding 2022) was manifested by the type of epiphytotics. At the same time, this method is not suitable for low prevalence diseases, as well as for diseases with short-term outbreaks. Obviously, in addition to the influence of weather and climatic conditions on the prevalence and development of diseases, it is necessary to take into account such factors as phytosanitary condition of soil and seed material, which did not appear in this work. This may serve as a material for further studies.

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АНАЛИЗ ФУНКЦИОНАЛЬНОЙ АКТИВНОСТИ КЛЕТОК ГРАНУЛЕЗЫ ОВАРИАЛЬНЫХ ФОЛЛИКУЛОВ СВИНЕЙ

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Функционирование клеток гранулезы овариальных фолликулов (ОФ) животных детерминирует рост и развитие ОФ и формирующегося в нем ооцита, что определяет их использование при моделировании состава сред для созревания ооцитов *in vitro* в клеточных репродуктивных технологиях. Цель исследования – с использованием комплексного тестирования показателей жизнеспособности клеток гранулезы (уровень апоптозов, продукция активных форм кислорода, функциональная активность митохондрий и липидома) и гормонального статуса жидкости фолликулов (уровень эстрадиола, тестостерона, антимюллерова гормона) определить особенности функциональной активности гранулезных клеток в фолликулах разного диаметра (< 3, 3–5, 5–8 мм) с целью оптимизации состава культуральных сред для экстракорпорального созревания ооцитов свиней. В ходе экспериментов обнаружено, что доля апоптотических клеток в ОФ возрастает в процессе роста диаметра ОФ (на 11% в ОФ диаметром 5–8 мм, на 4% в ОФ диаметром < 3 мм). Отмечено увеличение числа клеток с высоким содержанием активных форм кислорода (на 10% в ОФ диаметром 5–8 мм по сравнению с ОФ диаметром < 3 мм) и высокой митохондриальной активностью (на 12% в ОФ диаметром 5–8 мм по сравнению с ОФ диаметром < 3 мм). Обнаружены корреляции между группой клеток с высокой генерацией активных форм кислорода и уровнем апоптозов (0,500, $p < 0,05$), а также с высоким уровнем митохондриальной активности (0,500, $p < 0,05$). Концентрация эстрадиола в фолликулярной жидкости по мере роста ОФ повышалась (1,50 нг/мл в ОФ диаметром < 3 мм и 2,09 нг/мл в ОФ диаметром 3–5 мм, $p < 0,01$), почти не меняясь в ОФ диаметром 5–8 мм (2,11 нг/мл), как и концентрация тестостерона. Выявлены корреляции между долей клеток с высокой ИФ NileRed/ЛК (маркер функциональной активности липидных капель) и уровнем эстрадиола (0,998, $p < 0,05$), тестостерона (0,500, $p < 0,05$), антимюллерова гормона (–0,500, $p < 0,05$) в динамике фолликулогенеза, а также между концентрацией эстрадиола и долей клеток с высокой митохондриальной активностью (0,746, $p < 0,05$). Обнаружены корреляции между концентрациями эстрадиола и антимюллерова гормона, тестостерона и антимюллерова гормона (–1,000, $p < 0,05$), между уровнем апоптотических клеток и концентрацией антимюллерова гормона в фолликулярной жидкости (–1,000, $p < 0,05$). Результаты проведенного мониторинга показателей жизнеспособности и функциональной активности клеток гранулезы ОФ свиней дополняют имеющиеся сведения об особенностях их функционирования в динамике фолликулогенеза и могут быть использованы при моделировании систем экстракорпорального дозревания донорских ооцитов *Sus Scrofa Domesticus*.

Ключевые слова: гранулеза, *Sus Scrofa Domesticus*, митохондрии, активные формы кислорода, апоптоз, стероидные гормоны, антимюллеров гормон, липидные капли

ANALYSIS OF FUNCTIONAL ACTIVITY OF GRANULOSA CELLS OF PORCINE OVARIAN FOLLICLES

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Functioning of the granulosa cells of animal ovarian follicles (OF) determines the growth and development of the OF and the oocyte formed in it, which determines their use in modeling the composition of media for oocyte maturation *in vitro* in cell reproductive technologies. The purpose of the study is to determine the peculiarities of functional activity of granulosa cells in follicles of different diameters (< 3, 3–5, 5–8 mm) using complex testing of granulosa cell viability parameters (apoptosis level, production of reactive oxygen intermediates, functional activity of mitochondria and lipidome) and hormonal status of follicle fluid (estradiol, testosterone, anti-Müllerian hormone level) in order to optimize the composition of the culture media for in vitro maturation of pig oocytes. The experiments found that the proportion of apoptotic cells in the OF increased as the diameter of the OF increased (by 11% in OFs 5–8 mm in diameter, by 4% in OFs < 3 mm in diameter). There was an increase in the number of high reactive oxygen intermediates cells (by 10% in 5–8 mm diameter OF compared with < 3 mm diameter OF) and high mitochondrial activity (by 12% in 5–8 mm diameter OF compared with < 3 mm diameter OF). Correlations were found between the group of cells with high generation of reactive oxygen intermediates and the level of apoptosis (0.500, $p < 0.05$), as well as with high levels of mitochondrial activity (0.500, $p < 0.05$). Estradiol concentration in follicular fluid increased as the OF grew (1.50 ng/ml in OF < 3 mm diameter and 2.09 ng/ml in OF 3–5 mm diameter, $p < 0.01$), being almost unchanged in OF 5–8 mm diameter (2.11 ng/ml), as was the testosterone concentration. Correlations were found between the proportion of the cells with high NileRed/LD FI (a marker of lipid droplet functional activity) and the levels of estradiol (0.998, $p < 0.05$), testosterone (0.500, $p < 0.05$), and anti-Müllerian hormone (–0.500, $p < 0.05$) in the dynamics of folliculogenesis, as well as between estradiol concentration and the proportion of cells with high mitochondrial activity (0.746, $p < 0.05$). Correlations were detected between the concentrations of estradiol and anti-Müllerian hormone, testosterone and anti-Müllerian hormone (–1.000, $p < 0.05$), between the level of apoptotic cells and the concentration of anti-Müllerian hormone in follicular fluid (–1.000, $p < 0.05$). The results of the monitoring of the viability and functional activity of porcine OF granulosa cells supplement the available data on the peculiarities of their functioning in the dynamics of folliculogenesis and can be used in modeling the systems of in vitro maturation of donor oocytes of *Sus Scrofa Domesticus*.

Keywords: granulosa, *Sus Scrofa Domesticus*, mitochondria, reactive oxygen intermediates, apoptosis, steroid hormones, anti-Mullerian hormone, lipid drops

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INTRODUCTION

Cellular reproductive DNA technologies are one of the most important directions in the pig industry, including replication of high-value individuals by cloning, preservation of the gene pool of existing and endangered breeds, creation of a cryobank of gametes. The development of this area, in particular genome editing techniques, has led to an increased interest in the *Sus Scrofa Domesticus* species on the part of biotechnologists due to the possibility of using this species as a model object for understanding the course of various human diseases, testing pharmacological drugs, medical devices for humane medicine, xenotransplantation of organs, etc. [1]. To create intact, transgenic and cloned embryos, it is necessary to have donor oocytes with high quality ooplasts, which is achieved by creating *in vitro* adequate conditions for maturation of female gametes *in vivo*.

Follicular growth and oocyte maturation in mammalian ovaries are processes of cell differentiation accompanied by a complex of morphological, biochemical and molecular transformations of various components of the follicle, namely, the oocyte and somatic cells. Knowledge of the mechanisms and peculiarities of interaction and functioning of the structural components of the follicle *in vivo* will make it possible to create a model of the environment that meets the physiological "demands" of the developing female gamete [2]. The systems developed so far for maturation of donor oocytes of pigs provide not more than 40-45% of embryos at the final blastocyst stage, which indicates the insufficient efficiency of the composition of culture media[1]. The best results are achieved when using structural components of follicles (follicle wall, granulosa cells)[2]. However, technically, obtaining follicle walls pre-selected according to morphological criteria is a rather labor-intensive process, which complicates the work of embryotechnologists. The use of granulosa cells allows to replenish the culture media with products of their secretion. Since oocytes from follicles with a diameter of 3-5 mm, as shown in numerous studies, have a high potential for fertilization *in vitro*, hormonal profiling of the fluid isolated from follicles of different diameters and identification of the nature of apoptotic processes, generation of reactive oxygen species in conjunction with the analysis of the activity of cellular compartments of granulosa cells are of considerable interest.

Oocyte maturation is a complex process that is the result of hormonal interactions, can be regulated, and involves the coordination of multiple events that allow the resumption of meiosis and the acquisition of competence for further fertilization under *in vivo* conditions. Creating in vitro conditions maximally close to those described above has its own difficulties that require modification. One of the methods of modeling the systems of *in vitro* maturation of donor oocytes is the use of structural components of antral ovarian follicles, in particular granulosa cells, which makes it possible to significantly increase the fertilization rate of the *in vitro* matured oocyte[3, 4].

Granulosa cells are a component of a multidimensional biological system in follicles. Working through paracrine factors, as well as

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signaling through gap junctions, they are directly involved in the formation of the necessary microenvironment for oocyte growth and maturation [3, 4]. In addition, granulosa cells are the main producers of steroid hormones during folliculogenesis, increasing the production of estradiol by aromatization of androgens derived from theca cells [5], converting incoming cholesterol through enzymatic transformations into steroid hormones with the help of mitochondria [6]. In addition to cholesterol, granulosa cells contain other lipid derivatives, which are found as part of cell membranes and cell organelles (phospholipids, glycolipids, neutral lipids) [7], and are also stored in cells as energy reserves (triacylglycerides), acting as a powerful source of energy when needed [8].

The production of adenosine triphosphoric acid (ATP) as an energy substrate through mitochondrial oxidative phosphorylation is essential for the proliferation of granulosa cells during folliculogenesis [9]. Meanwhile, the byproduct of phosphorylation in the form of reactive oxygen intermediates (ROIs) in physiologically acceptable concentrations plays a vital role in ovarian physiological activity as a secondary messenger for cell signaling [10], and is involved in the regulation of the ovarian cycle, including meiosis [11], ovulation[5], maintenance of the corpus luteum [12] and regression [13]. In excessive amounts, ROIs can cause damage to mitochondrial and nuclear DNA, oxidation of some amino acids and lipid peroxidation, which, in turn, can induce cell death [14].

The purpose of the study was to identify the peculiarities of functional activity of granulosa cells in follicles of different diameters using complex testing of granulosa cell viability parameters (apoptosis level, ROI production, functional activity of mitochondria and lipidome) and hormonal status of follicle fluid (estradiol, testosterone, anti-Mullerian hormone content) in order to optimize the composition of culture media for in vitro maturation of pig oocytes.

In accordance with the set goal, the study addressed the following tasks:

1) assessment of granulosa cell viability parameters (apoptosis, ROI generation) sampled from follicles of different diameters (< 3, 3–5, 5–8 mm);

2) analysis of functional activity of lipidome and mitochondria of granulosa cells from follicles of the indicated diameter;

3) hormonal profiling (estradiol, testosterone, anti-Müllerian hormone) of follicle fluid of the declared diameter.

MATERIAL AND METHODS

Sixty-four postmortem ovaries from 32 6–8-month-old Landrace pigs were used in the experiments. The ovaries were obtained at the meat processing plant "Tosnensky" (Leningrad region), after which they were delivered to the laboratory of the All-Russian Research Institute of Farm Animal Genetics and Breeding. The material was selected at the stage of follicular growth. Ovaries without pathological changes were used. Based on the measurements with an electronic caliper, 2275 follicles were ranked by diameter < 3, 3-5, 5-8 mm. Follicular fluid and granulosa cells were aspirated from 20-30 follicles of each ovary, grouping them according to diameter, then centrifuged for 6 min at 300 rpm. The precipitate containing granulosa cells was then suspended in phosphate-buffer saline (PBS) for further cytogenetic analysis. The supernatant as follicular fluid was divided into aliquots and stored at -20 °C until further immunoenzymatic analysis for hormonal profiling (determination of testosterone, estradiol and Anti-Müllerian hormone levels).

The proportion of dead cells was determined using propidium iodide (PI) fluorochrome. Granulosa cells were washed twice in PBS buffer with sedimentation by centrifugation at 1200 rpm for 7 min. The cells were analyzed immediately after addition of PI to the tested

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samples until a final concentration of 5 μg/ml was obtained. The samples were analyzed on a CytoFlex flow cytometer (Beckman Coulter Life Sciences, USA).

The content of intracellular H_2O_2 was determined by flow cytometry using a CytoFlex flow cytometer. For this purpose, granulosa cells were washed twice in PBS buffer (1200 rpm, 7 min) and 2',7' dichlorofluorescein diacetate (DCFH-DA) was added at a final concentration of 5 μM/ mL followed by incubation for 30 min at 38.5 °C. Then the precipitate was washed twice from fluorochrome residues (1200 rpm, 7 min). Two populations of cells – with high and low hydrogen peroxide content – were distinguished by fluorescence analysis on a flow cytometer[6].

Mitochondrial activity of granulosa cells was evaluated using fluorescent dye TMRE (tetramethylrhodamine ethyl ester perchlorate) using CytoFlex flow cytometer. Tetramethylrhodamine ethyl ester (TMRE) is a cell-permeable positively charged red-orange dye (max excitation 549 nm, emission 574 nm) that accumulates in active mitochondria due to their relatively negative charge. Inactive or depolarized mitochondria have a reduced membrane potential (ΔψM) and therefore cannot retain TMRE, showing a low fluorescence signal. Granulosa cells were washed twice in PBS buffer followed by centrifugation at 1200 rpm for 7 min. The cell precipitate was resuspended in PBS and incubated with TMRE at a final concentration of 1 μM for 30 min at 38.5 °C. The concentration for the studied samples was selected empirically and according to the manufacturer's recommendations (Lumiprobe RUS Ltd, Russia). After incubation, the cells were washed from fluorochrome residues. When analyzing fluorescence on a flow cytometer, two populations of cells – with high and low membrane potential – were distinguished.

The functional activity of the lipidome was evaluated by the fluorescence intensity of the Nile Red/lipid droplet complex (NileRed/LD FI). Nile Red dye (Sigma-Aldrich, USA) was used to determine NileRed/LD FI in granulosa cells, which allows visualization of neutral and polar lipids. After dye addition triglycerides fluoresce in yellow color (580-596, fluorescence peak – 590 nm), polar lipids (phospholipid bilayers) – in orange spectrum (597-620, peak – 600 nm)[7].

After washing of granulosa in PBS-buffer, the cells were resuspended and fixed in 1% m paraformaldehyde at room temperature for 10 min. The fixed cells were washed twice with cold PBS and Nile Red dye was added to a working concentration of 10 μM, then incubated at room temperature for 10 min in the dark. The stained cells were washed twice with cold buffer, then resuspended in cold PBS. Three groups of cells with high, medium and low fluorescence intensity were analyzed.

The concentration of testosterone, estradiol and anti-Müllerian Hormone (AMH) was determined in follicular fluid using the reagent kit "SteroidIFA Testosterone", "SteroidIFA Estradiol" (Alkor Bio, Russia) and ELISA Kit for anti-Mullerian Hormone for Pigs (Cloud-Clone Corp., PRC). The experiment was carried out in six replications. All reagents used in the study, except for the above reagents, were produced by Sigma-Aldrich, plastic laboratory glassware - BD Falcon™ (USA).

The results were processed using Sigma Stat statistical program. Reliability of the difference between the compared mean values was assessed using the x2 criterion at three significance levels: $p < 0.05$, $p < 0.01$, $p < 0.001$, as well as the Spearman coefficient and the Student's criterion.

The scheme of the experiment is presented in Fig. 1.

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RESULTS AND DISCUSSION

In the course of the study, it was found that in the process of follicle growth and development there is a decrease in the indicators of granulosa cell viability, expressed in an increase in the level of the cells with signs of apoptosis (4% of cells at the apoptosis stage in follicles < 3 mm in diameter, 11% in follicles 5-8 mm in diameter, $p < 0.05$) (see Fig. 2). At the same time, the proportion of the cells with high ROI content tended to increase (in follicles 5-8 mm in diameter, this index increased by 10% compared to the follicles < 3 mm in diameter, $p < 0.05$), as well as the group of cells with high mitochondrial activity (12% increase in follicles 5-8 mm in diameter compared to the follicles < 3 mm in diameter, $p < 0.05$). In addition, significant positive correlations were found between the increase in the proportion of the cells with high ROI generation and the proportion of the apoptotic cells (0.500, $p < 0.05$), as well as the group of cells with high mitochondrial activity (0.500, $p < 0.05$).

In the cells of different tissues, lipid droplets are generally composed of neutral lipids (mainly triglycerides and cholesterol esters), they are substrates for energy production, act as signaling lipids, and serve as structural components for cell membranes [15].

At the initial stage of folliculogenesis, an increase in NileRed/LD FI was observed (10% increase in the proportion of the cells with high fluorescence intensity, $p < 0.05$) (see Fig. 3). At the same time, the number of cells with medium NileRed/LD FI significantly decreased by 10% by the stage of follicle growth to 3-5 mm ($p < 0.05$). In addition, a negative correlation was observed between the groups of cells with high and medium NileRed/LD FI (–1.000, $p < 0.05$). These values may indicate

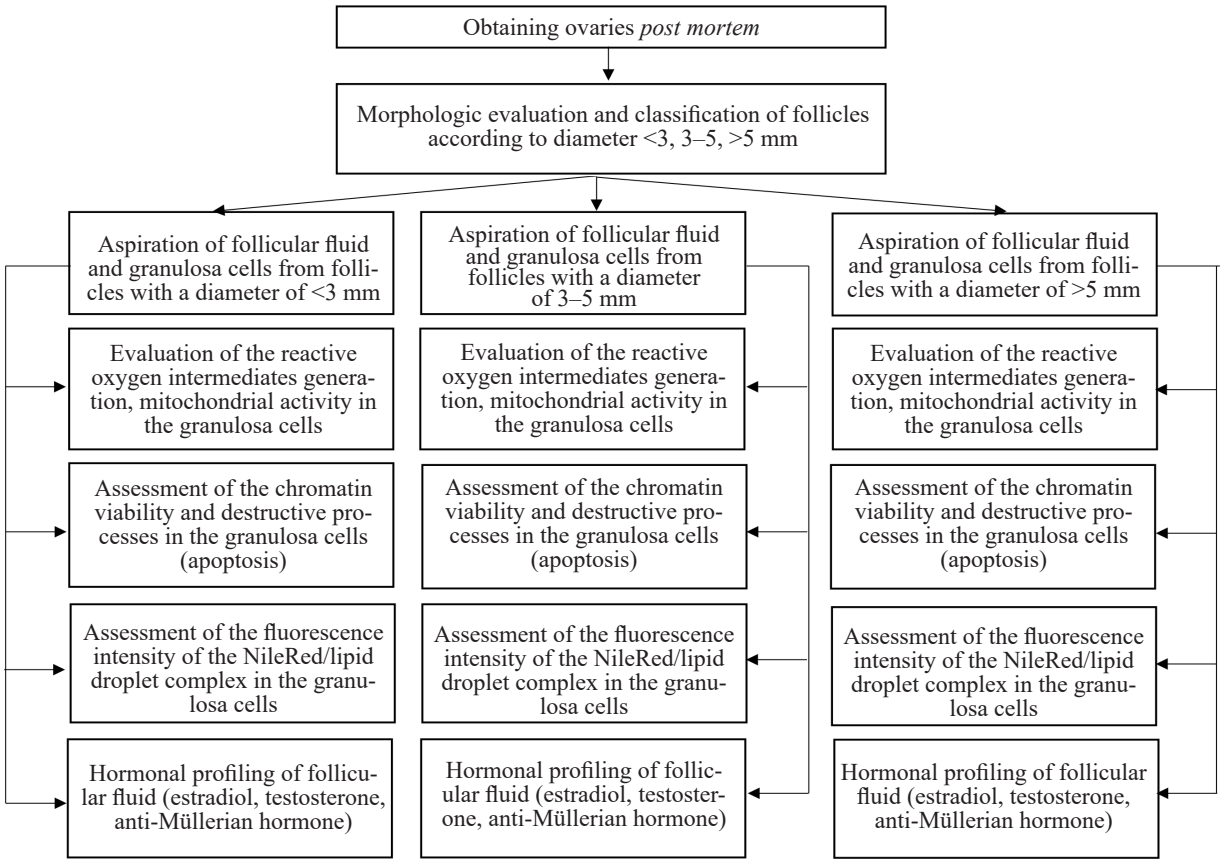

Рис. 1. Структурно-логическая схема эксперимента
Fig. 1. Structural and logical diagram of the experiment

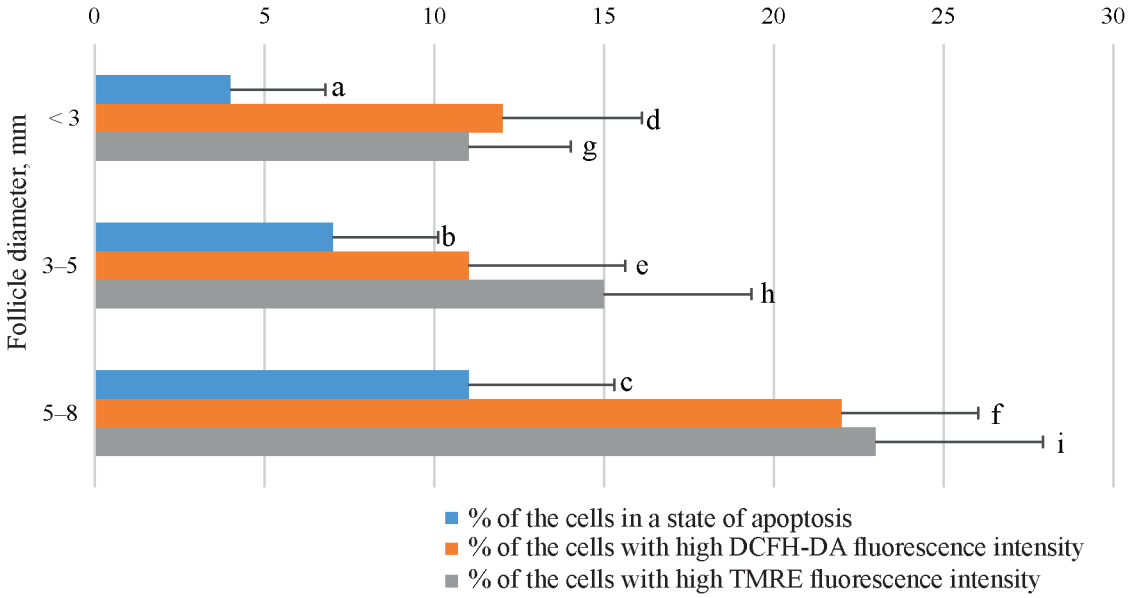

Рис. 2. Апоптотические процессы, генерация АФК и митохондриальная активность в клетках гранулезы из фолликулов разного диаметра:
a:c; a:d; a:e; a:g; d:f; d:i; e:f; f:g; c:f; g:i – $p < 0,05$; e:i – $p < 0,01$; a:f; a:h; a:i; b:f; b:i – $p < 0,001$; n – 125,434. Корреляция по Спирмену: a, b, c : d, e, f 0,500 ($p < 0,05$); d, e, f : g : h : i 0,500 ($p < 0,05$)

Fig. 2. Apoptotic processes, ROS generation and mitochondrial activity in granulosa cells from follicles of different diameters:
a:c; a:d; a:e; a:g; d:f; d:i; e:f; f:g; c:f; g:i – $p < 0.05$; e:i – $p < 0.01$; a:f; a:h; a:i; b:f; b:i – $p < 0.001$; n – 125.434.
Spearman correlation: a, b, c : d, e, f 0.500 ($p < 0.05$); d, e, f : g : h : i 0.500 ($p < 0.05$)

an active accumulation of lipid droplets by the time of active proliferation of granulosa cells, their fusion in cells and, as a consequence, an increase in fluorescence intensity. The proportion of cells with the lowest NileRed/LD FI showed no significant differences at all stages of follicle growth and development.

Lipids are one of the main sources of energy during oocyte maturation, fertilization and preimplantation development. In particular, cholesterol plays an important role as a precursor in steroidogenesis [16]. The production of steroid hormones (estradiol, testosterone) is an integral step in the formation of the necessary conditions for oocyte maturation and its further ovulation.

Estradiol concentration in the follicular fluid of ovarian follicles was found to increase during growth and development (1.50 ng/ml in follicles < 3 mm in diameter and 2.09 ng/ml in follicles 3-5 mm in diameter, $p < 0.01$), remaining almost unchanged by the end of follicle growth (2.11 ng/ml, $p < 0.01$) (see Fig. 4). A similar trend was observed in the testosterone concentration - an increase of 3.8 ng/ml in follicles 3-5 mm in diameter and 4.02 ng/ml in follicles over 5 mm in diameter compared to follicles < 3 mm in diameter ($p < 0.01$, $p < 0.001$). At the same time, no significant differences in the AMH level were detected.

Folliculogenesis is an energy-intensive process and requires bioenergetic support in the granulosa cells due to intensive proliferation, including through the functional activity of mitochondria involved in the cell cycle, providing most of the cellular ATP by oxidative phosphorylation. However, at the same time, active oxidative processes may act as initiators of cell death through intensive production of ROI [17]. In our study, a significant increase of cells with high levels of ROI in follicles with diameters < 3, 3-5 and 8 mm ($p < 0.05$) and high mitochondrial activity ($p < 0.05$) was observed, and a significant correlation between these parameters was found (0.500, $p < 0.05$), which may indicate intensive energy exchange

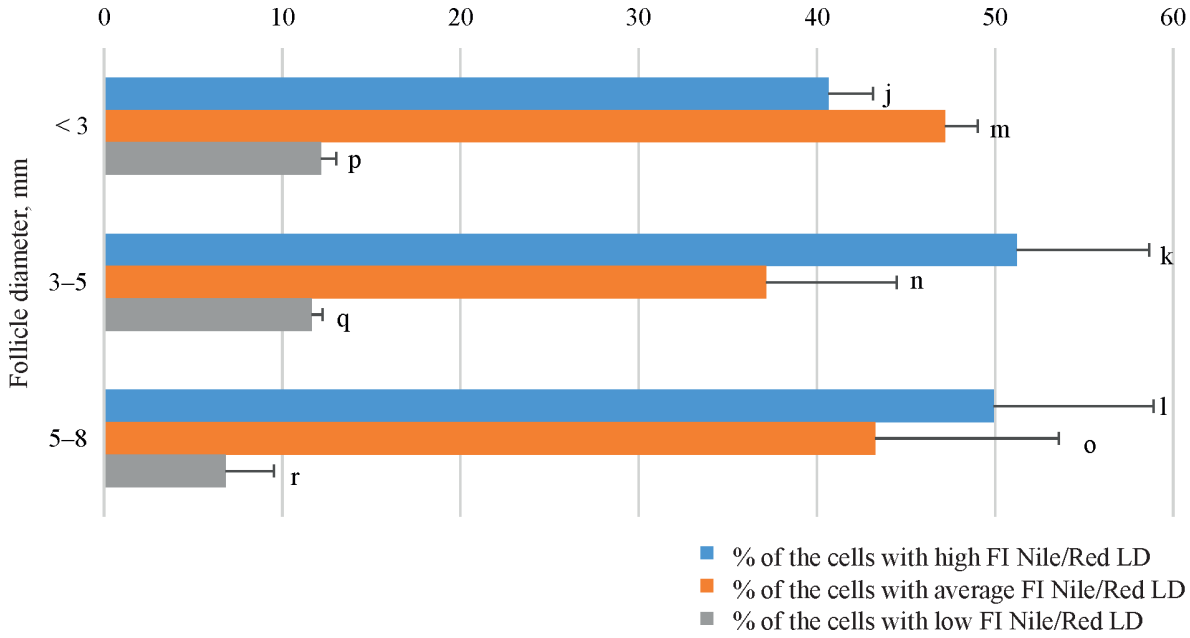

Рис. 3. Показатели функционирования липидома (интенсивность флуоресценции комплекса NileRed/ЛК) в клетках гранулезы в фолликулах разного диаметра:
j:k; j:l; m:n – $p < 0,05$; j:p; j:q; j:r; k:p; k:q; k:r; l:p; l:q; l:r; m:p; m:q; m:r; n:p; n:q; n:r – $p < 0,001$; n – 83,884. Корреляция по Спирмену: j, k, l : m, n, o –1,000 ($p < 0,05$)

Fig. 3. Indicators of lipidome functioning (fluorescence intensity of NileRed/lipid droplet complex) in granulosa cells in follicles of different diameters:
j:k; j:l; m:n – $p < 0.05$; j:p; j:q; j:r; k:p; k:q; k:r; l:p; l:q; l:r; m:p; m:q; m:r; n:p; n:q; n:r – $p < 0.001$; n – 83.884. Spearman correlation: j, k, l : m, n, o –1.000 ($p < 0.05$)

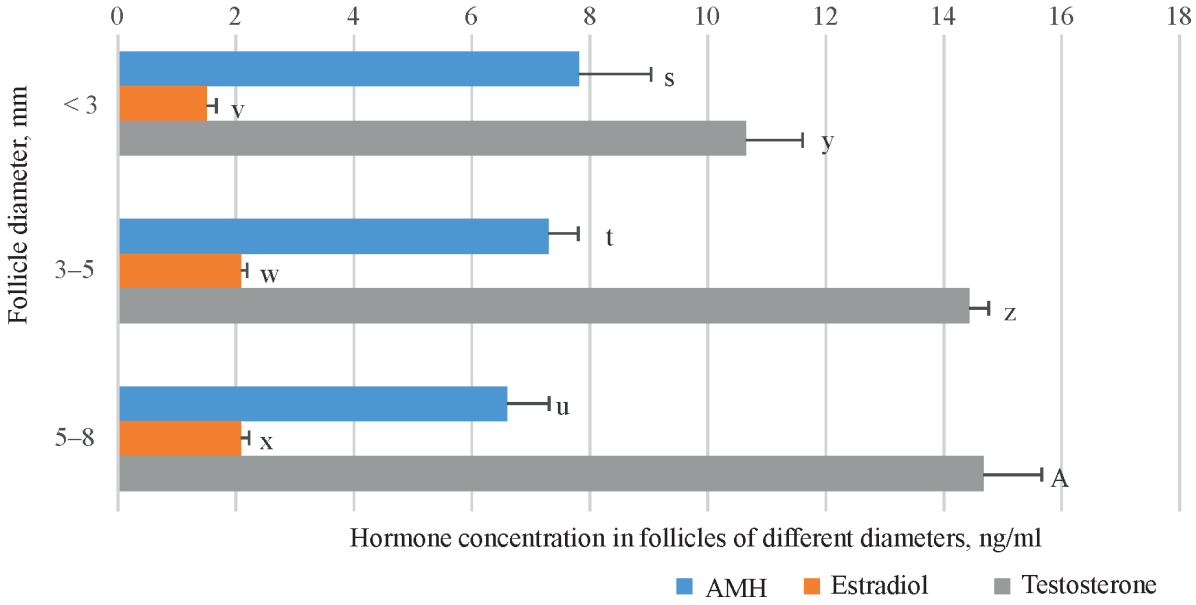

Рис. 4. Гормональный профиль жидкости из фолликулов разного диаметра:
v:w; v:x; y:A – $p < 0,01$; y:z – $p < 0,001$. Корреляция по Спирмену: a, b, c : d, e, f – функциональная обратная (–1,000); a, b, c : g : h : i – функциональная обратная (–1,000); d, e, f : g : h : i – функциональная прямая (1,000)

Fig. 4. Fluid hormonal profile from follicles of different diameters:
v:w; v:x; y:A – $p < 0.01$; y:z – $p < 0.001$. Spearman correlation: a, b, c : d, e, f – functional inverse (–1.000); a, b, c : g : h : i – functional inverse (–1.000); d, e, f : g : h : i – functional line (1.000)

in the granulosa cells during follicle maturation.

In the growing antral follicles, granulosa cells and theca cells actively proliferate and secrete various factors, produce hormones and absorb large amounts of lipids and cholesterol from plasma[8] [18]. Neutral lipids such as triacylglycerols and steryl esters serve as precursors of sex hormones. In our study, significant correlations were found between the proportion of the cells with high NileRed/LD FI in the granulosa cells and the concentrations of estradiol (0.998, $p < 0.05$), testosterone (0.500, $p < 0.05$) and AMH (–0, 500, $p < 0.05$) during follicle growth, there was a direct correlation between estradiol concentration and the proportion of the cells with high mitochondrial activity (0.746, $p < 0.05$), which confirms the data of D. B. Sreerangaraja Urs et al. [19].

Anti-Müllerian hormone is a dimeric glycoprotein of the transforming growth factor beta (TGF-β) superfamily produced directly by granulosa cells of preantral and early antral follicles up to 5-6 mm in diameter [20]. AMH plays an inhibitory role in the recruitment of primordial follicles, regulating the growth of preantral and small antral follicles by attenuating their response to follicle-stimulating hormone [21]. AMH is highly expressed in the granulosa cells of preantral and small antral follicles.

AMH has been shown to be a good endocrine marker of ovarian reserve in cattle[9] and mares [22] and a predictive marker of super ovulatory responses for oocyte retrieval and *in vitro* embryo production in cattle[10] and goats[11]. In pig ovaries, AMG has the same expression and localization pattern as in human, rodent and bovine ovaries [23]. AMHR2 protein was found to be expressed in the granulosa cells and theca cells of human and pig preantral and antral follicles [24].

Our data revealed an inverse functional correlation between estradiol and AMH, testosterone and AMH concentrations (–1.000, $p < 0.05$). In addition, we found an inverse functional correlation between the proportion of the granulosa cells in a state of apoptosis and the AMH content in follicular fluid (–1.000, $p < 0.05$). According to K.J. Hong et al. [25], AMH level correlates with the total number of retrieved oocytes and the success rate of *in vitro* fertilization. The number of mature oocytes was shown to be positively and strongly correlated with serum AMH level ($r = 0.719$, $p < 0.01$), but not with intrafollicular AMH level. AMH content in follicular fluid is thought to be positively correlated with serum AMH levels. K.J. Hong et al. [25] found approximately equal levels of AMH in follicular fluid of follicles of different diameters, which correlates with the data obtained by us.

CONCLUSION

As a result of a series of experiments to assess the functional activity of granulosa cells *in vivo* and *in vitro*, the peculiarities of their functioning in the process of growth of antral ovarian follicles of pigs were revealed.

The number of apoptotic cells in OF increased depending on the follicle diameter (by 4% in OF with diameter < 3 mm, by 11% in OF with diameter 5–8 mm). There was an increase in the proportion of cells with a high level of ROI (by 12% in OF < 3 mm diameter, by 22% in OF 5-8 mm diameter) and high mitochondrial activity (by 12% in OF 5–8 mm diameter compared to OF < 3 mm diameter).

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Correlations were found between the group of cells with high ROI generation and high apoptosis (0.500, $p < 0.05$), as well as with high mitochondrial activity (0.500, $p < 0.05$). An increase in the proportion of cells with high NileRed/LD FI (by 41% in OF < 3 mm diameter, by 50% in OF 5–8 mm diameter, $p < 0.05$) and a decrease in the number of cells with medium NileRed/LD FI (by 47% in OF < 3 mm diameter, by 37% in OF 3-5 mm diameter) were detected. A negative correlation between the groups of cells with high and medium NileRed/LD FI was shown (–1.000, $p < 0.05$).

The concentration of estradiol in follicular fluid increased in a diameter-dependent manner as the OF grew (1.50 ng/mL in OF < 3 mm in diameter and 2.09 ng/mL in OF 3-5 mm in diameter, $p < 0.01$), almost unchanged in OF 5–8 mm in diameter (2.11 ng/mL). The change in testosterone concentration had a similar trend. This index increased with follicle growth (10.65 ng/mL in OF with diameter < 3 mm and 14.43 ng/mL in OF with diameter 3–5 mm, $p < 0.01$) and remained almost at the same level in OF with diameter 5-8 mm (10.67 ng/mL). Correlations were found between the proportion of cells with high NileRed/LD FI (a marker of lipid droplet functional activity) and the concentrations of estradiol (0.998, $p < 0.05$), testosterone (0.500, $p < 0.05$), AMH (–0.500, $p < 0.05$) in the dynamics of folliculogenesis, as well as between the concentration of estradiol and the proportion of cells with high mitochondrial activity (0.746, $p < 0.05$). Correlations were found between estradiol and AMH, testosterone and AMH concentrations (–1.000, $p < 0.05$), between the level of apoptotic cells and AMH concentration in follicular fluid (–1,000, $p < 0,05$).

The revealed peculiarities of hormonal homeostasis and functional activity of pig granulosa cells in OF of different diameters supplement the available data on their role in the formation of oocytes competent for fertilization, the nature of interaction between steroid hormones and AMH, apoptotic processes and generation of ROI, as well as the functioning of cellular compartments (mitochondria, lipid droplets) of granulosa during prolonged cultivation. Based on the available data on high competence for *in vitro* maturation of donor pig oocytes isolated from follicles with a diameter of more than 3 mm [26] and based on the results obtained in the study, we recommend using a population of granulosa cells from follicles 3-5 mm in diameter with the addition of hormones (estradiol, testosterone) at concentrations determining high viability (2.9 and 14.43 ng/ml, respectively) when modeling the cultivation system for *in vitro* maturation.

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ВЛИЯНИЕ РАЗЛИЧНЫХ КОНЦЕНТРАЦИЙ ЭКСТРАКТА ДУШИЦЫ ОБЫКНОВЕННОЙ (*ORIGANUM VULGARE*) НА РУБЦОВОЕ ПИЩЕВАРЕНИЕ *IN VITRO*

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Остаточные противомикробные вещества, являющиеся результатом чрезмерного использования в кормах для животных стимулирующих рост антибиотиков, представляют собой новую угрозу для здоровья человека и состояния окружающей среды. Экстракты растений, содержащие эфирные масла, в частности экстракт *Origanum vulgare*, являются потенциальной альтернативой кормовым антибиотикам, способствуя росту продуктивного потенциала благодаря своим антимикробным и антиоксидантным свойствам. Они могут подавлять размножение патогенных микроорганизмов, вызывая конформационные изменения клеточных мембран. В связи с этим целью исследования стало изучение влияния различных дозировок экстракта *O. vulgare* на переваримость компонентов рациона и состав летучих жирных кислот в рубцовом содержимом в условиях *in vitro*. В эксперименте протестированы три дозировки экстракта душицы: 0,5; 1; 10 мл/л. Результаты проведенного исследования свидетельствуют о том, что малые дозы экстракта *O. vulgare* не приводят к метаболическим сдвигам в рубцовом пищеварении. Высокая доза (10 мл/л), напротив, снижает общее количество простейших. Средняя дозовая нагрузка (1 мл/л) способствует увеличению переваримости до 72,63%, концентрации инфузорий – до 555,56 тыс. шт./мл. Таким образом, на основании полученных данных установлено, что включение экстракта *O. vulgare* в дозе 1 мл/л обусловливает усиление метаболических процессов в рубце, что приводит к лучшему перевариванию кормов. Дозировка 10 мл/л, максимальная из тестируемых, обеспечила повышение концентрации летучих жирных кислот. Однако при внесении 1 мл/л экстракта *O. vulgare* произошли самые значимые изменения в концентрации летучих жирных кислот.

Ключевые слова: переваримость, летучие жирные кислоты, экстракт *Origanum vulgare*, инфузории, рубцовое пищеварение

EFFECT OF DIFFERENT CONCENTRATIONS OF OREGANO (*ORIGANUM VULGARE*) EXTRACT ON RUMEN DIGESTION *IN VITRO*

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Residual antimicrobial substances resulting from the overuse of growth-promoting antibiotics in animal feed pose an emerging threat to human health and the environment. Plant extracts containing essential oils in general, and in particular *Origanum vulgare* extract, are a potential alternative to feed antibiotics, contributing to the growth of productive potential, due to their antimicrobial and antioxidant properties. They can inhibit the reproduction of pathogenic microorganisms, causing conformational changes in cell membranes. In this regard, the purpose of the study was to study the effect of different dosages of *O. vulgare* extract on the digestibility of diet components and the composition of volatile fatty acids in the rumen content under *in vitro* conditions. Three dosages of the oregano extract were tested in the experiment: 0.5; 1; 10 ml/l. The results of the present study indicate that small doses of *O. vulgare* extract do not lead to metabolic shifts in the rumen digestion. High dose (10 ml/l), on the contrary, reduces the total number of protozoa. The average dose load of 1 ml/l leads to an increase in digestibility to 72.63% and the concentration of infusoria to 555.56 thousand pcs/

ml. Thus, based on the results obtained, it follows that the inclusion of the *O. vulgare* extract in a dose of 1 ml/l enhances metabolic processes in the rumen, leading to better digestion of feed. The dosage of 10 ml/l, the maximum of the tested, provided an increase in the concentration of the volatile fatty acids. However, when using 1 ml/l of *O. vulgare*, the most significant changes in the concentration of the volatile fatty acids occurred.

Keywords: digestibility, volatile fatty acids, *Origanum vulgare* extract, infusoria, rumen digestion

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INTRODUCTION

Essential oils (EO) added to animal feeds act as a natural alternative to antibiotics and growth stimulants. Some experiments have confirmed that the use of plant extracts is similar in results to the inclusion of antibiotics in feed [1]. However, the number of works proving the effect of EO on microbial fermentation in the rumen is limited.

It was previously shown that saponins, tannins and EO affect fermentation in the rumen, leading to the accumulation of nitrogen and reduction of ammonia concentration, which suggests the influence of these substances on the process of deamination of amino acids by rumen bacteria.

The sensitivity of rumen microorganisms to EO was assessed, as some data indicated that high consumption of EO caused digestive problems. High doses of EO added to rumen bacterial cultures *in vitro* decreased the total number of viable bacteria, at the maximum dose the only surviving species were small Gram-negative microorganisms.

After the ban of antibiotics as feed additives in the European Union, there has been a renewed interest in studying the effects and mechanisms of action of EO on microbial fermentation in the rumen. Over the last six years, studies on the effects of more than 25 different plant extracts on microbial fermentation in the rumen *in vitro* have been published. Among them *Lavandula angustifolia*, *Solidago virgaurea* and *Achillea millefolium* stimulated rumen fermentation, *Equisetum arvense*, *Armoracia rusticana* and *Salvia officinalis* inhibited the methanogenesis.

Origanum vulgare (oregano) is a perennial herbaceous plant growing in Eurasia and North Africa. The EO content of Greek oregano (*O. vulgare* ssp. *hirtum*) is quite high – about 4.0% of dry matter. Another subspecies of oregano, wild marjoram (*O. vulgare* ssp. *vulgare*), has a lower level of EO – from 0.2 to 1.0% of dry matter.

EO extracted from *O. vulgare* is an aromatic volatile oil with strong antibacterial and antioxidant properties [2, 3]. When added to feed, this oil helps to maintain a healthy microbiota by regulating the reproduction of beneficial microorganisms in the gastrointestinal tract and inhibiting the growth of pathogenic microflora. Researchers have found that EO improves rumen fermentation and feed digestion efficiency by targeting bacteria in the Prevotellaceae, Lachnospiraceae and Ruminococcaceae families.

In addition to influencing the diversity of microbiota, EO modulates the processes of methanogenesis (in particular, gas production in the

rumen). At the same time, more recent studies indicate that EO regulates the digestive metabolism of animals, improving protein metabolism and the structure of volatile fatty acids (VFAs) [4]. The action of *O. vulgare* leaves as an anti-methanogenic product with no adverse effect on fermentation in the rumen has been described *in vitro*. A single application of *O. vulgare* EO was tested, which reduced methane production in the rumen by improving the digestibility of the nutrients in the gastrointestinal tract.

The effect of EO on rumen function includes inhibition of deamination and methanogenesis, which provides a reduction in ammonia nitrogen and methane levels, respectively. Essential oils reduce acetate in the rumen while maintaining total VFAs production by increasing propionate and butyrate production [5].

In this regard, the purpose of our study was to investigate the effect of different dosages of the *O. vulgare* extract on in vitro digestibility and the VFAs composition in the rumen fluid.

MATERIAL AND METHODS

The experiment was conducted in the center "Nanotechnologies in Agriculture" (established in 2014 with the support of the Russian Science Foundation) and the Center for Collective Use of the Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences on the *in vitro* model "artificial rumen" (incubator unit "ANKOM Daisy II", Ankom Technology Corporation, USA). The above model allows simultaneous incubation of several samples of rumen fluid in sealed polyester bags in the same incubation vessel, which is constantly rotating at a temperature of 39.5 °C. In this method, the material disappearing from the bag during incubation is considered digested. The experiment was carried out in triplicate repeats.

In the course of the study, samples of the rumen contents were taken. The available samples were divided into control (without extract) and three experimental groups, in which different amounts of oregano extract were added to the rumen fluid: group 1 – 0.5 ml/l of the extract, group 2 – 1 ml/l, group 3 – 10 ml/l. To obtain the extract, 20 g of herb was poured into 200 ml of distilled water, then the mixture was kept on a water bath for 30 min with subsequent filtration.

Rumen fluid was sampled through a chronic rumen fistula in 10-month-old Kazakh white-headed bulls with a live weight of 250 kg. The main diet of the animals included 30.0% concentrates and 70.0% roughage. The samples were transported for 30 min, maintaining a temperature regime of 38.5–39.5 °C. The rumen fluid was stored in a closed vessel without air access until analyzed. Before use, it was shaken thoroughly, filtered through four layers of gauze, and incubated in an artificial rumen at a constant temperature of 39.5 °C for 48 h. At the end of incubation, the samples were washed and dried at 60 °C to constant weight.

The *in vitro* dry matter digestibility coefficient was calculated as the difference in masses of the bagged feed sample before and after incubation using the following formula:

$$K = (A - B)/C \times 100\%,$$

where K – feed dry matter digestibility coefficient, %; A – initial mass 1 (weight of the feed sample with an utricle is taken into account), mg; B – mass after two-stage incubation (weight of the feed sample with an utricle), mg; C – initial mass 2 (weight of the feed sample without the utricle mass), mg.

Counting of infusoria was carried out by microscopic method in a Goryaev counting chamber. For this purpose, 5 ml of filtered rumen fluid was taken into a tube and mixed with 0.1 ml of 45% formalin solution, providing fixation of infusoria. Then 0.9% NaCl, pre-stained with methylene blue solution, was added. Then, one drop of the studied liquid was introduced into the chamber with a Goryaev grid under the cover glass. The number of infusoria was counted in 100 large squares. The total number of infusoria was determined by the following formula:

$$x = 25 \times A,$$

where x is the number of infusoria in 1 mm^3; A is the number of counted infusoria.

The level of VFAs in the rumen contents was determined by gas chromatography with flame

ionization detection on a gas chromatograph "Crystallux-4000M". For the chromatograph with flame ionization detection and capillary column the necessary gas velocities were selected and the following parameters were set: programmable temperature increase of the column thermostat 60-260 °C, injector temperature 250 °C, detector temperature 250 °C. The analysis time was 40 min and the sample input was 1 mm^3. Solutions of acid mixtures at concentrations of 10, 25 and 50 $\mu g/cm^3$ were used as samples for calibration. At least two chromatograms of each solution were recorded, starting from the lower concentration.

Statistical analysis was performed using Microsoft Excel program. The obtained data were presented as: *M* (mean value) ± *m* (standard error of the mean). Reliability of differences was determined by Student's *t*-criterion. The results were considered reliable when $p \leq 0{,}05$.

RESULTS AND DISCUSSION

The degree of feed decomposition and the intensity of digestion directly determine the assimilation of the nutrients by animals [6]. The complex and diverse chemical composition of plants modulates the intensity of rumen processes and affects dry matter digestibility *in vitro* [7].

The obtained data show that dry matter digestibility in the control group was 64.36% (see Table 1). When different doses of *O. vulgare* extract were added, the considered index increased. Thus, in group 1 the digestibility coefficient increased by 5.00%, in group 2 – by 8.27, in group 3 – by 8.17% relative to the control. When comparing the experimental groups among themselves, it was found that the best results were recorded in the 2nd group (72.63%), the concentration of the extract in which was 1 ml / l.

The number of infusoria in rumen fluid of different groups was within close values. However, when the minimum concentration of the extract was added, an increase in the proportion of protozoa by 5.5% was observed; when the maximum dose was added, its decrease by 16.1% was observed compared to the control group. After application of 1 ml/l of *O. vulgare* extract, the number of infusoria increased by 35.89% relative to the control, which is the best result.

Herbs and their extracts can form cross-links between bacteria and feed, thereby increasing the rate of rumen fermentation and nutrient digestion [8].

Thus, the addition of 1 ml/l oregano extract to the rumen fluid promoted the growth of the number of infusoria in the rumen, thereby increasing the digestibility of feed dry matter.

Addition of different doses of O. vulgare extract resulted in certain changes in rumen fermentation (see Table 2). The application of 0.5 ml/l of the extract (group 1) reduced the formation of VFAs: acetic acid by 15.6%, propionic acid by 5.0% ($p \leq 0.05$), butyric acid by 2.3%, valerianic acid by 25.5% ($p \leq 0.05$), and caproic acid by 21.7% compared to the control. Application of the maximum of the tested doses (10 ml/l, group 3) caused an increase in the concentration of VFA. However, at a dosage of 1 ml/l (group 2), the most significant changes occurred relative to the control group, the concentration of the acids increased as follows: acetic acid by 47.5%, propionic acid by 51.4% ($p \leq 0.05$), butyric acid by 51.6%, valerianic acid by 32.4%, and caproic acid by 56.5% ($p \leq 0.05$).

It is known that VFAs are the main breakdown products of dietary carbohydrates and provide most of the energy precursors for metabolic processes in ruminants. O. vulgare extract can reduce VFA production by inhibiting bacte-

Табл. 1. Переваримость сухого вещества *in vitro* и число инфузорий в рубцовой жидкости

Table 1. The digestibility of dry matter *in vitro* and the number of infusoria in the rumen fluid

Group	Digestibility coefficient, %	Number of infusoria, thousand pcs./ml
Control	64,36 ± 1,08	400,00 ± 20,00
1st	69,36 ± 1,84	422,22 ± 21,10
2nd	72,63 ± 2,42	555,56 ± 27,78
3rd	72,53 ± 2,19	344,44 ± 17,22

Табл. 2. Концентрация летучих жирных кислот в рубцовой жидкости в эксперименте, мг/дм^3
Table 2. Concentration of volatile fatty acids in the rumen fluid in the experiment, mg/dm^3

VFA	Group			
	control	1st	2nd	3rd
Acetic	20,40 ± 1,02	17,20 ± 0,86	30,10 ± 1,50	28,40 ± 1,42
Propionic	14,00 ± 0,70	13,30 ± 0,66*	21,20 ± 1,06*	20,80 ± 1,04*
Butyric	17,21 ± 0,86	16,80 ± 0,84	26,10 ± 1,31	25,90 ± 1,29
Valeric	1,45 ± 0,07	1,08 ± 0,05*	1,92 ± 0,09	1,88 ± 0,09
Capronic	0,23 ± 0,01	0,18 ± 0,01	0,36 ± 0,01*	0,33 ± 0,01

*$p \leq 0,05$.

rial activity[1] [9]. In addition, different doses of oregano extract can reduce protozoan population, acetic acid level and total VFA[2] [10]. The maximum doses within the experiment caused a similar effect.

Essential oils, being secondary metabolites of plants, have been found not only to affect VFA production in general, but in particular to increase the production of butyric acid, which is generally considered to be better because it stimulates rumen metabolism [11, 12].

Thus, high doses (1 and 10 ml/l) of the plant extract had a positive effect on the rumen microbial fermentation, contributing to an increase in total VFA concentration.

CONCLUSION

Previous studies using *O. vulgare* have shown that oregano extract can influence rumen bacterial communities in ruminants by increasing feed digestibility while increasing VFA fermentation. However, a large number of rumen bacteria have yet to be cultured. It should be noted that the results obtained in the present experiment may have limitations. Future *in vivo* studies are needed to optimize the application rate of *O. vulgare* so that an effective increase in feed digestibility and fermentation in the rumen can be achieved.

Thus, when adding the maximum allowable dose of the extract (10 ml/l) there is a decrease in the total number of infusoria by 16.1%, at the same time the digestibility of dry matter of the feed remains high and is 72.53%. At introduction of the oregano extract in the minimum dosage there is an increase in both indicators, but to a lesser extent.

Thus, the optimal rate of introduction of the *O. vulgare* extract into the feed for future studies is 1 mg/l for adult animals, as the above concentration contributes to an increase in dry matter digestibility of the feed up to 72.63% and has a positive effect on microbial fermentation of the rumen.

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ВЛИЯНИЕ УРОВНЯ УГЛЕВОДОВ В РАЦИОНАХ КОРОВ ХОЛМОГОРСКОЙ ПОРОДЫ НА МОЛОЧНУЮ ПРОДУКТИВНОСТЬ И КАЧЕСТВО МОЛОКА

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Изучено влияние рационов с разным соотношением легкопереваримых углеводов на молочную продуктивность и качество получаемого молока высокопродуктивных коров холмогорской породы в период раздоя. Научно-производственный опыт на коровах проводился в Архангельской области в 2022 г. Отбор животных в контрольную и опытную группы проводился методом пар-аналогов при стойловой системе содержания. В исследованиях участвовало две группы коров: опытная и контрольная по 10 гол. в каждой. Длительность опыта составила 100 дней. В качестве углеводной добавки к рационам опытных животных использована углеводно-протеиновая смесь в количестве 1,5 кг на одну голову в сутки. Селекционный контроль качества молока на массовую долю жира, белка, лактозы, сухого вещества, сухого обезжиренного молочного остатка (СОМО), точки замерзания, мочевины и соматических клеток проводился на высокоскоростном инфракрасном анализаторе молока Bentley Combi 150 в лаборатории селекционного контроля качества молока. Выявлено, что сахаропротеиновое отношение в опытной группе было в 2 раза выше (0,58), чем в контрольной (0,29), за счет наличия в рационе углеводной добавки. Установлено увеличение молочной продуктивности животных на 4,6 и 0,14% уровня белка в молоке при включении в рацион углеводно-протеиновой смеси в количестве 1,5 кг на одну голову в сутки. Зафиксировано снижение на 3,2% уровня мочевины в молоке, указывающее на более эффективное использование животными аминокислот в процессе метаболизма при одном уровне всасывания аммонийных форм при увеличении уровня простых углеводов в рационе. Выявлено положительное влияние данной добавки на содержание сухого обезжиренного молочного остатка, увеличившегося в молоке коров опытной группы на 0,128 абсолютных % (абс.%). Применение углеводно-протеиновой смеси в количестве 1,5 кг на одну голову в сутки и контроль за качеством производимой продукции позволит увеличить молочную продуктивность коров, улучшить качество получаемой продукции, увеличить продуктивное долголетие молочного скота.

Ключевые слова: холмогорский крупный рогатый скот, молочная продуктивность, качество молока, уровень углеводов, углеводная добавка

EFFECT OF CARBOHYDRATE LEVEL IN THE DIETS OF KHOLMOGOR COWS ON MILK PRODUCTIVITY AND MILK QUALITY

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The effect of the diets with different ratio of easily digestible carbohydrates on milk productivity and milk quality of high-yielding Kholmogor cows during the milking period was studied. The research and production experiment on the cows was conducted in the Arkhangelsk region in 2022. Selection of animals in the control and experimental groups was carried out by the method of pair-analogues under the stall barn system. Two groups of cows participated in the research: experimental and control groups of 10 cows each. The experiment lasted for 100 days. Carbohydrate-protein mixture was used as a carbohydrate supplement to the diets of experimental animals in the amount of 1.5 kg per head per day. Selective milk quality control for mass fraction of fat, protein, lactose, dry matter, milk

solids non-fat (MSNF), freezing point, urea and somatic cells were determined on a Bentley Combi 150 high-speed infrared milk analyzer at the Selective Milk Quality Control Laboratory. It was found that the sugar-protein ratio in the experimental group was twice higher (0.58) than in the control group (0.29), due to the presence of carbohydrate supplementation in the diet. The increase in milk productivity of the animals by 4.6 and 0.14% of the protein level in milk at inclusion of carbohydrate-protein mixture in the amount of 1.5 kg per one head per day in the diet was established. A 3.2% decrease in the level of urea in milk was recorded, indicating a more efficient use of amino acids by the animals in the process of metabolism at the same level of absorption of ammonium forms while increasing the level of simple carbohydrates in the diet. The positive effect of this additive on the content of dry skim milk residue, which increased in the milk of the cows of the experimental group by 0.128 absolute % (abs.%), was revealed. Application of the carbohydrate-protein mixture in the amount of 1.5 kg per one head per day and control over the quality of produced products will increase the milk productivity of cows, improve the quality of products, increase the productive longevity of dairy cattle.

Keywords: Kholmogor cattle, dairy productivity, milk quality, carbohydrate level, carbohydrate supplement

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INTRODUCTION

Full nutrition of dairy cows is the basis of their high productivity, good health, normal reproductive function and good product quality at the lowest feed inputs. In domestic cattle breeding due to poor quality of forages and their shortage the realization of genetic potential of dairy productivity is 40–60% [1].

A unique feature of ruminants is that mono- and disaccharides from feed, formed during microbial breakdown under anaerobic conditions, are digested to volatile fatty acids (VFAs) and some other products. Digestion of carbohydrates is the main cause of low blood glucose concentration in ruminants. In the process of rapid fermentation of easily digestible carbohydrates in the rumen fluid of cows, the concentration of VFAs sharply increases, while the molar fraction of lactate and propionates increases, and pH decreases. This leads to significant changes in rumen metabolism and microbiocenosis of rumen contents. As a consequence, the concentration of fat in milk decreases, metabolism in the cow body is disturbed, which is eventually accompanied by pronounced pathological processes [2].

Carbohydrate metabolism in the rumen is affected by a number of reasons: source and level of fiber in the diet, type of feed, degree of plant lignification, cellulosolytic activity, level of easily digestible carbohydrates [3, 4].

The ratio and amount of VFAs is largely determined by the type and method of feed preparation. Diets rich in starch and sugar and concentrates contribute to the formation of propionic acid (15–20%). Propionic acid, which is the main precursor of glucose in the body (gluco-

neogenesis in the liver), is used in the synthesis of fats. An increase in feed proteins in the diet causes an increase in the amount of butyric acid (10–15%) [5]. Both the ratio of proteins and carbohydrates in the feed and the predominance of certain carbohydrates in it are important in the formation of various fermentation acids [6].

At deficiency in the diet of easily digestible carbohydrates, and also at the increased content of protein in the rumen of ruminants there is an increase in the formation of butyric acid, which is not only an energy material and a precursor of fat in milk, but also at its excessive amount contributes to the formation of some under-oxidized products of carbohydrate-fat metabolism [7].

Recently, different degrees of participation of easily digestible carbohydrates in the synthesis of bacterial protein and amino acids, as well as in the metabolism of minerals have been established. The most significant in this respect are the so-called structural carbohydrates: lactose, galactose, raffinose and mannose. The introduction of feeds containing these carbohydrates has a positive effect on mineral absorption, especially in young, pregnant and lactating animals. Sucrose, glucose, starch, fructose and maltose are energy carbohydrates [8].

In animal nutrition, much attention is paid to sugars, especially the process of glucose formation and utilization. Lack of glucose formation negatively affects carbohydrate and fat metabolism and the process of milk synthesis, especially in the last two months of pregnancy and the first two months of lactation, when the cow's body is most sensitive to the fullness of carbohydrate nutrition. Excess sugar in the diet is also undesirable. With prolonged intake of significant amounts of sugar, acetic acid, which is involved in the synthesis of milk fat, begins to be actively used to deposit body fat. This causes obesity in cows and, as a consequence, a decrease in milk productivity [9, 10].

Balancing of feeding rations for high-yielding cows both on the total content of easily digestible carbohydrates and taking into account their fractions and protected starch, as well as on the ratio of their easily hydrolyzable fraction to decomposable protein for optimal use of nitrogen in the body is a factor in increasing the efficiency of nutrient utilization, normalization of metabolic processes and growth of animal productivity [11, 12].

The purpose of the study was to establish the influence of diets with different ratio of easily digestible carbohydrates on milk productivity and milk quality of high-yielding Holmogory cows during the milking period.

MATERIAL AND METHODS

The research and production experiment on cows was conducted in OOO "Agrofirma (AF) "Kholmogorskaya", Kholmogorsky district, Arkhangelsk region. The studies were conducted according to the generally accepted methodology of A.I. Ovsyannikov[1] by the method of pair-analogs under the stall barn system.

Two groups of cows were involved in the research: experimental and control groups of 10 cows each. The duration of the experiment was 100 days. Carbohydrate-protein mixture in the amount of 1.5 kg per head per day was used as a carbohydrate supplement to the diets of experimental animals. The mixture consisted of dry beet molasses and sunflower cake, containing 49% carbohydrates and 9% protein in its composition.

During the experiment, the cows of the control group received the basic diet (BD) formulated by farm specialists and designed for an average daily milk production of 30 kg. The diet consisted of 43 kg of silage distributed to the cows daily, meat and bone meal, grass meal, carbohydrate-protein mixture and concentrates. Concentrate feeds were introduced into the diet at the rate of 300 g per liter milked.

Milk productivity was assessed by control milkings every ten days. Sampling was carried out in accordance with GOST R 52738-2007 "Milk and milk products. Terms and definitions" with the proposed normative references.

Selective control of milk quality for mass fraction of fat, protein, lactose, dry matter, MSNF, freezing points, urea and somatic cells was determined on a Bentley Combi 150 high-speed

[1] *Ovsyannikov A.I.* Fundamentals of experimental work in animal husbandry: textbook. Moscow: Kolos, 1976, 304 p.

infrared milk analyzer in the laboratory of selective control of milk quality at the Arkhangelsk Research Institute of Agriculture – Primorsky branch of the Federal Research Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Science.

The obtained results of scientific research were processed by the method of variation statistics using a standard statistical analysis package Microsoft Excel 2007 on a personal computer. The reliability of the obtained results was assessed using the Student's criterion.

RESULTS AND DISCUSSION

The analysis of chemical composition of forages in the OOO "AF "Kholmogorskaya" showed that harvested forages have low content of metabolizable energy (8.95 MJ) and crude protein (11.8%) and therefore expensive concentrated forages are used to cover the deficit of protein nutrition (see the table).

To increase the level of carbohydrates in the ration of experimental cows, a carbohydrate-protein mixture in the amount of 1.5 kg was included in addition to the basic ration; cows of the control group consumed only the basic general ration. The analysis of diets showed that the concentration of metabolizable energy in dry matter of diets of cows of control and experimental groups was: in the control group – 10.00 MJ, in the experimental group – 10.17 MJ. The concentration of crude protein in the diets of experimental and control groups was within the norm (15.6%). The sugar-protein ratio in the experimental group was 2 times higher (0.58) than in the control group (0.29), due to the presence of carbohydrate supplement in the ration.

The structure of the diet in terms of metabolizable energy in the groups had insignificant differences and amounted to: concentrated fodder – 41.0 -45.5%, roughage and silage –58.9-54.5%.

Milk productivity of cows is one of the main criteria, which allows to evaluate the balance and nutritional adequacy of feeding, as well as the productive effect of the diets in dairy cattle breeding.

The table presents milk quality indicators by groups of animals for the period of experience

Молочная продуктивность и качество молока исследуемых коров
Dairy productivity and milk quality of the cows under study

Indicator	Group	Control milking							
		1	2	3	4	5	6	7	8
Average daily milk yield, kg	Experimental	26,4 ± 2,6	26,7 ± 2,6	25,7 ± 2,2	25,0 ± 2,1	26,7 ± 2,5	26,6 ± 2,2	26,7 ± 2,6	23,6 ± 2,5
	Control	26,2 ± 2,3	25,1 ± 1,9	24,5 ± 1,7	23,8 ± 1,9	25,6 ± 1,3	25,1 ± 1,3	25,1 ± 1,9	22,7 ± 1,7
Fat content, %	Experimental	3,6 ± 0,2	3,3 ± 0,17	3,3 ± 0,22	3,2 ± 0,17	3,4 ± 0,27	3,2 ± 0,19	3,3 ± 0,16	3,3 ± 0,18
	Control	3,4 ± 0,14	3,4 ± 0,17	3,4 ± 0,25	3,2 ± 0,14	3,1 ± 0,15	3,2 ± 0,17	3,4 ± 0,17	3,3 ± 0,12
Protein content, %	Experimental	3,08 ± 0,09	3,03 ± 0,03	3,09 ± 0,06	3,05 ± 0,05	3,24 ± 0,09	3,16 ± 0,06	3,06 ± 0,02	3,17 ± 0,05
	Control	3,08 ± 0,12	2,99 ± 0,06	2,90 ± 0,03	2,81 ± 0,06	3,07 ± 0,05	2,97 ± 0,07	2,93 ± 0,03	3,03 ± 0,04
Urea content, mg%	Experimental	26,3 ± 1,16	26,8 ± 0,99	27,7 ± 1,27	26,0 ± 1,18	22,1 ± 0,47	22,1 ± 0,78	26,8 ± 0,99	19,6 ± 0,70
	Control	28,5 ± 1,42	27,0 ± 1,38	27,7 ± 1,03	27,6 ± 1,19	23,1 ± 0,71	23,2 ± 0,95	27,0 ± 1,38	19,7 ± 1,17
MSNF content, %	Experimental	8,985 ± 0,09	8,917 ± 0,05	9,005 ± 0,17	8,719 ± 0,08	8,972 ± 0,09	8,937 ± 0,08	8,917 ± 0,05	8,942 ± 0,09
	Control	8,959 ± 0,13	8,820 ± 0,09	8,715 ± 0,06	8,514 ± 0,10	8,793 ± 0,08	8,850 ± 0,09	8,820 ± 0,09	8,912 ± 0,13

in the context of control milkings, conducted 2 times a month.

It was noted that different ratio of the amount of easily digestible carbohydrates had a positive effect on milk productivity of cows of the experimental group. The difference in productivity between the experimental and control groups was 0.22-1.51 kg per head per day in favor of the animals of the experimental group consuming carbohydrate-protein mixture.

For such indicator of milk quality as mass fraction of fat, no significant differences between the groups were revealed.

The content of readily available carbohydrates in the carbohydrate-protein mixture is more directed to increase the content of propionic acid in the rumen, responsible for lactose and protein synthesis, and to decrease acetic acid, responsible for fat synthesis. The addition of carbohydrate supplementation to the diet of the experimental group significantly ($P = 0.95$) increased the protein content in milk by 0.14% on average over the period (see Fig. 1).

Such an important indicator as urea level in milk shows that in all groups the efficiency of feed protein conversion to milk protein was at different levels (see Fig. 2).

The milk of cows of the experimental group showed a slight decrease in the level of urea compared to the milk of cows of the control group, which indicates a more efficient use of amino acids in the process of metabolism at the same level of absorption of ammonium forms. Thus, the level of urea in milk of cows of the experimental group was lower by 3.2%.

The research results suggest that the milk solids non-fat content (MSNF) varies depending on the level of carbohydrates (see Fig. 3).

On the average for the period of the experiment the difference in MSNF in milk of the studied groups was 0.128 abs.% in favor of the animals of the experimental group.

CONCLUSION

The conducted research has shown that increase in the diet of easily accessible carbohydrates by 741 g, or by 112%, increases milk productivity of animals by 4,6% ($P = 0,95$), protein level by 0,14%, MSNF in milk by 0,128%. Reduction by 3.2% of urea level in milk of animals receiving carbohydrate supplement indicates a positive course of protein and carbohydrate metabolism in the body.

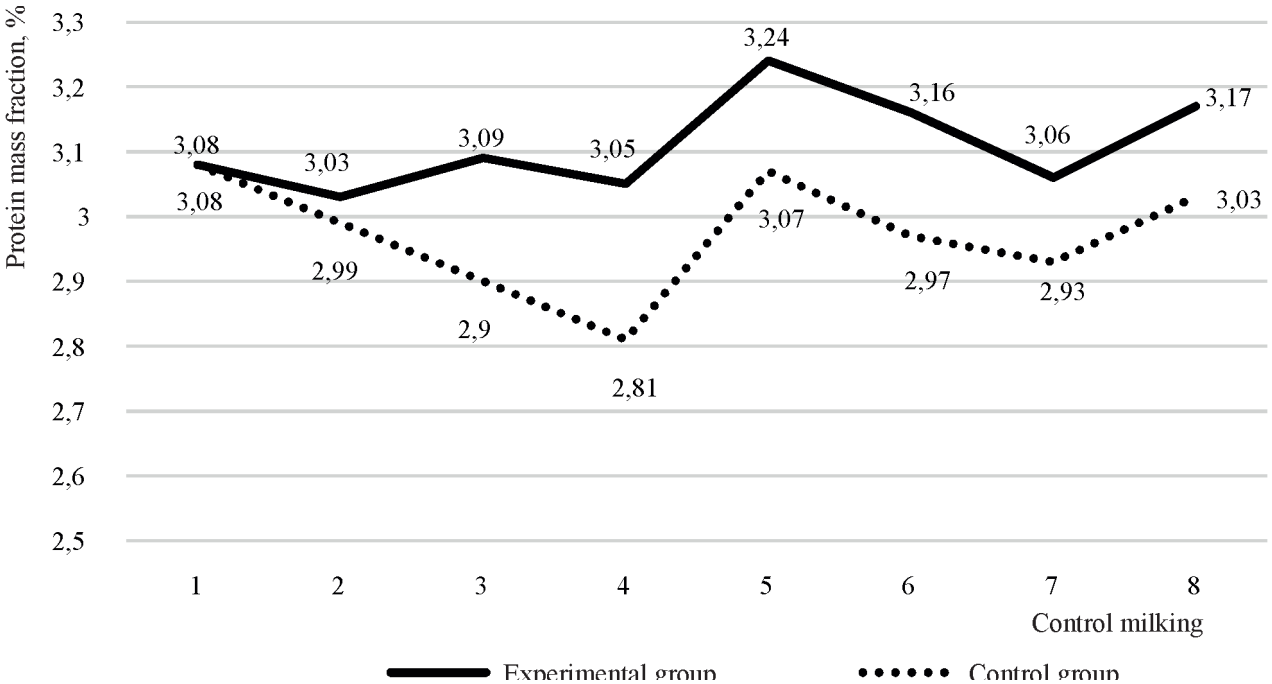

Рис. 1. Массовая доля белка в молоке исследуемых животных
Fig. 1. Mass fraction of protein in the milk of the tested animals

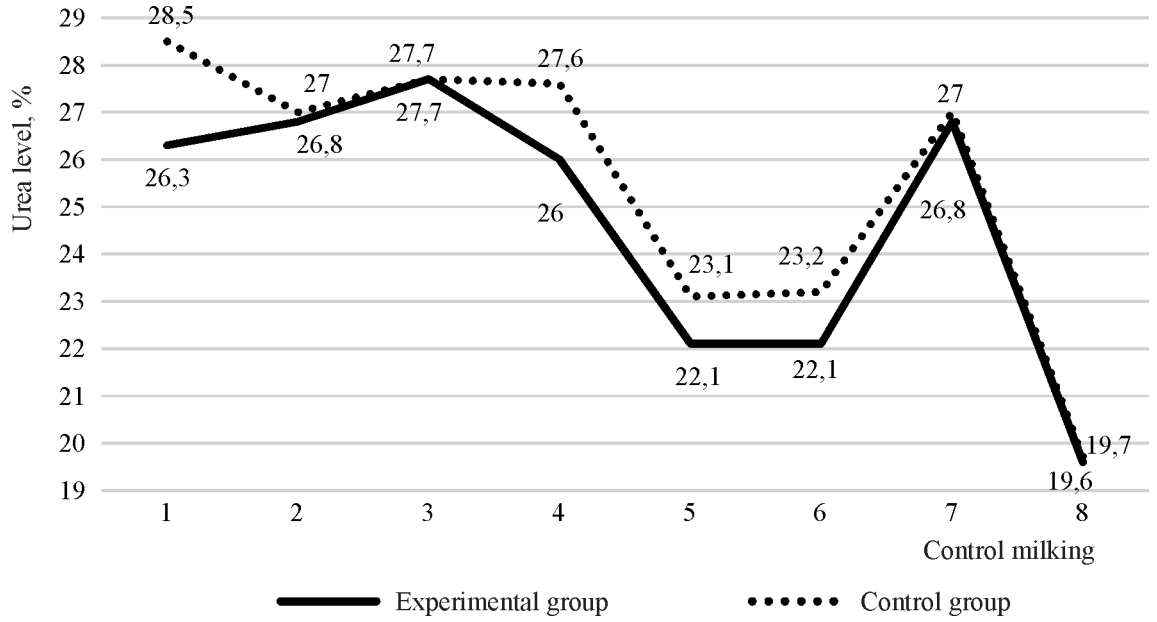

Рис. 2. Уровень мочевины в молоке исследуемых животных
Fig. 2. Urea level in the milk of the tested animals

The use of carbohydrate-protein mixture containing 49% of readily available carbohydrates in the amount of 1.5 kg per head per day and laboratory control over the quality of produced products will increase the milk productivity of cows and improve the quality of the obtained products.

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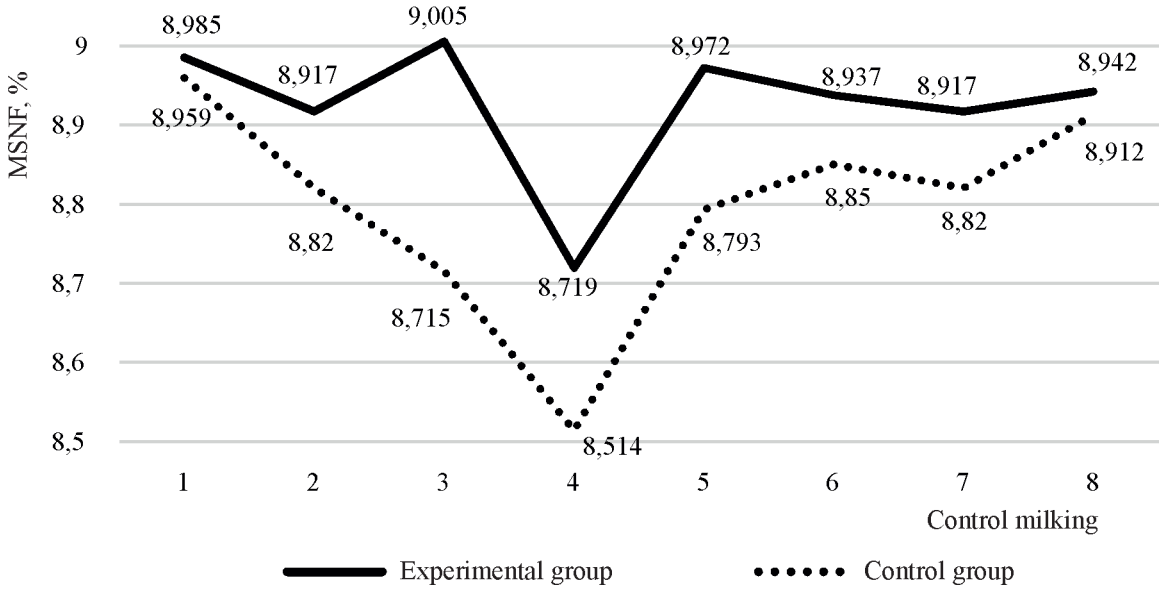

Рис. 3. Содержание СОМО в молоке исследуемых животных
Fig. 3. MSNF content in the milk of the tested animals

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МИКРОБИОЛОГИЧЕСКИЕ ОСОБЕННОСТИ И АНТИБИОТИКОЧУВСТВИТЕЛЬНОСТЬ КУЛЬТУР *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* (*MAP*), ВЫДЕЛЕННЫХ НА ТЕРРИТОРИИ ЗАПАДНОЙ СИБИРИ

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Представлены результаты исследований микробиологических особенностей культур стандартизированного и клинических штаммов, выделенных из биологического материала животных на территории Западной Сибири, относящихся к *Mycobacterium avium* subsp. *paratuberculosis* (*MAP*). Микробиологические исследования патогенов состояли из бактериоскопического метода (окраска мазков культур по Цилю – Нильсену) и культурального (обработка биоматериала методом А.П. Аликаевой с последующим посевом полученного осадка на яичные питательные среды Левенштейна – Йенсена и Финн-2 с микобактином). Кроме этого, применяли биохимические тесты с изолированными из материала культурами, включающие изучение роста колоний при 30, 37 и 42 °С, на среде с салицилатом натрия, с 5%-м хлоридом натрия, редукцию нитратов, определение амидазной, каталазной, арилсульфатазной активности, гидролиз твин-80, а также биологический метод, состоящий из внутривенного инфицирования суспензиями стандартизированного и клинических штаммов нелинейных белых мышей. Результаты исследований показали принадлежность культур клинических штаммов к 3-й группе микобактерий по классификации Раньона и идентичность их свойств со стандартизированным штаммом *M. paratuberculosis,* что позволяет отнести их к микобактериям паратуберкулеза. Изучение антибиотикочувствительности стандартизированного штамма *M. paratuberculosis* (Центрально-Любинский) и клинических штаммов выявило их восприимчивость ко всем использованным в исследованиях препаратам. При постановке биологической пробы у мышей, зараженных паратуберкулезными патогенами, масса тела была меньше, чем в контрольных группах. На вскрытии у животных выявлены увеличение легких, селезенки и печени, единичные гнойные очаги на печени, селезенке и брыжейке, печень имела мраморную окраску, слизистая оболочка тонкой кишки не изменена. Интенсивность роста культур из биоматериала (легкие, печень, селезенка) составила 2(++) – 3(+++) – 4(++++) балла, из слизистой оболочки тонкой кишки – 0(+/–) балла. Бактериоскопическое исследование мазков колоний культур исследуемых патогенов, выделенных из внутренних органов мышей опытных групп и окрашенных по Цилю – Нильсену, показало наличие единичных кислотоустойчивых зернистых палочек, расположенных группами или в виде «палисадника», что характерно для возбудителя паратуберкулеза. Биологический метод исследования на лабораторных животных выявил восприимчивость нелинейных белых мышей к исследуемым культурам и возможность воспроизведения на них экспериментальной паратуберкулезной инфекции.

Ключевые слова: паратуберкулез, штамм, микробиологические свойства, антибактериальные препараты

MICROBIOLOGICAL CHARACTERISTICS AND ANTIBIOTIC SENSITIVITY OF *MYCOBACTERIUM AVIUM* SUBSP. *PARATUBERCULOSIS* (*MAP*) ISOLATED ON THE TERRITORY OF WESTERN SIBERIA

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The results of the studies of microbiological features of cultures of standardized and clinical strains isolated from biological material of the animals on the territory of Western Siberia belonging to *Mycobacterium avium* subsp. *paratuberculosis* (*MAP*) are presented. Microbiological studies of the pathogens consisted of the bacterioscopic method (staining of smears of cultures according to Ziehl – Neelsen)

and the culture method (processing of biomaterial by the method of A.P. Alikaeva with subsequent sowing of the obtained sediment on Lowenstein-Jensen and Finn-2 egg nutrient media with mycobactin). In addition, biochemical tests with the cultures isolated from the material were used, including examination of colony growth at 30, 37 and 42 C, on the medium with sodium salicylate, with 5% sodium chloride, nitrate reduction, determination of amidase, catalase, arylsulfatase activity, hydrolysis of Tween-80 and a biological method consisting of intravenous infection with suspensions of standardized and clinical strains of non-linear white mice. The results of the studies showed that the cultures of clinical strains belonged to the 3rd group of mycobacteria according to the Runyon classification and their properties were identical to the standardized strain of *M. paratuberculosis*, which allows us to attribute them to mycobacteria of paratuberculosis. Antibiotic sensitivity studies of the standardized strain of *M. paratuberculosis* (Central-Lubinsky) and clinical strains revealed their susceptibility to all the drugs used in the studies. In a biological assay, mice infected with paratuberculosis pathogens had lower body weight than in the control groups. Autopsy revealed enlargement of lungs, spleen and liver, single purulent foci on liver, spleen and mesentery, and the liver was marbled, the mucosa of the small intestine is not changed. The growth intensity of cultures from the biomaterial (lungs, liver, spleen) was 2(++) to 3(+++) to 4(++++) points, the growth intensity of the cultures from the mucosa of the small intestine is 0(+/-) points. Bacterioscopic examination of smears of colony cultures of the studied pathogens isolated from the internal organs of mice of the experimental groups and stained by Ziehl – Neelsen staining showed the presence of single acid-fast granular bacilli arranged in groups or in the form of a "palisade", which is characteristic of the causative agent of paratuberculosis. The biological method of research on laboratory animals revealed the susceptibility of nonlinear white mice to the tested cultures and the possibility of reproducing experimental paratuberculosis infection on them.

Keywords: paratuberculosis, strain, microbiological properties, antibacterial drugs

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INTRODUCTION

Paratuberculosis (paratuberculous enteritis, Johne's disease) is a chronic infectious granulomatous enteritis of animals, which is a clinical manifestation of infection with *Mycobacterium avium* subsp. *paratuberculosis* (MAP). The disease is characterized by progressive diarrhea and, as a consequence, cachexia of animals, which may lead to their death. Paratuberculosis was first described in 1895 and is now considered endemic in farmed cattle worldwide, having a global impact on animal health and maintaining a high prevalence rate, resulting in significant economic losses in livestock production [1, 2]. The infection is largely asymptomatic and has various manifestations ranging from high prevalence and significant lethality to low prevalence and rare lethality[1,2] [3, 4].

Paratuberculous enteritis is one of the most difficult infections to control and diagnose because of its long incubation period, which can last from 6 months to 15 years. In addition, the pathogen is extremely resistant to environmental conditions, where it can survive for up to a year or more [5, 6].

[1]*Nielsena S.S., Toftb N.* Ante mortem diagnosis of paratuberculosis: A review of accuracies of ELISA, interferon-γ assay and faecal culture techniques // Veterinary Microbiology, 2008, vol. 129, issue 3–4, N 22, pp. 217–235. DOI: 10.1016/j.vetmic.2007.12.011.

[2]*Whittington R.J.* Progress towards understanding the spread, detection and control of Mycobacterium avium subsp paratuberculosis in animal populations // Australian Veterinary Journal, 2001, N 79 (4), pp. 267–278. DOI: 10.1111/j.1751-0813.2001.tb11980.

In this regard, the full range of vivo diagnostic methods (clinical manifestations of the disease) and microbiological methods are necessary to isolate the pathogen. These methods include microscopic, culture and morphological studies, biochemical tests based on the fact that in the process of metabolism some species of mycobacteria show different enzymatic activity, as well as the biological method of modeling paratuberculosis on laboratory animals (mice, rats, rabbits, guinea pigs)[3]. Proper selection and use of each of these diagnostic tests will ensure their success and may allow the establishment of a program to control the spread and treatment of paratuberculosis infection, since there is no definitive cure for *M. avium* subsp. *paratuberculosis*, the pathogen causing paratuberculosis. Antimicrobials used to treat paratuberculosis may help prevent infection in calves and reduce fecal excretion of MAP in infected adult cattle. Antibiotics can affect the course of paratuberculosis by reducing the concentration of bacteria in the intestinal lumen and altering the composition of the intestinal microflora. Frequent drug sensitivity testing of pathogens should be performed, as inadequate use of antibacterial drugs is the main cause of antibiotic resistance.

The use of microbiological isolation methods to characterize the pathogen of paratuberculosis will allow timely planning and implementation of effective anti-epizootic measures, which will make it possible to prevent the uncontrolled spread of this disease among animals [7-9]. Drug resistance of the pathogen to antibacterial drugs in paratuberculosis is a serious problem, therefore, effective schemes of paratuberculosis control should also be aimed at identifying the sensitivity of the pathogen to antimicrobial drugs in order to overcome resistance to already existing drugs.

The purpose of the work is to present the results of microbiological studies and to study the sensitivity to antibacterial drugs of cultures *M. avium* subsp. *paratuberculosis* (*MAP*).

MATERIAL AND METHODS

Studies of the standardized strain of *M. paratuberculosis* (Central-Lubinsky) and three clinical strains of mycobacteria isolated from biological material of animals were conducted in 2021-2023 at the Institute of Experimental Veterinary Science of Siberia and the Far East of the SFSCA RAS. Pre-sowing treatment of the biomaterial was carried out in accordance with GOST 26073-84 (ST SEV 3459-81) with subsequent sowing of the obtained sediment on Lowenstein -Jensen and Finn-2 egg nutrient media with mycobactin. Mycobactin is a growth factor, which is an extract from *Mycobacterium phlei*, containing substances necessary for nutrition and reproduction of *M. paratuberculosis* on artificial nutrient media. The use of two nutrient media simultaneously increases the percentage of mycobacterial isolation from pathologic material[4].

The isolated cultures were studied bacterioscopically by Ziehl – Neelsen staining of smears and evaluated for culture and morphological properties. Biochemical tests were performed: growth of cultures colonies at 30, 37 and 42 °C, on the medium with sodium salicylate and 5% sodium chloride, nitrate reduction, determination of amidase, catalase and arylsulfatase activity, hydrolysis of tween-80. Nitrate reductase activity was determined according to the method of Tsukamura, catalase activity was determined according to the method of G. Weyne (1962), hydrolysis of Tween-80 – according to the modified method of G. Weyne (1964). Weyne (1964), amidase activity of mycobacterium paratuberculosis was performed according to the Takae method[5].

Determination of sensitivity to antibacterial drugs was carried out by disk-diffusion method in accordance with the "Methodological guide-

[3]World Organization for Animal Health (OIE). Manual of diagnostic tests and vaccines for terrestrial animals; 7th ed. Vol. 2. Paris: OIE; 2012 (Version adopted in May 2014). Chapter 3.1.15, Paratuberculosis (Johne's disease), 2019, pp. 544–559.

[4]Guidelines for conducting preclinical studies of medicines. Moscow: Grif and K, 2012, part 1, 944 p.

[5]New methods of research of pathogens of anthropozoonoses. Tuberculosis: methodical recommendations. Moscow: RASKhN, 2003, 50 p.

lines for determining the sensitivity of microorganisms to antibacterial drugs" (2004), the studies were carried out in three repetitions. Inoculates of the pathogens under study were prepared according to the McFarland 0.5 turbidity standard (500 million microbial bodies in 1 ml of suspension), which were added 1.0 ml to the surface of a Petri dish with Lowenstein-Jensen egg nutrient medium with mycobactin, distributing evenly over the entire surface of the medium. The density of the suspension was measured on a densitometer – turbidity detector of suspensions DEN-1B. After inoculation, disks with 11 antibacterial drugs were applied to the surface of the nutrient medium: streptomycin, rifampicin, amikacin, kanamycin, ofloxacin, levofloxacin, clarithromycin, imipinem, moxifloxacin, ciprofloxacin, linezolid. The cultures were incubated at 37 ºC for 4 weeks. The cultures were reviewed every day, noting the appearance of mycobacterial colonies and areas of growth retardation. The biological method was carried out by infecting nonlinear white mice with a suspension of tested cultures intravenously in 4 experimental and 4 control groups, each containing 5 mice with an average weight of 23 g. To infect them, 1.0 ml of suspension containing 1 mg of bacterial mass of pathogen cultures dissolved in 1 ml of physiological solution was used. The mice were infected once, intravenously into the tail vein. The duration of the experiment was 120 days, after which a planned euthanasia with autopsy and biomaterial collection (lungs, liver, spleen, small intestine mucosa) was performed. The organs were processed by the method of A.P. Alikaeva[6] with sowing of the obtained suspensions on egg nutrient media of Lowenstein-Jensen and Finn-2 with mycobactin. The appearance of the colony growth was counted every 2 days during the first week, then once a week thereafter.

Crop growth intensity was considered according to the 4-point system proposed by G.N. Pershin (Order 558 of the Ministry of Health of the USSR from 08.06.1978, Moscow, 1978).

RESULTS AND DISCUSSION

The standardized strain Central-Lubinsky was isolated from the small intestine of cattle with changes characteristic of paratuberculous enteritis: pronounced folding and thickening of the mucosa. Primary growth of the culture on Lowenstein-Jensen and Finn-2 egg media with mycobactin was observed on the 8th-10th day; the colonies were beige in color, separate from each other with a depression in the center, rough consistency (R form). No growth was observed at 30 °C, abundant growth was observed at 37 °C, and scanty growth was observed at 42 °C. Growth was observed on the nutrient medium containing sodium salicylate. There was no resistance to 5% NaCl. Hydrolysis of Tween-80 was positive (day 6). Amidase and arylsulfatase activities and nitrate reduction were negative, the catalase activity was negligible.

Clinical strains were isolated from regional lymph nodes of the small intestine of pigs with the presence of caseous foci, from the small intestine of cattle with pathological changes characteristic of paratuberculous enteritis in the form of pronounced folding and thickening of the mucosa, and from the internal organs of crows. Bacterial culture colonies were identified on the basis of cultural, morphological and biochemical properties according to "Bergey's Bacterial Identifier" (2000). In the passages on Lowenstein-Jensen and Finn-2 egg media with mycobactin, growth of pathogens was observed on the 5th-8th day. The colonies were light beige in color, separate from each other and merged, rough and mucous consistency (R and S form). There was no colony growth at 30 °C, intense growth at 37 °C, and scanty growth at 42 °C. The appearance of growth on the nutrient medium containing sodium salicylate was observed on the 10th-11th day. There was no resistance to 5% NaCl. Hydrolysis of Tween-80 was positive (day 5-6). Amidase, arylsulfatase activity and nitrate reduction were negative. Catalase activity was weakly expressed. Based on the diagnostic tests performed, the isolated strains were

[6]Agricultural animals and poultry. Methods of laboratory diagnosis of tuberculosis: GOST 26072-89 (ST SEV 3457-81). Moscow: Publishing House of Standards, 1989, 13 p.

classified as group 3 mycobacteria according to the Runyon classification.

Bacterioscopic study of cultures of clinical strains showed the presence of acid-fast granular bacilli in the smears stained according to Ziehl-Neelsen, located in clumps in the form of a "palisade", which is a characteristic feature of *M. avium* subsp *paratuberculosis*.

Reduction in the time of colony growth of the studied pathogens of paratuberculosis at repeated passages on dense egg nutrient media occurs due to their adaptation to favorable conditions of cultivation. These conditions lead to a change in the amount of mycolic acids on the surface of bacterial cells, causing a change in the thickness and permeability of their membrane, which in turn leads to a change in the hydrophobicity of the cell wall and to an increase in the supply of the nutrients into the cell, affecting the rate and intensity of growth of the cultures. After the third and subsequent passages on dense egg nutrient media, R-subcultures of paratuberculosis pathogens acquired the ability to grow on the Pavlovsky potato medium even without mycobactin growth factor [10].

The phenotypic study of the sensitivity of standardized *M. para-tuberculosis* (Central-Lubinsky) and three clinical strains to antibacterial drugs revealed that the studied pathogens have sensitivity to all antibacterial drugs used in the studies: streptomycin, rifampicin, amikacin, kanamycin, ofloxacin, levofloxacin, clarithromycin, imipenem, moxifloxacin, ciprofloxacin, and linezolid (see the table). The zone of growth retardation of bacterial culture of the standardized strain *M. paratuberculosis* (Central-Lubinsky) around the drug disks on dense nutrient medium Lowenstein-Jensen with mycobactin ranged from 9, 2 ± 0.6 to 30.2 ± 0.1 mm, clinical strain 2608 – from 12.2 ± 0.3 to 25.2 ± 0.2 mm, clinical strain 905 – from 10.1 ± 0.1 to 22.6 ± 0.6 mm and clinical strain 6107 – from 10.6 ± 0.6 to 30.2 ± 0.1 mm.

The conducted biological studies showed that the body weight of mice in the experimental groups was lower than in the control groups. Autopsy of mice infected with paratuberculous pathogens revealed enlargement of lungs, spleen and liver, mucous membrane of the small intestine was not changed, there were single purulent foci on the liver, spleen and mesentery, the liver had a marbled color. Growth intensity of the cultures from biomaterial (lungs, liver, spleen) was 2(++) - 3(+++) - 4(++++) points, from small intestine mucosa - 0(+/-) points. Bacterioscopic examination of smears of the colony cultures of the tested pathogens isolated from the internal organs of mice of the experimental groups, stained by Ziehl-Neelsen staining, revealed single acid-fast granular bacilli arranged in groups or in the form of a "palisade", which is characteristic of the causative agent of paratuberculosis.

CONCLUSION

Microbiological studies of the cultures of clinical strains of paratuberculous mycobacteria isolated in Western Siberia have provided scientific information about their culture-morphological, biochemical and biological properties, which coincide with similar indicators of the standardized strain of *M. avium* subsp. *paratuberculosis* (*MAP*), which allows us to attribute them to mycobacteria of paratuberculosis. The biological method of research on laboratory animals revealed the susceptibility of nonlinear white mice to the tested cultures and the possibility of reproducing experimental paratuberculosis infection on them. The study of sensitivity to antibacterial drugs of the studied standardized and clinical strains showed their susceptibility to all antibacterial drugs used in the study.

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Антибиотикочувствительность, выявленная у стандартизированного и клинических штаммов *M. avium* subsp. *paratuberculosis (MAP)*
Antibiotic sensitivity detected in standardized and clinical strains of *M. avium* subsp. *paratuberculosis* (*MAP*)

Antibacterial preparation	Microorganism			
	Standardized strain	Clinical strain 2608	Clinical strain 905	Clinical strain 6107
Streptomycin	15,1 ± 0,4*	19,4 ± 0,6*	19,4 ± 0,6*	12,6 ± 0,3*
Rifampicin	14,1 ± 0,3*	18,2 ± 0,5*	18,2 ± 0,5*	12,7 ± 0,7*
Amikacin	20,4 ± 0,5*	18,1 ± 0,7*	18,1 ± 0,7*	15,4 ± 0,6*
Clarithromycin	30,2 ± 0,1*	25,2 ± 0,2*	25,2 ± 0,2*	30,2 ± 0,1*
Levofloxacin	9,2 ± 0,3*	12,5 ± 0,3*	12,5 ± 0,3*	13,2 ± 0,8*
Ofloxacin	15,3 ± 0,3*	14,1 ± 0,2*	14,1 ± 0,2*	14,4 ± 0,4*
Kanamycin	12,1 ± 0,6*	18,3 ± 0,1*	18,3 ± 0,1*	12,8 ± 0,5*
Imipenem	12,2 ± 0,1*	15,3 ± 0,7*	15,3 ± 0,7*	14,3 ± 0,7*
Ciprofloxacin	9,2 ± 0,6*	24,1 ± 0,4*	24,1 ± 0,4*	14,1 ± 0,1*
Linezodide	18,4 ± 0,3*	12,2 ± 0,3*	12,2 ± 0,3*	20,4 ± 0,4*
Moxifloxacin	10,6 ± 0,1*	17,4 ± 0,5*	17,4 ± 0,5*	10,6 ± 0,6*

* Statistically significant difference $p < 0,05$.

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ИССЛЕДОВАНИЕ ВЛИЯНИЯ ТЕХНОЛОГИЧЕСКИХ ФАКТОРОВ НА ПРОПУСКНУЮ СПОСОБНОСТЬ ДОЗАТОРА ПНЕВМАТИЧЕСКОЙ ПОСЕВНОЙ МАШИНЫ

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Одним из значимых направлений научных исследований в части совершенствования конструкции посевных машин и повышения их приспособленности к современным требованиям автоматизации является создание дозаторов, обеспечивающих корректировку нормы высева во время работы посевной машины. Специалистами Сибирского научно-исследовательского института механизации и электрификации сельского хозяйства разработан образец дозатора пневматической посевной машины, который использовался в качестве прототипа при проведении исследования. Применявшийся дозатор позволяет обеспечить плавную корректировку нормы высева семян в соответствии с картой-заданием, а также автоматическое отключение подачи семян в семяпровод при разворотах агрегата на краю поля для предотвращения перерасхода семян и повторного сева. Цель исследования – определение конструктивных и технологических параметров экспериментального дозатора пневматической посевной машины. Для достижения поставленной цели были сформулированы следующие задачи: 1) провести лабораторное исследование процесса дозирования семян в экспериментальном дозаторе пневматической посевной машины; 2) определить закономерности взаимного влияния значимых факторов на пропускную способность дозатора и описать их регрессионными уравнениями. В соответствии с перечисленными задачами изучен процесс дозирования семян на лабораторной установке. В ходе обработки экспериментальных данных получены регрессионные зависимости. По характеристикам полученных уравнений установлено, что наиболее значимое влияние на пропускную способность дозатора оказывает объем катушки дозатора. Максимальная производительность дозатора отмечается при частоте вращения ротора в пределах 80–100 об./мин. Наибольшая скорость подачи и исключение самопроизвольной утечки семян из дозатора в семяпровод достигаются при высоте затвора выгрузного окна дозатора в пределах 2 см. Результаты исследования могут быть использованы при доработке конструкции дозатора с целью применения его в качестве исполнительного механизма системы комплексной автоматизации посевного агрегата.

Ключевые слова: дозатор, семена, ротор, семяпровод, затвор, скорость подачи

INVESTIGATION OF THE INFLUENCE OF TECHNOLOGICAL FACTORS ON THE THROUGHPUT CAPACITY OF THE DISPENSER OF A PNEUMATIC SOWING MACHINE

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One of the significant directions of scientific research in terms of improving the design of sowing machines and increasing their adaptability to modern requirements of automation is the creation of dispensers that provide adjustment of the seeding rate during the operation of the sowing machine.

Specialists of the Siberian Research Institute of Mechanization and Electrification of Agriculture have developed a sample of a pneumatic sowing machine dispenser, which was used as a prototype in the study. The dispenser used allows for a smooth adjustment of the seed rate in accordance with the map task, as well as automatic shut-off of the seed supply to the seed line when the machine turns at the headland to prevent overconsumption of seeds and reseeding. The purpose of the research is to determine the design and technological parameters of the experimental dispenser of the pneumatic sowing machine. To achieve the set goal the following tasks were formulated: 1) to conduct a laboratory study of the process of seed metering in the experimental dispenser of the pneumatic seeding machine; 2) to determine the regularities of mutual influence of significant factors on the throughput capacity of the dispenser and describe them by regression equations. In accordance with the listed tasks the process of seed dosing on the laboratory unit was studied. In the course of experimental data processing, regression dependencies were obtained. According to the characteristics of the obtained equations, it is found that the most significant influence on the throughput capacity of the dispenser is the volume of the dispenser coil. Maximum performance of the dispenser is noted at rotor speeds in the range of 80–100 rpm. The highest feed rate and avoidance of spontaneous seed leakage from the dispenser into the seed pipe are achieved at a gate height of the dispenser discharge window within 2 cm. The results of the research can be used to finalize the design of the dispenser in order to use it as an actuator of the system of complex automation of the sowing unit.

Keywords: dispenser, seeds, rotor, seed deferens, gate, feed rate

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INTRODUCTION

Due to the increasing demand of agricultural production for new advanced seeding units equipped with precision farming system, the need to create actuators that will allow to ensure the adjustment of the working bodies of the machine during its operation is actualized [1-4]. The method of differentiated seeding taking into account the site peculiarities, when variable seeding rate is applied to each field plot separately, allows optimizing the seeding density in order to obtain the best agronomic and economic results [5–7].

One of the important directions of scientific research is the creation of reliable metering devices that provide controlled seed supply to the pneumatic system of the seeding machine[1] [8, 9]. The Siberian Research Institute of Mechanization and Electrification of Agriculture developed an experimental sample of a pneumatic sowing machine dispenser[2]. The dispenser allows to adjust the seeding rate in accordance with the map task and to carry out automatic shutdown of seed supply to the seed pipe at the headland turns of the machine to prevent overconsumption of seeds and exclude reseeding. The purpose and objectives of the study were determined to justify the parameters of the prototype.

The purpose of the research is to determine the design and technological parameters of the experimental metering unit of the pneumatic sowing machine.

[1]*Lepeshkin N.D., Mizhurin V.V., Zayats D.V.* Analysis of metering devices of seeding machines and identification of ways to improve them for sowing hard-to-sow grass seeds // Mechanization and electrification of agriculture: interdepartmental thematic collection, Minsk, 2018, pp. 114-119.

[2]Patent 2737206 C 1, Russian Federation. Seeding machine for precision agriculture. Application No. 2019120296 of 27.06.2019; published 26.11.2020.

Objectives of the study:

1) laboratory research of the seed metering process in the experimental sowing machine dispenser of a pneumatic sowing machine;

2) determination of the regularity of mutual influence of significant factors on the throughput capacity of the dispenser and their description by means of regression equations.

MATERIAL AND METHODS

The study was carried out [10-12] on an experimental laboratory installation (see Fig. 1). This unit is a frame on which the dispenser, receiving hopper, electric motor, actuator and unload receiver storage are installed. The dispenser is driven by an electric motor via a belt drive system.

The required speed of the dispenser was set using an inverter (*6*) ESO 1000 3PH AC 380V. The revolutions were monitored using a digital phototachometer "Megeon 18001". The weight of the loaded material was measured by electronic scales SWII-20. The time of passage through the dispenser of a given portion of grain was recorded with a stopwatch "CHRONOGRAPH".

The dispenser (see Fig. 2) consists of a housing, inside of which there is a rotor consisting of a drum with grooves for small seeds, a loading window flap connected by a leash to an actuator, and a drive. In the lower part of the dispenser there is a discharge window, inside of which a flap is built in (see Fig. 3). Changing the height of the gate affects the throughput capacity of the dispenser.

In the course of the study, two experiments were conducted. In the first experiment the influence of three significant variable factors on the throughput capacity of the dispenser (*Y*, kg/min) was determined:

Wheat grain was used as a working material. Experiments were carried out according to the planning matrix CFD 23 with threefold repetition (see Tables 1, 2).

Then the experimental data were processed, the first-order regression equation was calculated, statistically evaluated and analyzed.

Рис. 1. Лабораторная установка для проведения исследований с экспериментальным дозатором:
1 – дозатор; *2* – рама; *3* – ротор; *4* – электродвигатель; *5* – приводной ремень; *6* – частотный преобразователь; *7* – актуатор; *8* – приемный бункер; *9* – поводок; *10* – выгрузной приемник-накопитель

Fig. 1. Laboratory setup for conducting research with an experimental dispenser:
1 – dispenser; *2* – frame; *3* – rotor; *4* – electric motor; *5* – drive belt; *6* – frequency converter; *7* – actuator; *8* – receiving hopper; *9* – leash; *10* – unload receiver storage

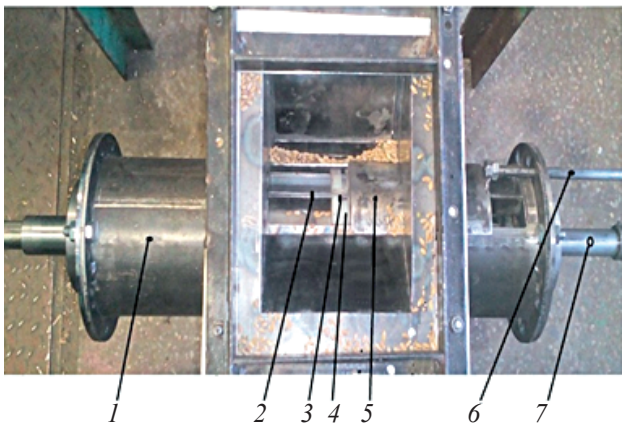

Рис. 2. Дозатор высевающего аппарата для сеялок точного земледелия:
1 – корпус; *2* – ротор; *3* – барабан ротора; *4* – желобки для высева мелкосемянных культур; *5* – заслонка загрузочного окна; *6* – поводок заслонки загрузочного окна; *7* – привод ротора

Fig. 2. Dispenser of the seeding apparatus for precision farming seeders:
1 – body; *2* – rotor; *3* – rotor drum; *4* – grooves for sowing small-seeded crops; *5* – loading window flap; *6* – loading window flap leash; *7* – rotor drive

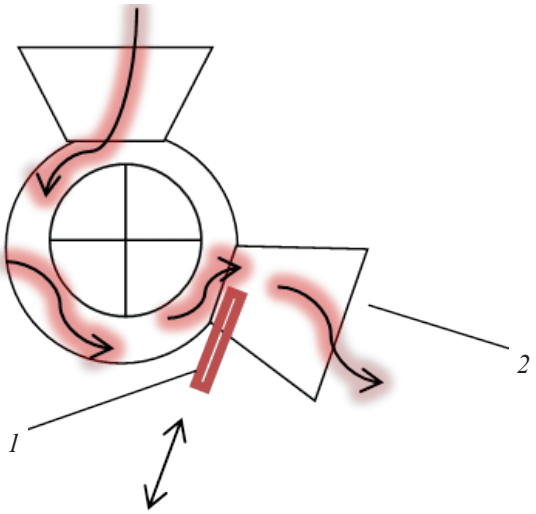

Рис. 3. Схема расположения затвора выгрузного окна дозатора:
1 – затвор выгрузного окна; *2* – выгрузное окно

Fig. 3. Layout of the flap of the discharge window of the dispenser:
1 – flap of the unloading window; *2* – unloading window

In the second experiment the dependence of the throughput capacity of the dispenser (*y*, kg/min) on the rotor speed and the height of the dispenser discharge window flap was additionally investigated. The volume of the dispenser coil was set constant - 0.375 dm^3. Wheat grain was used as a working material. The levels of variable factors were chosen as follows:

V – rotor speed, rpm

(V^- = 25, V^0 = 65, V^+ = 105);

H – flap height, cm

Табл. 1. Таблица кодирования факторов
Table 1. Factor coding table

Factor	Upper level (+)	Bottom level (–)	Center (0)	Variability interval	Dependence of the coded variable on the natural variable
V	105	25	65	40	$x_1 = \frac{V - 65}{40}$
U	0,600	0,150	0,375	0,225	$x_2 = \frac{U - 0{,}375}{0{,}225}$
H	5	1	3	2	$x_3 = \frac{H - 3}{2}$

Табл. 2. Исходная матрица планирования в кодированном виде с результатами опыта
Table 2. Initial planning matrix in encoded form with the results of the experiment

Experiment number	Factor under study			Experiment results		
	x_1	x_2	x_3	Y_1	Y_2	Y_3
1	+	+	–	11,892	11,890	12,075
2	–	+	–	4,095	4,114	4,154
3	+	–	–	2,785	2,849	2,783
4	–	–	–	1,101	1,075	1,073
5	+	+	+	26,182	25,413	25,411
6	–	+	+	7,855	7,928	7,926
7	+	–	+	6,554	6,359	6,357
8	–	–	+	2,431	2,413	2,409

(H^- = 1, H^0 = 3, H^+ = 5).

Experiments and data processing were performed using a second-order orthogonal plan matrix with one point at the center of the plan (see Tables 3 –5).

The experimental data were processed, the second-order regression equation was calculated, its statistical evaluation and analysis were performed.

RESULTS AND DISCUSSION

Based on the results of the first experiment, the regression equation was obtained in a coded form

$$Y = 7{,}80 + 3{,}91x_1 + 4{,}61x_2 - 2{,}81x_3 + 2{,}48x_1x_2 - 1{,}53x_1x_3 - 1{,}57x_2x_3 - 0{,}96x_1x_2x_3. \quad (1)$$

According to the data of the regression equation (1), a graph was constructed, which shows the influence of the studied factors on the throughput capacity of the dispenser (see Fig. 4).

According to the regression equation and the graph, it can be seen that the strongest influence on the throughput capacity of the dispenser (*Y*) is made by the volume of the dispenser coil (x_2),

Табл. 3. Таблица кодирования факторов
Table 3. Factor coding table

Factor	Upper level (+)	Bottom level (–)	Center (0)	Variability interval	Dependence of the coded variable on the natural variable
V	105	25	65	40	$x_1 = \frac{V-65}{40}$
H	5	1	3	2	$x_2 = \frac{H-3}{2}$

Табл. 4. Исходная матрица планирования в кодированном виде с результатами опыта
Table 4. Initial planning matrix in encoded form with the results of the experiment

Experiment number	Factor under study		Experiment results		
	x_1	x_2	y_1	y_2	y_3
1	+	+	7,982	8,207	8,131
2	–	+	2,736	2,710	2,693
3	+	–	16,111	15,263	15,000
4	–	–	5,058	5,029	5,000
5	+	0	14,262	15,000	15,273
6	–	0	4,915	4,888	4,943
7	0	+	6,041	5,649	5,541
8	0	–	10,610	11,154	11,013
9	0	0	10,235	10,741	10,610

Табл. 5. Матрица ортогонального плана второго порядка
Table 5. Second order orthogonal plan matrix

Plan content	Experiment number	x_0	x_1	x_2	$x_1 \times x_2$	$x_1' = x_1^2 - 0{,}666$	$x_2' = x_2^2 - 0{,}666$	$y_{ср}$
CCD kernel (CFD 2^K)	1	1	+	+	+	1/3	1/3	8,107
	2	1	–	+	–	1/3	1/3	2,713
	3	1	+	–	–	1/3	1/3	15,458
	4	1	–	–	+	1/3	1/3	5,029
Star points ($\alpha = 1$)	5	1	+	0	0	1/3	–2/3	14,845
	6	1	–	0	0	1/3	–2/3	4,915
	7	1	0	+	0	–2/3	1/3	5,744
	8	1	0	–	0	–2/3	1/3	10,926
CCD Center	9	1	0	0	0	–2/3	–2/3	10,529

as it has the largest absolute value of the coefficient. It is followed by rotor speed(x_1) and flap height (x_3). Signs of the coefficients show that with increasing rotor speed(x_1) and coil volume (x_2) the throughput capacity of the dispenser increases and decreases with increasing flap height (x_3).

Based on the data from the second experiment, a second-order regression equation in a coded form was compiled

$$y = 8{,}7 + 4{,}29x_1 - 2{,}48x_2 - 1{,}26x_1x_2 - 0{,}41x_1^2 - 1{,}58x_2^2. \quad (2)$$

The graphs of dependence of the throughput capacity of the dispenser on the rotor speed and height of the discharge window flap are shown in Figs. 5, 6.

According to the regression equation (2) and the graphs, it can be noted that the strongest influence on the throughput capacity of the dispenser (y) is exerted by the rotor rotation speed (x_1), the smallest – by the height of the discharge

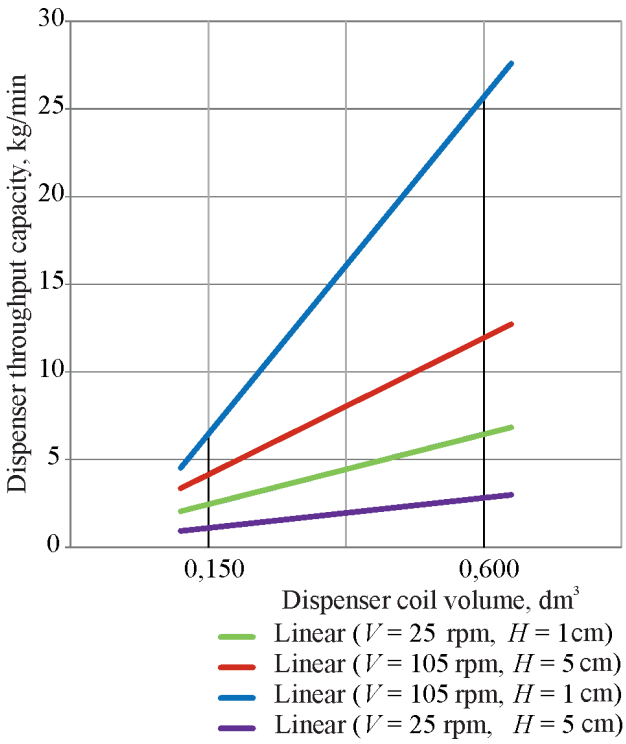

Рис. 4. Зависимость пропускной способности от объема катушки дозатора
Fig. 4. Throughput capacity dependence on the dispenser coil volume

window flap (x_2). Increasing the rotor speed(x_1) nonlinearly affects the increase in the throughput capacity of the dispenser, and vice versa, an increase of the discharge window flap height (x_2) leads to a nonlinear decrease in its throughput capacity.

The calculated data of the first and second experiments are in good agreement with each other.

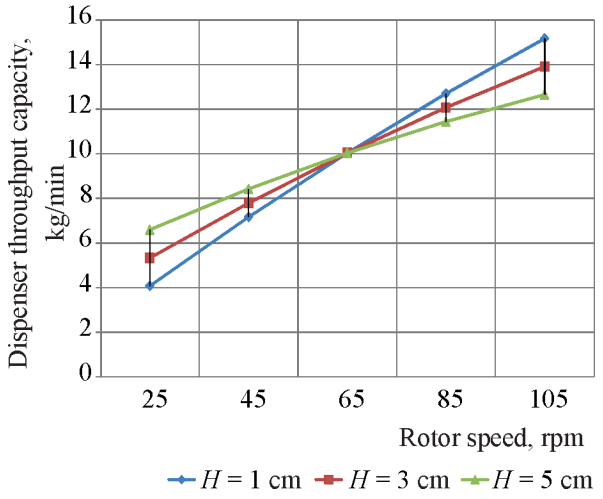

Рис. 5. Зависимость пропускной способности от скорости вращения ротора дозатора

Fig. 5. Throughput capacity dependence on the speed of rotation of the dosing rotor

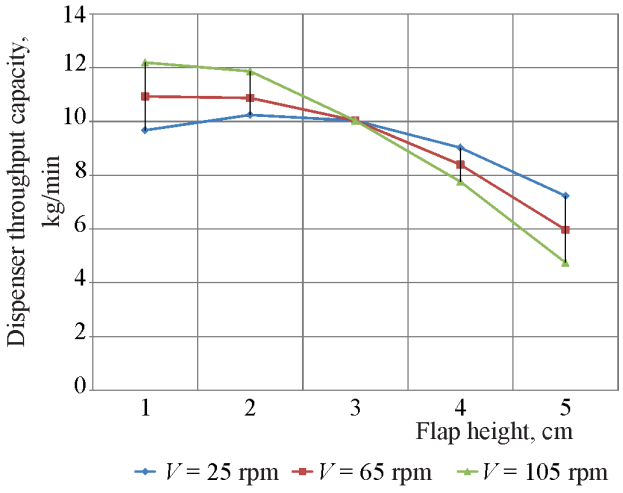

Рис. 6. Зависимость пропускной способности от высоты затвора дозатора

Fig. 6. Throughput capacity dependence on the height of the dispenser gate

CONCLUSIONS

1. As a result of the experimental data processing the regression equations describing the influence of technologically significant factors on the throughput capacity of the dispenser were drawn up.

2. According to the characteristics of the obtained regression equations, it can be seen that the most significant influence on the throughput capacity of the dispenser is the volume of the coil.

3. The maximum throughput capacity of the dispenser is noted at rotor speeds in the range of 80-100 rpm.

4. The highest feed rate and avoidance of spontaneous seed leakage from the dispenser into the seed pipe are achieved at the height of the dispenser discharge window flap within 2 cm.

5. The results of the research can be used to finalize the design of the dispenser in order to use it as an actuator of the system of complex automation of the sowing unit.

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БАЗАРБАЙ ОРАЗБАЕВИЧ ИНЕРБАЕВ

Ушел из жизни один из продолжателей нового научного направления Сибирского научно-исследовательского и проектно-технологического института животноводства – специализированного мясного скотоводства, доктор сельскохозяйственных наук, заслуженный изобретатель Российской Федерации Базарбай Оразбаевич Инербаев. Ему было всего 63 года.

Базарбай Оразбаевич – выпускник Алтайского сельскохозяйственного института и аспирантуры Сибирского научно-исследовательского института животноводства. Ученым много сделано: два заводских типа мясного скота в герефордской породе, один тип – в казахской белоголовой и один – в симментальской породе. По проектам руководимой им лаборатории построены фермы мясного скота в Томской, Новосибирской областях, в республиках Алтай и Хакасия созданы мясные племенные предприятия.

Последние научные работы Базарбая Оразбаевича посвящены совершенствованию технологий мясного скотоводства: двухфазная и трехфазная технологии для условий Сибири, обеспечивающие ритмичность производства мраморной говядины, в том числе для детского питания. Как специалист высокого класса Б.О. Инербаев был востребован во многих регионах России, в составе группы ученых из Австралии разрабатывал мясной кластер Мироторга в Брянской области. Благодаря его профессиональным и человеческим качествам вокруг него сформировался обширный круг друзей и единомышленников, которому будет не хватать этого скромного эрудированного человека.

Коллектив СибНИПТИЖа СФНЦА РАН выражает искренние соболезнования семье Базарбая Оразбаевича Инербаева, а также его родным и друзьям.

Уважаемые авторы!

Сообщаем, что на базе Федерального государственного бюджетного учреждения науки Сибирского федерального научного центра агробиотехнологий Российской академии наук создан совет по защите диссертаций на соискание ученой степени кандидата наук, на соискание ученой степени доктора наук 24.1.211.02 по научной специальности 4.2.3. Инфекционные болезни и иммунология животных (биологические науки, ветеринарные науки).

Приказ Министерства науки и высшего образования Российской Федерации «О выдаче разрешения на создание совета по защите диссертаций на соискание ученой степени кандидата наук, на соискание ученой степени доктора наук на базе Федерального государственного бюджетного учреждения науки Сибирского федерального научного центра агробиотехнологий Российской академии наук» № 1944/нк от 12.10.2023.

ЗЕМЛЕДЕЛИЕ И ХИМИЗАЦИЯ

РАСТЕНИЕВОДСТВО И СЕЛЕКЦИЯ

КОРМОПРОИЗВОДСТВО

ЗАЩИТА РАСТЕНИЙ

ЗООТЕХНИЯ И ВЕТЕРИНАРИЯ

МЕХАНИЗАЦИЯ, АВТОМАТИЗАЦИЯ, МОДЕЛИРОВАНИЕ И ИНФОРМАЦИОННОЕ ОБЕСПЕЧЕНИЕ

ПРОБЛЕМЫ. СУЖДЕНИЯ

ПЕРЕРАБОТКА СЕЛЬСКОХОЗЯЙСТВЕННОЙ ПРОДУКЦИИ

КРАТКИЕ СООБЩЕНИЯ

НАУЧНЫЕ СВЯЗИ

ИЗ ИСТОРИИ СЕЛЬСКОХОЗЯЙСТВЕННОЙ НАУКИ

ПАМЯТИ УЧЕНОГО

AUTHOR GUIDELINES

The guidelines are drawn up in accordance with the ethical principles, common for all the members of the scientific community, and the rules for publications in international and local scientific periodic magazines as well as in compliance with the requirements stipulated by the State Commission for Academic Degrees and Titles for the periodicals included in the List of Russian peer-reviewed scientific journals in which the major scientific outcomes of theses for the degrees of Doctor or Candidate of Sciences must be published.

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The article sent to the editorial board must correspond to the thematic sections of the journal "Siberian Herald of Agricultural Science":

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Plant growing and breeding	4.1.1. General agriculture and crop production 4.1.2. Plant breeding, seed production and biotechnology
Plant protection	4.1.3. Agrochemistry, soil science, plant protection and quarantine
Fodder production	4.1.1. General agriculture and crop production 4.1.2. Plant breeding, seed production and biotechnology 4.1.3. Agrochemistry, soil science, plant protection and quarantine
Zootechnics and veterinary medicine	4.2.3. Infectious diseases and animal immunology 4.2.4. Private zootechnics, feeding, technology of feed preparation and production of livestock products 4.2.5. Breeding, selection, genetics, and animal biotechnology
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The text of the manuscript is printed in Times New Roman font, type size 14 with 1.5 spacing, all margins 2.0 cm, page numbering at the bottom. The size of a manuscript should not exceed 15 pages (including tables, illustrations and bibliography); the articles placed in the sections "From dissertations" and " Brief reports" should not exceed 7 pages.

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Transliteration of the source = English name of the source

Monograph

Klimova E.V. *Field crops of Zabaikalya*. Chita, Poisk Publ., 2001, 392 p. (In Russian).

Part of a book

Kholmov V.G. Minimum tillage of coulisse-strip fallow for spring wheat with intensification of arable agriculture in southern forest-steppe of Western Siberia. *Resource-saving tillage systems*, Moscow, Agropromizdat Publ., 1990, pp. 230–235. (In Russian).

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