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СИБИРСКИЙ ВЕСТНИК СЕЛЬСКОХОЗЯЙСТВЕННОЙ НАУКИ

SIBIRSKII VESTNIK SEL'SKOKHOZYAISTVENNOI NAUKI

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ВЛИЯНИЕ ПОСЛЕДЕЙСТВИЯ МИНЕРАЛЬНЫХ УДОБРЕНИЙ ПРИ ВОЗДЕЛЫВАНИИ ЯЧМЕНЯ ПОСЛЕ ПОДСОЛНЕЧНИКА

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Представлены результаты исследований за 2021, 2022 гг. по влиянию последействия различных доз минеральных удобрений, вносимых под подсолнечник, на продуктивность и качество ярового ячменя сорта Камашевский. Полевые опыты заложены в условиях Ульяновской области на черноземе выщелоченном тяжелосуглинистом. Изучали четыре фона минеральных удобрений: N_0, N_{30}, $N_{30}P_{30}K_{30}$, $N_{60}P_{30}K_{30}$ кг д.в./га. Метеоусловия были контрастными в годы исследований, что позволило более полно оценить эффективность последействия удобрений. Технология возделывания ячменя включала весеннюю разделку растительных остатков подсолнечника дискатором и модульной бороной, посев зерновой сеялкой и прикатывание. Ячмень проявил наибольшую отзывчивость на последействие минеральных удобрений в дозе $N_{60}P_{30}K_{30}$ кг д.в./га. Урожайность зерна на данном варианте составила 2,11 т/га, что на 1,05 т/га выше по сравнению с неудобренным вариантом. На данном агрофоне получено более крупное зерно (масса 100 зерен составила 48,4 г, на контроле – 44,4 г) с высоким содержанием белка 12,7% (на контроле – 11,5%). Проведение корреляционно-регрессионного анализа позволило выявить прямую положительную взаимосвязь между накоплением сухого вещества и продуктивностью ячменя (R^2 = 0,96). Установлено, что с увеличением дозы азотных удобрений на каждые 10 кг д.в./га наблюдается повышение содержания сырого белка в зерне на 0,2%. Содержание белка в зерне зависело от условий влагообеспеченности года. Возделывание ячменя после подсолнечника агрономически целесообразно на фоне последействия минеральных удобрений. При отсутствии удобрений продуктивность ячменя после подсолнечника резко снижается. Кроме того, необходимы тщательный контроль за засоренностью посевов ячменя (в том числе в связи с появлением большого количества падалицы подсолнечника) и своевременное проведение химической прополки.

Ключевые слова: ячмень яровой, минеральные удобрения, последействие, продуктивность, сырой белок

INFLUENCE OF MINERAL FERTILIZERS AFTER-EFFECT WHEN CULTIVATING BARLEY AFTER SUNFLOWER

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The results of research for 2021, 2022 on the impact of the after-effect of different doses of mineral fertilizers applied to sunflower on productivity and quality of spring barley of the Kamashevsky variety are presented. Field experiments were laid in the conditions of the Ulyanovsk region on chernozem leached heavy loamy soil. Four backgrounds of mineral fertilizers were studied: N_0, N_{30}, $N_{30}P_{30}K_{30}$,

$N_{60}P_{30}K_{30}$ kg a.i./ha Meteorological conditions were contrasting in the years of research, which allowed a more complete assessment of the effectiveness of fertilizer after-effect. Barley cultivation technology included spring cutting of sunflower crop residues with a discator and a modular harrow, sowing with a grain drill and rolling. Barley showed the greatest responsiveness to the after-effect of mineral fertilizers at a dose of $N_{60}P_{30}K_{30}$ kg a.i./ha. Grain yield in this variant was 2.11 t/ha, which is 1.05 t/ha higher compared to the unfertilized variant. On this agricultural background, a larger grain was obtained (the weight of 100 grains was 48.4 g, on the control - 44.4 g) with a high protein content of 12.7% (on the control – 11.5%). Correlation and regression analysis revealed a direct positive relationship between dry matter accumulation and barley productivity (R^2 = 0,96). It was found that with an increase in the dose of nitrogen fertilizers for every 10 kg a.i./ha, an increase in the crude protein content of grain by 0.2% was observed. Protein content in grain depended on the moisture conditions of the year. Cultivation of barley after sunflower is agronomically expedient on the background of mineral fertilizers aftereffect. In the absence of fertilizers, the productivity of barley after sunflower sharply decreases. In addition, it is necessary to carefully control the weediness of barley crops (including the emergence of large amounts of sunflower fallen seed) and timely chemical weeding.

Keywords: spring barley, mineral fertilizers, after-effect, productivity, crude protein

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INTRODUCTION

Spring barley is the most important fodder crop occupying annually about 10% of the sown area in the Ulyanovsk region (95-104 thousand hectares) with a yield of 15-20 c/ha. It is important to cultivate it according to adaptive technology, taking into account the responsiveness of the crop to various agronomic practices – forecrops, doses and types of applied mineral fertilizers, crop protection system, etc. [1–4].

The role of a forecrop in crop cultivation technology is difficult to overestimate [5-9]. Studies [6] on leached chernozem showed that spring wheat and oats are the worst forecrops for barley due to the increase in weediness of crops, plant infestation by diseases and, as a consequence, a decrease in crop yields. Placement of barley on spring wheat and oats reduced the crude protein content by 0.8-0.9%.

Widespread sunflower crops and late dates of its harvesting do not always allow to till the soil in the autumn, which requires the study of the effectiveness of sunflower as a forecrop for grain crops from an agronomic point of view. Often agricultural producers practice cultivation of barley after sunflower. As experience shows, barley gives the same yield in direct sowing after sunflower with a stubble seeder as in traditional cultivation technology[1].

It is known that barley shows increased requirements to the level of mineral nutrition, because of this it is responsive to the direct action of mineral fertilizers and, first of all, starting doses [10-13]. Feeding during the growing season is ineffective due to the short growing season of the crop.

Applying mineral fertilizers to the preceding crop allows providing barley with accessible elements of mineral nutrition in the early

[1]Pat. No. 2714706 C1 Russian Federation, MPK A01C 7/00, No. 2019124821. Method of spring barley cultivation by direct sowing / A.L. Toigildin, D.E. Ayupov, A.S. Galkin; applicant – Ulyanovsk State Agrarian University named after P.A. Stolypin; applied 02.08.2019; published 19.02.2020.

development period [14–16]. However, in this case, it is important to assess the productivity of the crop depending on the background of mineral fertilizers of the preceding crop. With the high cost of mineral fertilizers, this issue becomes particularly relevant.

The purpose of the research is to present a comprehensive assessment of the after-effect of mineral fertilizers when cultivating barley after sunflower.

MATERIAL AND METHODS

In 2020 and 2021, studies were conducted to develop elements of sunflower cultivation technology using various doses of mineral fertilizers, then assessing the after-effect of the studied factors on the productivity of spring barley. Sunflower harvesting was carried out late (October – December). The field was not tilled in autumn. Soil preparation for sowing was done in the spring period: the first treatment with a disk harrow BDM 3 × 4, the second with a modular harrow BM-4.5. The beginning of crop germination was noted on May 18–25. Care for the crops during vegetation included protection from weeds in the tillering phase with the Balet, EC (in a dose of 0.4 l/ha) preparation.

The field experiment studied four backgrounds of mineral fertilizers in after-effect: N_0, N_{30}, $N_{30}P_{30}K_{30}$, $N_{60}P_{30}K_{30}$. The experiment was repeated three times, with systematic placement of the plots. The accounting area of the plot was 15 × 22.4 = 336 m². Barley sowing in 2021 was carried out on May 17, in 2022 – on May 9 with a grain seeder SZ-3.6 across the sowing of the previous crop without fertilizers, at a depth of 5–6 cm with a seeding rate of 4.5 million germinating seeds/ha. The harvest was carried out with a selective combine Sampo-500 at the stage of full ripeness, with further translation of the data to 100% purity and 14% moisture. As mineral fertilizers, azophoska with a content of $N_{15}P_{15}K_{15}$ and ammonium nitrate with a nitrogen content of 34.4 kg a.i./ha were used. Fertilizers were applied before sowing sunflower in the previous year.

The object of the study was a promising, zoned in the Middle Volga region medium-ripe variety of spring barley (*Hordeum vulgare* L.) grain-forage direction Kamashevsky. The variety is a steppe morpho-biotype, moderately resistant to fungal diseases, resistant to loose smut. It is prone to lodging with the application of high rates of nitrogen fertilizers and an excessive seeding rate. The protein content in the grain reaches 14%. The variety is valuable for quality[2].

Before sowing, the reserves of productive moisture were unsatisfactory (in the layer 0–10 cm – 4.7–6.5 mm, 0–30 cm – 17.9–21.5 mm). The low moisture reserve was also due to the fact that spring mechanical soil treatments were carried out for the disintegration of sunflower plant residues, which led to additional loss of moisture reserves.

From May to July, the sum of active temperatures amounted to 1947°, with the norm being 1600°. The intensely high temperature regime in June contributed to the accelerated pace of barley development. During the plant development period from the third ten-day period of May to the first ten-day period of August, 105.5 mm of precipitation fell, with the norm being 166 mm.

The vegetation period of 2022, on the contrary, was characterized by cool and rainy weather in May, moderate temperature regime and precipitation in June, intensive torrential rains in July, and hot, dry weather in August. The amount of precipitation in May was 65.7 mm with a norm of 39.0 mm (168% of the norm). The rains were significant, so early in the second ten-day period of May, a dangerous phenomenon was noted – soil overmoisture. In June, the weather was unstable: periods of warm, and on some days hot weather alternated with short periods of cooling. In July, the weather was predominantly very warm with rains of varying intensity.

[2]New super variety of barley Kamashevsky – what is its strength, the scientists are explaining. URL: https://www.agroxxi.ru/zhurnal-agromir-xxi/stati-rastenievodstvo/novyi-super-sort-jachmenja-kamashevskii-v-chem-ego-sila-rasskazyvayut-uchenye.html (accessed on 05.07.2023).

The maximum air temperature on the warmest days rose to 30–32 °C. The showers were local, accumulating 140 mm over the month (the long-term average norm is 69 mm). The sum of active temperatures from May to July was 1544°, with the norm being 1600°. During the development period of the plants from May to July inclusive, 250 mm of precipitation fell. In 2021, the HTC was 0.5, in 2022 – 1.6, with the norm being 1.0.

All registrations, observations, and analyses were conducted according to generally accepted methods and corresponding GOST standards. Mathematical processing of experimental data was carried out using methods of dispersion and correlation analysis.

RESULTS AND DISCUSSION

In all years of research, sunflower harvesting took place late (October – December) due to the prolonged ripening of the crop caused by prolonged precipitation in the autumn period. Consequently, the chopping of sunflower stubble was carried out in the spring period. It should be noted that shallow soil tillage (8–10 cm) caused the growth of sunflower volunteers, as well as weeds. Conducting chemical weeding with a tank mixture against perennial and annual weeds became a mandatory and effective agricultural practice.

The research results revealed a high responsiveness of the spring barley variety Kamashevsky to the after-effect of mineral fertilizers applied under sunflower in the preceding year, the effectiveness of which primarily depended on the moisture conditions of the year.

Depending on the background of mineral fertilizers, barley sowing significantly differed in the rates of biomass accumulation and in the content of nutrients in the plants (see Fig. 1, Table 1). The highest accumulation of dry matter was noted in the $N_{60}P_{30}K_{30}$ variant (56 c/ha), which was 1.9 times higher than the control (30 c/ha).

Due to insufficient moisture, the plants were in a suppressed state, with accelerated passage of interphase periods observed. For instance, the total nitrogen content in barley plants during the shooting stage varied from 2.32 to 2.66% and was assessed as low. By the end of vegetation, in terms of total nitrogen content in the vegetative mass of barley, the advantage was with the experimental variants – 1.27–1.37% (on the control – 1.16%). No clear dependence on the after-effect of mineral fertilizers was found for the total phosphorus and potassium content in the green mass of plants.

The after-effect of mineral fertilizers manifested in the improvement of nitrogen

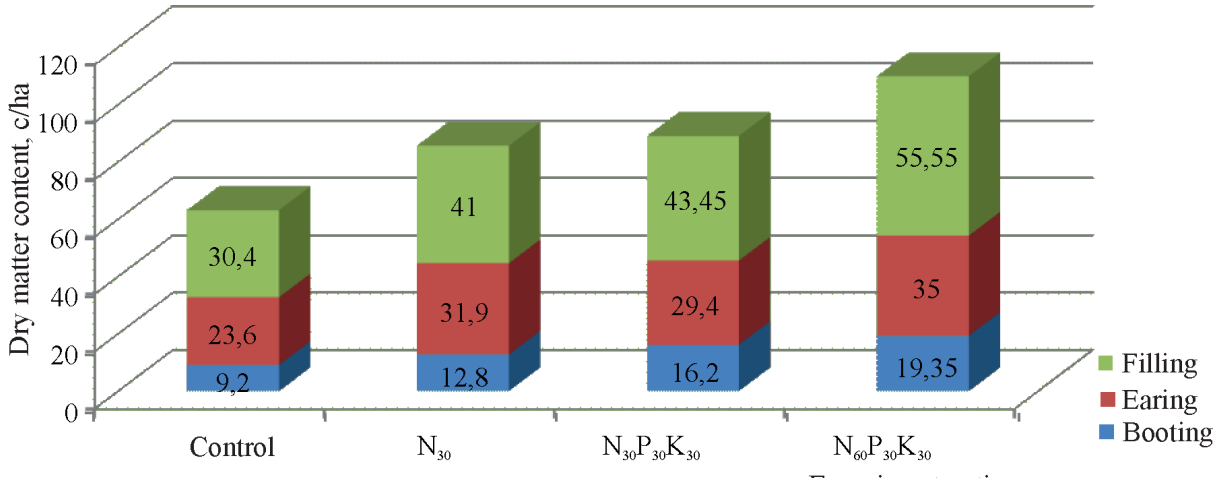

Рис. 1. Влияние последействия минеральных удобрений на накопление сухого вещества посевами ячменя

Fig. 1. Effect of mineral fertilizers on dry matter accumulation in barley crops

Табл. 1. Динамика элементов минерального питания в растениях ячменя по фазам развития
Table 1. Dynamics of mineral nutrition elements in barley plants by phases of development

Experiment option	Barley development phase								
	booting			earing			filling		
	N	P_2O_5	K_2O	N	P_2O_5	K_2O	N	P_2O_5	K_2O
N_0	2,32	1,48	5,59	1,47	1,06	2,58	1,16	1,11	1,68
N_{30}	2,66	1,24	5,42	1,37	0,95	2,66	1,37	0,89	1,50
$N_{30}P_{30}K_{30}$	2,42	1,37	4,56	1,57	1,02	2,42	1,33	0,98	1,63
$N_{60}P_{30}K_{30}$	2,43	1,2	4,59	1,30	0,93	2,63	1,27	0,96	1,47

nutrition in barley and, consequently, contributed to the formation of a larger above-ground mass compared to the unfertilized variant already at the initial stages of barley development (see Fig. 2).

Barley sowings on the variants with the application of mineral fertilizers had more intense coloration and stem density.

At the beginning of barley vegetation, a higher nitrate content was noted in the soil compared to the control (+2–5 mg/kg of soil to the control). The trend of increased nitrogen provision remained before the barley harvest.

Correlation-regression analysis showed a strong relationship between the accumulation of dry matter in plants (y) and the dose of nitrogen fertilizers (x) in after-effect. The linear dependence is described by equations of the type:

shooting $y = 0{,}17x + 9{,}31$ ($R^2 = 0{,}90$); (1)

earing $y = 0{,}19x + 24{,}27$ ($R^2 = 0{,}93$); (2)

filling $y = 0{,}42x + 30{,}0$ ($R^2 = 0{,}99$). (3)

Equation (3) shows that for every 10 kg a.i./ha of applied nitrogen in after-effect, there was an average increase in the dry matter accumulation in plants of 4.2 c/ha.

The productivity of barley significantly depended on the after-effect of mineral fertilizers applied under sunflower in the previous year, proportionally to the level of mineral nutrition (see Table 2).

Despite the fact that barley sowing in 2022 was carried out 8 days earlier than in 2021, the crop reached full ripeness 9 days later (August 5) due to increased precipitation.

In 2021, due to the late sowing date and dry conditions in June, low productivity of barley was formed. Nevertheless, a significant after-effect of mineral fertilizers was observed. The grain yield increase compared to the control was 0.24–0.33 t/ha (20.7–28.5%), with no significant differences between the fertilized variants. In 2022, a reliable increase in barley

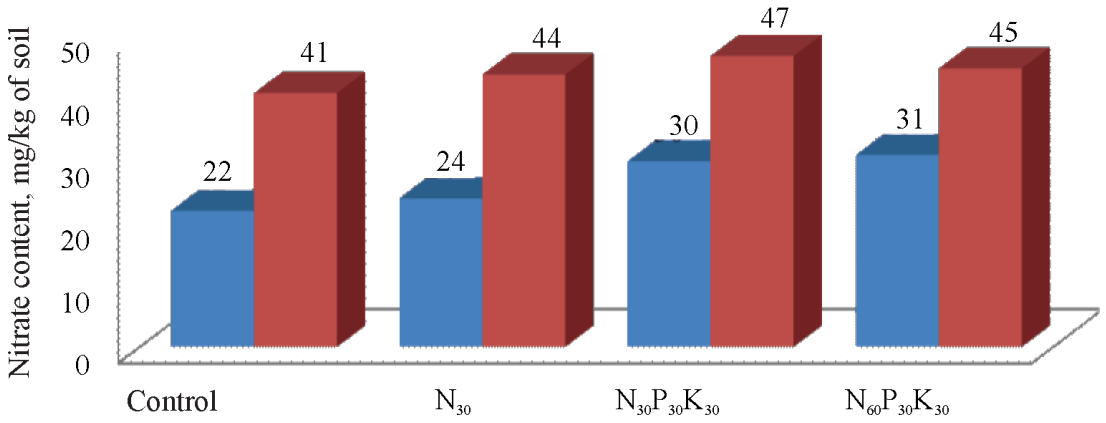

Рис. 2. Содержание нитратов в почве в зависимости от последействия минеральных удобрений, мг/кг почвы

Fig. 2. Nitrate content in soil depending on the after-effect of mineral fertilizers, mg/kg of soil

Табл. 2. Влияние последействия минеральных удобрений на продуктивность и качество ячменя (2021, 2022 гг.)

Table 2. Impact of the mineral fertilizers after-effect on productivity and quality of barley (2021, 2022)

Experiment option	Grain yield, t/ha				Weight of 1000 grains, g	Crude protein, %	Gross protein yield, kg/ha
	2021	2022	Average	± t/ha			
Control	1,16	0,95	1,06	–	44,4	11,5	122
N_{30}	1,4	1,31	1,36	+0,3	46,0	11,8	161
$N_{30}P_{30}K_{30}$	1,43	1,45	1,44	+0,38	45,9	11,9	172
$N_{60}P_{30}K_{30}$	1,49	2,72	2,11	+1,05	48,4	12,7	268
LSD_{05} 2021 p = 3,05%, 2022 p = 3,95%	0,14	0,19					

yield was also observed at all levels of mineral nutrition. The greatest responsiveness of barley was shown to the after-effect of $N_{60}P_{30}K_{30}$ kg a.i./ha (+1.77 t/ha).

On average, over the years of research, cultivating barley after sunflower, depending on the doses of mineral fertilizers in after-effect, allowed to obtain an additional 0.30–1.05 t of grain/ha compared to the unfertilized variant.

A direct positive correlation was established between the accumulation of dry biomass in plants and the productivity of barley, which is described by a linear equation of the type

$$y = 0{,}42x–2{,}97\ (R^2 = 0{,}96),$$

where y – barley yield; t/ha, x – dry biomass of plants, c/ha.

The application of mineral fertilizers contributed to the improvement of quantitative and qualitative indicators of the grain. For instance, the weight of 1000 grains on the control was 44.4 g, on the fertilized variants – 46.0–48.4 g. The largest grain was obtained in the variant with the after-effect of an increased dose of mineral fertilizers ($N_{60}P_{30}K_{30}$).

On average for 2021 and 2022, in terms of raw protein content in the grain, the advantage was also with the experimental variants. A direct positive correlation was found between the content of raw protein in barley grain and the dose of mineral nitrogen. The equation is as follows

$$y = 0{,}02x + 11{,}36\ (R^2 = 0{,}91),$$

where y – raw protein content in grain, %; x – dose of nitrogen fertilizers, kg a.i./ ha (the equation is valid for raw protein content of 11.5–12.7% and nitrogen doses of 0–60 kg a.i./ha). The equation shows that with an increase in the dose of nitrogen fertilizers by every 10 kg a.i./ ha, there is a 0.2% increase in the protein content.

Under the influence of the after-effect of mineral fertilizers, significant changes were observed in the structure of the barley yield (see Table 3).

The after-effect of fertilizers manifested in the formation of a greater number of productive stems in the experimental variants (25–98 pcs./m² higher than the control). An increase in the length of the ear by 0.4–2.4 cm, the grain weight per ear by 0.08–0.18 g, and the ear grain content by 1.4–3.3 pcs./plant were noted. An increase in plant height was directly proportional to the doses of mineral fertilizers applied under sunflower (44 cm in control, 48–59 cm in the experimental variants).

Correlation analysis of productivity elements revealed a direct positive relationship between barley yield and the number of productive stems (r = 0.99), length of the ear (r = 0.98), and the ear grain content (r = 0.97), as well as the weight of grain from one ear (r = 0.96) (see Table 4). The grain weight directly depended on its quantity from one ear (r = 1.0).

CONCLUSIONS

1. The studies revealed a high responsiveness of the spring barley variety Kamashchevsky when cultivated on leached chernozem to the after-effect of mineral fertilizers applied under sunflower.

Табл. 3. Структура урожая ячменя в зависимости от последействия минеральных удобрений
Table 3. Barley yield structure depending on the after-effect of mineral fertilizers

Experiment option	Barley yield structure indicator						
	Number of productive stems, pcs./m²	Tillering coefficient	Ear length, cm	Grain weight per ear, g	Number of grains, pcs./ plant	Straw weight, g/plant	Plant height, cm
Control	330	1,26	4,7	0,46	9,9	0,43	44
N_{30}	355	1,30	5,1	0,54	11,3	0,52	50
$N_{30}P_{30}K_{30}$	375	1,43	5,0	0,57	11,8	0,56	48
$N_{60}P_{30}K_{30}$	428	1,47	6,3	0,64	13,2	0,59	59

Табл. 4. Матрица коэффициентов корреляции между продуктивностью и элементами структуры урожая
Table 4. Matrix of correlation coefficients between productivity and elements of the yield structure

Indicatoe	Yield, t/ha	Number of productive stems, pcs./m²	Ear length, cm	Grain weight per plant, g	Number of grains per plant, pcs.	Straw weight from one plant, g	Plant height, cm
	1	2	3	4	5	6	7
1	1,0						
2	0,99*	1,0					
3	0,98*	0,95*	1,0				
4	0,96*	0,97*	0,89	1,0			
5	0,97*	0,98*	0,91	1,0**	1,0		
6	0,87	0,9	0,77	0,97*	0,96*	1,0	
7	0,98*	0,95	0,99*	0,92	0,94	0,82	1,0

* Significant at the level of p = 0,05.
** Significant at the level of p = 0,01.

2. Barley sowing on the background of fertilizers formed 11–26 c of dry matter/ha more than the control. Experimental plants throughout the vegetation period had a higher content of total nitrogen in the green mass.

3. The highest productivity of barley was found in the after-effect of $N_{60}P_{30}K_{30}$ kg a.i./ha, which provided an increase of 1.05 t/ha compared to the unfertilized variant. In the experimental variants, larger grains were obtained (weight of 1000 grains 46–48.4 g, in the control – 44.4 g) with a high protein content of up to 11.8–12.7% (in the control 11.5%).

4. The correlation-regression analysis revealed a direct positive relationship between the accumulation of dry matter and the productivity of barley ($R^2 = 0.96$). It was established that with an increase in the dose of nitrogen fertilizers by every 10 kg a.i./ha, there is an increase in the raw protein content in the grain by 0.2%.

5. Cultivating barley after sunflower is effective against the background of the after-effect of mineral fertilizers in the dose of N_{30}–$_{60}P_{30}K_{30}$ kg a.i./ha, which is reflected in the increase in productivity and quality of the grain.

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БИОЛОГИЯ И ЭКОЛОГИЯ *FORESTIERA NEO-MEXICANA* A. GRAY И ПЕРСПЕКТИВЫ ИСПОЛЬЗОВАНИЯ В НИЖНЕМ ПОВОЛЖЬЕ

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Изучены вопросы расширения ассортимента древесных и кустарниковых лесных насаждений в лесоразведении засушливых регионов. *Forestiera neo-mexicana* A. Gray является интродуцированным и маловстречаемым экзотическим тропическим растением в агролесомелиорации Волгоградской области. Место проведения исследования – коллекционные участки Волгоградского селекционно-семеноводческого комплекса в Кировском участковом лесничестве Волгограда, где произрастает *Forestiera neo-mexicana*. Проведена биоэкологическая оценка использования кустарника *Forestiera neo-mexicana* A. Gray в озеленении и защитном лесоразведении Нижнего Поволжья. С помощью методики фенологических наблюдений, разработанной сотрудниками Главного ботанического сада РАН (ГБС РАН), определены общая масса семян, морфометрические показатели плодов, динамика физиологического состояния кустарника. Биоэкологические свойства оценивали по пяти признакам: засухоустойчивость (шестибалльная шкала С.С. Пятницкого), зимостойкость (семибалльная шкала ГБС РАН), оценка интенсивности цветения и плодоношения методом В.Г. Каппера (пятибалльная шкала), жизненность (трехбалльная шкала). Согласно фенологическим наблюдениям, *Forestiera neo-mexicana* A. Gray в климатических условиях Волгоградской области проходит все фенологические фазы. При биоэкологическиой оценке свойств форестьеры по пяти признакам растение имеет высокий балл по интенсивности цветения и плодоношения (5 баллов), жизненности (5 баллов). Растение зимостойко (1 балл) и засухоустойчиво (1 балл). *Forestiera neo-mexicana* A. Gray может возделываться на участках, непригодных для общего землепользования, на пастбищных угодьях и использоваться в защитном лесоразведении.

Ключевые слова: *Forestiera neo-mexicana* A. Gray, интродукция, биоэкология, лесоразведение, ассортимент, перспективность

BIOLOGY AND ECOLOGY OF *FORESTIERA NEO-MEXICANA* A. GRAY AND PROSPECTS FOR USE IN THE LOWER VOLGA REGION

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The issues of expanding the assortment of tree and shrub forest plantations in afforestation of arid regions are studied. *Forestiera neo-mexicana* A. Gray is an introduced and under-recognized exotic tropical plant in the agroforestry of the Volgograd region. The study site was the collection plots of the Volgograd breeding and seed production complex in the Kirovsky district forestry of Volgograd where *Forestiera neo-mexicana* grows. Bioecological assessment of the use of *Forestiera neo-mexicana* A. Gray shrub in landscaping and protective afforestation of the Lower Volga region was carried

out. Using the methodology of phenological observations developed by the staff of the Main Botanical Garden of the Russian Academy of Sciences (MBG RAS), the total seed weight, morphometric indices of fruits and the dynamics of the physiological state of the shrub were determined. The bioecological properties were evaluated according to five traits: drought resistance (six-point scale of Pyatnitsky S.S.), winter hardiness (seven-point scale of the MBG RAS), evaluation of flowering and fruiting intensity by the method of V.G. Kapper (five-point scale), vitality (three-point scale). According to phenological observations, *Forestiera neo-mexicana* A. Gray passes all phenological phases in climatic conditions of the Volgograd region. In bioecological evaluation of the *Forrestiera* properties for five traits, the plant has a high score for flowering and fruiting intensity (5 points), vitality (5 points). The plant is winter hardy (1 point) and drought tolerant (1 point). *Forestiera neo-mexicana* A. Gray can be cultivated on the areas unsuitable for general land use, on pasture lands and can be used in protective forestry.

Keywords: *Forestiera neo-mexicana* A. Gray, introduction, bioecology, afforestation, assortment, prospects

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INTRODUCTION

Works on green construction and agroforestry reclamation in the Lower Volga region are inseparably linked with the use of a diverse assortment of woody plants [1–3]. Introducing new long-lived tree and shrub species into protective afforestation is a current task and requires a deep study of their biology, economic value, methods of reproduction, and implementation [4–6].

In the conditions of water resource scarcity in the dry steppe and semi-desert, the assortment of tree species for creating anti-erosion and pasture-protective strips can be expanded through the introduction of shrubs for greening and improving the ameliorative condition of urban landscapes [7–11].

Forestiera neo-mexicana A. Gray presents theoretical and practical interest regarding its resilience to the challenging forest-growing conditions of this region due to its morphophysiological features and economic-biological properties.

The genus *Forestiera Poir*. of the Oleaceae Lindl. family includes about 20 species, spread in the North and Central America. *Forestiera neo-mexicana* A. Gray is a multi-stemmed shrub reaching up to 3.5 m in height with an inverted-ovoid crown. The leaves are simple, elongated or inverted-ovoid, leathery, gray-green, up to 40 mm long and 8–18 mm wide. An interesting feature of forestiera is the orientation of its leaves relative to the sun: they turn following the sun so that the sun's rays glide along the surface

of the leaf laminas, preventing them from overheating[1, 2] [12].

The bioecology of *Forestiera neo-mexicana* A. Gray in the area of insufficient moisture is poorly studied. The shrub is quite rare in dendrological collections. In the North Caucasus, it is found only in Rostov-on-Don, in Sochi[3, 4], in the Lower Volga region – in the collections of the FSC Agroecology RAS, Volgograd[5]. It is occasionally cultivated as an ornamental shrub in household plots and gardens in the territory of the "Nizhnekhopyorsky" natural park [13].

The purpose of the study is to conduct a bioecological assessment of the shrub *Forestiera neo-mexicana* A. Gray to determine its potential for use in greening and improving the ameliorative condition of the urban landscapes in the Lower Volga region.

The research objectives are:

– to conduct a bioecological assessment of *Forestiera neo-mexicana* A. Gray based on five characteristics adopted in the study of the introduced species;

– to identify the dynamics of the physiological condition of the shrub during the vegetation period.

MATERIAL AND METHODS

The objects of the study were plants of *Forestiera neo-mexicana* A. Gray. Long-term introduction testing of this species was conducted in the collection plots of the Volgograd Selection and Seed Production Complex in the Kirov Forestry of Volgograd. The region is characterized by a sharply continental climate, distinguished by aridity, sharp fluctuations in air temperature (annual temperature amplitude – 32.0 °C, average annual – 7.6 °C), unstable moisture regime and its high variability (average annual precipitation – 350 mm). The summer period is characterized by low relative air humidity (down to 12–16%) with strong winds and dry winds (up to 55 days per year). The soils of the collection plots are light-chestnut medium-loamy with low humus content (0.54–0.94%). The combination of these factors hinders the introduction of many tree and shrub plants.

In 1998, 54 plants were planted, 53 survived, with a survival rate of 98.1%. The planting sites were spaced at 5 × 5 m. As of May 2023, over 78% have been preserved. The age of the plants is currently 24 years. The methodology of phenological observations of the Main Botanical Garden of the Russian Academy of Sciences (1975) was applied in the research. The average fruit weight was determined by weighing 100 fruits in triplicate on the VK-300 scales. Morphometric indicators of fruits – length and width – were determined with an accuracy of 0.01 mm (100 measurements for each type). According to GOST 13056.4–67 "Tree and shrub seeds. Methods for determining the weight of 1000 seeds" by weighing a sample of 250 seeds on the VK-300 electronic scales in two repetitions, the weight of 1000 seeds was determined.

The bioecological properties of the selected objects were visually assessed based on five characteristics traditionally used in the evaluation of the introduced species: drought resistance (S.S. Pyatnitsky six-point scale), winter hardiness (seven-point scale of the Main Botanical Garden of the Russian Academy of Sciences), assessment of the intensity of flowering and fruiting using the method of V.G. Kapper (five-point scale), vitality (three-point scale).

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The dynamics of the physiological state of the shrub during the vegetation period in terms of the pigment system of the green leaf (chlorophyll, flavonoids, anthocyanins) was determined using the DUALEX SCIENTIFIC device [14].

The obtained data from field and laboratory studies were statistically processed according to the methodology of G.N. Zaytsev and using the Microsoft Excel 2023 analysis package.

RESULTS AND DISCUSSION

The maximum temperature in 2023 was recorded in August (+38 °C), and the minimum in January (–20 °C). During the summer, air temperatures varied from +38 to +16 °C. In the winter months of 2023, the thermometer scale dropped to –20 °C and rose to +9 °C. The relative humidity in 2023 ranged from 38 to 87%. A drop to 38% was recorded in August, with the highest rate in January and February 2023 (87%). The total precipitation for the first half of 2023 amounted to 228 mm. The highest cumulative amount of precipitation fell in May (51.5 mm), with the least in June (27.5 mm). The average wind speed for the first half of the year was 4.9 m/s.

The data on the growth and condition of the plants testify to their high resistance to extreme environmental factors. The plants complete a full development cycle. The timing of the phenological phases is presented in Table 1.

The start of vegetation (mass swelling of buds) occurs in the first and second ten-day periods of April, early in spring, when there is no threat of early spring frosts. Leafing begins in the second ten-day period of April, simultaneously with shoot growth, which continues until the end of June. After the bush is covered with leaves, flowering is observed: male transparent-yellowish flowers bloom 2-3 days earlier than the female ones.

The flowering period varies from 15 to 20 days: it lengthens in cool weather and shortens in dry sunny conditions.

The plant is dioecious, with flower buds formed on shortened two-year shoots (see Fig. 1). Autumn leaf fall is recorded in mid-October. The introduced forestiera blooms and bears fruit abundantly, has good shoot-forming ability. Thus, the annual growth for 2023 averaged about 120 mm. Drought-resistant tree species never have large leaves and dense crowns.

Табл. 1. Данные сезонного развития *Forestiera neo-mexicana* A. Gray

Table 1. Data on the seasonal development of *Forestiera neo-mexicana* A. Gray

Phenological phase		Mean annual phenophase onset dates
Massive swelling of buds		05.04 ± 4,2
Bud bursting		11.04 ± 3,8
Full foliation		17.04 ± 4,0
End of shoot growth		25.06 ± 3,9
Full maturation of shoots		23.07 ± 4,1
Start of flowering	male	12.04 ± 3,6
	female	15.04 ± 4,0
End of flowering	male	26.04 ± 3,9
	female	30.04 ± 3,7
Mass ripening of fruits		27.07 ± 3,8
Massive foliage fall		11.10 ± 4,1
Duration of vegetation, days		189

Forestiera neo-mexicana is relatively consistent in diagnostic characteristics of the leaf structure and shape over most of its range in the southwestern United States, from California to New Mexico and in Western Texas (see footnote 2). In the conditions of the research region, the leaves are non-pubescent, round-oval to elongated, 10–40 mm in length and 5–18 mm in width (see Fig. 2).

Growth intensity is primarily related to soil and air humidity and the biological characteristics of the species. In 2023, the fruits of the studied shrub formed larger in size compared to the average multi-year data due to favorable conditions during the vegetation period. The mass of 1000 fruits was 55.6 g, which is 3.2 g higher than the average multi-year indicators (see Table 2).

Puc. 1. Генеративные органы
Fig. 1. Generative organs

Puc. 2. Листовые пластины
Fig. 2. Laminas

The ecological and biological study of the introduced *Forestiera neo-mexicana* A. Gray indicates that this multi-purpose subtropical culture has successfully acclimatized due to its resilience to biotic and abiotic factors. Overall, the shrub is quite winter-hardy (1 point) and drought-resistant (1 point), which suggests the possibility of its use in protective afforestation and greening of the region (see Table 3). Thus, the degree of adaptation of this shrub to climatic conditions is high. The seeds do not require special pre-treatment for growing seedlings in autumn and can be sown in the ground in late autumn or early spring. It also propagates well vegetatively by summer cuttings and hardwood cuttings.

One of the valuable economic qualities of *Forestiera neo-mexicana* A. Gray is the absence of root suckers (see footnote 5).

When selecting an assortment of economically valuable multi-purpose woody plants for creating agroforestry complexes and multifunctional greening plantations in the dry steppe zone, special attention is given to decorative characteristics (see Table 4).

Decorative features of *Forestiera neo-mexicana* A. Gray begin to appear during the period of

Табл. 2. Характеристика плодов
Table 2. Characteristics of the fruits

Weight of 1000 grains, g				Fruit size, mm			
fruits		seeds					
Average	2023	Average	2023	Average		2023	
				length	width	length	width
52,4 ± 0,06	55,6 ± 0,04	8,8 ± 0,03	8,9 ± 0,02	5,8 ± 0,02	3,7 ± 0,03	6,1 ± 0,01	4,2 ± 0,02

Табл. 3. Эколого-биологическая характеристика *F. neo-mexicana*
Table 3. Ecological and biological characteristics of *F. neo-mexicana*

Height, m	Winter hardiness	Drought resistance	Flowering	Fruiting	Viability
2,5–3,0	Does not frost over (1 point)	Does not react to drought (1 point)	Abundant - 100% of flowers or inflorescences bloomed on the plant (5 points)	Full, abundant - after abundant flowering, almost all 100% of the flowers set fruit that matured (5 points)	Good, the plant is well developed, has a healthy appearance, well-developed shoots, buds and leaves, normalizes their coloration, abundant or good flowering and fruiting (5 points)

mass flowering of male inflorescences of bright yellow color. The greatest decorative effect is observed as numerous bluish spindle-shaped fruits mature. At the end of the vegetation period, before leaf fall, forestiera plantings acquire bright yellow shades.

The preservation of plant vitality under insufficient water supply is closely related to the functioning of pigment systems (see Fig. 3).

Plants with high drought resistance lose less water and have a more stable chlorophyll – a protein-lipoid complex of plastids.

Seasonal changes in the content of chlorophylls, carotenoids, and anthocyanins confirm the above. Towards the end of the vegetation period, there is a decrease in the content of the sum of chlorophylls a + b and the level of the nitrogen balance index (NBI). An increase in Flav values by 10.9% and Anth by 57.7% indicates a specific protective response to a complex of unfavorable growing conditions.

CONCLUSION

The results of the study and analysis of the long-term introduction of the introduced species *Forestiera neo-mexicana* A. Gray allow determining a high degree of decorativeness, noting the plant's resistance to drought, high and low temperatures. The dynamics of the physiological state of the shrub during the vegetation period in terms of the pigment system of the green leaf indicates pronounced protective reactions

Табл. 4. Оценка декоративности *Forestiera neo-mexicana* A. Gray
Table 4. Assessment of decorativeness of *Forestiera neo-mexicana* A. Gray

Flowers	Fruits	Leaves		Stem and branches	Crown
		Shape	Coloring		
Gathered in short clusters 4-5, female – inconspicuous greenish with reduced stamens, male – distinguished by long stamens with bright yellow anthers	Small grayish-blue spindle-shaped	Plain, not pubescent, leathery, roundish-oval to elongated, gray-green	Green in the summer, yellow in the autumn	Multi-stemmed shrub with obovate crown	Sprawling, rounded shape, medium dense

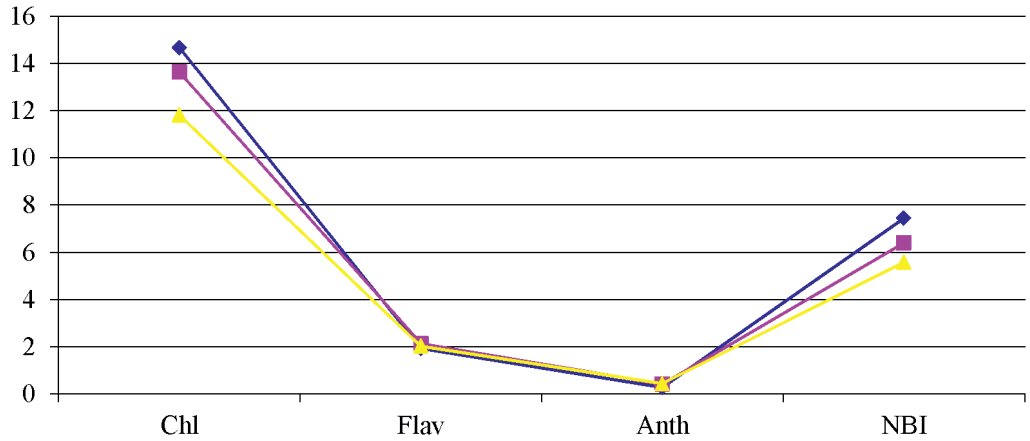

Рис. 3. Динамика физиологического состояния кустарника в течение вегетационного периода по показателям пигментной системы зеленого листа (Chl – хлорофилл, Flav – флавоноиды, Anth – антоцианы, NBI – индекс азотного баланса)

Fig. 3. Dynamics of the physiological state of the shrub during the growing season according to the indicators of the pigment system of the green leaf (Chl – chlorophyll, Flav – flavonoids, Anth – anthocyanins, NBI – nitrogen balance index)

of self-regulation due to features of water exchange – maintaining high absorptive capacity of root cells for water, economical use of moisture for transpiration, and rapid restoration of physiological functions under normalization of environmental conditions. All this has an adaptive value for increasing resistance to extreme environmental factors.

The analysis of the long-term introduction of the introduced species *Forestiera neo-mexicana* A. Gray allows recommending the shrub for use in green construction to strengthen the resilience of agroforestry landscapes with the aim of ecological optimization of protective afforestation in the Lower Volga region, as well as expanding the diversity of vegetation cover.

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ВЛИЯНИЕ СРОКОВ ПОСЕВА НА ФЕНОЛОГИЧЕСКОЕ РАЗВИТИЕ И УРОЖАЙНОСТЬ ЛЕКАРСТВЕННЫХ КУЛЬТУР В ЗАБАЙКАЛЬЕ

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Представлены результаты полевых и лабораторных исследований за 2020–2022 гг. по созданию агрофитоценозов ценных и перспективных лекарственных растений: расторопши пятнистой *(Silybum marianum)*, фенхеля обыкновенного *(Foeniculum vulgare*) и скорцонеры испанской (*Scorzonera hispanica* L.). Исследования выполнены на лугово-черноземной мучнисто-карбонатной почве (по гранулометрическому составу – легкий суглинок) на опытном поле при разных сроках посева в условиях лесостепной зоны Забайкалья. Работа посвящена изучению влияния сроков посева (II декада мая, II декада июня, II декада июля) на продолжительность межфазных периодов развития растений, линейный рост, облиственность, полевую всхожесть, сохранность растений и урожайность лекарственного сырья. Установлена возможность формирования различной урожайности лекарственных культур за счет различных сроков посева. Наибольшая урожайность лекарственного сырья сформирована в посевах 15 мая и 15 июня. У расторопши пятнистой урожайность зеленой массы составила 15,4–16,0 т/га, сухой массы – 2,46–2,56 т/га, семян – 1,69–1,71 т/га; фенхеля обыкновенного – зеленой массы – 43,0–43,2 т/га, сухой массы – 6,66–6,71 т/га; скорцонеры испанской – с сырой массой корнеплодов – 32,1 т/га, листьев – 10,7 т/га. Высота растений к моменту уборки расторопши пятнистой была 163–166 см, облиственность – 54–57%; фенхеля обыкновенного – 144–147 см и 50–54%; скорцонеры испанской – 39 см и 98% соответственно. Отмечено отсутствие пораженности лекарственных растений болезнями и вредителями. Все культуры устойчивы к полеганию и засухе (5 баллов) в условиях Забайкалья.

Ключевые слова: лекарственные растения, расторопша пятнистая, фенхель обыкновенный, скорцонера испанская, межфазные периоды, урожайность, адаптивность, развитие растений, сроки посева

INFLUENCE OF SOWING DATES ON THE PHENOLOGICAL DEVELOPMENT AND YIELD OF MEDICINAL CROPS IN TRANSBAIKALIA

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The results of field and laboratory studies for 2020, 2022 on the creation of agrophytocenosis of valuable and promising medicinal plants: milk thistle (*Silybum marianum*), common fennel (*Foeniculum vulgare*) and scorzonera (*Scorzonera hispanica* L.) are presented. The studies were carried out on meadow-chernozem mealy carbonate soil (light loam by granulometric composition) in the experimental field at different sowing dates in the conditions of the forest-steppe zone of Transbaikalia. The work is devoted to the study of the sowing dates influence (II ten-day period of May, II ten-day period of June, II ten-day period of July) on the duration of the interphase periods of plant development, linear growth, foliage, field germination, plant safety and yield of medicinal raw materials. The possibility of formation of different yields of medicinal crops due to different sowing dates has been established. The highest yield of medicinal raw material was formed in the crops of May 15 and June 15. Milk thistle had the yield of the herbage of 15.4–16.0 t/ha, dry mass – 2.46–2.56 t/ha, seeds – 1.69–1.71 t/ha; common fennel herbage – 43.0–43.2 t/ha, dry mass – 6.66–6.71 t/ha; scorzonera – with wet weight of root crops – 32.1 t/ha, leaves – 10.7 t/ha. Plant height by the time of harvesting of milk

thistle was 163–166 cm and 54–57%; common fennel – 144–147 cm and 50–54%; scorzonera – 39 cm and 98%, respectively. There was no infestation of medicinal plants by diseases and pests. All crops were resistant to lodging and drought (5 points) in the conditions of Transbaikalia.

Keywords: medicinal plants, milk thistle, common fennel, scorzonera, interphase periods, yield, adaptability, plant development, sowing dates

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INTRODUCTION

Medicinal plants hold significant importance in the national economy. Due to their widespread occurrence and valuable properties, medicinal plants have been used since ancient times. The arsenal of medicinal plant-based drugs has been created as a result of the long historical experience of peoples around the world and the research efforts of numerous scientists [1–5]. Compared to synthetic drugs, plant-based preparations possess a broader and gentler therapeutic action, lower toxicity, and minimal side effects. Eco-products based on medicinal plants are used not only in the production of medical and veterinary drugs but also in the food industry, cosmetology, perfumery, etc. The huge demand for medicinal raw materials in China is due to the fact that this method of treatment is "in the blood" of the Chinese people, as the country has existed for many millennia and traditional medicine has become part of Chinese philosophy. The book on medicinal plants "Ben Cao", dated 2600 BC, describes 900 plants, many of which are still widely used in many countries today. The global market for medicinal drugs and dietary supplements is worth hundreds of billions of dollars and its capitalization volumes are growing every year. In global medical practice, there is a steady trend towards the use of therapeutic and prophylactic drugs of plant origin. In Russia, the domestic pharmaceutical industry's need for medicinal raw materials is not fully met. Significant volumes of plant medicinal raw materials are imported, although many species were previously grown and harvested in our country, particularly in Transbaikalia. In this context, the revival and development of medicinal plant cultivation in the Russian Federation at the current stage and in the future is a relevant task. Cultivating medicinal crops has great national economic significance, as it not only addresses the pharmaceutical industry's supply issues but also has social importance related to employment and import substitution [4].

Among the promising sources of medicinal remedies are lady's thistle (*Silybum marianum*), common fennel (*Foeniculum vulgare*), and Spanish salsify (*Scorzonera hispanica* L.). The medicinal raw materials of these plant species have healing properties for many diseases. For instance, lady's thistle (*Silybum marianum*) is used for liver diseases, gallbladder issues, improving metabolism, and in cases of poisoning. Therapeutic properties are not only in the fruits but also in the leaves, stems, and roots of the plant. Lady's thistle fruits contain about 3% silymarin, which is also present in the stems, roots, and leaves[1, 2] [6, 7]. Spanish salsify (*Scorzonera hispanica* L.) is beneficial and medicinal. Its healing properties include calming the nervous

[1]*Kshnikatkina A.N., Alenin P.G., Kshnikatkin S.A., Voronova I.A.* Milk thistle: Issues of biology, cultivation, application. Penza: EPD PSAA, 2016, 325 p.

[2]*Alenin P.G., Kshnikatkin S.A., Voronova I.A.* Productive process of seed agrophytocenoses of milk thistle, burnet polygam in the conditions of the forest-steppe of the Middle Volga region // Volga Region Farmland, 2017, N 1 (42), pp. 2-9.

system, alleviating insomnia, normalizing blood sugar levels, regulating heart rhythm, stopping the development of osteoarthritis and gout, and being useful in oncological diseases, liver cirrhosis, and atherosclerosis. The medicinal raw materials of black salsify (roots, leaves) are a source of antioxidants[3] [8–12]. Common fennel (*Foeniculum vulgare*) helps eliminate toxins and harmful substances from the body. Preparations made using common fennel are widely used in medicine. Various forms are used: infusions, decoctions, powder, oil. Crushed dry leaves serve as an effective expectorant[4] [13].

The content of biologically active substances in plant organs significantly depends on the growing conditions, the duration of vegetation, and the mass and size of these organs. In the complex of technological practices for cultivating medicinal crops, the timing of sowing plays a significant role. Sowing dates influence the yield and quality of the product to the extent that they coincide with favorable environmental conditions (in terms of moisture and heat supply) for plant growth, development, and crop formation. Through different sowing dates, it is possible to reduce the plants' dependence on unfavorable environmental factors and form a good yield of medicinal raw materials.

In the conditions of the Transbaikalia region, the sowing dates for medicinal plants have not been previously studied. Therefore, research to determine the optimal sowing dates for medicinal crops - lady's thistle, common fennel, and Spanish salsify - is timely, relevant, and has scientific and practical value.

The purpose of the research is to determine the optimal sowing dates that allow for high yields of medicinal raw materials (lady's thistle, common fennel, Spanish salsify) in the forest-steppe zone of Transbaikalia.

MATERIAL AND METHODS

The research was conducted from 2020 to 2022 at the experimental field of the East Siberia Research Institute of Veterinary Science - a branch of the Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences, located in the Ingodinskaya-Chita forest-steppe. The study examined the influence of the sowing dates on the growth, development, and yield of medicinal plants such as lady's thistle (*Silybum marianum*), common fennel (*Foeniculum vulgare*), and Spanish salsify (*Scorzonera hispanica* L.).

The soil at the experimental site is meadow-chernozem sandy loam with carbonate content, granulometric composition – light loam. The soil solution of the arable horizon is slightly acidic, while the subsoil horizon is neutral. The organic matter content in the 0–20 cm soil layer is 3.67%, with total nitrogen at 0.21%. The availability of mobile phosphorus is low, and exchangeable potassium is medium. The sowing area of each plot is 20 m², with an accounting area of 10 m² and a four-fold repetition of the experiments. The layout is systematic.

Mineral fertilizers were applied before sowing at the rate of $N_{60}P_{60}K_{60}$. Sowing was carried out mechanically using a CH-16 seeder in a row method with 30 cm spacing between rows at three different times: in the second ten-day period of May, the second ten-day period of June, and the second ten-day period of July. The seeding rate for lady's thistle was 18 kg/ha, for common fennel 10 kg/ha, and for Spanish salsify 12 kg/ha, with a seed planting depth of 3–4 cm. For uniform sowing, the seeds were mixed with granulated superphosphate (at a ratio of 1:3). All registrations and observations were carried out in accordance with methodological guides[5–10].

[3]*Galyuk, N.G.* Processing of inulin-containing raw materials for inulin and its derivatives / N.G. Galyuk, N.D. Lukin, T.S. Puchkova, D.M. Pihalo // Achievements of science and technology of AIC, 2017, vol. 31, N 8, pp. 76-79.

[4]*Karomatov I.D., Muzaffarova S.K., Turaev P.T.* Therapeutic properties of fennel // Biology and Integrated medicine, 2017, N 9, pp. 23-43.

[5]Methodological instructions for conducting field experiments with forage crops. Moscow, 1983, 197 p.

[6]Experimental work in field farming. Leningrad, 1982, 190 p.

[7]*Dospekhov B.A.* Methodology of field experiment. Moscow, 1985, 357 p.

[8]Methodology of state variety testing of agricultural crops. Moscow, 1985, 267 p.

[9]Instruction for zonal agrochemical laboratories on fodder and plant analysis. Moscow, 1968, 56 p.

[10]GOST 34221-2017 Seeds of medicinal and aromatic crops. Sort and sowing qualities. Technical conditions. Moscow: STANDARDINFORM, 2017, 23 p.

The climate of the area is sharply continental with a cold, low-snow winter, a hot summer, and a lack of atmospheric precipitation. The average annual precipitation is 330–380 mm, with the majority (85–90%) falling in the warm period, the maximum in July-August, and the minimum in May-June. Overall, the regime is characterized by variability in moisture. Years with good moisture are followed by satisfactory and often dry ones. The sum of temperatures above 10 °C during the summer months is 1500…1800°, with a high average daily temperature in July of 19.1 °C.

The weather conditions during the vegetation periods of 2020–2022 were mainly rainy and warm. From April to September, precipitation totaled 320.2; 349.0 and 406.0 mm against the long-term average norm of 276.0 mm. Exceeding the long-term average was 44.2; 73.6; 130 mm, or 16.0; 26.6; 47.0%. The average daily air temperature for this period exceeded the norm by 0.7; 0.9 and 1.9 °C with a long-term average of 11.2 °C. Hydrothermal coefficients (HTC) for the months of the vegetation periods on average were: in May – 1.0, June – 1.2, July – 2.6, August – 1.1, September – 2.4 units. According to these coefficients, May, June, and August are characterized as sufficiently moistened, while July and September – as excessively moistened.

Overall, the weather conditions that developed during the vegetation period contributed to the timely emergence of seedlings, good plant development, and the formation of sufficiently high yields of the studied crops in agro-phytocenoses.

The studied medicinal crops were resistant to diseases and pests. No diseases or damage caused by pests and diseases were noted.

RESULTS AND DISCUSSION

The research established that the hydrothermal conditions and biological characteristics of the culture significantly influenced the development of the studied crops, the timing of phenological phases, and their duration.

For the spring sowing date of May 15th, the average daily air temperature during the sowing-emergence period was 8.8 °C, significantly affecting soil warming and the emergence of seedlings. Seedlings of medicinal plants in the May 15th sowings appeared on the 23rd–25th day after sowing. June sowings of medicinal herbs accelerated the emergence of seedlings by 8–9 days, and July sowings by 16–18 days (see Table 1). The period from emergence to budding (branching) for lady's thistle was 50 to 55 days, and for common fennel 52 to 62 days. The duration of the emergence-flowering period for May and June sowings for lady's thistle was 70–73 days, for common fennel 77-81 days; in June sowings, this period for lady's thistle was 3 days shorter, for common fennel 4 days. In July sowings, the plants did not reach the flowering phase. Seed ripening in lady's thistle was achieved only in May and June sowings, with a period of 91—95 days.

The interphase period from emergence to root formation in Spanish salsify in May sowings was 118 days, in June sowings 110 days. In July sowings, harvesting was carried out 55 days after emergence (at the stage of plant row closure – beginning of root crops formation).

In sowings of lady's thistle and common fennel, field germination ranged from 77–78 to 86–87%, and for Spanish salsify from 75 to 81% (see Fig. 1), increasing from the early sowing date (May 15th) to the later ones (June 15th by 3-4% and July 15th by 6–9%). Plant survival in medicinal herb crops was quite high, at 98-99%.

Weed infestation in spring sowings during emergence (with one pre-sowing cultivation) was high, at 97–114 plants/m²; June 15th (with two pre-sowing cultivations) at 73-80 plants/m²; July 15th (with three pre-sowing cultivations) at 32–37 plants/m². Before harvesting, the overall level of weed infestation in sowings was low, ranging (depending on sowing dates) from 15-16 to 2-8 plants/m², decreasing from early to later sowing dates.

The maximum height of medicinal plants at the time of harvesting was reached in sowings of May 15th and June 15th: for lady's thistle 163–166 cm, for common fennel 144–147 cm, for Spanish salsify 39 cm, which was higher than in the July 15th sowings by 97–100, 82-85, and 12–13 cm respectively for the crops; the

Табл. 1. Продолжительность межфазных периодов лекарственных растений, дни (среднее за 2020–2022 гг.)

Table 1. Duration of the interphase periods of medicinal plants, days (average for 2020–2022)

Culture	Periods							
	Sowing – sprouts	Sprouts – 4 – 6 pairs of true leaves	Sprouting – closing of crop	Sprouting – budding (branching)	Sprouting – flowering	Sprouting – seed ripening, beginning of fruit formation	Sprouting – leaf rosette, root formation	Sprouting – harvesting
Sowing May 15								
Lady's thistle	23	–	–	55	73	95	–	95
Common fennel	25	–	–	62	81	85	–	85
Spanish salsify	25	26	48	–	–	–	118	118
Sowing June 15								
Lady's thistle	14	–	–	51	70	91	–	91
Common fennel	17	–	–	52	77	81	–	81
Spanish salsify	17	22	44	–	–	–	110	110
Sowing July 15								
Lady's thistle	7	–	–	55	–	–	–	55
Common fennel	7	–	–	55	–	–	–	55
Spanish salsify	7	21	41	–	–	55	–	55

leaf coverage was 54-57%, 50–54%, and 98% respectively. In the late sowing date (July 15th), the height of the plants was the lowest, being 66, 62, and 27 cm for the respective crops, with high leaf coverage – 64, 61, and 98% (see Fig. 2).

In assessing the plants' response to drought, where the methodology is based on the yellowing of basal leaves and loss of turgor, it was noted that the studied crops did not suffer from drought (drought resistance – 5 points).

The assessment of medicinal crops showed that under the conditions of the vegetation periods, lady's thistle and common fennel in the May 15th and June 15th sowings formed maximum productivity: herbage – 15.40–16.00; 43.00–43.20 t/ha; dry matter – 2.46-2.56; 6.66–6.71 t/ha; seeds (for lady's thistle) – 1.69–1.71 t/ha, with good qualities – 1000 seed weight – 31–32 g, fat content in seeds – 23.6–25.2% (see Tables 2, 3).

In the July 15th sowings, the yield of medicinal raw materials was less: herbage by 12.5-13.1 t/ha (or 81.7-81.9%); dry matter by 2.00-2.10 t/ha (or 81.3-82.0%); seeds did not form in this sowing period for lady's thistle.

In agro-phytocenoses of Spanish salsify, root crops and leaves were accounted for (see Table 3). The highest total yield of medicinal raw materials (42.8 t/ha) of Spanish salsify was formed in the spring sowing (May 15th): including root crops – 32.1 t/ha, leaves – 10.7 t/ha, total biomass 42.8 t/ha, exceeding the later sowings by 9.5-29.0 t/ha (or 22.2-68.0%).

Important indicators of the yield structure of Spanish salsify root crops are their diameter and length. The largest (18.5 mm in diameter) and longest (28 cm) root crops were formed in the May 15th sowings (over 118 days of vegetation) (see Table 3).

In the May 15th sowings, the highest yield of root crops was 32. 1 t/ha, which was 22.4% higher than in the June 15th sowings, and 95% higher than in the late (July 15th) sowing, confirming the yield structure. A close positive correlation (r = 0.98) was noted between the yield and the diameter of the root crops, and the yield and the length of the root crops.

CONCLUSIONS

1. In the forest-steppe zone of Transbaikalia, the most favorable conditions for the growth, phenological development, and formation of medicinal raw material yields – for lady's thistle (*Silybum marianum*) and common fennel (*Foeniculum vulgare*) – are the second ten-day period of May (May 15th) and the second ten-day pe-

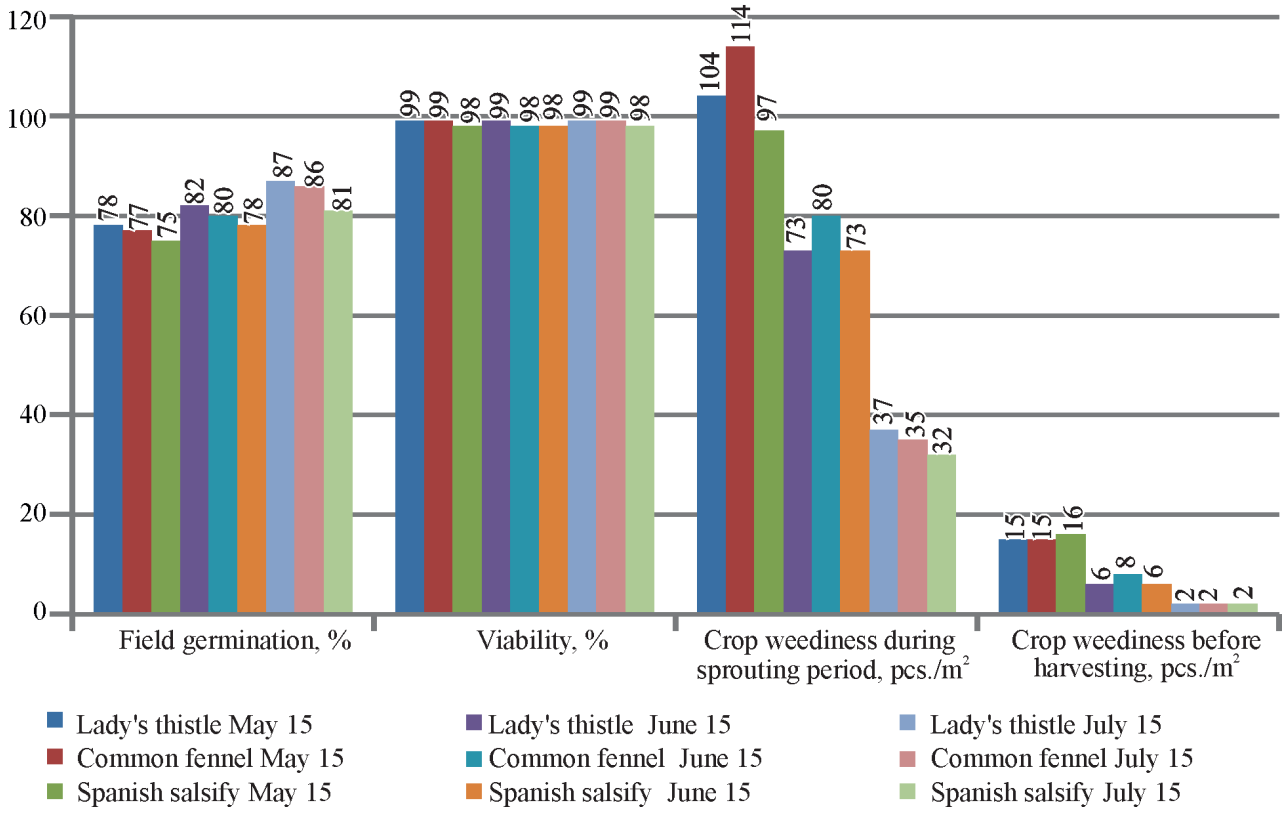

Рис. 1. Полевая всхожесть, сохранность и засоренность посевов лекарственных растений в разные сроки посева: 15 мая, 15 июня, 15 июля (среднее за 2020–2022 гг.)

Fig. 1. Field germination, safety and contamination of medicinal plant crops at different sowing dates: May 15, June 15, July 15 (average for 2020–2022)

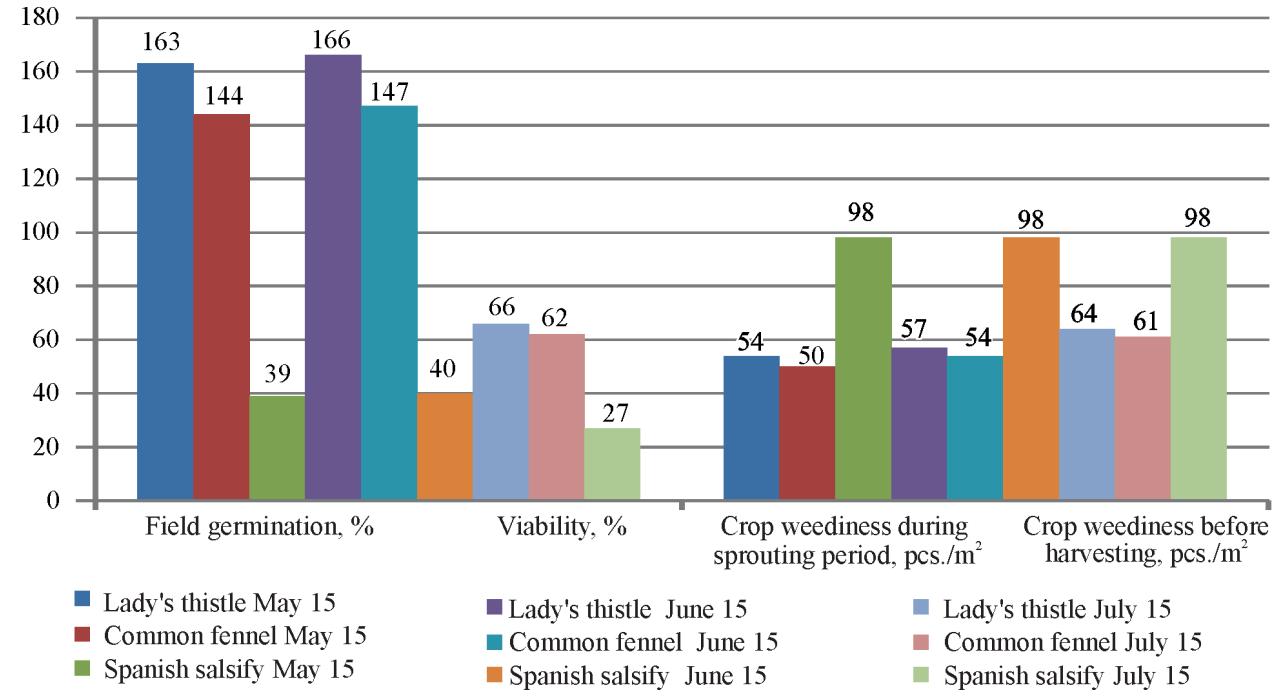

Рис. 2. Высота и облиственность лекарственных растений в разные сроки посева: 15 мая, 15 июня, 15 июля (среднее за 2020–2022 гг.)

Fig. 2. Height and foliage of medicinal plants at different sowing dates: May 15, June 15, July 15 (average for 2020–2022)

Табл. 2. Урожайность лекарственного сырья расторопши пятнистой и фенхеля обыкновенного (среднее за 2020–2022 гг.)
Table 2. Yield of medicinal raw materials of milk thistle and fennel (average for 2020–2022)

Culture	Sowing date	Yield, t/ha			Weight of 1000 seeds, ha	Fat content in seeds, %
		Herbage	Dry matter	Seeds		
Lady's thistle	15 May	15,40	2,46	1,69	32,0	25,2
	15 June	16,00	2,56	1,71	31,0	23,6
	15 July	2,89	0,46	–	–	–
$LSD_{0,5}$		2,22	0,28			
Common fennel	15 May	43,00	6,66	–	–	–
	15 June	43,20	6,71	–	–	–
	15 July	10,47	1,54	–	–	–
$LSD_{0,5}$		1,88	0,96			

Табл. 3. Структура и урожайность биомассы скорцонеры испанской (среднее за 2020–2022 гг.)
Table 3. Structure and yield of scorzonera biomass (average for 2020–2022)

Culture	Sowing date	Yield, t/ha			Root crop length, cm	Root crop diameter, mm
		Root crops	Leaves	Total weight		
Spanish salsify	May 15	32,1	10,7	42,8	28	19,0
	June 15	24,9	8,4	33,3	25	17,2
	July 15	1,6	12,2	13,8	10	7,0
$LSD_{0,5}$		1,42				

riod of June (June 15th), and for Spanish salsify (*Scorzonera hispanica* L.) – the second ten-day period of May (May 15th). These sowing dates ensure the highest yields of medicinal raw materials for lady's thistle – herbage 15.4–16.0 t/ha, dry matter – 2.46–2.56 t/ha, seeds – 1.69–1.71 t/ha; for common fennel – herbage – 43.0–43.2 t/ha; dry matter – 6.66–6.71 t/ha; for Spanish salsify yield with raw mass of root crops – 32.1 t/ ha, leaves – 10.7 t/ha.

2. The duration of interphase periods for medicinal plants at these sowing dates was for lady's thistle: sowing – emergence – 14–23 days, emergence – budding – 51–55 days, emergence – flowering – 70–73 days, emergence – seed ripening (harvesting) – 91–95 days. For common fennel, the respective durations were 17–25 days from sowing to emergence, 52–62 days from emergence to budding, and 77–81 days from emergence to flowering. For Spanish salsify, the intervals were 17 days from sowing to emergence, 21 days until the formation of 4–6 pairs of true leaves, 41 days until plant closure in rows, and 118 days from emergence to the formation of root crops (harvest).

3. The height of lady's thistle plants at the time of harvest was 163–166 cm, with a leaf coverage of 54–57%; for common fennel, it was 144–147 cm and 50–54%; and for Spanish salsify, it was 39 cm with a leaf coverage of 98%.

4. The medicinal plants were not affected by diseases or pests. All crops were resistant to lodging and drought (5 points) under the conditions of Transbaikalia.

5. Weed infestation in the crops (depending on the sowing dates) ranged from 15–16 to 2–8 plants/m², decreasing from the early sowing date to the later ones.

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НАСЛЕДОВАНИЕ ЦЕННЫХ ПРИЗНАКОВ ПРОДУКТИВНОСТИ ГИБРИДНЫХ КОМБИНАЦИЙ МЕСТНЫХ И ИНОСТРАННЫХ СОРТОВ ОВСА В УСЛОВИЯХ ЗАУРАЛЬЯ

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Представлены результаты изучения характера наследования ценных признаков гибридов первого поколения, полученных от скрещивания иностранных и отечественных сортов овса в условиях Северного Зауралья. Эксперимент проводили на опытном поле Научно-исследовательского института сельского хозяйства Северного Зауралья в 2019 и 2020 гг. Объектом исследования являлись 18 гибридных популяций F1 и шесть родительских сортов: Талисман, Отрада, Фома, Sang, Solidor и Ensiler. Оценку элементов продуктивности осуществляли согласно методике Всероссийского института генетических ресурсов растений им. Н.И. Вавилова. Для статистической обработки данных использовали методику Б.А. Доспехова. Характер наследования фенотипических признаков определяли по G.M. Beil, R.E. Atkins. Анализ элементов структуры урожая продемонстрировал отсутствие преимущества сортов иностранной селекции перед генотипами, полученными в Северном Зауралье. Установлено, что по озерненности метелки и массе 1000 зерен сорт Отрада не уступает сортам Sang, Solidor и Ensiler, а Фома – превосходит их. Определено, что скрещивания между сортами местной и иностранной селекции имеют высокий процент удачи (42–68%), что обусловливает их перспективность в селекционном процессе. Выявлено, что 50% гибридных комбинаций характеризуются депрессией по признаку «высота растений» – степень фенотипического доминирования в данном случае варьирует от −22,2 до −3,1 ед. Скрещивание Фомы с иностранными сортами обеспечило сверхдоминирование по высоте метелки, ее озерненности и массе 1000 зерен. По степени фенотипического доминирования элементов продуктивности выделены следующие перспективные гибридные комбинации: Ensiler × Отрада, Sang × Отрада, Фома × Sang. У перечисленных комбинаций отбор высокопродуктивных генотипов можно проводить со второго поколения. Осуществлять отбор перспективных линий среди гибридных комбинаций, где одной из родительских форм являлся сорт Фома, рекомендуется в более поздних поколениях. Гибриды первого поколения, полученные от скрещивания сорта Талисман с сортами Ensiler, Sang и Solidor, не имеют преимуществ перед родительскими формами и неперспективны для селекционного процесса.

Ключевые слова: гибридизация, характер наследования, элементы продуктивности, гетерозис, генетическая депрессия, модель сорта

INHERITANCE OF VALUABLE PRODUCTIVITY TRAITS OF HYBRID COMBINATIONS OF LOCAL AND FOREIGN OAT VARIETIES IN THE CONDITIONS OF THE TRANS-URAL REGION

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The results of studying the character of inheritance of valuable traits of the first-generation hybrids obtained from crossing foreign and domestic varieties of oats in the conditions of the Northern

Trans-Urals are presented. The experiment was conducted on the experimental field of the Scientific Research Institute of Agriculture for Northern Trans-Ural Region in 2019 and 2020. The object of the study were 18 F1 hybrid populations and 6 parent varieties: Talisman, Otrada, Foma, Sang, Solidor and Ensiler. Productivity elements were evaluated according to the methodology of the N.I. Vavilov All-Russian Institute of Plant Genetic Resources. B.A. Dospekhov's methodology was used for statistical data processing. Inheritance of phenotypic traits was determined according to G.M. Beil and R.E. Atkins. Analysis of the yield structure elements demonstrated the lack of advantage of foreign selection varieties over the genotypes obtained in the Northern Trans-Urals. It has been established that the Otrada variety is not inferior to Sang, Solidor and Ensiler varieties in terms of panicle ear grain content and the thousand-kernel weight, and Foma is superior to them. It has been determined that crosses between the varieties of local and foreign selection have a high percentage of success (42–68%), which makes them promising in the breeding process. It has been revealed that 50% of hybrid combinations are characterized by depression in the trait "plant height" – the degree of phenotypic dominance in this case varies from –22.2 to –3.1 units. Crossing of Foma with foreign varieties ensured overdominance in the panicle height, its grain content and the thousand-kernel weight. According to the degree of phenotypic dominance of the productivity elements, the following promising hybrid combinations have been identified: Ensiler × Otrada, Sang × Otrada, Foma × Sang. In the above combinations, selection of high-yielding genotypes can be carried out from the second generation onwards. It is recommended to select promising lines among hybrid combinations, where one of the parental forms is the Foma variety, in the later generations. Hybrids of the first generation obtained from crossing the Talisman variety with Ensiler, Sang and Solidor varieties have no advantages over the parental forms and are unpromising for the breeding process.

Keywords: hybridization, nature of inheritance, elements of productivity, heterosis, genetic depression, variety model

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INTRODUCTION

The foundation of any country's food security lies in the adequate provision of agricultural produce, including feed for livestock and poultry farming. Therefore, grain forage crops are currently as significant as wheat, rice, soy, and corn. In the last 70 years, the yield of agricultural crops has increased more than threefold thanks to the implementation of a scientifically based system of agriculture. Such significant achievements in the agro-industrial complex were only possible with the transition to modern intensive varieties, whose genetic potential for productivity is incomparably higher.

According to Rosstat (Russian Federal State Statistics Service), in 2022, the sown area of oats in the Russian Federation was 2.16 million hectares, including 235.5 thousand hectares in the Ural Federal District. To meet the growing needs of livestock and poultry farming, it is

necessary to significantly increase the gross oat harvest. Consequently, an increase in sown areas and enhancement of agricultural crop yields will be required. However, the most fertile fields are currently occupied by strategic or marginal crops, and reducing their sown areas is impractical. Therefore, the expansion of oat sowing areas or other grain forage crops is possible only through the plowing of less fertile lands. Increasing yields through the use of agrochemicals (including mineral fertilizers) is the most promising approach in the modern world. The solution to increasing grain harvest lies in creating new varieties capable of forming high yields and utilizing the bioclimatic potential of the region.

In the State Register of Breeding Achievements, foreign oat varieties account for 10%. These varieties are characterized by high yield and grain quality. Modern domestic oat varieties also possess high potential productivity, almost equal to that of foreign varieties. However, the actual yield in different regions of the country remains quite low, not to mention the quality of the harvested grain. There are many reasons for this, but overall they boil down to non-compliance with cultivation technology and the lack of variety plasticity to a wide range of soil and climatic conditions. According to G.A. Batalova et al.[1], the reason for the low productivity of modern oat varieties also lies in their incorrect zoning by admission regions. One way to solve the problem of low oat yields is to use local breeding varieties as parental forms, which are characterized by good resistance to unfavorable soil and climatic conditions, high ecological plasticity, as well as high-yielding foreign varieties possessing the necessary economically valuable properties (high ear grain content of the panicle and grain size, short-stemmedness, etc.).

A modern variety should effectively realize its genetic potential under various natural and anthropogenic environmental factors, possessing significant resistance to stress of both abiotic and biotic nature [1].

The purpose of the research is to create promising hybrid populations by crossing oats of foreign and domestic breeding, followed by their evaluation for key economically valuable properties.

MATERIAL AND METHODS

The study was conducted at the Research Institute of Agriculture for Northern Trans-Ural Region, in the forest-steppe zone of the Trans-Urals, according to the methodology of state varietal testing[2]. The soil of the experimental site was dark gray forest podzolized, with typical regional morphological characteristics and primary physical-chemical properties [2, 3]. The soil and climatic conditions of the site closely resemble the agricultural zone of the Ural Federal District, allowing for an assessment of the potential of hybrid combinations and oat varieties involved in the breeding work.

Six varieties of local and foreign breeding were used in the experiment (see Table 1). The combinations for crossings were selected based on the principle of eco-geographical remoteness of the parent forms.

Hybridization was conducted in field conditions. During the beginning of the panicle emergence phase (when two to three spikelets appear from the leaf sheath), mechanical castration was performed. The upper and lower spikelets were removed from the panicle, leaving four to six of the most developed spikelets in the middle part. Castration was done in the morning, from 5 to 8 a.m. local time. On the 4th to 5th day, forced pollination was carried out according to the hybridization scheme. Pollen was collected on the day of pollination, from 9 a.m. to 12 p.m. Three mature anthers were placed in each flower. The panicle was then enclosed in a paper isolator. Crossing was carried out on ten panicles of each variety. The seeds of hybrid combinations were sown the following year according to the scheme ♀ – F1 – ♂ in three replications. Ten seeds of parent varieties and hybrids were placed on each

[1]*Batalova G.A., Lisitsyn E.M., Changzhong Ren, Andreev N.P., Tuliakova M.V., Shevchenko S.N., Malco A.M.* Breeding oats in the European North-East of Russia // Achievements of science and technology of AIC, 2016, vol. 30, N 1, pp. 21-24.

[2]Methodology of state variety testing of agricultural crops. Moscow, 1985, Issue 1, 270 p.

Табл. 1. Сорта овса отечественной и иностранной селекции, используемые в гибридизации
Table 1. Oat varieties of domestic and foreign selection used in hybridization

Number in the experiment	Variety	Sort	Country of origin	Source of description
1	Ensiler	*mutica*	USA	4
2	Sang	»	Sweden	4
3	Solidor	»	Germany	4
4	Otrada	»	Russia	5
5	Talisman	»	»	6
6	Foma	»	»	7

row. The distance between the rows was 20 cm, and the sowing depth was 5–6 cm. The sowing period was the second ten-day period of May. Agronomic practices in the experiments were standard for the northern forest-steppe of the Trans-Urals [8].

Phenological observations, assessment of crop condition, and recording of yield structure elements were conducted according to methodological instructions for studying the world collection of barley and oats[3].

The inheritance of traits was calculated using the formula by G.M. Beil, R.E. Atkins:

$$hp = (F1 - MP)/(HP - MP),$$

where *hp* is the degree of phenotypic dominance; *F*1 is the trait expression in F1 plants; *MP* is the average trait expression of both parental forms; *HP* is the most expressed trait of the parental form.

Hybrid grouping according to the degree of phenotypic trait expression was done using the classification by G.M. Beil, R.E. Atkins[4], according to which the following types of gene interactions are possible: $hp > 1$ – heterosis (positive over-dominance); $0.5 < hp < 1.0$ – positive dominance; $-0.5 < hp < 0.5$ – intermediate inheritance; $-1 \leq hp \leq -0.5$ – negative dominance; $hp < -1$ – depression (negative over-dominance).

For statistical analysis of the research results, the method of variance analysis was used in accordance with the methodology of B.A. Dospekhov[5].

RESULTS AND DISCUSSION

The hybridization of domestic and foreign oat varieties was conducted in 2019, a year characterized by favorable weather conditions throughout the entire vegetation period. As a result, 18 hybrid combinations were obtained (see Table 2). The success rate was quite high for oats, varying from 42% (Solidor × Talisman) to 68% (Otrada × Solidor).

It was noted that when using the Ensiler variety as the maternal plant in crossing with local varieties, the formation of hybrid seeds was maximal (61–68%), while using the same variety as the paternal plant resulted in a significantly lower outcome of 51–57% ($F_{fact} > F_{theor}$). The lowest success rate (42–48%) was recorded in the combinations where the Solidor variety was used as the maternal plant. However, when used in combinations with Talisman and Otrada as the paternal plant, the seed setting increased to 60 and 68%, respectively. In the Foma × Solidor combination, the success rate remained low.

The highest seed set (55–68%) was observed in the combinations where local varieties served as maternal plants. However, varietal characteristics were evident: Foma (♀) when pollinated by foreign varieties had a lower seed set (47–51%), while Otrada's rate varied from 55 to 68%.

[3]Methodological guidelines for the study of the world collection of barley and oats. St. Petersburg, 2012, 64 p.

[4]*Beil G.M., Atkins R.E.* Inheritance of guantitative characters in grain sorghum // Iowa State Journal Science, 1965, vol. 39, N 6, pp. 165–179.

[5]*Dospekhov B.A.* Methods of field experiment (with the basics of statistical processing of research results). 5th ed., supplemented and revised. Moscow: Agropromizdat, 1985, 351 p.

Табл. 2. Гибридные комбинации
Table 2. Hybrid combinations

Crossbreeding catalog	Hybrid combination (♀ × ♂)	Percentage of luck
1 × 4	Ensiler × Otrada	65
1 × 5	Ensiler × Talisman	68
1 × 6	Ensiler × Foma	61
2 × 4	Sang × Otrada	54
2 × 5	Sang × Talisman	51
2 × 6	Sang × Foma	60
3 × 4	Solidor × Otrada	45
3 × 5	Solidor × Talisman	42
3 × 6	Solidor × Foma	48
4 × 1	Otrada × Ensiler	57
4 × 2	Otrada × Sang	55
4 × 3	Otrada × Solidor	68
5 × 1	Talisman × Ensiler	56
5 × 2	Talisman × Sang	52
5 × 3	Talisman × Solidor	60
6 × 1	Foma × Ensiler	51
6 × 2	Foma × Sang	47
6 × 3	Foma × Solidor	50

There are many reasons for the reduction in hybrid seed set. Primarily, this includes unsuitable weather conditions: during the flowering period of oats in Siberia, the weather is usually hot and dry, leading to premature pollen sterilization. In our case, hybridization occurred within short periods, neutralizing the weather differences. According to G.L. Petrov and E.Y. Petrova[6], the success of oat hybridization also depends on the anatomical features of the flower structure.

Plant height is an important phenotypic trait used in the breeding of cereal crops. For oats, this trait is significant, as it affects the plants' resistance to lodging [9]. Modern research has shown a direct correlation between short-stemmedness and grain productivity [10]. Therefore, breeders need to balance between resistance to lodging and oat yield.

The average height of the local varieties was 77–80 cm, with significant variation: Otrada ranged from 72 to 98 cm, Talisman from 75 to 85 cm (see Fig. 1). The Foma variety had the smallest height range, from 74 to 80 cm.

Foreign varieties did not significantly differ in height from local genotypes. Average heights were as follows: Ensiler – 82 cm, Solidor – 85 cm, and Sang – 87 cm. However, the range of height variation in the experiment was significantly smaller, being ± 5% of the average values.

The first-generation hybrid populations (F1) exhibited varying heights relative to each other and to the parent forms. The tallest were hybrid combinations 6 × 3; 4 × 1; 4 × 3; 6 × 1; 3 × 6; 2 × 6; 5 × 3; 1 × 6, where the inheritance of this trait followed the pattern of over-dominance (h_p > 1.0). The height of such hybrids ranged from 88 cm (Otrada × Solidor) to 105 cm (Talisman × Solidor), with the degree of phenotypic dominance being 3.4 and 7.8 units, respectively.

Depression, caused by the effect of negative over-dominance (h_p < -1), was noted in F1 of the following hybrid combinations: 1 × 5; 2 × 5; 3 ×5; 5 × 2; 4 × 2; 6 × 2; 5 × 1, which could indicate the expression of the short-stemmedness gene. In the crosses of Ensiler × Talisman and Sang × Talisman, the highest degree of depression was observed: h_p was equal to -5.0 and -3.5 units, respectively. The height of these hybrids was 70 and 78 cm, respectively.

Hybrids obtained by crossing local varieties (♀) with the Sang variety (♂) maintained height at the level of the maternal forms, making them promising for further breeding in the conditions of Western Siberia [11]. A similar effect was noted in combinations 1 × 4 and 2 × 4, where Otrada served as the male parent. The height of

[6]*Petrov G.L., Petrova E.Yu.* Study of flowering biology and pollination methods of oats in the conditions of the Northern Trans-Urals // Natural and Technical Sciences, 2017, N 2 (104), pp. 13-14.

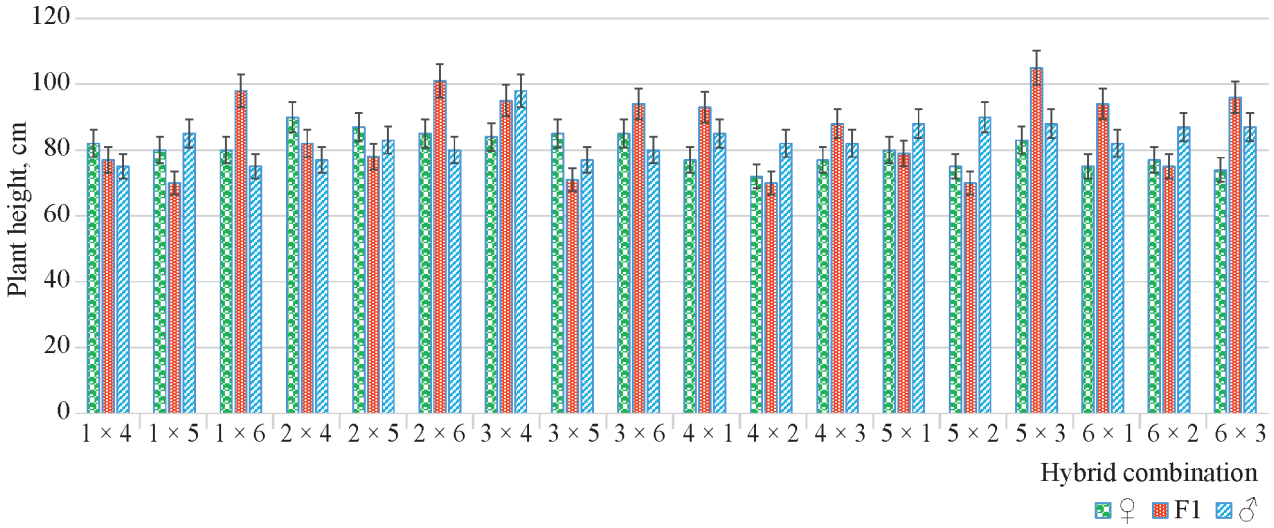


Рис. 1. Высота растений у родительских сортов и гибридов первого поколения при 5%-й погрешности
Fig. 1. Plant height of the parent varieties and hybrids of the first generation of oats at a 5% error limit

these hybrids was 77 and 82 cm, respectively. The degree of phenotypic dominance was −0.4 and −0.2 units, which corresponds to intermediate inheritance and makes these combinations promising for the breeding process.

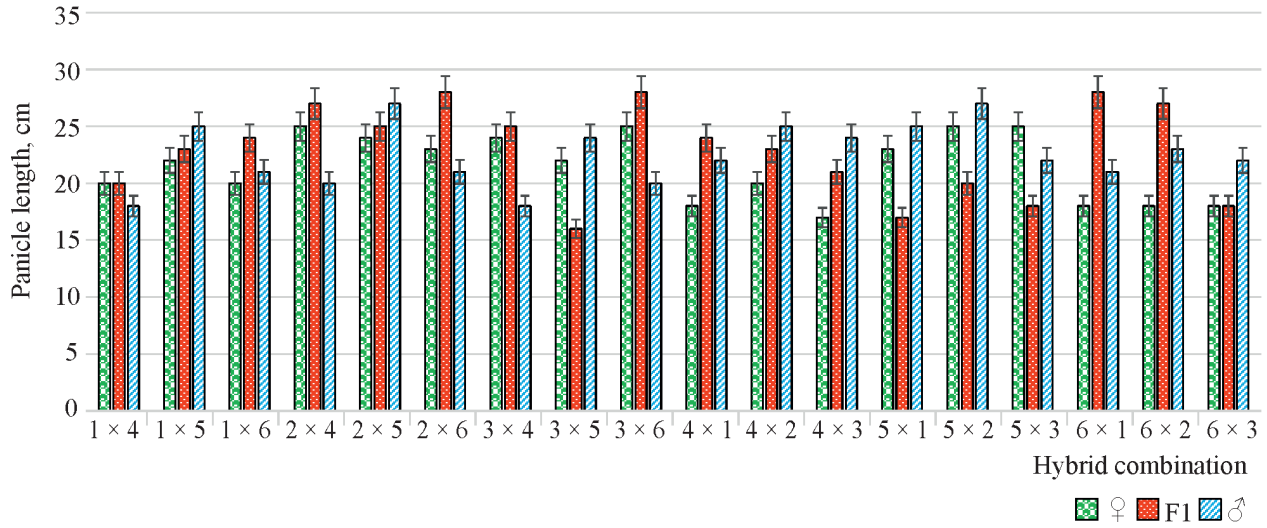
The panicle length of the Talisman variety varied from 23 to 27 cm (average in the experiment – 25 cm), which was the maximum result among the studied local varieties (see Fig. 2). The Otrada and Foma varieties had shorter panicle lengths – from 17 to 20 cm (average in the experiment – 19 cm). The Ensiler variety, in terms of the phenotypic trait "panicle length," was close to Talisman: the indicator ranged from 20 to 25 cm, with an average panicle length in the experiment of 22 cm. The Sang variety recorded the maximum average panicle length of 25 cm, varying from 23 to 27 cm, identical to the local variety Talisman. Solidor differed significantly in panicle length variation – from 18 to 24 cm, which is undesirable for modern intensive varieties. The average panicle length was 21 cm.

The first-generation hybrids obtained in the study exhibited a wide range of panicle lengths. The minimum length (16 cm) was observed in the hybrids of Solidor × Talisman, where the effect of negative over-dominance was manifested. A similar expression of depression ($h_p < -1$) was noted in the hybrid combinations 3 × 5; 5 × 1; 5 × 2; 5 × 3; 6 × 3. Hybrids with a heterosis effect ($h_p > 1$), whose panicle length significantly exceeded that of the parent varieties ($F_{fact} > F_{theor}$), included combinations 1 × 4; 3 × 4; 2 × 4; 4 × 1; 3 × 6; 6 × 2; 6 × 1; 2 × 6; 1 × 6. The greatest phenotypic over-dominance effect was observed in hybrid combinations where Foma was one of the parents – 6 × 1; 2 × 6 and 1 × 6, with panicle lengths ranging from 24 to 28 cm.

Intermediate inheritance of panicle length was identified in hybrids resulting from crosses of Ensiler × Talisman, Sang × Talisman, Otrada × Solidor, and Otrada × Sang. The panicle length of these hybrids varied from 21 to 25 cm, which was comparable to the Talisman variety.

M.N. Fomina et al. [11] have proven that oat yield is closely correlated with the “ear grain content of the main panicle” indicator. Therefore, breeders pay significant attention to this trait in creating new varieties for Western Siberia [12].

The average ear grain content of the main panicle of Talisman in the experiment was 33 pcs., with a variation range of 28–38 pcs. The modern variety Otrada showed higher values: the ear grain content of the main panicle reached 35–42 pcs., with an average value of 39 pcs. The maximum ear grain content of the main panicle among the local varieties was recorded for Foma – 43 pcs., with a value range from 38 to 47



Puc. 2. Длина метелки у родительских сортов и гибридов первого поколения при 5%-й погрешности
Fig. 2. Panicle length of the parent varieties and hybrids of the first generation of oats at a 5% error limit

pcs., which was significantly higher than Otrada (LSD_{05} = 3 pcs.).

Foreign selection varieties in the forest-steppe conditions of Zauralye did not have an advantage over local genotypes in terms of ear grain content in the panicle. The minimum grain count was noted for Ensiler and Sang – 34 and 32 grains, respectively. These varieties also stood out for their large range of variation in grain count in the panicle – 8 and 12 grains, respectively. The Solidor variety was at the level of Otrada in terms of ear grain content, both in average value and in the range of variation.

Regarding the "ear grain content of the main panicle" trait, more than half of the hybrids showed over-dominance ($h_p > 1$). The maximum degree of phenotypic dominance was noted in combinations 2 × 6; 3 × 4 and 6 × 3 (see Fig. 3). The ear grain content of the main panicle of these hybrids varied from 44 to 57 pcs. The hybrid population resulting from the cross of Ensiler and Foma was particularly interesting, as it managed to maintain the ear grain content of the panicle at the level of the local variety. The degree of phenotypic dominance was equal to 1.0 units.

First-generation hybrids of combinations 5 × 1; 3 × 5; 2 × 5; 5 × 3, and 4 × 3 were characterized by negative over-dominance ($h_p < 1.0$). The maximum effect of genetic depression was recorded in hybrids of Talisman × Ensiler, Solidor × Talisman, and Sang × Talisman, with h_p values of -4.31 and -3.91, respectively. It's important to note the combination 4 × 3, where Otrada and Solidor were used as parent forms: in this case, the ear grain content of the panicle remained at the level of the local variety.

First-generation hybrids obtained from crossing Otrada and the foreign variety Sang (4 × 2; 2 × 4) were characterized by positive dominance ($0.5 < h_p < 1.0$) and intermediate inheritance of the trait ($-0.5 < h_p < 0.5$), allowing their inclusion in the breeding process from the second generation [13].

Similarly, the combination 5 × 2, where Talisman was used as the female parent, produced hybrids that had the same ear grain content as the parent forms.

Grain weight per panicle, a trait directly correlated with productivity, is always considered in developing models for cereal and legume varieties [14, 15]. Among the studied oat varieties, Talisman had the lowest grain weight per panicle (1.0 g) with good uniformity of values (see Fig. 4).

The difference between the minimum and maximum was 0.2 g. Otrada and Foma had higher values – 1.5 and 1.8 g, respectively (LSD_{05} = 0.2). The average grain weights per panicle for the foreign varieties varied slightly – from 1.4 (Sang) to 1.6 g (Ensiler). They also differed in a larger range of values – a variation range of 0.5 g.

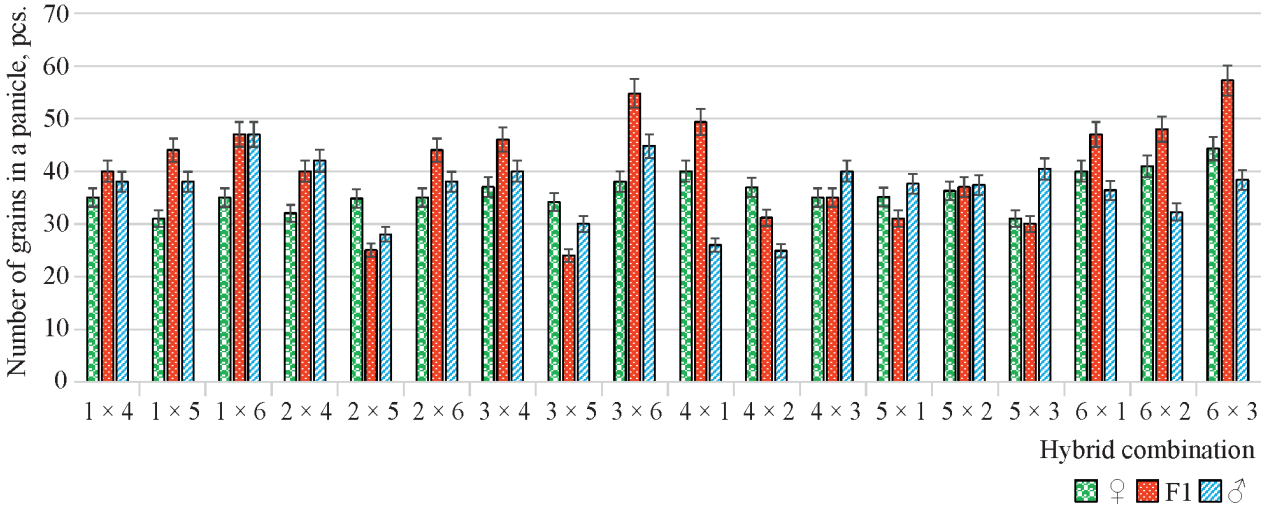


Рис. 3. Озерненность главной метелки у родительских сортов и гибридов первого поколения при 5%-й погрешности

Fig. 3. Grain content of the main panicle of parent varieties and hybrids of the first generation of oats at a 5% error limit

Inheritance of the "grain weight per main panicle" trait in 11 out of 18 F1 hybrid populations followed a pattern of over-dominance – h_p ranged from 1.0 (2 × 5; 3 × 4) to 7.0 (1 × 6). It's noteworthy that true heterosis was observed when crossing any foreign variety with Foma: the degree of phenotypic dominance varied from 3.0 to 7.0 units.

Additionally, three hybrid combinations (4 × 3; 5 × 3; 3 × 5) with negative dominance were identified, where h_p ranged from −1.00 to −2.12 units. In these combinations, the variety Solidor was used as one of the parental forms. Negative dominance was also observed in hybrids from combinations 5 × 1; 1 × 5, and 5 × 2, where the local variety Talisman was one of the parental forms.

In oat breeding, it's important to use genotypes with large grains. According to several scientists[7] [16, 17], grain size and its geometri-

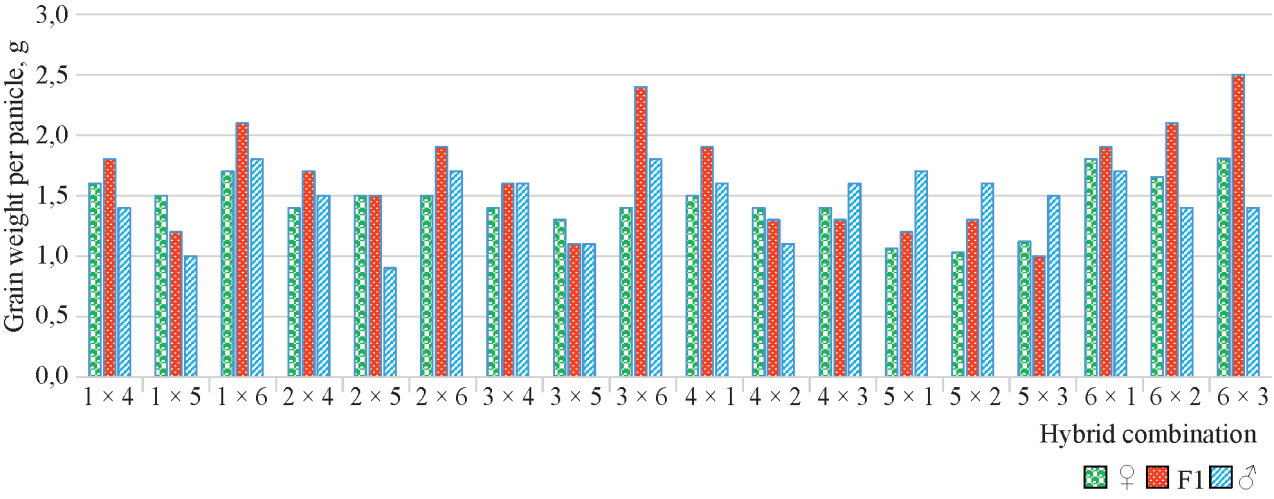


Рис. 4. Средняя масса зерна с метелки у родительских форм и гибридов первого поколения

Fig. 4. Average grain weight per panicle in parental forms and hybrids of the first generation

[7] *Ivanova Yu.S., Fomina M.N., Loskutov I.G.* Source material for the creation of high-protein varieties of oats in the zone of the Northern Trans-Urals // Proceedings on applied botany, genetics and breeding, 2017, vol. 178, N 2, pp. 38-47.

cal characteristics are 70-75% dependent on the genotype. The weight of 1000 grains not only determines the feed value of a variety but also its food value for humans. Moreover, cultivating large-grain oat varieties significantly reduces harvest and sorting losses [18]. In risky farming conditions, sowing oats with a weight of 1000 grains more than 35 g ensures uniform germination, helping to mitigate the negative impact of abiotic factors [19, 20]. This is particularly relevant for the Ural Federal District, where oat acreage is gradually increasing.

The variety Talisman used in the experiment was characterized by a low weight of 1000 grains (see Fig. 5). On average, this indicator was 29.1 g, varying from 27.3 to 30.4 g. Although Talisman had a consistent weight of 1000 grains, Otrada, despite having a higher weight (35.3 g), had a wider range between its minimum and maximum values.

Among the local varieties, Foma was the largest-grained – the average weight of 1000 grains was 40.7 g with minor variation (39.5–42.0 g). Such outstanding indicators characterize Foma as an intensive type variety.

The grain of foreign varieties was larger than that of the local genotypes. In the forest-steppe conditions of the Trans-Urals, the Solidor variety had an average weight of 1000 grains of 36.9 g, varying from 36.9 to 38.1 g. Sang and Ensiler belong to large-grained oat varieties: in their case, the average weight of 1000 grains was 43.4 and 46.2 g. The identified variability of values is considered low, so these varieties can be classified as intensive types.

First-generation hybrids, which showed clear signs of heterosis and a high degree of phenotypic dominance ($h_p > 1.0$), had a very large weight of 1000 grains – ranging from 43.7 (6 × 3; 3 × 6) to 48.7 g (6 × 2; 2 × 6). These hybrid combinations involved the local variety Foma and two foreign varieties – Sang and Solidor.

During hybridization, populations (4 × 3; 3 × 4; 5 × 1; 5 × 2) were obtained, which exhibited the effect of negative overdominance ($h_p < -1$). Their weight of 1000 grains was initially lower than the values of the parent forms – from 27.4 to 33.9 g. Maximum depression was noted in the offspring of the varieties Otrada and Solidor. In the hybridization of Otrada (♀) with Solidor and Sang (♂), hybrids with intermediate inheritance were obtained, caused by the additive effect of genes: h_p was −0.4 and 0.18 respectively. Hybrid combinations 1 × 5; 5 × 3; 3 × 5 were characterized by negative dominance – the weight of 1000 grains of first-generation hybrids was close to the paternal parental forms (Talisman and Solidor).

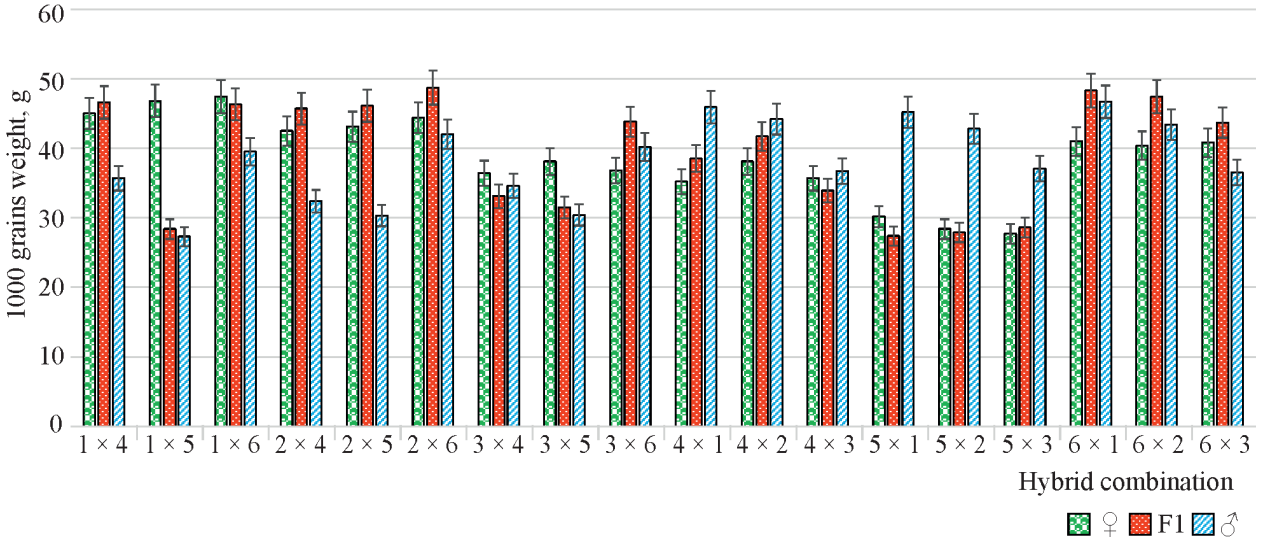


Рис. 5. Масса 1000 зерен у родительских сортов и гибридов первого поколения при 5%-й погрешности

Fig. 5. Thousand kernel weight of the parent varieties and hybrids of the first generation of oats at a 5% error limit

The conducted studies showed that the most valuable combinations in oat breeding are those where the first-generation hybrids dominate traits related to productivity and plant height.

According to the correlation analysis of the degree of phenotypic dominance, the main productivity indicators of oats (weight of 1000 grains, ear grain content per panicle, weight of grain per panicle) do not have a close dependence on plant height – $r = 0.19…0.33$ (see Table 3).

Therefore, the selection of hybridological material can be guided by productivity elements, taking into account their potential short-stemmed shape. Hybrids of the first generation obtained by crossing foreign and local oat varieties were selected based on a complex of traits. The most promising for the selection process were: Ensiler × Otrada (1 × 6), Sang × Otrada (2 × 4), Foma × Sang (6 × 2). These hybrid combinations had a lower height compared to the parental forms and predominantly showed a heterosis effect on productivity elements. Hybrid populations from the crossings Solidor × Foma (3 × 6), Foma × Ensiler (6 × 1), Foma × Solidor (6 × 3), Solidor × Foma (3 × 6) and Sang × Foma (2 × 6) were significantly taller than the parental forms, but also characterized by positive overdominance of yield structure elements, making them promising for breeding in the forest-steppe conditions of the Trans-Urals.

Other first-generation hybrids obtained during the study can be considered as sources of individual economically valuable traits. The least valuable were the hybrids obtained by crossing Otrada (♀) with the Solidor (♂) variety, as well as Talisman with all foreign varieties.

Табл. 3. Степень фенотипического доминирования по некоторым показателям продуктивности у гибридов овса первого поколения

Table 3. The degree of phenotypic dominance for some indicators of productivity in the hybrids of the first-generation oats

Crossbreeding catalog	Hybrid combination	Plant height, cm	Panickle length, cm	Number of grains in a panickle, pcs.	Grain weight per panicle, g	Weight of 1000 grains, g
1 × 6	Ensiler × Otrada	–0,4	1,0	2,3	3,0	11,0
1 × 5	Ensiler × Talisman	–5,0	–0,3	2,7	–0,2	–0,3
1 ×4	Ensiler × Foma	8,2	7,0	1,0	7,0	8,8
2 × 4	Sang × Otrada	–0,2	1,8	0,6	5,0	2,0
2 × 5	Sang × Talisman	–3,5	–0,3	–1,9	1,0	–10,3
2 × 6	Sang × Foma	7,4	6,0	5,0	3,0	11,7
3 × 4	Solidor × Otrada	0,6	1,3	5,0	1,0	2,5
3 × 5	Solidor × Talisman	–2,5	–7,0	–3,9	–1,0	–8,7
3 × 6	Solidor × Foma	4,6	2,2	4,0	4,0	2,9
4 × 1	Otrada × Ensiler	3,0	2,0	2,3	6,1	3,7
4 × 2	Otrada × Sang	–1,4	0,2	0,0	0,3	–0,4
4 × 3	Otrada × Solidor	3,4	0,1	–1,0	–2,1	1,9
5 × 1	Talisman × Ensiler	–1,3	–7,0	–4,3	–0,6	–3,0
5 × 2	Talisman × Sang	–1,7	–6,0	0,3	–0,1	–4,6
5 × 3	Talisman × Solidor	7,8	–3,7	–1,2	–1,6	–0,1
6 × 1	Foma × Ensiler	4,4	5,7	4,9	3,0	2,1
6 × 2	Foma × Sang	–1,4	2,6	2,6	4,5	8,2
6 × 3	Foma × Solidor	2,4	–1,0	5,3	4,4	3,7

CONCLUSION

It was established that the foreign and local varieties used in the study cross well and produce viable seeds. The overall success rate was 45-65%. The most successful crosses occurred when using Ensiler and Otrada as the female parent – 65% and 60%, respectively. The lowest success rate was noted when using Solidor and Foma as the female parent variety – 45% and 49%, respectively. As a result, the most promising hybrid combinations for further breeding work were identified: Ensiler × Otrada, Sang × Otrada, Foma × Sang, where selection of highly productive genotypes can be carried out from the second generation. The degree of phenotypic dominance of these hybrids varied in the range of 3.0 to 5.0, corresponding to positive overdominance. The number of grains per main panicle was 40-48, with a weight of 1000 grains being 45.7-47.4 g. In combinations such as Ensiler × Foma, Foma × Ensiler, Solidor × Foma, Sang × Foma, selection would be more effective in later generations. The hybrids obtained exhibited a pronounced heterosis effect only for individual yield structure elements. The weight of grain per main panicle in these hybrids varied from 1.9 to 2.4 g, and the number of grains from 44 to 55. Crossbreeding Talisman with foreign varieties (Ensiler, Sang, Solidor) is not promising, as the F1 hybrids obtained do not demonstrate an effect of improving economically valuable properties in the first generation. In hybrid combinations where Talisman was one of the parental forms, negative overdominance was observed for the main productivity indicators: number of grains, weight of grain per plant, and weight of 1000 grains. Other hybrid oat combinations can be used only in breeding for individual traits and properties.

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ЗАЩИТА РАСТЕНИЙ СОИ ОТ ФИТОФАГОВ В УСЛОВИЯХ ПРИМОРСКОГО КРАЯ

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Изучена эффективность предпосевной обработки семян сои и применения биорациональных инсектицидов в снижении численности основных вредителей сои в условиях Приморского края. Представлены результаты использования инсектицидов и биопрепаратов для регуляции численности доминантных вредителей сои. Исследования проведены в 2020 и 2021 гг. В полевых экспериментах изучена эффективность инсектицидных протравителей Имидор Про, КС (2,0 л/т) и Табу, ВСК (1,0 л/т), биоинсектицидов Фитоверм, КЭ (0,16 л/га), Проклэйм, ВРГ (0,3 кг/га), Бацикол, Ж (15 л/га), Биослип БВ, Ж (2 л/га), Биослип БТ, П (2 кг/га) против листоеда соевого полосатого (*Medythia nigrobilineatus* Motsch.) и плодожорки соевой (*Leguminivora glycinivorella* Mats.). Предпосевная обработка семян инсектицидами Имидор Про и Табу снижала поврежденность растений сои в фазу всходов жуками *Medythia nigrobilineatus* по сравнению с контролем на 94,0–98,2%. Протравливание семян препаратами на основе имидаклоприда обеспечивало эффективную защиту посевов культуры против вредителя в фазы всходы – ветвление. Высокую биологическую эффективность (71,1–98,8%) на 5–10-е сутки после обработки против листоеда соевого полосатого показали биорациональные инсектициды на основе аверсектина С и *Bacillus thuringiensis.* Поврежденность семян сои при использовании биоинсектицидов против *Leguminivora glycinivorella* составила 1,9–3,0% в сравнении с 5,6% в контроле. Проведенные исследования свидетельствуют о перспективности применения препаратов биологического происхождения для контроля численности доминантных вредителей в посевах сои.

Ключевые слова: соя, насекомые-фитофаги, инсектициды, биорациональные инсектициды, биологическая эффективность

PROTECTION OF SOYBEAN PLANTS FROM PHYTOPHAGES UNDER CONDITIONS OF THE PRIMORSKY TERRITORY

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The effectiveness of pre-sowing treatment of soybean seeds and application of biorational insecticides in reducing the number of major pests of soybean in the conditions of the Primorsky Territory have been studied. The results of using insecticides and biopreparations to regulate the number

of dominant pests of soybean have been presented. The studies were conducted in 2020 and 2021. The efficiency of insecticidal protectants Imidor Pro, SC (2 l/t) and Tabu, WSC (1.0 l/t), bioinsecticides Fitoverm, EC (0, 16 l/ha), Proclaim, WSG (0.3 kg/ha), Batsikol, L (15 l/ha), Biosleep BW, L (2 l/ha), Biosleep BT, P (2 kg/ha) against two-striped leaf beetle (*Medythia nigrobilineatus* Motsch.) and soybean pod borer (*Leguminivora glycinivorella* Mats.) were studied. Pre-sowing seed treatment with insecticides Imidor Pro and Tabu reduced damage of soybean plants in the sprouting phase by *Medythia nigrobilineatus* beetles compared to the control by 94.0–98.2%. Seed dressing with imidacloprid-based preparations provided effective protection of crops against the pest in the sprouting – branching phase. Biorational insecticides based on avermectin C and *Bacillus thuringiensis* showed high biological efficacy (71.1–98.8%) on the 5–10th day after treatment against the two-striped leaf beetle. Soybean seed damage when bioinsecticides were used against *Leguminivora glycinivorella* was 1.9–3.0% compared to 5.6% in the control. The conducted studies testify to the prospect of using the preparations of biological origin to control the number of dominant pests in soybean crops.

Keywords: soybean, phytophagous insects, insecticides, biorational insecticides, biological effectiveness

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INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is the dominant agricultural crop in the Primorsky Territory. The sown areas in the region are gradually increasing: in 2021, they amounted to 277,000 hectares, and in 2022 – 345,000 hectares.

The main factors limiting the yield growth of this crop are harmful organisms: phytophages of various families, phytopathogens of fungal, bacterial, and viral nature, and weeds. The fauna of soybean pests in the region is represented by polyphagous species. These include sod webworm, noctuid moths, soybean yellow butterfly, aphids, bugs, and polyphagous soybean leaf beetle. Among the dominant and most widespread are specialized pests of the crop: two-striped leaf beetle (*Medythia nigrobilineatus* Motsch. (= *Paraluperodes suturalis nigrobilineatus* Motsch.)), soybean pod borer (*Leguminivora glycinivorella* Mats.)[1]. In other regions of Russia and abroad, the dominant soybean pests include the cotton budworm (*Helicoverpa armigera* Hbn.), lima-bean pod borer (*Etiella zinckenella* Tr.), and dusky stink bug (*Nezara viriduta* L.) [1–3].

Two-striped leaf beetle causes significant damage to the crop's seedlings. Both larvae and adult beetles are harmful. The beetles gnaw pits on the lower side of the cotyledons and sometimes damage young stems. Harmfulness of the phytophage increases in dry warm weather. The damage caused by the pest during this phase can lead to plant death. The larvae live in the soil, penetrate the nodules, and feed on their contents. Damage to the nodules reduces soil nitrogen enrichment, decreasing soy's role as a forecrop in crop rotation. The damage caused to soybean plantings by the soybean pod borer results in not only yield losses but also a reduction in seed quality. Inside the pods, caterpillars eat the seeds, and damage to the hilum and embryo often leads to a complete loss of germination [4].

[1]*Mashchenko N.V.* The most common pests of soybean in the Amur region and measures to combat them: method. manual. Blagoveshchensk: Publishing house DEI "Zeya", 2012, 32 p.

Increasing soybean productivity and improving crop quality depend on the effectiveness of protecting the crop from harmful organisms. Therefore, the issue of protecting soybeans from pests remains relevant. Most protective measures for soybean crops are based on chemical means, the range of which is constantly being improved, and the share of combined preparations is increasing [5–7]. Pre-sowing seed treatment is the most environmentally safe and effective way to use pesticides for regulating the numbers and reducing the harmfulness of phytophages at the early stages of plant development [8–10]. Using biological means of protection is one way to reduce the pesticide load on the agroecosystem. Research conducted in our country and abroad shows the prospects of using bioinsecticides based on actinomycetes, *Bacillus thuringiensis*, *Beauveria bassiana* against pests in soybean crops [1, 8, 11–12].

Research is needed to assess their effectiveness in specific agroclimatic conditions to include biological-origin products in the soybean plant protection system.

The purpose of the study is to investigate the effectiveness of pre-sowing soybean seed treatment and the use of bio-rational insecticides in reducing the number of main soybean pests in the Primorsky Territory.

MATERIAL AND METHODS

Research on protecting soybean crops from key phytophages was carried out at the Federal Scientific Center of Agricultural Biotechnology of the Far East named after A.K. Chaiki. The subjects of the study were *Medythia nigrobilineatus* Motsch. (Coleoptera: Chrysomelidae) and *Leguminivora glycinivorella* Mats. (Lepidoptera: Tortricidae). The study examined the influence of pre-sowing treatment of soybean seeds with the products Imidor Pro, SC (imidacloprid, 200 g/l; AO "Shchelkovo Agrokhim") and Tabu, WSC (imidacloprid, 500 g/l; AO "August") at usage rates of 2.0 and 1.0 l/t on the phytosanitary state of soybean crops. During vegetation, the plants were treated in the first ten-day period of June in the full emergence phase and in the first ten-day period of August in the flowering – bean formation phases. The following means of protecting soybean crops from phytophages were studied: Batsikol, L (*Bacillus thuringiensis*, FSBSI VNIISKhM), Biosleep BW, L (*Beauveria bassiana*, OOO "Organic Park"), Biosleep BT, P (*Bacillus thuringiensis*, OOO "Organic Park"), Fitoverm, EC (avermectin C, 50 g/l, OOO SBC "Farmbiometservice"), Proclaim, WSG (amamectin benzoate, 50 g/kg, OOO "Syngenta"), Biokill, EC (abamectin, 10 g/l, OOO "Vashe Khozyaistvo").

The effectiveness of bioinsecticides was studied in comparison with the use of the insecticide Espero, SC (imidacloprid, 200 g/l + alpha-cypermethrin, 120 g/l; AO "Shchelkovo Agrokhim"). The Primorskaya 86 soybean variety was used in the experiment. Sowing was carried out in the third ten-day period of May in 2020 and 2021. The forecrop was cereal crops, with a four-fold repetition, and the plot area was 10.8 m². The working fluid usage rate for seed treatment was 10 l/t, and for spraying vegetating plants, it was 400 l/ha. Counts of the number of two-striped leaf beetle and the damage caused by soybean pod borer to the soybean pods were carried out in accordance with the guidelines[2]. The biological effectiveness was calculated using the Abbott formula. The harvest was manually collected in the first ten-day period of October. The biological yield of soybean seeds (g/m²) was determined in four samples from an area of 0.25 m² in each repetition of all the variants of the experiment and recalculated per hectare. Statistical data processing was conducted according to B.A. Dospekhov[3].

RESULTS AND DISCUSSION

The settlement of soybean crops by two-striped leaf beetle was noted in the early first ten-day period of June in 2020 and 2021. The conducted counts showed that the pre-sowing

[2]Methodological guidelines for registration tests of insecticides, acaricides, molluscicides and rodenticides in agriculture / edited by V.I. Dolzhenko. SPb.: VIZR, 2009, 321 p.

[3]*Dospekhov B.A.* Methodology of field experiment (with the basics of statistical processing of research results). Moscow: Kolos, 1985, 336 p.

treatment of soybean seeds with Tabu and Imidor Pro preparations contributed to a decrease in seedling damage by the pest by 94.0% and 98.2% respectively, compared to the control (see Table 1).

High temperature regime and periodic precipitation in the second ten-day period of June in the years of research contributed to the activity and harmfulness of the phytophage.

The degree of leaf damage to soybean plants in the control group was 1.7 points, while in the variants with seed treatment, this indicator was significantly lower by 1.4 points. The biological effectiveness of insecticides against the pest ranged from 81.0% to 83.7%. In 2020, heavy rains at the end of the third ten-day period of June restrained the activity of the phytophage in the soybean crops, while hot, dry weather in 2021 was favorable for the development of two-striped leaf beetle. The effectiveness of the insecticides 14-21 days on average was at the level of 73.2% to 76.0%. No significant differences between the variants were noted.

The conducted assessments of soybean plant damage by the two-striped leaf beetle have shown that seed treatment with imidacloprid-based preparations provides effective protection for crop plantings against the pest from the germination to branching stages.

When treating mature soybean plants in the full emergence stage, bioinsecticides Batsikol and Fitoverm, applied on the 5th day after treatment, exhibited effectiveness against *M. nigrobilineatus* at the level of the chemical pesticide Espero (see Table 2). In the variants using the Biosleep product based on *B. bassiana* and *B. thuringiensis*, the effectiveness was significantly lower, ranging from 61.7% to 76.2%. On the

Табл. 1. Биологическая эффективность обработок семян сои протравителями против *M. nigrobilineatus* Motsch. (среднее за 2020, 2021 гг.)

Table 1. Biological effectiveness of soybean treatments by protectants against *M. nigrobilineatus* Motsch. (average for 2020 and 2021)

Experiment option	Average score of plant damage after adult emergence in the control by days of counting				Decrease in damage relative to the control by days of registration, %			
	3rd	7th	14th	21st	3rd	7th	14th	21st
Control	1,1	1,7	0,5	0,7	–	–	–	–
Imidor Pro, SC 2,0 l/t	0,05	0,3	0,1	0,2	94,0	83,7	76,0	74,0
Tabu, WSC, 1,0 l/t	0,03	0,3	0,2	0,1	98,2	81,0	73,2	75,9
LSD_{05}	0,4	0,5	0,1	0,1	4,7	3,8	7,1	7.1

Табл. 2. Биологическая эффективность биоинсектицидов против *M. nigrobilineatus* Motsch. в посевах сои (среднее за 2020, 2021 гг.)

Table 2. Biological effectiveness of bioinsecticides against *M. nigrobilineatus* Motsch. on soybean sowings (average for 2020 and 2021)

Experiment option	Preparation consumption rate, l/ha, kg/ha	Reduction of pest population relative to the control after treatment by days of counting, %		
		5th	10th	15th
Espero, SC	0,2	100 ± 0	92,4 ± 1,2	63,1 ± 1,7
Biosleep BW, L	2,0	61,7 ±1,0	54,3 ± 4,0	26,8 ± 1,5
Biosleep BT, P	2,0	76,2 ±2,5	71,1 ± 1,2	46,3 ± 0,9
Batsikol, L	15,0	98,8 ± 0,7	75,5 ± 2,6	46,2 ± 1,4
Fitoverm, EC	0,16	96,7 ± 1,0	84,9 ± 1,1	41,5 ± 1,1
LSD_{05}	–	4,5	6,3	4,0

Табл. 3. Биологическая эффективность препаратов в борьбе с соевой плодожоркой (среднее за 2020, 2021 гг.)

Table. 3. Biological effectiveness of preparations against *Leguminivora glycinivorella* Mats. (average for 2020 and 2021)

Experiment option	Preparation consumption rate, l/ha, kg/ha	Damaged beans, %	Reduced bean damage relative to the control, %	Yield, t/ha
Control	–	5,6	–	1,6
Biosleep BW, L	2,0	3,0	46,4 ± 1,1	1,8
Biosleep BT, P	2,0	2,6	53,6 ± 1,6	1,8
Proclaim, WSG	0,3	2,0	64,3 ± 1,2	1,9
Biokill, EC	0,4	1,9	66,0 ± 0,4	2,0
Espero, SC	0,2	0,9	83,9 ±1,3	2,2
LSD_{05}	–	0,3	2,6	0,2

10th day after treatment, Fitoverm demonstrated relatively high effectiveness (84.9%), while the reduction in pest population in variants using the Biosleep BT and Batsikol products was 71.1% and 75.5%, respectively. The lowest effectiveness (54.3%) was observed when using Biosleep BW. The conducted assessments have shown that the application of the above-mentioned preparations provides effective protection for soybean plants at the vulnerable emergence stage.

Treating soybean plants with preparations during the flowering and pod formation stages aims to reduce damage by the soybean pod borer. As the research has shown, when using bioinsecticides, the number of damaged soybean pods (1.9–3.0%) by soybean pod borers was significantly lower compared to the control (5.6%) (see Table 3). The highest effectiveness (64.3% and 66.0%) was observed with the preparations based on amamectin benzoate (Proclaim) and abamectin (Biokill). There were no significant differences between these variants. The effectiveness of Biosleep based on *B. bassiana* and *B. thuringiensis* was lower. Significant differences were observed in the reduction of soybean pod damage when using these two preparations. The use of the chemical pesticide Espero reduced pod damage by the pest by 83.9% compared to the control.

The use of bioinsecticides ensured the preservation of grain yield, with yield increases ranging from 0.2 to 0.4 tons per hectare.

CONCLUSIONS

1. As a result of the conducted research, the effectiveness of insecticidal and bioinsecticidal seed treatments in reducing the population of the two-striped leaf beetle and soybean pod borer damage has been established.

2. The use of Imidor Pro, SC, Tabu, WSC preparations contributed to reducing soybean emergence damage by *M. nigrobilineatus* compared to the control by 94.0–98.8%. Imidacloprid-based seed treatments provided effective protection for crop plantings against the two-striped leaf beetle in the germination to branching stages.

3. A single spraying of mature soybean plants with Fitoverm, Batsikol, Biosleep BT, and Biosleep BW preparations resulted in a reduction in the population of *M. nigrobilineatus* by 5–10 days compared to the control by 54.3–98.8%. Under the influence of Biosleep BW, Biosleep BT, Proclaim, and Biokill preparations, soybean pod damage by soybean pod borer decreased by 46.4–66.0%. The flight of butterflies and egg laying by soybean pod borers were delayed, and the protective effect of bioinsecticides lasted up to 14 days. Therefore, when using bio-rational insecticides, it is necessary to increase the frequency of treatments: the first treatment against *L. glycinivorella* should be carried out in the first ten-day period of August, and the second one after 2 weeks.

4. The results of the conducted research indicate the potential for using bio-preparations

based on actinomycetes *B. bassiana* and *B. thuringiensis* against pests in soybean crops to reduce the pesticide load on agroecosystems.

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СРАВНИТЕЛЬНОЕ ИЗУЧЕНИЕ ПРИМЕНЕНИЯ ИФА С МОЛОКОМ И СЫВОРОТКОЙ КРОВИ ДЛЯ ДИАГНОСТИКИ БРУЦЕЛЛЕЗА КРУПНОГО РОГАТОГО СКОТА

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Иммуноферментный анализ как более чувствительный метод позволяет выявить специфические антитела в пробах сборного молока. С учетом доступности и простоты выполнения данного метода исследование методом ИФА сборного молока в хозяйствах может стать важнейшим элементом в системе противобруцеллезных мероприятий по части контроля за эпизоотическим состоянием в хозяйствах. Для скрининговой экспресс-диагностики бруцеллеза крупного рогатого скота разработана методика постановки иммуноферментного анализа с молоком коров. Иммуноферментный анализ с сывороткой молока является специфичным, чувствительным, простым в постановке, учете и интерпретации результатов методом. Установлено, что условия хранения и транспортировки проб молока, соответствующие значениям комнатной температуры и приводящие к сквашиванию, не оказывают влияния на уровень специфических противобруцеллезных иммуноглобулинов в течение 8 сут, что снимает вопрос о применении холодовой цепи при транспортировке до места проведения анализа проб молока, подлежащих исследованию. При отработке оптимальной пробоподготовки разница в специфическом сигнале при постановке ИФА между сывороткой молока, полученной при высокоскоростном центрифугировании, и сывороткой молока, полученной методом сквашивания в течение 24 ч, составляла менее 10%. В связи с этим для подготовки проб сыворотки молока при исследовании ИФА выбрано скоростное центрифугирование. Изучены возможности применения ИФА с сывороткой молока на вакцинированном и не вакцинированном против бруцеллеза поголовье крупного рогатого скота. Молоко и кровь для исследования в ИФА необходимо брать через 6 мес и более после вакцинации (в инструктивные сроки). Установлен высокий уровень корреляции между данными ИФА с молоком и данными ИФА с сывороткой крови вне зависимости от эпизоотического или иммунного статуса (благополучные и неблагополучные стада, привитые и непривитые животные), который составил 86,8–92,0%.

Ключевые слова: бруцеллез, крупный рогатый скот, поствакцинальная диагностика, сыворотка крови, молоко, иммуноферментный анализ

COMPARATIVE STUDY OF THE ELISA USE WITH MILK AND BLOOD SERUM FOR BOVINE BRUCELLOSIS DIAGNOSIS

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Enzyme immunoassay, being a more sensitive method, makes it possible to identify specific antibodies in samples of combined milk. The ELISA study of harvested milk in farms can become an

important element in the system of anti-brucellosis measures regarding the control of the epizootic state in farms taking into account the availability and ease of implementation of this method. For screening express-diagnostics of bovine brucellosis the method of enzyme immunoassay with milk of cows has been developed. ELISA with milk serum is specific, sensitive, easy to formulate, account for and interpret the results. It has been found that the conditions of storage and transportation of milk samples corresponding to room temperature values and leading to fermentation do not affect the level of specific anti-viral immunoglobulins for eight days, and the question of the use of a cold chain during transportation of milk samples to be examined to the place of analysis can be withdrawn from the agenda. When working out the optimal sample preparation, the difference in the specific signal when setting the ELISA between the milk serum obtained by high-speed centrifugation and the milk serum obtained by fermentation for 24 hours was less than 10%. Therefore, high-speed centrifugation was chosen for the preparation of milk serum samples during the ELISA study. The possibilities of using ELISA with milk serum on vaccinated and non-vaccinated cattle against brucellosis have been studied. Milk and blood for testing in the ELISA should be taken 6 months or more after vaccination (within the instructional time frame). A high level of correlation was established between ELISA data with milk and ELISA data with blood serum, regardless of epizootic or immune status (satisfactory and unfavorable herds, vaccinated and unvaccinated animals), which amounted to 86.8–92.0%.

Keywords: brucellosis, cattle, post-vaccination diagnostics, blood serum, milk, enzyme immunoassay

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INTRODUCTION

Brucellosis in agricultural animals still prevails in our country. There is still a risk of human infection, making the problem of completely eradicating this infection relevant.

Multiple vaccinations pose a challenge for post-vaccination diagnosis. Therefore, a complex of tests is used for brucellosis diagnosis, including agglutination reaction (AR), complement fixation test (CFT), indirect hemagglutination reaction (IHA), immunodiffusion reaction (IDR) with O-PS antigen, enzyme-linked immunosorbent assay (ELISA), and others. The subject of research is blood serum [1–3]. It is important to find simple, easily accessible express methods for monitoring the well-being of farms regarding brucellosis [4]. Tests using milk can be one such method[1]. Studying milk using the ring test (RT) and IHA allows for a simple sample collection method for brucellosis diagnosis, which does not cause stress to animals. It also involves the examination of the mammary gland, which is one of the sites of entry, localization, and spread of the infection in brucellosis [5, 6].

The most promising research methods are the ring test and enzyme-linked immunosorbent assay with milk, which have received high praise from domestic and foreign researchers [7–9]. The ring test is the most widely used method for preliminary examination of brucellosis in cattle herds and individual animals. Titre fluctuations in individual udder quarters indicate brucellosis infection, while identical titres in all udder quarters are more likely post-vaccination titres. The

[1]*Popova T.G.* Diagnostic value of research methods for brucellosis of milk of cows reimmunized with brucellosis vaccines (strain 19 and strain 82): Extended abstract of candidate's thesis in Veterinary Medicine, Novosibirsk, 1990, 16 p.

FAO/WHO Expert Committee on Brucellosis recommends this reaction for widespread use in all countries as an additional method [5, 6, 10].

ELISA can be used for mass screening of cattle herds and for making a final diagnosis in individual animals[2]. Compared to other methods for detecting antigens and antibodies, enzyme-linked immunosorbent assays have several advantages: high sensitivity, specificity, reproducibility of results, stability when storing all necessary reagents (up to a year or more), ease of conducting the reaction, and the ability to use minimal volumes of the material being tested, as well as instrumental (qualitative and quantitative) recording of the reaction and the possibility of automating all its stages [11, 12]. The material for examination by the enzyme-linked immunosorbent assay can be udder secretion from dry cows, colostrum, and milk[3]. ELISA with milk is conducted in the same way as with blood serum [13].

It has already been proven that the use of ELISA for diagnosing brucellosis in agricultural animals is quite effective. In a comparative study of cows using this test, antibodies were detected in blood serum 50–72% more than in AR, CFT, AR with rivanol, and RBT (Rose Bengal Test) combined, and in milk 5–6 times more than in the ring test [8, 13].

ELISA can determine antibody titres, which is especially important when examining vaccinated animals, as it is necessary to distinguish post-vaccination reactions from those occurring during natural brucellosis [11, 14, 15].

Enzyme-linked immunosorbent assay is a more sensitive method, allowing the detection of specific antibodies in bulk milk samples. Considering the accessibility and simplicity of this method, research using ELISA with bulk milk on farms can become a crucial element in the brucellosis control system regarding the epidemiological status in farms [11, 14].

The purpose of the study is the comparative examination of the developed ELISA test with milk and with blood serum in brucellosis of cattle.

MATERIAL AND METHODS

The studies were conducted in the laboratory of optimization of anti-epizootic systems of the Institute of Experimental Veterinary Science of Siberia and the Far East of the SFSCA RAS.

To develop a methodology for obtaining milk serum for setting up milk-based enzyme-linked immunosorbent assay (ELISA) for the diagnosis of brucellosis in cattle, milk samples were collected from vaccinated and unvaccinated cows. Blood samples were simultaneously collected from the same animals for comparative analysis.

Milk serum was obtained from milk through high-speed centrifugation and clotting, which was used for the ELISA. Blood serum was obtained using traditional methods. ELISA with milk serum and blood serum was carried out in the classical manner. The reaction results were recorded using a spectrophotometer, and optical density was measured at a wavelength of 450 nm.

In parallel with the serum milk and blood serum research, a mandatory complex of serological tests for brucellosis diagnosis was conducted, including agglutination reaction, complement fixation reaction with S antigen, and immunodiffusion reaction with O-PS antigen.

The epizootological analysis of bovine brucellosis was carried out on the basis of statistical data, materials of veterinary records and reports, results of laboratory studies conducted in veterinary laboratories, and literature data.

RESULTS AND DISCUSSION

During the optimization of sample preparation, it was found that the difference in specific signal in the ELISA between milk serum obtained through high-speed centrifugation and milk serum obtained by clotting for 24 hours was less than 10%, which is not essential for analysis (see Table 1).

[2]*Verkovsky O.A.* Laboratory diagnostics of infectious diseases of cattle using immunoenzyme analysis (leukosis, foot-and-mouth disease, brucellosis) // Veterinaria Kubani, 2007, N 2, pp. 11-12.

[3]*Vanzini V.R., Aguirre N., Lugaresi C.I., Echaide S.T., Canavesio V.G., Guglielmone A.A., Marchesino M.D., Nielsen K.* Evaluation of an indirect ELISA for the diagnosis of bovine brucellosis in milk and serum samples in dairy cattle in Argentina // Preventive veterinary medicine, 1998, N 3, pp. 211–217. DOI: 10.1016/s0167-5877(98)00080-4.

Табл. 1. Уровень сигнала в ИФА специфических противобруцеллезных иммуноглобулинов молока при различных типах пробоподготовки

Table 1. The signal level in the ELISA of specific anti-brucellosis immunoglobulins of milk in various types of sample preparation

Sample number	Sample type	High-speed centrifugation, $D_{450, O.E.}$	Fermentation, $D_{450, O.E.}$
1	Negative	0,154	0,168
2	»	0,122	0,136
3	»	0,126	0,132
4	»	0,137	0,144
5	Positive	1,857	1,890
6	»	0,560	0,578
7	»	0,823	0,843
8	»	0,455	0,623
9	»	2,216	2,237
10	»	0,445	0,534
11	»	1,235	1,362
12	»	1,583	1,603
13	»	1,129	1,341
14	»	1,529	1,627
15	»	0,481	0,503
16	»	0,639	0,681
17	»	0,885	0,912
18	»	2,251	2,282
19	»	0,772	0,781
20	»	0,981	0,923
21	»	1,112	1,182
22	»	0,991	1,121
23	»	1,651	1,668
24	»	1,225	1,284

Research on the duration of the preservation of specific immunoglobulins in milk serum during transportation and storage at room temperature was conducted. It was established that the level of the specific signal in positive milk serum in the ELISA remained unchanged for 7–8 days, after which a decrease was observed, leading to non-reactivity in low-titer samples (see Table 2).

Research was also conducted to study the correlation between ELISA with milk and ELISA with blood serum. Previously, it was established that ELISA is a specific and sensitive method for diagnosing brucellosis in cattle. The results of the mandatory complex of serological tests were also taken for comparison.

In the first group of animals from a disease-free farm, 50 cows that had been repeatedly vaccinated against brucellosis were selected. Samples of milk and blood were simultaneously taken from them shortly after the latest revaccination (1.5 months after).

In the analysis of vaccinated animals using milk-based ELISA, positive results were obtained in 46 samples (92%), while in ELISA with blood serum, positive results were obtained in all 50 samples (100%). Only in four milk samples, the results were questionable, with positive results in the blood serum samples, resulting in a correlation of 92.0% of the examined samples (see Table 3).

When comparing the results obtained with traditional blood serum tests, it was found that only 16 animals showed low titers in the agglutination reaction (AR), which is characteristic of post-vaccination reactions. The results of complement fixation tests with S antigen and immunodiffusion with O-PS antigen were negative.

This group of animals was vaccinated against brucellosis, and blood and milk were collected from them at early non-standard intervals. Therefore, the positive results in both milk-based ELISA and blood serum ELISA can be attributed to the higher sensitivity of the method. It also becomes evident that milk and blood from vaccinated animals for immunoenzyme analysis should be collected after 6 months or more following vaccination.

39 cows were selected in the second group of animals, and samples of milk and blood were

Табл. 2. Уровень сигнала в ИФА специфических противобруцеллезных иммуноглобулинов молока в различные сроки инкубации

Table. 2. Signal level in the ELISA of specific anti-brucellosis immunoglobulins of milk at different incubation periods

Incubation, days	Sample number											
	1	2	3	4	5	6	7	8	9	10	11	12
1	1,857	0,560	0,823	0,455	2,216	0,445	1,235	1,583	1,129	1,529	0,481	0,639
2	1,861	0,564	0,818	0,455	2,231	0,443	1,238	1,579	1,220	1,534	0,501	0,649
3	1,868	0,568	0,842	0,458	2,239	0,454	1,309	1,603	1,258	1,561	0,534	0,651
4	1,867	0,561	0,839	0,463	2,225	0,461	1,287	1,591	1,129	1,553	0,498	0,648
5	1,849	0,571	0,835	0,458	2,258	0,449	1,277	1,599	1,209	1,549	0,511	0,643
6	1,872	0,567	0,846	0,465	2,256	0,462	1,289	1,584	1,131	1,551	0,489	0,650
7	1,864	0,564	0,829	0,467	2,273	0,458	1,301	1,595	1,142	1,521	0,490	0,649
8	1,853	0,569	0,801	0,461	2,254	0,449	1,278	1,601	1,132	1,499	0,488	0,647
9	1,812	0,559	0,811	0,435	2,234	0,438	1,263	1,584	1,113	1,541	0,485	0,635
10	1,801	0,551	0,802	0,448	2,012	0,421	1,235	1,579	1,108	1,488	0,451	0,621
11	1,785	0,502	0,783	0,425	1,983	0,398	1,176	1,521	0,961	1,112	0,455	0,599
12	1,631	0,463	0,731	0,385	1,832	**0,335**	0,890	1,381	0,633	0,899	0,401	0,457
13	1,402	0,364	0,632	**0,322**	1,788	**0,301**	0,670	1,278	0,563	0,654	0,378	0,384
14	1,299	**0,320**	0,554	**0,301**	1,701	**0,256**	0,598	1,012	0,488	0,599	**0,322**	**0,341**

Note. Samples with an ELISA result as unreactive are marked in bold type.

taken simultaneously. This group of animals had never been vaccinated against brucellosis. As the research showed, a significant number of diseased animals were detected in the herd, which was considered unfavorable for brucellosis.

In the unvaccinated population of cattle, positive results were obtained in 34 out of 39 milk samples (87.2%) in milk-based ELISA and in 37 out of 38 blood serum samples (97.2%) in ELISA with blood serum. A correlation of 86.8% was registered in 33 out of 39 examined samples (see Table 4).

Analyzing the results obtained, it can be concluded that regardless of the epizootic or immune status (disease-free and unfavorable herds, vaccinated and unvaccinated animals), the correlation between milk-based ELISA and blood serum ELISA ranges from 86.8% to 92.0%.

CONCLUSIONS

1. It has been established that the enzyme-linked immunosorbent assay (ELISA) diagnostic test system developed by the Institute of Experimental Veterinary Science of Siberia and the Far East (SFECA RAS) in collaboration with OOO RPC Sibbiotest is suitable for determining specific anti-brucellosis immunoglobulins in milk serum.

2. It is shown that storage and transportation conditions of milk samples that result in clotting

Табл. 3. Результаты исследования молока и сыворотки крови на вакцинированном против бруцеллеза поголовье крупного рогатого скота (благополучное стадо)

Table 3. Results of milk and blood serum testing on brucellosis-vaccinated cattle (healthy herd)

Item No.	Inventory No.	ELISA with milk		ELISA with blood serum		Other serologic tests		
		Indicator	Result	Indicator	Result	AR	CBR-S	IDR
1	KZF192340333	2,272	Positive	1,861	Positive	Negative	Negative	Negative
2	KZF191261907	1,744	»	2,011	The same	»	The same	The same
3	KZF192340331	2,232	»	2,288	»	50 ME*	»	»
4	KZF192340343	1,565	»	1,960	»	Negative	»	»
5	KZF192340386	2,302	»	1,561	»	50 ME*	»	»
6	KZF192340334	1,852	»	2,080	»	Negative	»	»
7	KZF192359717	1,828	»	2,148	»	»	»	»
8	KZF192037298	0,830	»	2,210	»	»	»	»
9	KZF192340335	1,527	»	2,040	»	50 ME*	»	»
10	KZF192340328	1,017	»	2,088	»	Negative	»	»
11	KZF192359715	1,619	»	1,913	»	50 ME*	»	»
12	KZF192340382	0,897	»	1,419	»	Negative	»	»
13	KZF191088986	1,428	»	1,875	»	»	»	»
14	KZF191088635	2,113	»	2,424	»	50 ME*	»	»
15	KZF191088978	0,770	»	1,866	»	50 ME*	»	»
16	KZF192340348	1,290	»	1,415	»	Negative	»	»
17	KZF189138202	1,393	»	2,036	»	»	»	»
18	KZF192359721	2,283	»	2,016	»	»	»	»
19	KZF192340395	1,604	»	2,294	»	»	»	»
20	KZF191261835	2,296	»	1,898	»	»	»	»
21	KZF191089174	1,342	»	1,874	»	»	»	»
22	KZF191261922	1,016	»	2,108	»	»	»	»
23	KZF190473568	2,191	»	2,181	»	50 ME*	»	»
24	KZF192340332	0,540	Doubtful	1,558	«	Negative	«	«
25	KZF189857819	1,196	Positive	1,771	«	50 ME*	«	«
26	KZF192340392	0,906	»	1,647	»	50 ME*	»	»
27	KZF189211198	1,959	»	2,191	»	Negative	»	»
28	KZF191089171	1,898	»	2,023	»	50 ME*	»	»
29	KZF191089163	1,001	»	1,536	»	50 ME*	»	»
30	KZF189857842	0,946	»	2,126	»	50 ME*	»	»
31	KZF190096431	1,729	»	1,935	»	50 ME*	»	»
32	KZF190121536	2,113	»	2,086	»	Negative	»	»
33	KZF191261920	1,955	»	2,364	»	50 ME*	»	»
34	KZF192340369	1,897	»	1,931	»	Negative	»	»
35	KZF192340387	1,367	»	1,951	»	»	»	»
36	KZF192359725	0,843	»	1,502	»	»	»	»
37	KZF191089025	1,615	»	1,900	»	50 ME*	»	»
38	KZF191126039	1,529	»	2,089	»	Negative	»	»
39	KZF192367987	1,908	»	2,258	»	»	»	»
40	KZF192340381	0,923	»	1,112	»	»	»	»
41	KZF189429652	0,648	Doubtful	1,969	»	»	»	»
42	KZF192340337	2,074	Positive	2,223	»	»	»	»
43	KZF190096491	1,161	»	1,512	»	»	»	»
44	KZF191261908	0,373	Doubtful	1,409	»	»	»	»
45	KZF191261840	1,975	Positive	1,914	»	»	»	»
46	KZF190096500	1,084	»	2,264	»	»	»	»
47	KZF189429656	0,317	Doubtful	1,525	»	»	»	»
48	KZF190096419	0,720	Positive	2,043	»	»	»	»
49	KZF192340336	0,987	»	1,499	»	50 ME*	»	»
50	KZF192368020	2,212	»	1,967	No serum	Negative	»	»

* The result of AR 50 IU on brucellosis vaccinated cattle (in a healthy herd) is considered doubtful.

Табл. 4. Результаты исследования молока и сыворотки крови на не вакцинированном против бруцеллеза поголовье крупного рогатого скота (неблагополучное стадо)

Table 4. Results of milk and blood serum testing on bovine cattle not vaccinated against brucellosis (unfavorable herd)

Sample number	Inventory No.	ELISA with milk		ELISA with blood serum		Other serologic tests		
		Indicator	Result	Indicator	Result	AR	CBR-S	IDR
1	90097334**	2,139	Positive	2,275	Positive	Negative	1 : 10+++	Negative
2	90097053**	2,292	The same	2,321	The same	200 ME*	1 : 20++++	+48 ч
3	90633070**	2,193	»	2,317	»	Negative	1 : 5+++	Negative
4	90097363**	2,274	»	2,357	»	200 ME*	1 : 20++++	+24 ч
5	90097050**	2,146	»	2,322	»	200 ME*	1 : 20++++	+24 ч
6	90097064**	2,165	»	2,107	»	50 ME	1 : 5+++	Negative
7	90097056**	2,132	»	2,151	»	200 ME*	1 : 10++	»
8	90321543	0,142	Negative	1,208	»	Negative	Negative	»
9	90097357**	2,235	Positive	2,112	»	50 ME	1 : 20++++	»
10	90097337**	2,310	The same	2,401	»	200 ME*	1 : 20++	+48 ч
11	90097095**	2,100	»	2,276	»	Negative	1 : 20++++	Negative
12	90097324**	2,293	»	2,311	»	50 ME	1 : 5++++	«
13	90097078**	2,369	»	2,391	»	200 ME **	1 : 20++++	+24 ч
14	90097089**	0,458	Doubtful	1,553	»	50 ME	1 : 20++++	Negative
15	90097079**	2,364	Positive	2,382	»	200 ME*	1 : 20++++	+24 ч
16	90324551	0,072	Negative	0,241	Negative	Negative	Negative	Negative
17	90097371**	2,214	Positive	2,361	Positive	100 ME*	1 : 20++++	+24 ч
18	91073268**	0,018	Negative	2,252	The same	50 ME*	1 : 10++++	+48 ч
19	90097372	1,658	Positive	2,151	»	Negative	Negative	Negative
20	90097039**	2,098	The same	2,344	»	100 ME*	1 : 20+++	»
21	90097355**	2,417	»	2,334	»	200 ME *	1 : 20++++	»
22	90097336**	2,172	»	2,352	»	200 ME*	1 : 20++++	+24 ч
23	90097076**	2,390	»	2,453	»	200 ME*	1 : 20++++	+24 ч
24	90633071**	2,178	»	2,372	»	200 ME*	1 : 20+++	+24 ч
25	90097331**	2,035	»	2,450	»	50 ME	1 : 20+++	+24 ч
26	90097342**	2,207	»	2,208	»	50 ME	1 : 5++++	Negative
27	90097333**	2,259	»	2,405	»	100 ME*	1 : 20++++	+24 ч
28	90097062**	2,285	»	2,322	»	100 ME**	1 : 20+++	+48 ч
29	90097325**	2,232	Positive	2,358	Positive	50 ME	1 : 20++++	Negative
30	90321801**	2,302	The same	2,361	The same	50 ME	1 : 5++++	The same
31	90097055**	2,167	»	2,156	»	100 ME*	1 : 10++	»
32	90097362**	2,291	»	2,301	»	50 ME	1 : 20+++	»
33	90321768**	1,903	»	2,325	»	100 ME*	1 : 10+++	+48 ч
34	90097351**	2,203	»	2,260	»	200 ME*	1 : 20+++	+24 ч
35	90097356	0,670	Doubtful	1,189	»	Negative	Negative	Negative
36	90097048**	2,102	Positive	2,325	»	50 ME	1 : 10++	То же
37	97073220**	2,207	The same	2,421	»	100 ME*	1 : 20++++	+24 ч
38	91073265**	2,238	»	2,322	»	100 ME*	1:20++++	+48 ч
39	89202667**	2,330	»	2,347	Not studied	Negative	1 : 10+++	Negative

* The result of AR 100 IU and above and/or CBR 1 : 5 and above on unvaccinated against brucellosis cattle (in an unfavourable herd) is considered positive..

** The animals were recognized as sick and sent to slaughter.

at room temperature do not affect the level of specific anti-brucellosis immunoglobulins for at least 8 days. This eliminates the need for a cold chain during transportation of the milk samples to the testing site.

3. It has been found that in a vaccinated population of cattle against brucellosis, the correlation between milk-based ELISA and blood serum ELISA is 92.0%. It should be noted that milk and blood for ELISA analysis should be collected after 6 months or more following vaccination (according to the instructions).

4. In an unvaccinated population of cattle against brucellosis, the correlation between milk-based ELISA and blood serum ELISA is 86.8%, with results matching in 33 out of 39 samples.

5. Regardless of the epizootic or immune status (disease-free and unfavorable herds, vaccinated and unvaccinated animals), the correlation between milk-based ELISA and blood serum ELISA ranges from 86.8% to 92.0%.

6. The high correlation percentage provides grounds for using milk initially for brucellosis diagnosis, and if positive results are obtained, then blood can be collected from such animals for comprehensive brucellosis testing.

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ЭФФЕКТИВНОСТЬ ПРИМЕНЕНИЯ ТКАНЕВОГО БИОСТИМУЛЯТОРА ПРИ ВЫРАЩИВАНИИ ТЕЛОК

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Представлены материалы исследования эффективности применения тканевого биостимулятора в технологии выращивания ремонтных телок. Эксперимент проведен в условиях Алтайского края на четырех группах телочек приобского типа черно-пестрой породы живой массой 51,3 ± 1,48 кг в возрасте 1 мес. В каждой группе было по 10 гол. Продолжительность опыта составила 18 мес. Животным контрольной группы каждый месяц вводили подкожно физиологический раствор: с 1-го по 5-й месяц – в дозе 3,0 мл/гол., с 6-го по 11-й месяц – 6 мл/гол., с 12-го по 15-й месяц – 12,0 мл/гол. и с 16-го по 18-й месяц – 15,0 мл/гол. Телкам опытных групп делали инъекции тканевого биостимулятора по следующим схемам: в 1-й опытной группе – с 1-го по 5-й месяц – в дозе 2,0 мл/гол., с 6-го по 11-й месяц – 4 мл/гол., с 12-го по 15-й месяц – 8,0 мл/гол. и с 16-го по 18-й месяц – 10,0 мл/гол.; во 2-й опытной группе – с 1-го по 5-й месяц – в дозе 3,0 мл/гол., с 6-го по 11-й месяц – 6 мл/гол., с 12-го по 15-й месяц – 12,0 мл/гол. и с 16-го по 18-й месяц – 15,0 мл/гол.; в 3-й опытной группе – с 1-го по 5-й месяц – в дозе 4,0 мл/гол., с 6-го по 11-й месяц – 8,0 мл/гол., с 12-го по 15-й месяц – 16,0 мл/гол., с 16-го по 18-й месяц – 20,0 мл/гол. Биостимулятор изготовлен из боенских отходов и субпродуктов пантовых оленей. Схема его использования, применяемая во 2-й опытной группе животных, оказалась наиболее эффективной и способствовала повышению массы тела у ремонтных телок до 14% ($p < 0,001$), среднесуточного прироста – до 33% ($p < 0,001$), абсолютного прироста – до 23% ($p < 0,001$) и относительного прироста – до 2% ($p < 0,05$).

Ключевые слова: крупный рогатый скот, ремонтные телки, тканевый препарат, живая масса, абсолютный прирост, среднесуточный прирост, относительный прирост, интенсивность роста

EFFICIENCY OF TISSUE BIOSTIMULANT APPLICATION IN GROWING HEIFERS

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The materials of research on the effectiveness of tissue biostimulant application in the technology of breeding replacement heifers are presented. The experiment was conducted in the conditions of the Altai Territory on four groups of heifers of the Priobsky type of the Black-and-White breed with a live weight of 51.3 ± 1.48 kg at the age of 1 month. Each group had ten heads. The experiment lasted for 18 months. Animals of the control group were injected subcutaneously with physiological solution every month: from the 1st to the 5th month – at a dose of 3.0 ml/head, from the 6th to the 11th month – 6 ml/head, from the 12th to the 15th month – 12.0 ml/head and from the 16th to the 18th month – 15.0 ml/head. Injections of tissue biostimulant were given to the heifers of the experimental groups according to the following schemes: in the 1st experimental group – from the 1st to the 5th month – at a dose of 2.0 ml/head, from the 6th to the 11th month – 4 ml/head, from the 12th to the 15th month – 8.0 ml/head and from the 16th to the 18th month – 10.0 ml/head; in the 2nd experimental group – from the 1st to the 5th month – at a dose of 3.0 ml/head, from the 6th to the 11th month – 6 ml/head, from the 12th to the 15th month – 12.0 ml/head and from the 16th to the 18th month – 15.0 ml/head; in the 3rd experimental group – from 1st to 5th month – at a dose of 4.0 ml/head, from the 6th to the 11th month – 8.0 ml/head, from the 12th to the 15th month – 16.0 ml/head, from the 16th to the 18th month – 20.0 ml/head. Biostimulant is made of slaughter house tankage and by-products of the antler deer.

The scheme of its application, used in the 2nd experimental group of animals, was the most effective and contributed to the increase in body weight in replacement heifers up to 14% ($p < 0.001$), average daily gain – up to 33% ($p < 0.001$), absolute gain – up to 23% ($p < 0.001$) and relative gain – up to 2% ($p < 0.05$).

Keywords: cattle, replacement heifers, tissue preparation, live weight, absolute gain, average daily gain, relative gain, growth intensity

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INTRODUCTION

Increasing the growth rate of replacement heifers allows for a significant reduction in the time it takes to raise cows, increases the live weight of first-calving heifers, and results in higher milk yields in their first lactation. In practical farming, it is essential to plan for the intensity of heifer growth, which ensures that the animals achieve live weights corresponding to breed standards at all age periods [1].

The organization of the replacement heifer rearing process, where animals are inseminated at a younger age, and calving occurs at 23–24 months, is a necessary condition for intensive milk production technology, which enhances milk productivity and economic indicators of the farm [2].

The technology for raising replacement heifers, which promotes the expression of the animals' inherited productive traits, must be economically viable. An essential condition for realizing the genetic potential of animals is the intensity of their growth [3].

In industrial livestock production, animals of modern dairy breeds and types are characterized by genetically determined high productivity. At the same time, this makes them exceptionally susceptible to the influence of adverse environmental factors. Therefore, when raising replacement young stock, it is necessary to create optimal conditions for their maintenance and feeding [4, 5].

Enhancing metabolic processes opens up reserve opportunities for increasing agricultural production without raising feed costs by identifying factors that contribute to the better utilization of the genetic potential. In this regard, methods related to the use of biologically active preparations as means of reducing the adverse effects of external factors on the organism and acting as regulators of metabolism to improve the efficiency of using the basic diet are used. This approach ensures the development of the feed base, selection, and genetic engineering [6].

Tissue preparations are among the biological growth stimulators. The use of biostimulants has a positive effect on animals (from immune system correction to stimulation of the body's enzymatic and hormonal systems). The use of biogenic stimulants in raising young livestock contributes to a reduction in feed costs, a decrease in the duration of rearing, an increase in herd survival, and an increase in the industry's profitability [7–9].

The purpose of the study is to investigate the effectiveness of using a tissue biostimulator in the technology of raising replacement heifers.

MATERIALS AND METHODS

The scientific and economic experiment was conducted in 2020 and 2021 at the Uchhoz Prigorodnoe AO in the Industrial District of Barnaul, Altai Territory, Russia. The experiment scheme is presented in the table.

To conduct the experiment based on the principle of analogs, four groups of replacement heifers were formed, each consisting of 10 heads. When selecting, age (1 month) and live weight (51.3 ± 1.48 kg) were taken into account. The duration of the experiment was 18 months.

The material for the tissue biostimulator was obtained from: uteri with fetuses (2–3 months), placenta, liver, spleen, mesenteric lymph nodes and interpleural space, collected under aseptic conditions during slaughter. The animals were healthy.

The live weight of replacement heifers was determined by individual weighing on VEP-X-N scales with an accuracy of 1 kg, starting from the 1st month, and then every month during the growth period, until the replacement young cattle reached the age of 18 months. Based on the live weight data of heifers in the age dynamics, the average daily, absolute, and relative weight gain for each month of growth were calculated using the generally accepted formula.

The obtained data were subjected to biometric processing using Microsoft Excel 2016 software. The reliability of the experiment results compared to the control group was calculated using the *t*-Student criterion for independent samples.

RESULTS AND DISCUSSION

Rearing replacement young cattle is one of the most important technological moments in the dairy cattle industry, as successful rearing of young cattle is the basis for high production performance [10]. The dynamics of live weight of replacement young cattle of the experimental groups are presented in Fig. 1.

From the analysis of the data presented in Fig. 1, it can be concluded that the introduction of a tissue biostimulator in different doses to replacement young cattle contributed to an increase in live weight at the age of 2 months in the 1st experimental group by 3.7%, in the 2nd group by 5.0%, in the 3rd group by 4.9% ($p < 0.05$). At the age of 3 and 4 months, the heifers of the 3rd experimental group showed the highest live weight, surpassing the control by 7.8% ($p < 0.01$) and 9.8% ($p < 0.001$), respectively. Animals in the 1st and 2nd experimental groups in the considered age periods also outperformed the control by 5.6–9.6% ($p < 0.001$). At the age of 5–6 months, the highest live weight was observed in the calves of the 2nd experimental group, to which the tissue biostimulator was administered at a dose of 3 ml/head. By this value, they exceeded the control by 11.4% ($p < 0.001$) and 12.6% ($p < 0.001$). Heifers of the 1st exper-

Схема научно-хозяйственного эксперимента
Scheme of the scientific and economic experiment

Group	*n*	Preparation	Age of replacement heifers at drug administration, months	Dose of subcutaneous injection of the drug, ml/goal.	Frequency and interval of drug administration
Control	10	Physiological solution	1–5 6–11 12–15 16–18	3,0 6,0 12,0 15,0	18 times with an interval of 30 days
Experimental:					
1-я	10	Tissue biostimulant	1–5 6–11 12–15 16–18	2,0 4,0 8,0 10,0	The same
2-я	10	The same	1–5 6–11 12–15 16–18	3,0 6,0 12,0 15,0	»
3-я	10	»	1–5 6–11 12–15 16–18	4,0 8,0 16,0 20,0	»

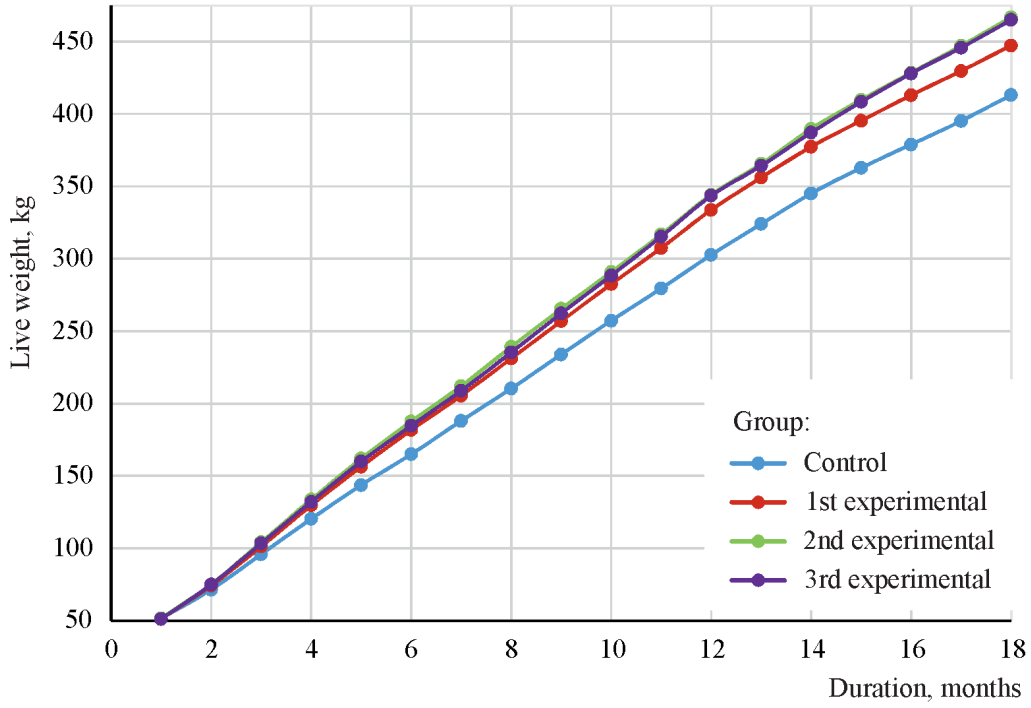

Рис. 1. Динамика живой массы ремонтного молодняка, кг
Fig. 1. Dynamics of the live weight of replacement young animals, kg

imental group in terms of live weight at the age of 5 and 6 months exceeded the animals of the intact group by 8.8% ($p < 0.001$) and 10.1% ($p < 0.001$), respectively. The analogs of the 3rd experimental group exceeded by 11.2% ($p < 0.001$) and 11.9% ($p < 0.001$), respectively.

In the age periods of 7–11 months of growth, the young cattle of the 2nd experimental group showed the highest live weight, exceeding the control counterparts by 12.0–13.2% ($p \leq 0.001$). Heifers of the 1st and 3rd experimental groups in the same age periods had a live weight greater by 9.3–12.8% ($p \leq 0.001$) compared to the control.

Animals in the experimental groups at the age of 12 months exceeded the control group by 10.2% ($p < 0.001$), 13.2% ($p < 0.001$), and 13.4% ($p < 0.001$), respectively.

The live weight of replacement heifers in the experimental groups at the age of 13–14 months was at a higher level compared to similar values in the control group. In the 1st experimental group, it was higher by 9–10% ($p < 0.001$), in the 2nd group by 13% ($p < 0.001$), and in the 3rd group by 12% ($p < 0.001$).

The highest live weight from the 15th to the 18th month of growth was observed in the heifers of the 2nd experimental group, which was 13% ($p < 0.001$) higher than in the control. In these age periods, the replacement young cattle of the 1st and 3rd experimental groups in terms of live weight exceeded the intact group by 8–13% ($p < 0.001$).

The indicators for average daily weight gain of replacement young cattle are presented in Figure 2. The analysis of the dynamics of average daily weight gains (see Fig.2) shows that in the 1–3-month period, the highest average daily weight gain was observed in the young cattle of the 3rd experimental group, which was 20% and 16% ($p < 0.001$) higher than in the control.

For replacement heifers of the 1st and 2nd experimental groups, daily weight gains during the 1–2-month period increased by 11% and 18% ($p < 0.05$), and during the 2–3-month period, by 11% and 16% ($p < 0.001$), respectively, compared to the control. In the 3–4-month period, the average daily weight gains of replacement young cattle in the 1st, 2nd, and 3rd experimental groups exceeded the control by 16–17% ($p < 0.001$), respectively. The highest growth intensity in the age periods of 4–5 and 5–6 months was observed in the heifers of the 2nd experimental

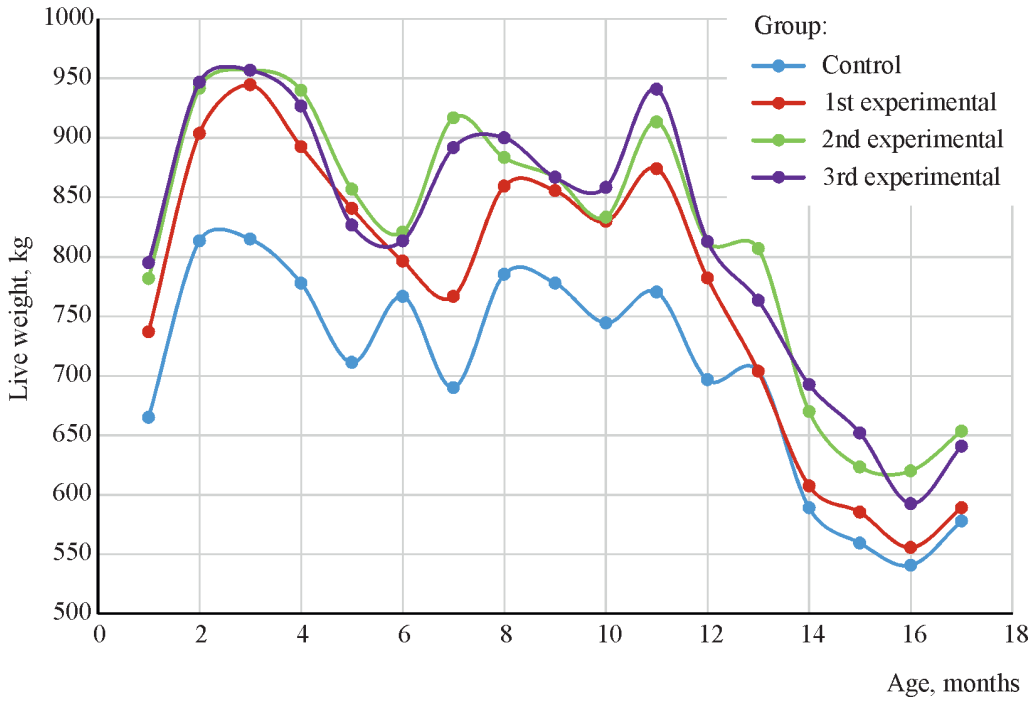

Рис. 2. Динамика среднесуточных приростов живой массы ремонтного молодняка, г
Fig. 2. Dynamics of average daily increases in the live weight of replacement young animals, g

group, which exceeded the control counterparts by 21% ($p < 0.001$) and 20% ($p < 0.001$), respectively. The young cattle of the 1st and 3rd experimental groups also showed the highest average daily weight gain values, by 15% and 19% ($p < 0.001$), compared to the control animals.

In the growth periods of 6–8 months, the highest indicators of average daily weight gain were observed in the young cattle of the 2nd experimental group, which exceeded the control group by 7–33% ($p < 0.001$). Replacement heifers of the 1st and 3rd experimental groups also outperformed the intact animals by 4–29% ($p < 0.001$) in terms of these indicators. In the growth periods of 7–12 months, the animals of the 3rd experimental group showed the highest growth intensity, surpassing the control by 11–22% ($p < 0.001$). Animals of the 1st and 2nd experimental groups also outperformed the control by 9–17% in the same age periods.

The value of average daily weight gain for heifers of the 2nd and 3rd experimental groups at the age of 12 to 14 months was 8–17% ($p < 0.001$) higher compared to the control. For the young cattle of the 1st experimental group, the average daily weight gains in the considered age periods were at a higher level, up to 12% ($p < 0.001$), than in the control group. From the 14th to the 18th month of growth, the average daily weight gains in the animals of the experimental groups exceeded the control by 2–18% ($p < 0.01$).

The mechanism of action of the obtained biogenic stimulators is based on the biological activity of the substances they contain (amino acids, peptides, nucleic acids, polysaccharides, phospholipids, vitamins, microelements, etc.). They stimulate the reactions of cellular and humoral immunity, increase non-specific resistance of the body, activate metabolic processes, and have antioxidant and stress-protective effects. The main role in the mechanism of action of tissue preparations is assigned to the neurohumoral and humoral systems, the basis of which is the central nervous system and the hypothalamic-pituitary complex. It has been established that the main role in changing the body's resistance to external influences belongs to the nervous system, its adaptive-trophic function. The hypothalamic-pituitary complex regulates neuroendocrine activity and maintains the body's homeostasis [11, 12].

The anabolic nature of metabolism during the period of rapid growth leads to changes and redistribution of major metabolic flows towards tissue-building processes [13, 14]. When tissue biostimulators are used, there is an activation of metabolic processes. This occurs because the primary point of action of tissue preparations is the reception of conversion of mechanical, chemical, and other irritants into nervous signals, directly related to the central nervous system and all links of the neurohumoral apparatus, which determine the diversity of physiological manifestations of the stimulating substrate's action [15].

CONCLUSION

The use of tissue biostimulators in the technology of rearing replacement heifers contributed to the increase in their growth intensity. The best results were achieved by the heifers of the 2nd experimental group, to which the tissue biostimulator was administered at a dose of 3.0 ml/head from the 1st to the 5th month of growth, 6.0 ml/head from the 6th to the 11th month, 12.0 ml/head from the 12th to the 15th month, and 15.0 ml/head from the 16th to the 18th month. This led to an increase in live weight by 14% ($p < 0.001$), average daily weight gain by 33% ($p < 0.001$), absolute weight gain by 23% ($p < 0.001$), and relative weight gain by 2% ($p < 0.05$).

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БИОТЕХНОЛОГИИ ТУТОВОГО ШЕЛКОПРЯДА КАК БАЗИС БИОИНДУСТРИАЛЬНОЙ ПЛАТФОРМЫ. УЛУЧШЕНИЯ НА ЭТАПЕ ВОСХОДЯЩЕГО ПРОЦЕССА (USP)

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Молекулярная инженерия – это метод инженерии «снизу вверх» для создания функциональных материалов и устройств с использованием молекул и атомов в качестве строительных блоков. В 2000 г. Япония стала первой страной в мире, которая генетически модифицировала тутового шелкопряда (*Bombyx mori*, далее *B. mori)*. Последующее за этим развитие исследований в области разработки новых материалов расширили возможности использования продукции шелководства, характеризуя эту ситуацию как «революция в шелководстве». В Российской Федерации молекулярная инженерия в науках о жизни направлена на решение задач по разработке технологических платформ мирового уровня с целью создания инструментов для получения новых молекул (биополимеров, белков, ферментов), биопродуктов, клеток и организмов. Изучены основные подходы «снизу вверх», применяемые на этапе восходящего процесса (USP) в шелководстве для улучшения производственно-экономических показателей и качественных характеристик сырья. Разнообразие способов улучшения включает: использование искусственной питательной среды; молекулярную инженерию, основанную на методах транзиентной экспрессии или стабильной трансформации зародышевой линии; генетические методы селекции; управление размножением и др. Преимущества тутового шелкопряда (*B. mori*): низкая стоимость разведения, значительно более высокий выход продукции по сравнению с другими системами экспрессии белка – способствуют его использованию в качестве эффективного продуцента рекомбинантных белков, антимикробных пептидов и биологически активных веществ. Биотехнологии этапа USP позволяют получать новые виды сырья для последующего преобразования в нисходящем процессе (DSP) для получения широкого спектра продуктов, способствующих улучшению качества жизни людей. Комплекс биотехнологических решений составляет современный базис биоиндустриальной платформы тутового шелкопряда.

Ключевые слова: тутовый шелкопряд, искусственная питательная среда, молекулярная инженерия, рекомбинантные белки, антимикробные пептиды

MULBERRY SILKWORM BIOTECHNOLOGY AS THE BASIS OF A BIOINDUSTRIAL PLATFORM. IMPROVEMENTS ON THE UPSTREAM PROCESSING STAGE (USP)

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Molecular engineering is an upstream engineering method for creating functional materials and devices using molecules, and atoms as building blocks. In 2000, Japan became the first country to genetically modify silkworms (*Bombyx mori*, hereinafter referred to as *B. mori*). The subsequent research in the development of new materials expanded the possibilities of using sericulture products,

characterizing this situation as a "sericulture revolution". In the Russian Federation, molecular engineering in life sciences is aimed at solving the tasks of developing world-class technological platforms to create tools to produce new molecules (biopolymers, proteins, enzymes), bioproducts, cells and organisms. The main "bottom-up" approaches applied in the upstream process (USP) stage of silk production to improve the production and economic performance and quality characteristics of raw materials have been studied. The variety of improvement methods includes: the possibility of using artificial nutrient medium, molecular engineering based on transient expression techniques or stable germline transformation, genetic selection methods, breeding management, etc. The advantages of the mulberry silkworm (*B. mori*) such as low breeding costs, significantly higher production yield compared to other protein expression systems, favor its use as an effective producer of recombinant proteins, antimicrobial peptides and biologically active substances. USP stage biotechnologies enable the production of new raw materials for downstream processing (DSP) to produce a wide range of products that contribute to improving the quality of human life. A set of biotechnological solutions forms the modern basis of the mulberry silkworm bioindustrial platform.

Keywords: mulberry silkworm, artificial nutrient medium, molecular engineering, recombinant proteins, antimicrobial peptides

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INTRODUCTION

Being the only fully domesticated species of invertebrate, *B. mori* has the potential to help understand the similarities and differences in numerous processes, including domestication in vertebrates and plants. Domestication of mulberry silkworm has affected various characteristics, such as the cocoon shell weight: in *B. mori*, it is 0.5 g, which is 10 times more than in *B. mandarina* (wild mulberry silkworm) – 0.04–0.07 g[1][1].

In 2004, a preliminary genome sequence of the domesticated mulberry silkworm was presented[2]. The analysis of the mulberry silkworm genome project systematically demonstrated key genes related to economic traits, growth, development, sex regulation, and mulberry silkworm disease resistance. An international research group led by Southwest University (State Key Laboratory of Silkworm Genome Biology, Chongqing Engineering and Technology Research Center for Novel Silk Materials, Chongqing 400716) completed an accurate mulberry silkworm genome map in 2008[3] and a map of genetic information variability in 40 silkworm genomes in 2009[4] as part of international coop-

[1]*Ômura S.* Research on the behavior and ecological characteristics of the wild silkworm, Bombyx mandarina // Bull. Seric. Exp. Sta. Jpn. 1950, vol. 13, pp. 7–130.

[2]*Xia Q. et al*. A draft sequence for the genome of the domesticated silkworm (Bombyx mori) // Science. 2004. DOI: 10.1126/ science.1102210.

[3]International Silkworm Genome Consortium et al. The genome of a lepidopteran model insect, the silkworm Bombyx mori // Insect biochemistry and molecular biology, 2008, vol. 38, N 12, p. 1036–1045. DOI: 10.1016/j.ibmb.2008.11.004.

[4]*Xia Q., Guo Y., Zhang Z., Li D., Xuan Z., Li Z., Wang J*. Complete resequencing of 40 genomes reveals domestication events and genes in silkworm (Bombyx) // Science, 2009, vol. 326, N 5951, pp. 433–436. DOI: 10.1126/science.1176620.

eration implementing the "Three-step Silkworm Genome Project."

Transgenic technology has been widely used in fundamental research on mulberry silkworms and in the creation of industrial materials, especially in improving silk production and quality, enhancing antiviral genetic characteristics, and in research and development of bioreactors, which accelerated the introduction of genetically modified materials [2].

The "bottom-up" materials design opens up significant possibilities for creating predictable functional results. Recombinant DNA technology provides a systematic approach to replicating, modifying, and evaluating peptide combinations of silk core structure, and then to biosynthesizing polymers based on silk according to a given design. Post-biosynthesis processing allows for the use of another dimension of material design through controlled or auxiliary assembly. Integration of biosynthesis, processing, multiscale modeling, and experimental testing provides a path to creating *de novo* silk-based materials with individual properties [3].

The research goal is to systematize current information on a set of improvements used in the upstream processing stage (Upstream Processing – USP) to obtain new options for silk production raw materials and products.

MATERIAL AND METHODS

Derived products of *B. mori* (larvae (caterpillars), cocoon shells, pupae) include antimicrobial peptides (AMP); chitin and chitosan; various silk variants: with improved characteristics (a combination of reliable mechanical properties and high biocompatibility), recombinant silk (recombinant spidroin), and artificial super-strong silk (superior to natural spider silk). The mulberry silkworm itself serves as a platform for a fast and cost-effective process of obtaining recombinant proteins, including monoclonal antibodies and vaccines. These products will find wide application in Russia as a functional biomaterial for drug and gene delivery, chemotherapy drug delivery, wound healing, tissue engineering, flexible electronics, bio-inks for 3D bioprinting, cosmetics and nutraceuticals, catalysts, and more.

RESULTS AND DISCUSSION

Methods of improvement

Baseline improvement – artificial nutrient medium

The development of *B. mori* biotechnologies currently cannot proceed without the use of artificial nutrient medium (ANM or artificial diet), which allows for the mitigation of seasonality factors in the cultivation of silkworms using mulberry leaves. For countries with a temperate climate, including the Russian Federation (RF), this improvement is crucial, as the traditional method of silkworm rearing only allows for two rounds of feeding during the summer-autumn period. The population density of silkworms grown on ANM is almost 2-3 times higher than when cultivated on mulberry leaves, significantly reducing the total production area required for cultivation. Substantial reduction in labor costs for cultivation significantly improves production process indicators, and further scale-up allows for achieving economies of scale. In the scientific field, ANM intensifies research work with subsequent commercialization of its results.

In 2023, the Silkworm Research Station is working on developing its own composition of an artificial diet, which includes mulberry leaf powder, as well as other components from plant raw materials. Susceptibility to the artificial diet was tested on over 40 strains of nine geographical groups from the living collection of the Silkworm Research Station of domestic and foreign collections. Test feedings of caterpillars, originally planned as screening for susceptibility to ANM for silkworm strains and for subsequent work with selected strains, showed that most strains exhibited a pronounced response to this type of nutrient medium. High viability rates during cultivation on ANM were demonstrated by 60% of the strains in the biocollection.

Solving one of the fundamental key tasks in developing and creating a functional ANM that allows for year-round scientific research work with mulberry silkworms and, with subsequent

optimization of the cultivation process, significantly reduces the gap between Russia and leading countries in the field of mulberry silkworm biotechnology: Japan, China, and the USA.

Molecular engineering. B. mori transgenesis

Transient (viral) expression

The domesticated silkworm *B. mori* represents an insect model of great scientific and economic value. Besides establishing stable transformation of the embryonic line using the PiggyBac vector, technically feasible methods for gene delivery *in vivo* and transient gene expression using viral vectors, especially Sindbis viruses and baculoviruses, have been developed. The recombinant baculovirus, the multiple nucleopolyhedrovirus *Autographa californica* (AcMNPV), typically used for large-scale protein production in permissive cell lines or insects, was used for transferring foreign genes into specific peptidergic cells of *B. mori in vivo*[5]. However, a drawback of using *in vivo* AcMNPV is its pathogenic effects on mulberry silkworms. Larvae or pupae of sensitive strains usually die within 1-2 weeks after AcMNPV injection. These developmental changes in larvae were prevented by disrupting the nonessential early gene encoding ETG[6, 7]. As targeted gene expression is essential for functional analysis of neuropeptide genes and their receptors, baculovirus-mediated gene transfer can serve as a reliable approach in reverse genetic studies of mulberry silkworms. Gene transfer methods and other reverse genetics methods provide powerful tools for functional analysis of genes and their products, as well as for elucidating the molecular mechanisms underlying a wide range of biological processes. Successful transgenesis of an additional insect species, *B. mori*, opens up new prospects in fundamental and applied research. One of the promising approaches to studying gene functions is transient expression of foreign genes using viral vectors. In these cases, somatic transformation, transgenes do not stably integrate into the host genome, and the successful gene transfer depends on the virus's ability to infect target tissues of the permissive host. Simple injection of a viral construct into the host at any stage of development provides excellent opportunities for introducing various genes or molecular markers into target cells or tissues and studying the functional outcomes of their expression. Thus, viral systems are relatively simple and efficient tools for reporter analysis or physiological and behavioral analyses (see footnote 5).

Sindbis Virus (SINV) is currently used as a highly effective transducing agent in insect biology. However, SINV-mediated expression of ectopic genes or RNA interference may be limited by tissue tropism during viral infection. For example, some organs in *B. mori*, such as the gonads, Malpighian tubules, and larval epidermis, are resistant to SINV infection[8]. There are also limitations to SINV-based vectors, including instability of recombinant clones after multiple passages in cultured cells[9]. Furthermore, the virus infects mammalian cells, making SINV infection potentially hazardous to humans.

The first successful introduction of genes into *B. mori* was achieved using recombinant BmSNPV-mediated *in vivo* expression of genes. The expression of the chorion genes (BmSNPV - *B. mori* nuclear polyhedrosis virus) is controlled by their own regulatory elements. Infection of mulberry silkworm pupae with a recombinant virus

[5]*Daubnerová I., Roller L., Žitňan D*. Transgenesis approaches for functional analysis of peptidergic cells in the silkworm Bombyx mori // General and comparative endocrinology, 2009, vol. 162, N 1, pp. 36–42. DOI: 10.1016/j.ygcen.2008.11.028.

[6]*Shikata M., Shibata H., Sakurai M., Sano Y., Hashimoto Y., Matsumoto T*. The ecdysteroid UDP-glucosyltransferase gene of Autographa californica nucleopolyhedrovirus alters the moulting and metamorphosis of a non-target insect, the silkworm, Bombyx mori (Lepidoptera, Bombycidae) // Journal of general virology, 1998, vol. 79, N 6, pp. 1547–1551. DOI: 10.1099/0022-1317-79-6-1547.

[7]*Guo T.Q., Wang J.Y., Guo X.Y., Wang S.P., Lu C.D*. Transient in vivo gene delivery to the silkworm Bombyx mori by EGT-null recombinant AcNPV using EGFP as a reporter // Archives of virology, 2005, vol. 150, pp. 93–105. DOI: 10.1007/s00705-004-0383-y.

[8]*Uhlirova M., Foy B.D., Beaty B.J., Olson K.E., Riddiford L.M., Jindra M*. Use of Sindbis virus-mediated RNA interference to demonstrate a conserved role of Broad-Complex in insect metamorphosis // Proceedings of the National Academy of Sciences, 2003, vol. 100, N 26, pp. 15607–15612. DOI: 10.1073/pnas.2136837100.

[9]*Foy B.D., Myles K.M., Pierro D.J., Sanchez-Vargas I., Uhlířová M., Jindra M., Olson K.E*. Development of a new Sindbis virus transducing system and its characterization in three Culicine mosquitoes and two Lepidopteran species // Insect molecular biology, 2004, vol. 13, N 1, pp. 89–100. DOI: 10.1111/j.1365-2583.2004.00464.x.

led to transient and tissue-specific chorion expression[10]. I. Daubnerová et al. (2009) used the convenient Bac-to-Bac ® baculovirus expression system (Invitrogen) to introduce genes and transient genetic manipulations into permissive strains of mulberry silkworm to elucidate questions regarding neuropeptide signaling required for normal development and behavior (see footnote 5).

B. mori larvae have been used for decades as bioreactors for producing recombinant proteins. In 1985, S. Maeda et al. reported the production of human alpha-interferon (IFN-α) in the hemolymph of mulberry silkworm larvae using BmNPV (*Bombyx mori* nucleopolyhedrovirus) containing the gene encoding human alpha-interferon, controlled by the polyhedrin promoter[11]. Many eukaryotic proteins have been expressed in *B. mori* larvae and purified. In general, the level of expression of recombinant proteins in silkworm larvae is higher than in insect and animal cell cultures.

The BmNPV bacmid system, constructed by Motohashi et al. in 2005[12], requires only the injection of BmNPV bacmid DNA into mulberry silkworm larvae and pupae, which provides rapid expression of recombinant proteins as it eliminates the need to prepare a baculovirus solution by transfection compared to the baculovirus expression system using cultured cells. Furthermore, this BmNPV bacmid system significantly reduces the time required for recombinant protein production through expression in mulberry silkworms. To improve protein expression, modified BmNPV bacmids were constructed: the BmNPV-CP bacmid, a hybrid nucleopolyhedrovirus (HyNPV)[13] bacmid.

Among the available expression systems are *Escherichia coli* (*E. coli*), eukaryotic cells, including mammalian cells (e.g., human 293 cells, CHO hamster cells, etc.), and yeast (e.g., *Pichia pastoris*, *Saccharomyces cerevisiae*, etc.). The baculovirus expression vector system (BEVS) has many advantages for expressing these proteins, including high expression levels using strong promoters (polyhedrin and P10), post-translational modifications similar to those generated in mammalian cell expression systems, and lower costs compared to them.

The *B. mori* expression system is a BEVS in which silkworms are used as bioreactors for producing recombinant proteins instead of cell lines. However, the construction, amplification, and purification of recombinant BmNPV virus using silkworm cell lines require a lot of time and specialized methods, as well as the AcNPV system (Autographa californica nucleopolyhedrovirus, a hybrid virus AcMNPV). As a solution to this problem, virus-free transgenic silkworm technology is available for stable expression of recombinant proteins[14].

A. Usami et al. (2011), using a hybrid baculovirus system, compared the expression of 45 recombinant proteins from six categories using two models: mulberry silkworm (larvae and pupae) and the Sf9 cell line. In total, 45 proteins were successfully expressed; obtaining a hybrid baculovirus was unsuccessful for one protein, and two proteins did not express. A similar expression pattern was observed in both mulberry silkworm cells and Sf9 cells, with double and multiple bands detected in immunoblotting of precipitates from both hosts. Degraded proteins were only found in the mulberry silkworm system, especially in the larvae. Obtaining mulberry silkworm larvae (caterpillars) was more efficient, with one silkworm producing approxi-

[10]*Iatrou K., Meidinger R.G.* Tissue-specific expression of silkmoth chorion genes in vivo using Bombyx mori nuclear polyhedrosis virus as a transducing vector // Proceedings of the National Academy of Sciences, 1990, vol. 87, N 10, pp. 3650–3654. DOI: 10.1073/pnas.87.10.3650.

[11]*Maeda S., Kawai T., Obinata M., Fujiwara H., Horiuchi T., Saeki, Y., Furusawa M.* Production of human α-interferon in silkworm using a baculovirus vector // Nature, 1985, vol. 315, N 6020, pp. 592–594.

[12]*Motohashi T., Shimojima T., Fukagawa T., Maenaka K., Park E.Y.* Efficient large-scale protein production of larvae and pupae of silkworm by Bombyx mori nuclear polyhedrosis virus bacmid system // Biochemical and biophysical research communications, 2005, vol. 326, N 3, pp. 564–569. DOI: 10.1016/j.bbrc.2004.11.060.

[13]*Kato T., Kajikawa M., Maenaka K., Park E.Y.* Silkworm expression system as a platform technology in life science //Applied microbiology and biotechnology, 2010, vol. 85, pp. 459–470. DOI: 10.1007 / s00253-009-2267-2.

[14]*Kajikawa M.* Silkworm Baculovirus expression system for molecular medicine //Journal of Biotechnology & Biomaterials, 2012, vol. 9, N 01, p. 1. DOI: 10.4172/2155-952X.S9-005.

mately 70 times more protein than 106 Sf9 cells in 2 ml of culture medium[15].

The development and improvement of baculovirus-based expression systems continue to this day. For example, H. Yagi et al. (2020) presented data on the development of a previously established method for isotopic labeling of glycoproteins for nuclear magnetic resonance (NMR) studies using mulberry silkworm larvae raised on an artificial diet [4]. J. Wei et al. (2022) presented the results of creating a new baculovirus expression system – mulberry silkworms, in which the inoculation of purified occlusion bodies was proposed by direct spraying them onto mulberry leaves for large-scale industrial production [5].

Stable transformation of the embryonic line (transgenesis)

The PiggyBac transposon-based vector has been successfully used for the transformation of *B. mori.* The transgene was stably transmitted to the next generation through normal Mendelian inheritance[16]. One popular method for obtaining transgenic mulberry silkworms is injection, obtained from a PiggyBac transposon plasmid with a target construct into silkworm eggs. Several recombinant proteins are expressed in the mulberry silkworm's silk gland and produced in cocoons at levels ranging from one to several hundred milligrams per microgram of cocoon mass. Transgenic silkworm technology can be used to modify host strains for the baculovirus expression system. There are two different methods for using transgenic silkworms. One is the expression of beneficial genes for the production of recombinant proteins, such as molecular chaperones and enzymes that modify proteins, regardless of their origin. The other method is the suppression or knockout of harmful genes using RNA interference or gene targeting (see footnote 13).

Mulberry silkworm has the ability to synthesize a large amount of silk proteins in its silk gland. The mechanism of silk protein synthesis has been widely studied at the molecular level[17, 18]. Silk consists of two proteins called fibroin and sericin. Fibroin is the main component of silk fibers, while sericin is a kind of sticky protein that covers the surface of the fibers. Fibroin accounts for about 75% of all silk proteins and is produced in the posterior silk gland (PSG). The remaining 25% is sericin, which is synthesized in the middle silk gland (MSG). Fibroin contains three different proteins, called heavy (H) and light (L) chains of fibroin, as well as fibrohexamerin (FHX), produced in a molar ratio of 6:6:1, respectively[19]. The character of silk as a fiber is determined by the large H-chain of fibroin, with a molecular weight of 350-400 kDa. The L-chain of fibroin and FHX are small proteins with a molecular weight of about 25 kDa.

The system for obtaining recombinant proteins in transgenic mulberry silkworms utilizes the silk synthesis system in the silk gland and the silk genes, which are highly expressed in the silk gland. So far, for the production of PSG (posterior silk gland), production systems using the L- and H-chains of fibroin and FHX genes have been used. In MSG (middle silk gland), two different systems using the sericin 1 gene have been developed, each with its own advantages and disadvantages depending on the purpose of protein production. Proper selection of the production system for recombinant protein is required for the appropriate use of transgenic silkworms. The systems can be categorized as follows: recombinant protein production system using the L-chain of fibroin gene, GAL4/UAS binary transgene expression system, FHX gene

[15]*Usami A., Ishiyama S., Enomoto C., Okazaki H., Higuchi K., Ikeda M., Nagaya H*. Comparison of recombinant protein expression in a baculovirus system in insect cells (Sf9) and silkworm // The journal of biochemistry, 2011, vol. 149, N 2, pp. 219–227. DOI: 10.1093/jb/mvq138.

[16]*Tamura T., Thibert C., Royer C., Kanda T., Eappen A., Kamba M., Couble P*. Germline transformation of the silkworm Bombyx mori L. using a PiggyBac transposon-derived vector // Nature biotechnology, 2000, vol. 18, N 1, pp. 81–84. DOI: 10.1038/71978.

[17]*Mizuno S*. Regulation of fibroin gene expression and secretion of fibroin in the silk gland //Seikagaku. The Journal of Japanese Biochemical Society, 1987, vol. 59, N 12, pp. 1308–1320.

[18]*Julien E*. Silk gland development and regulation of silk protein genes //Comprehensive molecular insect science, 2005, vol. 2, pp. 369–384.

[19]*Inoue S., Tanaka K., Arisaka F., Kimura S., Ohtomo K., Mizuno S*. Silk fibroin of Bombyx mori is secreted, assembling a high molecular mass elementary unit consisting of H-chain, L-chain, and P25, with a 6: 6: 1 molar ratio //Journal of Biological Chemistry, 2000, vol. 275, N 51, pp. 40517–40528. DOI: 10.1074/jbc.M006897200.

expression system, recombinant protein production system using the H-chain of fibroin gene, expression system involving the sericin 1 gene promoter, BmNPV ie1 gene, and hr enhancer sequence[20].

Transgenic mulberry silkworms can be used as bioreactors for the production of recombinant proteins. They can be easily obtained by using the PiggyBac DNA transposon as a vector through the injection of helper and vector plasmid DNA into eggs immediately after egg laying. Recombinant protein production systems have been constructed using silk genes expressed in the silk gland. The PSG expression system is suitable for producing genetically modified silk. Silk obtained from transgenic silkworms can be used to produce tissues and biomaterials for medical purposes. The MSG system is suitable for the production of recombinant proteins that can be used for pharmaceutical purposes. The latter system can yield up to 4 mg of recombinant protein per silkworm. Transgenic silkworms have several important properties that make them good candidates for use as bioreactors. Silk glands represent a highly efficient system for producing large quantities of proteins, with a production capacity of over 500 mg of silk protein per larva. In addition, the larval fat body can synthesize about 100 mg of hemolymph/larva protein. Other advantages include the low cost of raising silkworms in a short time, which is required to obtain transgenic silkworms (60 days) [6].

B. mori genetic manipulation technologies include transposon-based technologies, gene integration technologies (insect DNA-type transposons and site-specific recombination), gene expression technologies (heat-inducible expression system), Gal4/upstream activating sequence expression system, tetracycline-inducible/tetracycline-off expression system, gene silencing technologies based on transgenic RNA interference, and trap gene and enhancer technologies. Genome editing technologies include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated 9 (CRISPR/Cas9) [7].

Y. Wang et al. (2015) successfully constructed the MGES (multigene expression system), which allows the generation of silk with two or more additional valuable functions, such as wound healing and antibacterial activity, biocompatibility and tissue regeneration, or high strength and durability due to the co-expression of multiple functional genes [8]. Z. Li et al. (2022) presented data on the creation of a targeted expression system using targeted insertion mediated by a transcription activator-like effector nuclease (TALEN), which allows the production of up to 3.1% (mass/mass) EGFP protein in the cocoon shell. Using this strategy, the production of an important human epidermal growth factor (hEGF) was significantly increased in both middle silk glands and cocoon shells, more than 15 times higher than canonical PiggyBac-based transgenesis [9].

The gene that needs to be introduced into the mulberry silkworm is injected into the egg immediately after the butterfly lays the eggs. Upon injection, it integrates into the chromosomal DNA of the mulberry silkworm. Larvae hatched from the eggs reproduce into adult individuals, and inheritance occurs in the next generation after mating and egg laying with injected silkworms. Selected transgenic silkworms are used to reproduce new lines of transgenic animals capable of passing on genetic information. Offspring of recombinant silkworms can be obtained indefinitely. For this reason, a single injection into silkworm eggs (germs) is sufficient[21]. N. Yamada et al. (2023) developed a gene engineering injection method for diapausing strains of silkworms using dimethyl sulfoxide (DMSO). The method is easy to perform and reliable, allowing its application to various diapausing strains of silkworms, including hybrid combinations of

[20] *Tatemastu K., Sezutsu H., Tamura T.* Utilization of transgenic silkworms for recombinant protein production //J Biotechnol Biomaterial S, 2012, vol. 9, pp. 1–8. DOI: 10.4172/2155-952X.S9-004.

[21] *Nobuo Kuwabara.* Breeding of Genetically Modified Silkworms by Sericulture Farmers //Bulletin of the" SEKAITO"-Silk Powered Innovation Incubator of the Gunma Prefectural World Heritage Center / Gunma Prefectural World Heritage Center "The power of raw silk to change the world" Institute, 2022, N 2, pp. 25–34. https://www.sbj.or.jp/wp-content/uploads/file/sbj/9306/9306_ tokushu_2.pdf.

Japanese, Chinese, European origins, and mutant bivoltine and polyvoltine strains [10].

Genetic methods of selection and sex regulation

Reproduction management of B. Mori

By using parthenogenetic clones as mothers and crossing them with strains marked by sex, clonal-breed hybrids with 100% purity can be obtained. In Uzbekistan, hybrids of strains APK × C-5, 9PK × C-5, 9PK × C-10, parthenogenetic clones, and sex-determined color egg strains of mulberry silkworms, created artificially as a means of silkworm reproduction management, outperformed control hybrids in terms of reproductive parameters and viability [11].

Ablation

For the genetic engineering of mulberry silkworms, microinjection of genetic material into eggs without diapause is required. Besides the fact that diapause can be useful for maintaining transgenic lines, a drawback of this technology is that most standard silkworm strains and experimental lines produce diapausing eggs. N. Yamada et al. (2022) investigated ablation (removal of a biological structure or functionality, genetic ablation being another term referring to the suppression of gene expression, where gene expression is canceled by changing or deleting genetic sequence information) of the subesophageal ganglion (SG) in female pupae, which is the source of the hormone necessary for initiating egg diapause as a means of canceling diapause[22]. It was shown that SG ablation is a reliable method for obtaining diapause-free eggs. Additionally, the issue of lower fecundity in females with SG ablation was resolved by injecting pilocarpine into mating females. The suitability of diapause-free eggs laid by females with SG ablation for transgenesis, targeted mutagenesis, and induction of parthenogenetic development was also investigated. The results demonstrated that SG ablation is a useful and straightforward method for expanding the possibilities associated with silkworm genetic engineering [12].

Genetic hybridization of transgenic silkworms

D. Long et al. (2021) proposed a strategy, called "light clothing" based on pruning redundant additive structural domains, to genetically hybridize a highly active functional fusion POI to silk-based materials using transgenic mulberry silkworm-based biosynthesis platforms. The process of genetic hybridization from transformation of the germline of *B. mori*, generation, backcrossing, screening and molecular identification of transgenic mulberry silkworms allowed, compared to conventional expression systems, a significant increase in the activity of fusion POIs without compromising the genetic hybridization process. This increased activity of fused POIs may be due to the removal of unnecessary post-translational modifications and increased structural similarity to native POIs. Compared to the direct incorporation of commercially available POIs into silk-based materials, these genetically hybridized silk-based materials not only have comparable POI activity, but also provide additional advantages, particularly in logistics and in the absence of the need for cold chain storage [13].

Pathogen infection and transgenesis for obtaining antimicrobial peptides (AMPs)

Some authors have described over 30 AMPs in silkworms, classified into groups such as cecropins, attacins, moricins, gloverins, lebocins, enbocins, and defensins; most of these AMPs are effective against both gram-positive and gram-negative bacteria, as well as other micro-

[22]Heritage Center "The power of raw silk to change the world" Institute. 2022, N 2, pp. 25–34. https://www.sbj.or.jp/wp-content/ uploads/file/sbj/9306/9306_tokushu_2.pdf.

[23]*Tanaka H., Ishibashi J., Fujita K., Nakajima Y., Sagisaka A., Tomimoto K., Yamakawa M.* A genome-wide analysis of genes and gene families involved in innate immunity of Bombyx mori // Insect biochemistry and molecular biology, 2008, vol. 38, N 12, pp. 1087–1110. DOI: 10.1016/j.ibmb.2008.09.001.

[24]*Tanaka H., Yamakawa M*. Regulation of the innate immune responses in the silkworm, Bombyx mori // Invertebrate Survival Journal, 2011, vol. 8, N 1, pp. 59–69.

organisms[23, 24] [14]. AMPs are highly sought-after therapeutic compounds due to their characteristics, including low toxicity to humans and animals, high specificity, and improved effectiveness against target microbes compared to conventional antibiotics, and most importantly, the fact that most microbes cannot develop resistance to AMPs. M. Mastore et al. (2021) used *B. mori* as a model organism to assess the suitability of hemolymph as a source of AMPs. After simple purification steps, the plasma was analyzed and tested against various strains of gram-positive and gram-negative bacteria. The results showed that partially purified silkworm plasma could be a promising source of AMPs that can be used in locally applied preparations without additional expensive purification stages. Furthermore, preliminary tests were conducted on the possibility of storing these molecules under non-refrigerated conditions. The results obtained from storing the preparations at temperatures above room temperature (25°C) did not show significant loss of effectiveness against *E. coli* [15].

Mulberry silkworms are infected with pathogens to isolate AMPs during their fifth instar, which lasts for 6-8 days, providing enough time for infection development. Mulberry silkworm AMPs are low molecular weight proteins (<50 amino acid residues; <10 kDa, with rare exceptions), most of which exhibit a broad-spectrum activity against various microorganisms. Additionally, the fat content in mulberry silkworm bodies reaches its peak during this stage, which is the primary source of AMPs[25] [16]. After infection, immunocompetent tissues are lysed in a suitable buffer to extract proteins and are subjected to various chromatographic methods, such as ion-exchange chromatography, gel filtration chromatography and reversed-phase which is high-performance liquid chromatography (HPLC), for purification [17].

Transgenesis for obtaining antimicrobial peptides (AMPs)

Transgenesis in mulberry silkworms has been expanded by incorporating antimicrobial peptides into silk proteins. Transgenic silk fused with antimicrobial peptides CEC B or MOR inhibited the growth of *E. coli*. Furthermore, the silk yarn retained its antibacterial properties against Escherichia coli. Silk fibers maintained the activity and antimicrobial molecules of MOR after refining processes [18].

Direct introduction of artificial additives: direct feeding method

Production of fluorescent silk is an example of using both transgenesis and the direct feeding method. Silk fibroin, transgenically hybridized with fluorescent proteins, can be processed and regenerated into various forms with nano- and microstructures for use in optics, electronics, optoelectronics, and medicine, as it is transparent, mechanically stable, edible, biocompatible, and implantable into the human body. Another way to produce fluorescent silk is by using direct delivery methods based on the absorption of fluorescent dye molecules in the silkworm's natural silk glands.

Generally, fluorescent-colored silk can be obtained by using a modified silkworm leaf diet containing molecules of common dyes in the textile industry: rhodamine dyes (e.g., sulforhodamine) and azo dyes (e.g., brilliant yellow, Congo yellow)[26]. Molecular weight is a factor that influences the absorption of dye molecules by the biochemical pathways of mulberry silkworms. A molecular weight below 400 g/mol is required for the efficient transport of dyes into the mulberry silkworm's biochemical pathways and for the production of naturally colored silk fibers [19].

H. Xu et al. (2019) demonstrated the improvement of mechanical characteristics of new silk fibers obtained by feeding silkworms puri-

[25] *Kajiwara H., Itou Y., Imamaki A., Nakamura M., Mita K., Ishizaka M.* Proteomic analysis of silkworm fat body // Journal of Insect Biotechnology and Sericology, 2006, vol. 75, N 2, pp. 47–56. DOI: 10.11416/jibs.75.47.

[26] *Tansil N.C., Li Y., Teng C.P., Zhang S., Win K.Y., Chen X., Han M.Y.* Intrinsically colored and luminescent silk //Advanced Materials, 2011, vol. 23, N 12, pp. 1463–1466. DOI: 10.1002/adma.201003860.

fied and biocompatible single-walled and multi-walled carbon nanotubes (CNTs). Increased CNT content not only promoted the self-assembly of silk fibers into buffer nodes but also increased the conductivity of the graphitized silk. Overall, these coating and purification strategies provide a potentially straightforward method for obtaining natural silk fibers with high mechanical characteristics [20].

Direct feeding of silkworms or spiders with artificial additives can be a practical method for producing reinforced silk fibers, allowing for easy production of silk containing functional additive nanomaterials. This is possible in part because silkworms have an open cardiovascular system. All their organs swim in hemolymph, which is a mixture of lymph and blood cells that surrounds all tissues. Thus, the unique anatomy of silkworms is very useful for the production of functional silk using direct feeding methods (oral exposure and consumption) of nanomaterials. Nanoscale additives can diffuse from the digestive tract into the hemolymph, and then into glands and other tissues. Specifically, if nanomaterials are introduced orally, they are absorbed in the digestive tract, pass through the membrane barrier, and circulate in the hemolymph and cells [21].

Combination of transgenesis, direct feeding, and reverse breeding methods

Silk fibroin containing unnatural amino acids was obtained by feeding transgenic *B. mori* with para-chloro, para-bromo, and para-azido-substituted analogs of L-phenylalanine (Phe) *in vivo*. This was accomplished by expressing a mutant phenylalanyl-tRNA synthetase with expanded substrate recognition capabilities in the silk glands. The azido groups incorporated into fibroin served as chemical markers for click chemistry both in solubilized and solid (fibrous) states. The azides survived the boiling alkaline solution degumming process required for complete removal of the sericin layer. This demonstrates that silk fibroin containing AzPhe (synthetic amino acid) can be a universal platform for producing "clickable" silk materials in various forms[27]. Practical application of AzidoSilk was hindered by low productivity. Later, Y. Tian et al. (2022) presented data on the derivation of a new transgenic line of B. mori for mass production of AzidoSilk using the conventional reverse breeding method. The newly obtained *B. mori* line, created after five rounds of reverse breeding, yielded 2.6 times more AzidoSilk per larva with a 25% increase in productivity [22].

Aggregate biotechnological improvements of B. Mori

The life cycle of *B. mori* includes five main stages of development: egg (embryo) (incubation period from 10 to 14 days); larva (caterpillar) - development period - 20-25 days (for 3-instar breeds) and 24-39 days (for 4-instar breeds); cocoon (spinning 3-5 days); pupa (stage duration from 10 to 15 days); butterfly (the mating process (papilionage) takes place for several hours). The main improvements made at various stages of the life cycle and the target products for subsequent processing are presented in the figure.

Improvements
Egg Stage

(1) Transgenesis – for obtaining recombinant proteins, inclusion of unnatural amino acids (UAA), for subsequent genetic hybridization, the injection method of genetic engineering of diapause breed eggs.

(2) Transgenesis to obtain antimicrobial peptides (AMP).

(3) Artificial reproduction: ameiotic and meiotic parthenogenesis, gynogenesis, androgenesis, polyploidy; methods: physical and chemical influence: heating, ionizing radiation, special poisons, etc.; microsurgery, shaking, etc.; cloning.

Caterpillar stage

(1) Basic improvement – the use of artificial nutrient medium (ANM).

[27] *Teramoto H., Kojima K*. Production of Bombyx mori silk fibroin incorporated with unnatural amino acids // Biomacromolecules, 2014, vol. 15, N 7, pp. 2682–2690. DOI: 10.1021/bm5005349.

(2) Transient system – DNA injection of BmNPV bacmid and others into larvae, expression and production of recombinant proteins in silkworm hemolymph.

(3) Transgenesis – recombinant proteins are expressed in the silk gland.

(4) Transient and stable expression systems – inoculation of purified occlusion bodies by direct spraying on ANM or oral infection.

(5) Infection with pathogens to obtain antimicrobial peptides (AMP).

(6) Direct introduction of artificial additives (direct feeding method).

Cocoon stage

(1) Transgenesis – production of target recombinant proteins in the cocoon shell: fibroin and sericin layers.

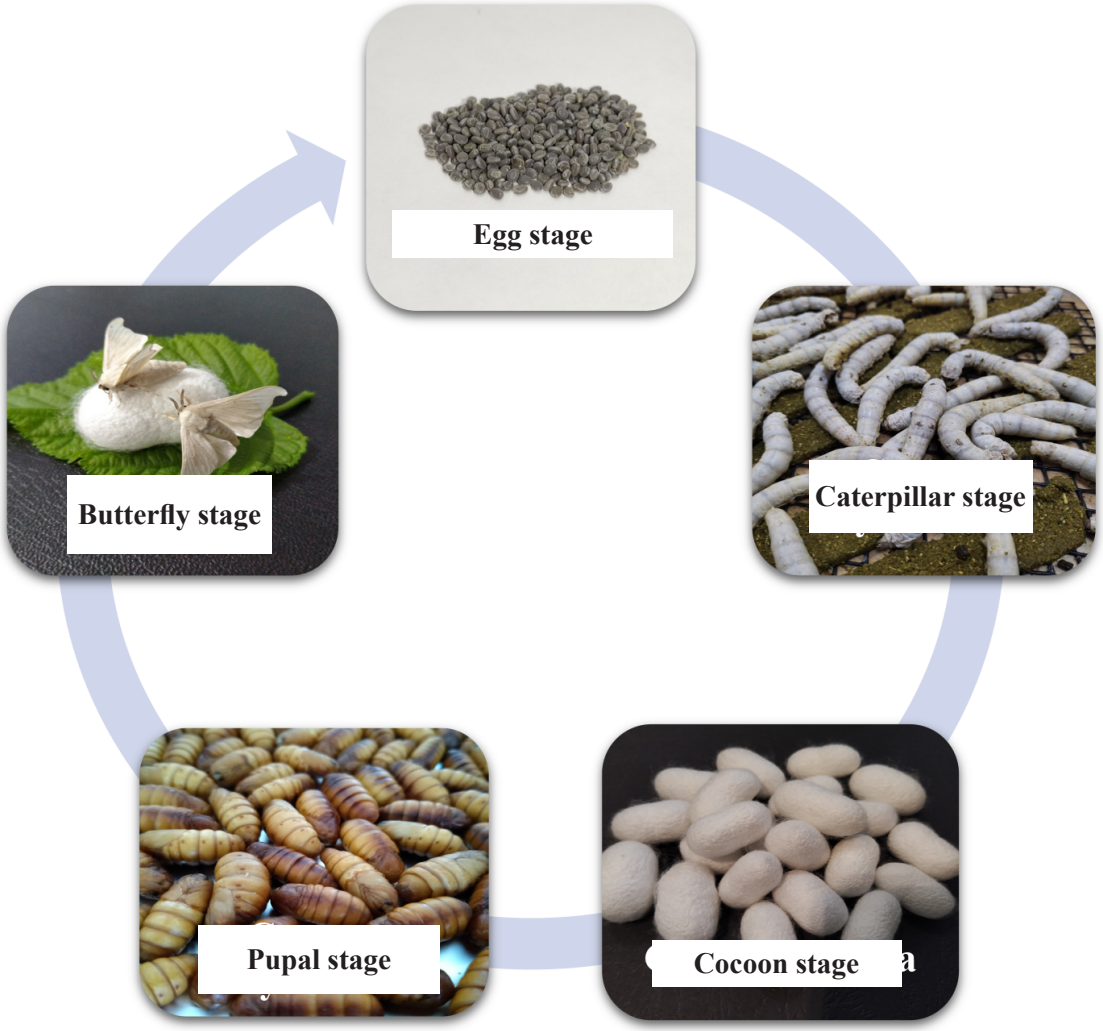
(2) Transgenesis – obtaining recombinant silk threads.

(3) Direct feeding – obtaining silk threads with improved mechanical characteristics.

Pupal stage

(1) Transient system – DNA injection of BmNPV bacmid and others into pupae, expression, and production of recombinant proteins.

(2) Ablation – to cancel diapause.



Жизненный цикл и улучшения *B. mori*
Life cycle and improvements of *B. mori*

Butterfly stage

(1) Genetic hybridization – to obtain new lines (breeds) with improved properties.

(2) Genetic hybridization of transgenic silkworms – reverse breeding.

CONCLUSION

The variety of biotechnological solutions used at various stages of the mulberry silkworm's life cycle is aimed at qualitative and quantitative improvements in content and reproduction, obtaining new types of raw materials, and, in the downstream stage, new products. Both the larva, cocoon shell, and pupa of *B. mori* can be used to produce recombinant proteins. The possibility of combining transgenesis, direct feeding, and reverse breeding methods further expands the variability and efficiency in obtaining new target products. The complex of improvements provides the basis for a biotechnological platform based on *B. mori*, the scaling of which transforms it into a bio-industrial platform for the modern pharmaceutical industry, consisting of functional biomaterials for drug and gene delivery, wound treatment, and healing, biomaterials for tissue engineering, flexible electronics, bio-inks for 3D bioprinting, cosmetics, and nutraceuticals, as well as for the industry of new enhanced textile materials.

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ИММУНОГЕНЕТИЧЕСКАЯ ХАРАКТЕРИСТИКА ПОРОД ОВЕЦ СИБИРСКО-ДАЛЬНЕВОСТОЧНОГО РЕГИОНА

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Проведен сравнительный анализ восьми пород овец (полугрубошерстных, грубошерстных, полутонкорунных и тонкорунных), разводимых в Сибирско-Дальневосточном регионе. В исследованиях использовали данные по иммуногенетическому тестированию овец в племенных хозяйствах за ряд лет. Тестирование проводилось с применением 14 специфических сывороток – реагентов. Изучены аллельный профиль овец, их сходство и различие, связанные с филогенезом и предшествующей селекцией. Все исследованные породы имели отличительный аллельный профиль. В каждой породе выявлены как часто, так и редко встречающиеся антигены. На основании частот антигенов рассчитан индекс генетического сходства (*r*), который был выше в породах одного направления продуктивности, например между породами буубэй и эдильбаевской (r = 0,912), и ниже – между породами, селекция которых проводилась изолированно друг от друга. Самый низкий индекс генетического сходства выявлен между бурятской и западно-сибирской мясной (r = 0,707). Породы одного направления продуктивности из разных регионов также имеют отличительные особенности. Индекс генетического сходства между грубошерстными породами (буубэй и эдильбаевская) составляет 0,912; между полугрубошерстными (бурятская и агинская) он находится на уровне 0,739; между полутонкорунными (горноалтайская и западно-сибирская мясная) – 0,845; между тонкорунными (кулундинская и забайкальская) – 0,902. С использованием кластерного анализа генетических дистанций определены взаимоотношения пород, их происхождение, филогенез. Западно-сибирская мясная и кулундинская образовали один кластер, буубэй и бурятская также вошли в один кластер. Более отдаленными породами оказались агинская (зугалайский тип) и эдильбаевская.

Ключевые слова: овца, порода, антиген, частота, индекс генетического сходства

IMMUNOGENETIC CHARACTERISTICS OF SHEEP BREEDS OF THE SIBERIAN-FAR EASTERN REGION

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A comparative analysis of eight breeds of sheep (medium-wool, coarse-wooled, semifine-wool and fine-wool) bred in the Siberian-Far Eastern region was carried out. The research used the data on immunogenetic testing of sheep in breeding farms for a number of years. The testing was performed using 14 specific sera – reagents. The allelic profile of sheep, their similarities and differences related to phylogeny and previous selection were studied. All breeds studied had a distinctive allelic profile. Both frequent and infrequent antigens were identified in each breed. Based on antigen frequencies, an index of genetic similarity (*r)* was calculated, which was higher in the breeds of the same productivity direction, e.g. between Buubei and Edilbaevskaya breeds (r = 0.912), and lower between

the breeds that were selected in isolation from each other. The lowest index of genetic similarity was found between the Buryat and West Siberian meat breeds ($r = 0.707$). Breeds of the same productivity direction from different regions also have distinctive features. The index of genetic similarity between coarse-wooled breeds (Buubei and Edilbaevskaya) is 0.912; between medium-wool breeds (Buryat and Aginskaya) it is at the level of 0.739; between semifine-wool breeds (Gorno Altai and West Siberian meat breeds) – 0.845; between fine-wool breeds (Kulunda and Zabaikalskaya) – 0.902. Using the cluster analysis of genetic distances, the relationships of breeds, their origin, and phylogeny were determined. West Siberian Meat and Kulunda breeds formed one cluster, Buubei and Buryat breeds were also included in one cluster. More distant breeds were Aginskaya (Zugalai type) and Edilbaevskaya.

Keywords: sheep, breed, antigen, frequency, index of genetic similarity

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INTRODUCTION

In Russia during the 2000s, there was a partial shift in sheep breeding due to the reduction in the sheep population. Due to market conditions, the number of fine-wool sheep breeds decreased by 2.3 times, semifine-wool by 4.3 times, while the population of coarse-wooled sheep breeds increased by 4.1 times. However, the most numerous sheep population belongs to the fine-wool productivity direction, with 15 breeds representing 54% of the total sheep population in Russia. Semifine-wool breeds account for only 4.6%, and the population of semi-coarse-wooled sheep is even smaller at 1.2%. In Russia, 17 breeds fall under the coarse-wooled productivity direction, accounting for 33% of the sheep population[1].

The observed annual decrease in the sheep population may lead to the loss of adaptive qualities to local climate and feeding conditions, as well as a reduction in genetic diversity. To study these processes, various types of genetic markers are used, including blood groups, protein polymorphism, microsatellite profiles, and whole-genome analysis, which can be used to study the population-genetic structure of individual breeds, populations, their variability during crossbreeding, and generational monitoring. Currently, microsatellite profiling [1, 2] is considered one of the most convenient, informative, and suitable for mass analysis.

The use of blood groups, which were in demand due to their informativeness, codominant inheritance type, and suitability for mass analysis, allowed solving some problems in the selection of farm animals, such as verifying the accuracy of recording the origin of young animals. In addition, immunogenetic analysis was widely used in establishing the phylogeny of breeds, similarities, and differences between populations, herds, and age-sex groups.

Studies [3] have shown that the highest genetic similarity is found between Black-and-White Holsteinized bulls and Ayrshire bulls (0.902), while the lowest is between Jersey and

[1] *Shichkin G.I., Safina G.F., Amerkhanov H.A., Chernov V.V., Grigoryan L.N., Khmelevskaya G.N., Ravicheva A.V., Stepanova N.G.* Yearbook on breeding work in sheep and goat breeding in farms of the Russian Federation (2021). FSBSI ARRIPW, Moscow, 2022, 325 p.

Ayrshire bulls (0.561). Immunogenetic analysis demonstrated differences in an isolated population of French Rambouillet sheep and Spanish Merinos, from which they originated. In a closed herd, only those alleles were fixed that were detected in Spanish Merinos with frequencies of 0.90 and 0.80[2].

With the help of blood groups and other genetic markers, one of the best domestic fur-bearing breeds, the Romanov breed, has been thoroughly characterized in comparative assessment with other breeds[3].

Immunogenetic analysis has found application in determining mono- and dizygosity of offspring of farm animals [4]. Studies [5] have shown the influence of the environment on the level of homozygosity of a herd for serum blood proteins, which may be a consequence of the mechanism of maintaining polymorphism, having adaptive value in unfavorable conditions.

The paper shows the allele profile of sheep crossbreeds and identifies desirable alleles associated with live weight, meat productivity, and productive longevity [6, 7]. There are reports of using blood groups for effective selection of mating pairs based on the genetic similarity index of parents[4, 5] [8]. It has been shown that in the formation of the allele pool of offspring, from 50% to 80% of the dominant alleles of the maternal herd of cattle are involved [9].

Considering that blood groups were the predominant genetic markers, during their active use in the selection of farm animals, a substantial amount of factual material has been accumulated on the genetic structure of different breeds, changes in allele and genotype frequencies under the influence of selection and the environment, and more detailed studies on blood group characteristics and the laws of their inheritance. As a result of the analysis of immunogenetic structures of eight species of ruminants, the similarity of some antigen loci of different species (sheep, goats, cattle) has been established [10].

Studies [3] provide data on the concentration, dynamics, and stability of erythrocyte alleles in cattle. It is shown that only 10% of alleles are stable, while about 30-35% are subject to elimination and genetic drift.

The purpose of the research is to analyze and summarize the allele pool of sheep of different breeds, taking into account the direction of productivity, their similarities and differences, and the relationships between them in the conditions of the Siberian-Far Eastern region.

MATERIAL AND METHODS

The research material consisted of blood samples collected from eight different breeds of sheep with various productivity directions, bred in the Siberian-Far Eastern region (Altai and Trans-Baikal Territory, Republic of Buryatia). The studied breeds included: coarse-wooled sheep breeds - Buubei (BUB) and Edilbayevskaya (EDL), semi-coarse-wooled sheep breeds - Buryat (BUR) and Aginskaya sheep of the Zugalai type (AGZ), semifine-wool sheep breeds – Gorno Altai breed of the Prikatunsky type (GAP) and West Siberian meat breed (WSM), and fine-wool sheep breeds – Zabaykalskaya of the Khangil type (ZBH) and Kulunda breed (KUL). A total of 2134 individuals were studied.

Immunogenetic testing was conducted using monospecific reagents from the bank of the Immunogenetics and DNA Technology Laboratory of the All-Russian Scientific Research Institute of Sheep and Goat Breeding. Testing covered six blood group systems (*A, B, C, D, M*, and *R-O*), including 14 erythrocyte factors (*Aa, Ab, Bb, Bd,*

[2]*Nguyen T.C., Morera L., Llanes D., Leger P.* Sheep blood polymorphism and genetic divergence between French Rambouillet and Spanish Merino: roleofgeneticdrift // Anim.Genet, 1992, N 23 (4), pp. 325–332. DOI: 10.1111/j.1365-2052.1992.tb00154.x.

[3]*Marzanov N.S., Komkova E.A., Malyuchenko O.P., Alekseev Ya.I., Ozerov M.Y., Kantanen Y., Lobkov V.Y., Marzanova L.K., Astafieva E.E., Petrov S.N., Kolpakov I.N., Andryukhin A.P., Adamyan K.K., Marzanova S.N.* Characterization of the allelofond of Romanov sheep breed by different types of genetic markers // Problems of Productive Animal Biology, 2015, N 2, pp. 23-40.

[4]*Vitanova O.I., Chizhova L.N.* Physiological and biochemical status of young sheep obtained from parents with different values of the index of antigenic similarity // Collection of Scientific Works of the Stavropol Research Institute of Animal Husbandry and Fodder Production, 2007, vol 3, N 3-3, pp. 10-12.

[5]*Skokova A.V., Barnash E.N., Sharko G.N., Yakubova E.V., Shumaenko S.N.* Productivity of young sheep depending on the index of genetic similarity of parents // Collection of Scientific Works of the Stavropol Research Institute of Animal Husbandry and Fodder Production, 2014, N 7, pp. 145-149

Be, Bi, Bg, Ca, Cb, Ma, Mb, R, O, and *Da*). The study was carried out through hemolysis and agglutination reactions at the Aginsk Veterinary Laboratory and the Laboratory of Biotechnology of the Siberian Research and Technological Design Institute of Animal Husbandry, SFSCA RAS.

The frequencies of antigens were calculated using the method of L.A. Zhivotovsky and A.M. Mashurov (1974). Genetic distance and genetic similarity were calculated using Nei's formulas (1972). Based on these calculations, genetic distance dendrograms were constructed between sheep breeds using the application program PAST version 3.25[6].

RESULTS AND DISCUSSION

To study the genetic characteristics of sheep of different breeds, groups were formed according to the following productivity directions: coarse-wooled, semi-coarse-wooled, semifine-wool, and fine-wool. The least numerous breeds in our study were Buubei, West Siberian meat, and Kulunda breeds, which account for only 1% of the total sheep population bred in the Siberian-Far Eastern region.

Buubei and Edilbayevskaya breeds belong to the coarse-wooled category, and their breeding histories have no common origin. The Buubei breed was created through long-term selective and breeding work by reintroducing indigenous Buryat sheep from Inner Mongolia, China[7]. The breed is becoming increasingly popular due to its good meat productivity and high meat quality [11, 12].

The Edilbayevskaya breed originates from Kazakhstan and is adapted to various meat sheep farming zones. The breed has three types of animals that differ in body structure and productivity[8]. Despite the same productivity direction, the breeds have significant differences, presumably due to previous selection (see Table 1).

For most antigens, differences were found. In the coarse-wooled meat breed Buubei, antigens *Bd, Bi, Ma*, and *R* were more frequent (by 0.235, 0.106, 0.100, and 0.052, respectively) compared to the coarse-wooled meat breed Edilbayevskaya. Conversely, the frequency of antigens *Ab, Bb, Be, Ca, Cb, Mb*, and *O* was higher in the Edilbayevskaya breed by 0.325, 0.089, 0.223, 0.252, 0.588, 0.354, and 0.098, respectively, compared to Buubei breed ($p \leq 0.05$, $p \leq 0.001$). The genetic similarity index between these breeds is $r = 0.910 \pm 0.014$.

Buryat and Aginskaya (Zugalai type) breeds belong to the semi-coarse-wooled sheep category. The Aginskaya breed was created by crossing the Zabaykalskaya fine-wool ewes with Kuchugursky fine-wool and Kazakh semi-coarse-wooled rams [13]. The Zugalai type of the Aginskaya breed was obtained through initial crossbreeding of the Aginskaya sheep with Kazakh semi-coarse-wooled rams of the Bayys type [14].

The Buryat breed was created with the participation of four breeds, with fine-wool Zabaykalskaya sheep of the Buryat type as the maternal foundation, which were crossed with Kazakh semi-coarse-wooled and Kuchugursky coarse-wooled rams. Subsequently, three-breed crossbred ewes were used, mating them with Baydaragsky rams of the fine-wool breed[9].

Table 2 presents data on the frequency of blood antigens in semi-coarse-wooled sheep of the Buryat and Aginskaya breeds (Zugalai type).

Immunogenetic analysis revealed that the Buryat breed of sheep has a higher frequency of antigens *Bd, Bi, Bg, Ma*, and *O* compared to the Aginskaya breed of the Zugalai type. In contrast, the Aginskaya breed of the Zugalai type surpasses in the frequency of the antigens *Ab, Be, Ca*, and *Cb* ($p \leq 0.05$, $p \leq 0.001$). The genetic similarity index of semi-coarse-wooled sheep is 0.739 ± 0.131.

[6] *Hammer O., Harper D.A.T., Ryan P.D.* PAST: Palaeontological Statistics software for education and data analysis // Palaeontologia Electronica, 2001, N 4 (1), p. 9.

[7] *Merzlyakov A.A., Danilov M.B.* Study of the composition and technological properties of meat of sheep indigenous breed "Buubei" // Education and Science: Materials of the national scientific-practical conf. Ulan-Ude, 2023, pp. 40-45.

[8] *Davletova A.M., Kosilov V.I.* Constitutional-productive types of Edilbaev sheep // Izvestiya Orenburg State University, 2013, N 1 (39), pp.102-104.

[9] *Biltuev S.I.* Creation of type and breed of sheep in specific environmental conditions of Western Siberia and the Republic of Buryatia: Ulan-Ude: Publishing house of BSSA named after V.R. Filippov, Ulan-Ude, 2010, 240 p.

Табл. 1. Частота антигенов крови грубошерстных овец буубэй и эдильбаевской пород

Table 1. Frequency of blood antigens in coarse-wooled sheep of the Buubei and Edilbaevskaya breeds

Antigen	BUB (*n* = 298)	EDL (*n* = 199)
Aa	0,691 ± 0,019	0,683 ± 0,023
Ab	0,107 ± 0,013	0,432 ± 0,025***
Bb	0,765 ± 0,017	0,854 ± 0,018***
Bd	0,742 ± 0,018***	0,507 ± 0,025
Bi	0,362 ± 0,020***	0,256 ± 0,022
Bg	0,399 ± 0,020	0,362 ± 0,024
Be	0,440 ± 0,023	0,663 ± 0,024***
Ca	0,386 ± 0,020	0,638 ± 0,024***
Cb	0,191 ± 0,016	0,779 ± 0,021***
Ma	0,738 ± 0,018***	0,638 ± 0,024
Mb	0,520 ± 0,020	0,874 ± 0,017***
R	0,198 ± 0,016*	0,146 ± 0,018
O	0,721 ± 0,018	0,819 ± 0,019***
Da	–	0,387 ± 0,024

Here and in Tables 2–4.
* $p \leq 0,05$.
** $p \leq 0,01$.
*** $p \leq 0,001$.

Табл. 2. Частота антигенов крови полугрубошерстных овец бурятской и агинской пород

Table 2. Frequency of blood antigens in medium-wool sheep of the Buryat and Aginskaya breeds

Antigen	BUR (*n* = 70)	AGZ (*n* = 356)
Aa	0,400 ± 0,041	0,449 ± 0,028
Ab	0,043 ± 0,017	0,489 ± 0,027***
Bb	0,714 ± 0,038	0,820 ± 0,016*
Bd	0,457 ± 0,042***	0,014 ± 0,037
Bi	0,400 ± 0,041***	0,191 ± 0,034
Bg	0,543 ± 0,042*	0,407 ± 0,036
Be	0,314 ± 0,039	0,688 ± 0,021***
Ca	0,329 ± 0,040	0,772 ± 0,018***
Cb	0,114 ± 0,027	0,458 ± 0,028***
Ma	0,529 ± 0,042***	0,034 ± 0,037
Mb	0,586 ± 0,042	0,652 ± 0,022
R	0,200 ± 0,034	0,166 ± 0,034
O	0,729 ± 0,038***	0,140 ± 0,035
Da	0,029 ± 0,014	0,008 ± 0,036

Gorno-Altai (Prikatunsky type) and West Siberian meat sheep breeds belong to the semi-fine-wool category. The Prikatunsky type of the Gorno-Altai breed of sheep was created in two stages. In the first stage, fine-wool and coarse-wooled ewes with different types of wool were crossed with rams of the Romni-Marsh breed until the second and third generations were obtained. Then, crossbred sheep of the desired type were bred within the breed[10].

The West Siberian meat breed was created based on the Kulunda short-tailed breed and its crossbreds with rams of the improved meat type of the Southern meat breed[11]. The compared breeds are bred in different eco-geographical conditions.

Semifine-wool sheep of the Gorno-Altai breed of the Prikatunsky type are characterized by a high frequency of occurrence of nine antigens - *Ab, Bb, Bd, Bi, Bg, Be, Ca, R*, and *O* compared to the West Siberian meat breed. Differences in frequencies range from 0.254 to 0.680 ($p \leq 0.05$, $p \leq 0.001$) (see Table 3). The genetic similarity index of semifine-wool sheep is 0.845 ± 0.0014.

The Kulunda fine-wool breed has been improved for a long time through "folk" selection methods, but starting from 1981, it was crossed

[10]*Podkorytov A.T.* Prikatunsky meat-wool type // Achievements of science and technology of AIC, 2006, N 2, pp. 30-31.

[11]*Katamanov S.G., Ulyanov A.N., Kulikova A.Y., Aboneev V.V., Selkin A.I., Afanasyeva A.I., Katamanov Yu. G., Loboda N.L., Moroz V.A., Trukhachev V.I., Katamanov A.S., Chebotaev A.N., Chmyryov M.A.* West Siberian meat breed // Sheep, goats, wool business, 2012, N 3, pp. 6-12.

with the Grozny breed to improve its wool quality, and later with the Stavropol Manych merinos[12].

In the creation of the Khangil type of the Zabaykalskaya breed, three breeds were involved at different times: Zabaykalskaya (maternal foundation), Australian, and Manych merinos[13].

The immunogenetic profile of the fine-wool sheep of the Kulunda and Zabaykalskaya breeds (Khangil type) also shows significant differences in the frequency of antigens (see Table 4).

For Kulunda fine-wool sheep, antigens *Aa, Ab, Bb, Bi, Bg, Ca, Cb, Ma, Mb, R* (50% or more) are characteristic, while antigens *Bd, O, Da* (less than 40%) are rarely present. The frequency of antigen factors *Ab, Bi, Be, Cb, Ma, Mb*, and *R* in this breed exceeds these indicators in the Zabaykalskaya of the Khangil type breed by 0.410, 0.161, 0.353, 0.253, 0.149, 0.150, 0.403, and 0.273, respectively. The Zabaykalskaya of the Khangil type breed, on the other hand, is characterized by a higher frequency of the antigens *Bb, Ca, O* - by 0.209, 0.293, 0.523 ($p \leq 0.01$, $p \leq 0.001$). The genetic similarity index of fine-wool sheep is 0.902 ± 0.022.

Comparative assessment of breed similarity expressed through genetic similarity indices and genetic distances is presented in Table 5.

The highest genetic similarity index was found between the Buubei and Buryat breeds (0.9681), which, in our view, can be explained

Табл. 3. Частота антигенов крови полутонкорунных овец горноалтайской и западно-сибирской мясной пород

Table 3. Frequency of blood antigens in semifine-wool sheep of the Gorno-Altai and West Siberian meat breeds

Antigen	WSM (*n* = 116)	GAP (*n* = 573)
Aa	0,543 ± 0,046***	0,150 ± 0,015
Ab	0,595 ± 0,046	0,894 ± 0,013***
Bb	0,500 ± 0,046	0,993 ± 0,003***
Bd	0,345 ± 0,044	0,998 ± 0,002***
Bi	0,603 ± 0,045	0,956 ± 0,009***
Bg	0,172 ± 0,035	0,609 ± 0,020***
Be	0,138 ± 0,032	0,747 ± 0,018***
Ca	0,465 ± 0,046	0,894 ± 0,017***
Cb	0,836 ± 0,034	0,769 ± 0,017
Ma	0,474 ± 0,046***	0,251 ± 0,018
Mb	0,595 ± 0,045	0,607 ± 0,020
R	0,405 ± 0,046	0,659 ± 0,019***
O	0,147 ± 0,033	0,827 ± 0,016***
Da	0,612 ± 0,045***	0,353 ± 0,020

Табл. 4. Частота антигенов крови тонкорунных овец кулундинской и забайкальской пород

Table 4. Frequency of blood antigens in fine-wool sheep of the Kulunda and Trans-Baikal breeds

Antigen	KUL (*n* = 122)	ZBH (*n* = 400)
Aa	0,541 ± 0,045	0,495 ± 0,025
Ab	0,893 ± 0,028***	0,483 ± 0,025
Bb	0,771 ± 0,038	0,980 ± 0,005***
Bd	0,311 ± 0,042**	0,150 ± 0,033
Bi	0,631 ± 0,044***	0,278 ± 0,030
Bg	0,853 ± 0,032	0,873 ± 0,013
Be	0,443 ± 0,045***	0,190 ± 0,032
Ca	0,639 ± 0,043	0,932 ± 0,009***
Cb	0,632 ± 0,044***	0,483 ± 0,025
Ma	0,705 ± 0,041**	0,555 ± 0,024
Mb	0,631 ± 0,044***	0,228 ± 0,031
R	0,811 ± 0,035***	0,538 ± 0,024
O	0,385 ± 0,044	0,910 ± 0,011***
Da	0,336 ± 0,043	0,318 ± 0,029

[12]*Selkin I.I., Katamanov A.S.* Fecundity of mothers and safety of young stock of Kulunda fine-fleece breed // Collection of Scientific Works of the Stavropol Research Institute of Animal Husbandry and Fodder Production, 2009, vol. 2, N 2-2, pp. 81-84.

[13]*Khamiruev T.N., Volkov I.V.* New wool and meat type in the Transbaikalian thin-cross breed of sheep - Khangilskiy // Zootechnia, 2015, N 4, pp. 6-7.

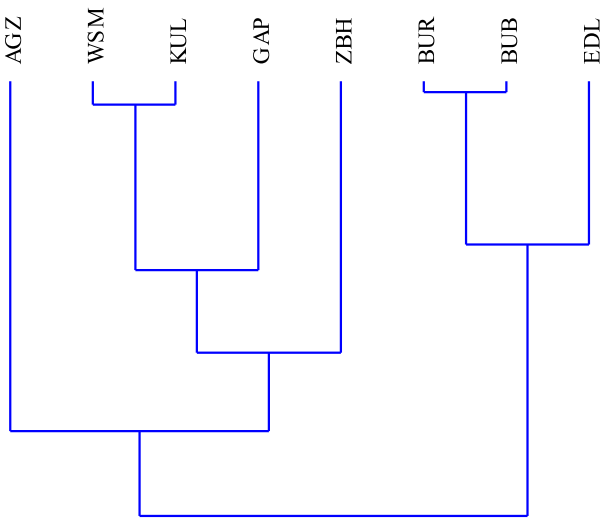
by the fact that the maternal foundation of the Buryat breed of sheep was the Buryat type of the Zabaykalskaya fine-wool sheep. The latter were bred based on indigenous Buryat sheep, on the basis of which the Buubei breed was created through reintroduction from Inner Mongolia, China.

The West Siberian meat breed was created based on the Kulunda breed, which is reflected in the level of genetic similarity between the breeds (0.963).

The genetic similarity index is higher in breeds with the same productivity direction and lower in different ones. For example, Buubei and Edilbayevskaya breeds of sheep have a genetic similarity index of 0.912, while with the semi-coarse-wooled Aginskaya breed, it is 0.719.

Cluster analysis of genetic distances showed the relationships between the studied breeds (see the figure).

West Siberian and Kulunda formed one cluster, Buubei and Buryat also entered one cluster. More distant were the Zugalai type of the Aginsky breed (semi-coarsed wool direction) and Edilbaevskaya breed (coarse-wooled).



Дендрограмма генетических дистанций
Dendrogram of genetic distances

CONCLUSION

Sheep breeds of the Siberian and Far Eastern region have distinctive features on blood group alleles, conditioned by breed peculiarities, previous genesis and, possibly, natural and climatic conditions. Breeds of the same direction of productivity from different breeding zones have the

Табл. 5. Матрица генетических расстояний между породами
Table 5. Matrix of genetic distances between the breeds

Breed	BUB	EDL	BUR	AGZ	WSM	GAP	KUL	ZBH
BUB		0,912	0,968	0,719	0,730	0,815	0,809	0,823
EDL	0,092		0,897	0,867	0,867	0,872	0,871	0,862
BUR	0,033	0,109		0,739	0,707	0,831	0,826	0,862
AGZ	0,330	0,143	0,302		0,754	0,813	0,834	0,800
WSM	0,315	0,143	0,347	0,282		0,845	0,963	0,823
GAP	0,205	0,137	0,185	0,207	0,168		0,903	0,847
KUL	0,212	0,138	0,191	0,182	0,038	0,102		0,902
ZBH	0,195	0,149	0,149	0,223	0,195	0,166	0,103	

Note. Genetic similarity indices are above the diagonal (r), under the diagonal are the genetic distances (DN); BUB – Buubei, EDL – Edilbayevskaya, BUR – Buryat, AGZ – Aginskaya – Zugalai type, WSM – West Siberian meat, GAP – Gorno Altai – Prikatunsky type, KUL – Kulunda, ZBH – Zabaikalskaya – Khangil type

same level of similarity and difference as interbreed (0.845-0.910). The interbreed difference ranges from 0.707 to 0.968.

The dendrogram of genetic distances of the studied breeds showed that breeds close in origin - West Siberian and Kulunda - formed one cluster, Buubei and Buryat breeds were also included in one cluster. Breeds of the same direction of productivity - Kulunda, Gorno-Altai, Aginskaya (Zugalai type) - form close subclusters, the index of genetic similarity of which is in the range of 0.845-0.903.

Thus, blood group antigens can serve as reliable genetic markers in studying the genetic profile of breeds, monitoring its change under selection pressure.

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ХАРАКТЕРИСТИКА ЗАРАЖЕННОСТИ И СТРУКТУРА ГЕЛЬМИНТОКОМПЛЕКСОВ ЛОШАДЕЙ В ПРОВИНЦИЯХ ГОРНОГО АЛТАЯ

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Целью исследования явилось изучение зараженности и структурных особенностей гельминтокомплексов лошадей в провинциях Горного Алтая, существенно отличающихся природно-климатическими и орографическими условиями местности. Пробы фекалий от спонтанно инвазированных гельминтами животных Центрального, Северного, Западного и Юго-Восточного Алтая исследовали оволярвоскопическими методами с последующим расчетом показателей встречаемости (экстенсивность инвазии – ЭИ, уровень экстенсивности инвазии – $ЭИ_у$), интенсивности заражения (индекс обилия – ИО, уровень индекса обилия – $ИО_у$) и индекса паразитокомплекса (ИП). Результаты многолетних исследований (2019–2023) свидетельствуют о том, что в формировании гельминтокомплекса пищеварительной системы лошадей Горного Алтая участвуют паразиты двух классов: Nematoda (подотряды Strongylata, Ascaridata, Rhabditata, Oxiurata) и Cestoda (подотряд Anoplocephalata). Во всех провинциях в нозологическом профиле гельминтозов доминируют стронгиляты, зараженность ими животных и их доля в гельминтокомплексах (ЭИ = 68,6–93,1%, ИП = 86,0–90,5) значительно превышают эти показатели для нематод подотряда Ascaridata (ЭИ = 6,2–16,5%, ИП = 4,6–8,1) и для цестод подотряда Anoplocephalata (ЭИ = 2,5–11,8%, ИП = 2,1–5,8). На фоне повсеместного распространения основных гельминтозов желудочно-кишечного тракта лошадей в условиях физико-географических провинций наблюдаются отличия в структуре гельминтокомплексов и интенсивности инвазированности животных гельминтами. Уровень зараженности лошадей Центрального Алтая стронгилятами достоверно выше относительно зараженности животных Северного и Западного Алтая. Инвазированность однокопытных Юго-Восточного Алтая стронгилятами (ЭИ = 68,6%) и аноплоцефалятами (ЭИ = 2,5%, ИП = 2,1) минимальна и соответственно в 1,5 и 2,8–4,7 раза ниже, чем в других провинциях. Однако здесь выявлены существенные внутризональные отличия в инвазированности лошадей нематодами – зараженность ими в горно-лесной зоне Юго-Восточного Алтая сопоставима с зараженностью в Центральном Алтае и значимо выше, чем в высокогорной степной зоне. В то же время показатели $ЭИ_у$ и $ИО_у$ для лошадей высокогорной степной зоны Юго-Восточного Алтая статистически ниже, чем у животных Северного и Центрального Алтая. Установлено, что уровень зараженности и структура гельминтокомплексов лошадей в основном обусловлены разнообразием природно-климатических и орографических характеристик горных территорий.

Ключевые слова: гельминты пищеварительной системы, лошади, структура гельминтокомплекса, экстенсивность и интенсивность инвазии, физико-географические провинции

CHARACTERIZATION OF INFESTATION AND STRUCTURE OF HORSE HELMINTH COMPLEXES IN THE PROVINCES OF THE ALTAI MOUNTAINS

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The purpose of the study was to investigate the infestation and structural features of the helminth complexes of horses in the provinces of the Altai Mountains which differ significantly in natural,

climatic and orographic conditions of the area. Fecal samples from spontaneously helminth-infested animals of the Central, Northern, Western and South-Eastern Altai Mountains were examined by ovolarvoscopic methods with the following calculation of occurrence indices (invasion intensity – II, level of invasion intensity – II_{level}), infestation intensity (abundance index – AI, level of abundance index – AI_{level}) and parasitocomplex index (PI). The results of long-term studies (2019–2023) indicate that parasites of two classes are involved in the formation of the helminth complex of the digestive system of horses in the Altai Mountains: Nematoda (suborders Strongylata, Ascaridata, Rhabditata, and Ochycrata) and Cestoda (suborder Anoplocephalata). Strongylates dominate in the nosological profile of helminthoses in all provinces: the degree of their infestation among animals and their proportion in the helminth complexes (II = 68.6–93.1%, PI = 86.0–90.5) are significantly higher than the same indicators for the suborder Ascaridata (II = 6.2–16.5%, PI = 4.6–8.1) and for the cestodes of the suborder Anoplocephalata (II = 2.5–11.8%, PI = 2.1–5.8). Against the background of ubiquitous distribution of the main helminths of the gastrointestinal tract of horses in the conditions of physiographic provinces, differences in the structure of helminth complexes and intensity of helminth infestation of animals are observed. The level of infestation of horses in the Central Altai with strongyles is significantly higher relative to the infestation of the animals in the Northern and Western Altai. The infestation of ungulates of South-Eastern Altai with strongylates (II = 68.6%) and anoplocephalates (II = 2.5%, PI = 2.1) is minimal and, respectively, 1.5 and 2.8–4.7 times lower than in other provinces. However, there are significant intrazonal differences in the nematode infestation of horses – their infestation in the mountain-forest zone of the South-Eastern Altai is comparable to that in the Central Altai and is significantly higher than in the high-mountain steppe zone. At the same time, the II_{level} and AI_{level} indices for horses of the high-mountain steppe zone of the South-Eastern Altai are statistically lower than in the animals of the Northern and Central Altai. It was found that the level of infection and the structure of helminth complexes of horses are mainly determined by the diversity of natural-climatic and orographic characteristics of mountainous territories.

Keywords: helminths of the digestive system, horses, structure of helminthocomplex, extensiveness and intensity of infestation, physico-geographical provinces

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Conflict of interest

The authors declare no conflict of interest.

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INTRODUCTION

The natural climatic conditions of the Gorno-Altai region are favorable for the development of horse herd farming. Nevertheless, parasitic infestations, including gastrointestinal helminthic diseases, are the factors limiting the increase in the number and productivity of animals.

Gastrointestinal helminths in horses are characterized by a significant diversity of species and are the most common and significant multicellular parasites of horses worldwide[1, 2] [1–6]. In the populations of whole-hoofed animals, complex mixed invasions caused by the parasitism of helminth agents manifest as diarrhea, colic, decreased performance, and in some cases, can lead to a lethal outcome[3, 4] [7].

The issues of epizootiology of invasive diseases in horses have been discussed in many works, but most of the research has only considered individual species or groups of parasites and used data from the western part of Russia[5, 6] [8–10]. Publications on this issue based on the materials from the Siberian region are limited, and the helminthiases prevalent in this territory, including the Gorno-Altai region, are insufficiently studied[7] [11.

The purpose of the study is to characterize the infection and structural features of horse helminth complexes in the Gorno-Altai provinces, which significantly differ from each other in natural-climatic and orographic conditions.

MATERIAL AND METHODS

The study was conducted in 2019-2023 in the farms of nine administrative districts located in four physiographic provinces of the Gorno-Altai region: Northern (Maiminsky, Choisky districts, part of Shebalinsky district), Central (Chemalsky, Ust-Koksinsky, Ongudaisky, Shebalinsky districts), Western (western part of Ust-Kansky district and Charishsky district of the Altai Territory), and Southeastern Altai (Kosh-Agachsky, Ulagansky districts).

Fecal samples obtained from spontaneously helminth-infected horses were examined using classical parasitological methods - helminthocoproscopic according to Fülleborn and helminthocopro-larvoscopy according to Berman-Orlov. Differential diagnosis of nematodes was carried out taking into account the morphometric features of eggs and invasive larvae (third stage). Identification of tapeworms was based on the size of eggs and characteristics of the pear-shaped apparatus[8, 9] [12]. In total, 1963 samples of biomaterial were studied, including 1053 and 910 samples by coproovoscopy and copro-larvoscopy methods, respectively.

The structural features of the gastrointestinal helminth complexes of whole-hoofed animals from different natural-geographical provinces of the Gorno-Altai were determined using the parasite complex index (PCI), reflecting the importance of the species, genus, or other taxon in the helminthological profile of animals[10]. When

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[8]*Kapustin V.F.* Atlas of the most common helminths of farm animals, Moscow: Selkhozgiz, 1953, 140 p.

[9]*Cernea M., Madeira de Carvalho L.M., Cozma V., Raileanu S., Cristina L., Silberg R.* Atlas of Diagnosis of Equine Strongylidosis, Cluj-Napoca, 2008, pp. 71–109.

[10]*Marchenko V.A., Efremova E.A., Vasilieva E.A.* Structure of helminthocenosis of cattle in the Altai Mountains // Russian Journal of Parasitology, 2008, N 3, pp. 18-23.

calculating the PCI, only parasites that are most common and pathogenic for horses, as well as reliably identifiable based on the morphometric features of their propagative forms - eggs or invasive larvae, were considered.

The assessment of animal infection with helminths was carried out based on the results of coprological studies and the calculation of the following indicators:

1) extensiveness of invasion (EI, %) – the proportion of infected animals among those examined;

2) intensity of invasion (II, eggs/g, larvae/g) – the average number of eggs/larvae per one infested animal in 1g of feces;

3) abundance index (AI, eggs/g, larvae/g) – the average number of eggs/larvae per one examined animal in 1g of feces;

4) level of invasion intensity (II_{level}, %) – the average value of II indicators in samples (tests);

5) level of abundance index (AI_{level} AI_{level}, eggs/g, larvae/g) – the average value of AI indicators in the samples.

The significance of differences in the infection of animals was established by comparing the values of II_{level} and II_{level} with the calculation of the Mann-Whitney *U*-test.

RESULTS AND DISCUSSION

In the territory of the Gorno-Altai, gastrointestinal helminthiases in horses are widespread and occur in the form of mixed infections (see Tables 1, 2). Formation of the gastrointestinal helminth complex in horses involves parasites from the classes Nematoda and Cestoda, including five suborders - Strongylata, Ascaridata, Rabditata, Oxiurata, and Anoplocephalata (Cestoda, Cyclophyllidea). Due to the fact that a specific method for diagnosing oxiurates was not used in the study, the indicators of their infection have a random character and are not reflected in the subsequent analysis. Ascaridates are represented by a single species, *Parascaris equorum*, which is widespread. Nematodes of the suborder Rhabditata (Schitwood, 1933) – *Strongyloides westeri* – were found in horses in the Central and Western Altai. The community of nematodes of the suborder Strongylata, family Strongylidae, is characterized by significant taxonomic diversity and includes representatives of the subfamily Strongylinae, including nematodes of the genera *Strongylus, Craterostomum, Triodentophorus, Oesophagodontus*, as well as strongylates of the subfamily Cyathostominae (Trichonematidae), including *Gyalocephalus, Poteriostomum*. In addition, in the examined fecal samples, larvae of strongylates from the family Trichostrongylidae were identified as *Trichostrongylus axei*. According to literature data, trichostrongylosis in horses is widespread[11, 12] [13, 14], but it is registered in the Gorno-Altai region for the first time. The infestation of horses with trichostrongylids in the Central, Northern, and Southeastern Altai is low, at 9.8%, 4.1%, and 27.6%, respectively. In the literature, there is also no mention of the distribution of triodontophores, esophagodonts, craterostomes, gyalocephals, and poteriostomes in the region.

It has been found that in all the provinces, representatives of the class Nematoda with slight zonal variability of the PI indicators (94.1–97.9) dominate in the helminth complexes of horses. Among nematodes, parasites of the suborder Strongylata prevail. The infection of animals with them and their share in helminth complexes not only in the republic (II = 86.8–86.9%, PI = 87) but also in all physiographic provinces of Altai (II = 68.6–93.1%, PI = 86.0–90.5) significantly exceed these indicators for the suborder Ascaridata (II = 13.2%, PI = 7.8 and II = 6.2–16.5%, PI = 4.6–8.1) and for cestodes of the suborder Anoplocephalata (II = 8.8%, PI = 5.2 and II = 2.5–11.8%, PI = 2.1–5.8).

The core of the gastrointestinal helminth complexes consists of cyathostomins, characterized by maximum indicators of horse infestation

[11] *Rehbein S., Visser M., Winter R.* Prevalence, intensity and seasonality of gastrointestinal parasites in abattoir horses in Germany // Parasitology Research, 2013, vol. 112, N 1, pp. 407–413.

[12] *Skryabin K.I., Shikhobalova N.P., Schultz R.S.* Fundamentals of nematodology. Trichostrongylidae of animals and man. Moscow, 1954, vol. 3, pp. 43-55.

and maximum PI values (see Tables 1, 3). At the same time, lower indicators characterize the representatives of strongylins of the genus *Strongylus – Strongylus (Alfortia) edentatus*, *Strongylus equinus*, with minimum values for *Strongylus (Delafondia) vulgaris*: II = 4.5–16.3%, PI = 3.7–8.0 (see Tables 2, 3).

The data obtained by us are consistent with the results of many researchers who note that the distribution of strongyles of the family Strongylidae ("large strongyles"), especially representatives of the genus *Strongylus* spp., and the infestation of horses with them are significantly lower. Currently, cyathostomins, on the contrary, are considered the main agents of equine helminthiases and are widespread worldwide[13-15].

Significant species diversity of strongylates, their ability to complete their life cycle without the involvement of an intermediate host, and the resistance of their propagative forms to adverse environmental factors explain the widespread distribution of the representatives of this suborder and the maximum indicators of animal infestation by them.

Regarding tapeworms, based on the morphometric characteristics of their eggs, they were classified into two species: *Anoplocephala perfoliata*, which is widespread, and *Paranoplocephala mamillana*, which was registered only in the Central and Northern Altai in one and two samples, respectively. Such results are consistent with the data of other researchers confirming the dominant position of *Anoplocephala perfoliata* among tapeworms (see footnote 15).

In the provinces with differing natural-climatic and orographic conditions, quantitative indicators of infestation by major helminth species are not identical.

The conditions in the Central Altai, where horse breeding is more developed and 49.1% of the total horse population of the republic is concentrated[16], are the most favorable for the life cycle of helminths. In this province, extensive areas of mid-mountain landscapes with modifications of steppe, forest, and meadow landscapes are presented, characterized by rich botanical composition of grass and snow-poor pastures. In this territory, the infestation of animals with

Табл. 1. Инвазированность лошадей в провинциях Алтая гельминтами желудочно-кишечного тракта (овоскопия)

Table 1. Infestation of horses in the Altai provinces by gastrointestinal helminths (ovoscopy)

Province	II, %				AI, e/g	*N*	II_{level}, %	AI_y, e/g
	Total	ST	PAR	ANOPL			ST	
Central Altai, *n* = 491	93,3 ± 1,1	93,1 ± 1,1	16,5 ± 1,6	11,8 ± 1,4	562,8 ± 35,1	19	89,6 ± 4,9	503,2 ± 74,3
Northern Altai, *n* = 312	93,6 ± 4,3	92,6 ± 1,5	12,5 ± 1,9	7,1 ± 1,5	311,2 ± 29,8	15	92,4 ± 3,3	328,2 ± 57,1
Western Altai, *n* = 129	78,9 ± 4,0	78,7 ± 4,1	6,2 ± 2,1	7,8 ± 2,3	202,9 ± 40,7	7	85,4 ± 6,8	299,9 ± 138,4
South-Eastern Altai, *n* = 121	68,6 ± 4,2	68,6 ± 4,2	9,1 ± 2,6	2,5 ± 1,4	565,2 ± 102	8	73,2 ± 9,1	429,6 ± 218,2
Republic of Altai, *n* = 1053	87,5 ± 1,0	86,9 ± 1,0	13,2 ± 1,0	8,8 ± 0,9	422,3 ± 22,8	49	83,3 ± 6,9	410,6 ± 186,1

Note. N – number of samples; n – number of tests; ST – helminths of the suborder Strongylata; PAR –Parascaris equorum nematodes; ANOPL – cestodes of the suborder Anoplocephalata.

[13]*Traversa D., Milillo P., Barnes H., von Samson-Himmelstjerna G., Schurmann S., Demeler J., Otranto D., Lia R.P., Perrucci S., Frangipane di Regalbono A., Beraldo P., Amodie D., Rohn K., Cobb R., Boeckh A.* Distribution and species-specific occurrence of cyathostomins (Nematoda, Strongylida) in naturally infected horses from Italy, United Kingdom and Germany // Veterinary Parasitology, 2010, vol. 168, pp. 84–92.

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[15]*Ryu S.H., Bak U.B., Kim J.G., Yoon H.J., Seo H.S., Kim J.T., Park J.Y., Lee C.W.* Cecal rupture by Anoplocephala perfoliata infection in a thoroughbred horse in Seoul Race Park, South Korea // Journal of Veterinary Science, 2001, vol. 3 (2), pp. 189–193.

[16]Statistical Yearbook of the Altai Republic. Gorno-Altaisk, 2016, 41 p.

Табл. 2. Инвазированность лошадей в провинциях Алтая стронгилятами желудочно-кишечного тракта (лярвоскопия)
Table 2. Infestation of horses in the Altai provinces by gastrointestinal strongyles (larvoscopy)

Province	II, %					AI, l/g	*N*	II_{level}, %	AI_{level}, l/g
	Total	Cyatho-sto-minae	Strongylinae						
			St. equin.	*St. edent.*	*St. vul.*				
Central Altai, *n* = 417	94,9 ± 1,1	93,0 ± 1,2	33,8 ± 2,3	31,9 ± 2,3	16,3 ± 1,8	15,5 ± 1,9	18	89,9 ± 4,2	14,7 ± 3,5
Northern Altai, *n* = 255	85,1 ± 2,2	84,7 ± 2,3	24,7 ± 2,7	20,0 ± 2,5	6,7 ± 1,6	9,4 ± 1,2	14	85,0 ± 7,0	10,6 ± 2,6
Western Altai, *n* = 127	74,8 ± 3,8	74,8 ± 3,8	18,1 ± 3,4	18,9 ± 3,5	8,7 ± 2,5	4,2 ± 0,6	7	78,1 ± 9,7	3,7 ± 1,1
South-Eastern Altai, *n* = 111	73,8 ± 4,2	69,4 ± 4,4	23,4 ± 4,0	13,5 ± 3,3	4,5 ± 1,9	10,9 ± 4,9	8	75,4 ± 8,1	12,1 ± 5,4
Republic of Altai, *n* = 910	86,8 ± 1,1	85,3 ± 1,2	27,8 ± 1,5	24,5 ± 1,4	10,2 ± 1,0	10,5 ± 0,8	47	82,2 ± 1,1	10,3 ± 2,3

Note. N – number of samples; n – number of tests; types: St. equin. – Strongylus equinus, St. edent. – Strongylus edentatus, St. vul. – Strongylus vulgaris.

Табл. 3. Структура гельминтокомплекса лошадей в провинциях Алтая (лярвоскопия)
Table 3. Structure of the helminth complex of horses in the Altai provinces (larvoscopy)

Province	PI					
	Cyatost.	*St. equin.*	*St. edent.*	*St. vul.*	PAR	ANOPL
Central Altai, *n* = 417	45,7	16,6	15,7	8,0	8,1	5,8
Northern Altai, *n* = 255	54,4	15,9	12,8	4,3	8,0	4,6
Western Altai, *n* = 127	55,6	13,5	14,1	6,5	4,6	5,7
South-Eastern Altai, *n* = 111	56,7	19,1	11,0	3,7	7,4	2,1
Republic of Altai, *n* = 910	50,2	16,4	14,4	6,0	7,8	5,2

Note. *n* – number of samples; strongyles: Cyatost. – subfamily Cyathostominae, *St. equin*. – *Strongylus equinus, St. edent. – Strongylus edentatus, St. vul. – Strongylus vulgaris*; PAR – nematodes *Parascaris equorum*; ANOPL – cestodes of the suborder Anoplocephalata.

strongyles, according to ova-larval research, is the highest – 93.1% and 94.9%, and the indicators of the abundance index (AI) and AI_{level} are also the highest – 562.8 eggs/gram, 15.5 larvae/gram, and 503.2 eggs/gram, 14.7 larvae/gram (see Tables 1, 2). Horses in this province are more infected with Parascaris (II = 16.5%, PI = 8.1) and cestodes of the suborder Anoplocephalata (II = 11.8%, PI = 5.8) than in other areas (see Tables 1, 3).

The infestation of equids with intestinal nematodes as a whole, including strongyles (93.6% and 85.1%) and ascarids (II = 12.5%, PI = 8.0), in the horse farms of the Northern Altai does not significantly differ from the level of infestation in the Central Altai. However, the AI and AI_{level} indicators for nematodes of the suborder Strongylata are 1.5–1.8 times lower – 311.2 and 328.1 eggs/gram, respectively. The infestation with Anoplocephalidae is also almost 2 times lower here (II = 7.1%), and the share of cestodes in the helminth complex structure is 4.6.

In the Western Altai, the infestation of animals with ascarids, anoplocephalids, and strongyles was 6.2 (PI = 4.6), 7.8 (PI = 5.7), and 74.8%, respectively. The degree of infestation of horses with nematodes of the suborder Strongylata (AI = 202.9 eggs/gram, AI_{level} = 299.9 eggs/gram) in this territory is 3.0 and 1.5 times lower than in the Central and Northern Altai. The re-

sults of ova-larval research are supported by larvalscopy data (see Table 2), which also indicate significant differences in the intensity of infestation of the horse population in the provinces.

The most pronounced changes in the structural-functional characteristics of helminth complexes are recorded in horses in the Southeastern Altai. In this area, the infestation of animals with strongyles and anoplocephalids is minimal compared to other provinces. The infestation of equines with intestinal nematodes, including strongyles, is 68.6 and 73.8%, and with tapeworms (Cestoda) – 2.5% (PI = 2.1), which is 1.5 and 2.8–4.7 times lower, respectively, than in other provinces (see Tables 1–3).

According to the results of ova-larval research, on the territory of the Southeastern Altai, despite lower extensiveness of equid infestation with strongyles, the intensity of infestation (AI_{level}) is 429.6 eggs/gram and 12.1 larvae/gram, which is comparable to similar indicators of animal infestation in the Central Altai – 503.2 eggs/gram and 10.6 larvae/gram. This fact is explained by the non-uniform intra-zonal distribution of strongyles, which is due to significant natural-climatic differences in the territory, where all natural zones from high-mountain forests and meadow landscapes to semi-deserts and tundra are represented. The infestation of one-toed ungulates in the mountain-steppe zone with nematodes of the suborder Strongylata (II = 62.4%, AI = 144.1 eggs/gram and II = 66.2%, AI = 4.7 larvae/gram) is 1.3 and 4.0–6.3 times lower than in animals in the mountain-forest zone (II = 83.3%, AI = 903.6 eggs/gram and EI = 89.2%, AI = 18.6 larvae/gram).

At the same time, the values of II and PI are comparable to II_{level} and AI_{level} values, which confirm our conclusions about the influence of the diversity of natural-climatic conditions within the highest-altitude province of Altai on the infestation of animals by helminths. Unlike other provinces, Southeastern Altai shows a wide range of infestation values in the samples (ova microscopy: II = 40.0–100.0%, AI = 7.4–1758.5 eggs/gram; larval microscopy: II = 45.0–100.0%, AI = 0.6–44.5 larvae/gram). Furthermore, in this province, all indicators of infestation of animals by nematodes of the suborder Strongylata in the mountain-forest zone (valleys of the Argut and Chulushman rivers) are significantly and significantly higher than in the high-mountain steppe zone. At the same time, the level of extensiveness of infestation (EI_{level}) and the level of intensity of infestation (II_{level}) of horses in the high-mountain steppe zone of Southeastern Altai are statistically lower than in the animals from Northern and Central Altai (see Table 4).

It has also been found that in the structure of the helminth complexes of horses in the highland steppes and highland forests of Southeastern Altai, despite the clear dominance of cyathostomes with PI of 58.3 (II = 59.5%) and 56.9 (II = 89.2%), subdominants are Parascaris (PI = 11.6, EI = 11.8%) and Trichostrongylus (PI = 13.8, II = 21.6%).

In contrast to other provinces, where the structure of the whole-hoofed animals helminth complex has a ratio of 16.2–20.7: 1.0-1.7: 1.0–1.2 for strongyles, ascarids, and anoplocephalids, in Southeastern Altai, the proportion of strongyles and Parascaris is significantly higher, resulting in the following ratio – 46.6: 3.5: 1.0.

The low values of PI (2.1) and infestation (2.5%) by anoplocephalids in horses in the high-mountain territories of Southeastern Altai, characterized by the lowest heat supply and low precipitation levels compared to other physiographic provinces of the region, are most likely determined by the low population density of oribatid mites - intermediate hosts of cestodes.

Overall, there are no significant differences in the extensiveness of infestation between the provinces of the Altai Mountains according to ova microscopy. However, the level of abundance of helminth eggs in the samples obtained from horses in Central Altai is significantly higher than in animals living in Northern and Western Altai (see Table 5). Additionally, it is established that the results of larval microscopy are comparable to ova microscopy data (see Table 5).

We have also not recorded significant differences in the values of EIu in horses from different provinces. However, the indicators of the abundance of helminth larvae and eggs in the samples from equids of Central Altai are significantly higher than in samples from animals in

Табл. 4. Достоверность различий показателей уровня зараженности лошадей Юго-Восточного Алтая кишечными стронгилятами по *U*-критерию Манна–Уитни ($U_{эмп}/U_{крит}$), оволярвоскопия
Table 4. Reliability of differences in the level of infestation of horses in the South-Eastern Altai by intestinal strongyles according to the Mann–Whitney *U*-test (U_{emp}/U_{crit}), ovolarvoscopy

Natural zone	*n*	Ovoscopy				Larvoscopy			
		II_{level}, %	U_{crit}	AI_{level}	U_{crit}	II_{level}, %	U_{crit}	AI_{level}	U_{crit}
Mountain-forest (middle mountains)	3	100,0	0/1**	928,8	2/3*	100,0	0/1**	24,4	1/3**
Mountain-steppe (highlands)	4	57,1		186,5		60,7		4,6	

Note. *$p \le 0,05$; **$p \le 0,01$; *n* – the number of samples.

Табл. 5. Достоверность различий показателей уровня зараженности лошадей кишечными стронгилятами по *U*-критерию Манна–Уитни ($U_{эмп}/U_{крит}$)
Table 5. Reliability of differences in the levels of infestation of horses with intestinal strongylates according to the Mann–Whitney *U*-test (U_{emp}/U_{crit})

Province	Northern Altai	Central Altai	Western Altai	South-Eastern Altai
	n = 15	*n* = 19	*n* = 7	*n* = 8
		Ovoscopy		
Northern Altai	0	141/94	37/28	35/33
Central Altai	94/94*	0	39/37	49/44
Western Altai	40/28	36/37*	0	23/13
South-Eastern Altai	48/33	60/44	25/13	0
		Larvoscopy		
Northern Altai	0	122/82	39/26	41/31
Central Altai	110/82	0	49/35	49/30
Western Altai		31/35*	0	24/13
South-Eastern Altai		61/41	22/13	0

Note. *$p \le 0.05$; *n* – number of samples; data above zero row – level of invasion extensiveness; data below zero row – level of abundance of helminth eggs (ovoscopy) and larvae (larvoscopy).

Western Altai (see Table 5).

On the territory of the Altai Mountains, parasitic infestations of horses occur in the form of mixed infestations. At the same time, the mosaic of ecological conditions in the mountainous region determines the structural features of the nosological profile of animal helminthiases and the degree of infestation by helminths in individual provinces.

CONCLUSION

In the structure of the helminth complexes of the digestive system of horses in different physiographic provinces of the Altai Mountains, strongyles dominate. The infestation of animals with strongyles (II = 68.6-94.9%, AI_{level} = 299.9-503.2 eggs/gram) and their proportion in helminth complexes (PI = 86.0-90.5) significantly exceed the corresponding indicators for the nematodes of the suborder Ascaridata (II = 6.2-16.5%, PI = 4.6-8.1) and cestodes of the suborder Anoplocephalata (II = 2.5-11.8%, PI = 2.1-5.8). Equine infestations are widespread, but differences in the intensity of animal infestation by helminths of individual taxonomic groups and in the structure of helminth complexes are recorded within provinces, with the most pronounced differences observed in horses from Southeastern Altai. Here, the infestation of horses with strongyles (68.6%) and anoplocephalids (2.5%) is 1.5 and 2.8-4.7 times lower than in other provinces, but the intensity of infestation with strongyles is maximal (AI = 565.3 eggs/gram, AI_{level} = 429.6 eggs/gram), which is due to intra-zonal differences in the ecological conditions of the highlands.

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РАЗРАБОТКА НОВОГО МЕТОДА ОЦЕНКИ ЭМБРИОНОВ В ЯЙЦЕ ПТИЦЫ ДО ЕГО ИНКУБАЦИИ

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Сформулированы и обоснованы требования к методам определения пола эмбриона в яйце в соответствии с ужесточением ранее принятых норм отбраковки петушков при инкубации. Проведен анализ новых разрабатываемых методов определения и отбраковки эмбрионов яиц в течение 7 дней их инкубации, описаны их преимущества и недостатки. Выявлены два неинвазивных метода, которые имеют определенную перспективу коммерческого внедрения в отрасль птицеводства (инфракрасная спектроскопия и компьютерное зрение). Цель исследования – определить возможности неинвазивного метода определения пола эмбриона в яйце до инкубации на основании интеллектуального анализа предложенных морфометрических признаков яйца птицы. Впервые разработан метод определения полового диморфизма, основанный на анализе параметров асимметрии яйца по трем пространственным координатам, определяемым методами компьютерного зрения с применением машинного обучения. Разработана экспериментальная установка оценки жизнеспособности и создание необходимых условий для проведения инкубации и вывода цыплят для подтверждения осуществления предлагаемого метода. В состав ее входят smart инкубатор «Умная наседка», брудер, тепловизионная микрокамера TE-Q1, маслонаполненный радиатора POLARIS модели PRE T 0915, увлажнитель воздуха Ergopower ER 604, бактерицидный облучатель-рециркулятор воздуха DEFENDER 2-15C, термогигрометр RGK TH-30 и ноутбук. При получении изображений в установке использован цифровой фотоаппарат Canon EOS 2000D EF-S 18-55 III Kit с современной CMOS-матрицей (22,3 × 14,9 мм) и мощным процессором. Геометрическую пространственную цифровую модель каждого яйца программным путем искусственно разбивали на множество элементов, по которым определяли асимметрию формы яйца. При этом по измеренным линейным размерам каждого элемента определяли их индексы формы, площадь, объем и периметр. Проведена инкубация 72 оплодотворенных яиц курицы кросса Dekalb White. После инкубации удалось достоверно определить пол 38 цыплят. Применение методов машинного обучения при решении задач бинарной классификации для малой выборки (38) с большой размерностью исходного набора признаков позволило получить три окончательные модели со значением точности метрик достоверности AUC = 73–72% и F1 = 69–72%: Random Forest классификатор с 4 оценщиками и максимальной глубиной 3; классификатор случайного леса с 10 оценщиками и максимальной глубиной 5 и классификатор AdaBoost с 4 оценщиками дерева решений и максимальной глубиной 3. Экспериментальное подтверждение взаимосвязи асимметрии формы яйца с его половым диморфизмом позволит приблизиться к решению мировой научной проблемы достоверного определения пола яйца до инкубации.

Ключевые слова: яйцо, инкубация, эмбрион, диморфизм, определение, методы, компьютерное зрение

DEVELOPMENT OF A NEW METHOD FOR EVALUATING EMBRYOS IN A BIRD EGG BEFORE INCUBATION

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The requirements to the methods of embryo sex determination in an egg have been formulated and substantiated in accordance with the tightening of the previously accepted norms of cockerel culling during incubation. New methods under development for identifying and culling of egg embryos within 7 days of incubation have been analyzed, and their advantages and disadvantages have been described. Two non-invasive techniques have been identified that have some potential for commercialization in the poultry industry (infrared spectroscopy and computer vision). The purpose of the study is to determine the possibilities of a non-invasive method for determining the sex of an embryo in an egg prior to incubation based on intelligent analysis of the proposed morphometric features of poultry eggs. The scientific novelty of the research lies in the fact that for the first time a method of determining sexual dimorphism based on the analysis of egg asymmetry parameters by three spatial coordinates determined by computer vision methods with the use of machine learning has been developed. An experimental unit for viability assessment and establishment of the necessary conditions for incubation and hatching of chicks has been developed to validate the implementation of the proposed method. It includes a smart incubator "Smart Nest", a brooder, a thermal imaging micro-camera TE-Q1, an oil-filled radiator POLARIS model PRE T 0915, an air humidifier Ergopower ER 604, a bactericidal air irradiator-recirculator DEFENDER 2-15C, a thermohygrometer RGK TH-30 and a laptop. For image acquisition, the setup utilized a Canon EOS 2000D EF-S 18-55 III Kit digital camera with a state-of-the-art CMOS sensor (22.3 × 14.9 mm) and a powerful processor. The geometric spatial digital model of each egg was artificially divided into a set of elements by software, by which the asymmetry of the egg shape was determined. In doing so, their shape indices, area, volume and perimeter were determined from the measured linear dimensions of each element. Incubation of 72 fertilized eggs of Dekalb White cross hen was carried out. Following the incubation, it was possible to reliably determine the sex of 38 chicks. Applying machine learning methods in solving binary classification problems for a small sample (38) with high dimensionality of the initial feature set yielded three final models with accuracy metrics AUC = 73–72% and F1 = 69–72%: Random Forest classifier with 4 evaluators and maximum depth of 3; Random Forest classifier with 10 evaluators and maximum depth of 5 and AdaBoost classifier with 4 decision tree evaluators and maximum depth of 3. Experimental confirmation of the relationship between the egg shape asymmetry and its sexual dimorphism will make it possible to approach the solution of the world scientific problem of reliable determination of the egg sex before incubation.

Keywords: egg, incubation, embryo, dimorphism, definition, methods, machine vision

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INTRODUCTION

Every year, more than 7 billion domestic birds undergo culling of male chicks after the incubation period [1]. Since this procedure is typically carried out using methods that are considered inhumane, such as maceration and suffocation in a carbon dioxide environment, it raises public concerns about the unethical practices of food producers [2]. In response to this, several European countries (Germany, France) have implemented laws prohibiting the mass culling of day-old chicks since 2022[1]. Switzerland plans to introduce a similar ban in 2026. Producers are required to use technologies to determine the sex of the chick before it hatches and prevent the hatching of male chicks during incubation. Furthermore, starting in 2024, some European countries plan to ban the destruction of live embryos in bird eggs after the 6th day of incubation[2]. This is due to the presence of heartbeats in developing embryos as early as the 3rd day of incubation and the gray area of embryonic pain limit on the 7th day of chicken egg incubation (see footnote 2).

Determining the sex of an embryo in an egg before incubation remains an unresolved issue [3]. In light of these new trends, a promising method for determining the sex of an embryo in an egg should:

– preserve the integrity of the eggshell or the embryo, i.e., be a non-invasive method;

– absence of a negative impact on the embryo in the egg and on chicks during their hatching and further development;

– allow for the culling of incubation eggs before incubation or within 6-7 days when the likelihood of pain limit in the developing embryo is low, making it ethically acceptable;

– be fast-acting, so it can be applied to a large number of eggs in commercial incubators;

– avoid the use of complex foreign technologies, technical, and other means during its implementation;

– possess the necessary reliability or accuracy in predicting sexual dimorphism in the egg;

– no labor-intensive operations or complex analytical procedures that would increase its commercial implementation costs but offer the prospect of its use directly in the incubators of domestic poultry farms.

In this regard, when analyzing existing methods for assessing sexual dimorphism in bird embryo eggs and developing a new method through experimental research, this study takes into account the requirements outlined above.

The research objective is to define the possibilities of a non-invasive method for determining the sex of an embryo in an egg before incubation based on the intelligent analysis of proposed morphometric features of bird eggs.

MATERIAL AND METHODS

The analysis of over 100 sources on existing methods and means for assessing the sexual dimorphism of bird embryos in eggs during incubation (up to 7 days) revealed five main methods (see Table 1). Among the methods capable of culling incubation eggs within 7 days, we can include infrared spectroscopy (IR) using an optical transmission spectrometer UV-VIS-NIR [4] and computer (technical) vision using a neural network of a genetic algorithm backpropagation error (GA-BPNN) [5].

These methods, to some extent, correspond to the requirements outlined above for their implementation in the poultry industry for assessing sexual dimorphism in embryos before they develop nociception.

However, transmission spectrometry requires[3]:

[1]*Gschweng D.* In Deutschland ist es seit 2022 verboten, Küken zu schreddern // Die Industrie umgeht das Verbot. 2023. 11.01. https://www.infosperber.ch/gesundheit/ernaehrung/bruderhaehne-toeten-verboten-nun-sterben-sie-im-ausland/ (дата обращения 15.09.2023).

[2]*Akiyama R., Matsuhisa A., Pearson J.T., Tazawa H.* Long-term measurement of heart rate in chicken eggs // Comp. Biochem. Physiol. Part A Mol. Integr. Physiol, 1999, vol. 124, Issue 4, pp. 483–490.

[3]Infrared spectroscopy: https://microbiologynote.com/ru/принцип-инфракрасной-ИК-спектроскопии-инструментальное-применение/ (accessed on 15.09.2023).

Табл. 1. Существующие методы определения пола эмбриона в ранние дни инкубации

Table 1. Existing methods for determining the sex of an embryo in the early days of incubation

Sex determination method	Special feature	Determination day	Significance, %	Advantages	Disadvantages	Source
Infrared spectroscopy	Transmission spectroscopy (wavelength range 360-1000 nm)	7^{th}	From 84,29 to 87,14	Non-invasive method	High labor intensity of sex determination	4
Computer (technical) vision	Determination of the structure and distribution of blood vessels in the volume of the incubated egg, neural network GA-BPNN	4th	From 89,00 to 78,58 for double and single uncovered layers	Non-invasive method, realized with the help of inexpensive technical means, early date of sex determination	The reliability of sex prediction depends on the coloration of the incubated egg, the presence of inhomogeneous inclusions on its shell, the need for strict orientation for image acquisition	5
Raman spectroscopy	Embryo blood sampling, laser excitation wavelength - 785 nm	3,5th	Up to 90	Early timing of sex determination, sufficient reliability of prognosis	Invasive method: opening the egg shell, which affects the development of the embryo	6, 7
Fluorescence spectroscopy	Embryo blood collection, laser wavelength - 785 nm	3,5th	Up to 90	The same	The same	8
"Two-wavelength" fluorescence spectroscopy	Taking embryo blood, opening the egg at the blunt end of the egg	4th	Up to 96	Early sex determination, inner membrane of the egg is not damaged, high reliability of prediction	Invasive method: opening of the egg shell at its blunt end	9

– exclusion of any external light, electronic noise, minimal vibrations, and other interferences within the analysis zone;

– placing the specimen under study in a specially designed cell that should not contain materials absorbing infrared radiation (plastic, glass);

– regulation of microclimate parameters, especially relative humidity of the air, as the accuracy of analytical procedures of such a spectrometer depends on changes in these conditions.

Therefore, the procedure for analysis by the IR spectrometry method is time-consuming, complex, labor-intensive, and less attractive for commercial implementation.

The use of computer vision reduces the costs associated with labor-intensive processes, related to the increasing intensification of poultry production. These methods will provide a large volume of diverse data for assessing viability and predicting the sexual characteristics of embryos in eggs for subsequent analysis [3, 5].

Regarding the developed method for determining the structure and volume distribution of blood vessels in the incubated egg, it does not have high accuracy in determining the gender (see Table 1). First, the eggshell's surface must have a purely white uniform surface, without specks and foreign inclusions; otherwise, this introduces additional errors in determining the gender of the embryo [10]. Second, the accuracy of determination is influenced by physiological and biochemical processes occurring during the embryo's development, as the composition of the egg's components changes significantly during incubation. For example, during incubation, there is a reduction in the mass of protein, especially from the 3rd to the 5th day (from 50.38% to 21.44%), and by the 7th day, the yolk accounts for 65.4% of the egg's mass[4].

In the implementation of the method for assessing the gender of embryos based on the characteristics of the vascular structure, the use of a light-controlled digital camera is required due to the loss of light permeability of the liquid in the egg during incubation [11]. A robotic system for egg identification, brief extraction from the incubator, uniform placement on a tray identical throughout the batch of incubated eggs, illuminated from a light source, and obtaining a precision image of the vessels is also necessary. For detection and image acquisition, an additional system is required, which, using a three-coordinate executive mechanism, will detect blood vessels and obtain a clear image of their structure. It should be noted that in the early days of incubation, the vascular structure is barely distinguishable. The size and distribution characteristics of the vessels on the surface are different because these characteristics change depending on the area of egg projection in the obtained image. Furthermore, during the process of obtaining images, it is necessary to wait for the yolk, which is more viscous than the protein, to stop making oscillatory movements and to select the projection that captures the vascular system. Therefore, the costs of implementing this method are comparable to the costs of developing the existing robotic system Ella by the German company Seleggt, with a capacity of 3600 eggs per hour, which allows for the determination of the sex of future chicks on the 8th-10th day of incubation with 98% accuracy using an invasive method (see Figure 1)[5].

A method is proposed based on the idea of using morphometric features of the egg to determine the sex of the embryo [12]. In the development of the considered approach, which is based on determining the ratio of the longitudinal to transverse linear size of the egg, the emphasis is placed on determining the asymmetry of morphometric features of the egg concerning spatial coordinates. It can be assumed that the character of the asymmetry of chicken eggs concerning spatial coordinates with embryos of male and female sexes is different.

The tendency towards symmetry of shape in living organisms is a well-known fact and is explained by the reduction of entropy in ordered systems. The shape of future chick eggs may be more symmetrical than that of future roosters,

[4]Physiology of the developing embryo: https://studref.com/534356/agropromyshlennost/fiziologiya_razvivayuschegosya_ embriona#:: (accessed on 15.09.2023).

[5]In-ovo sexing: https://en.wikipedia.org/wiki/In-ovo_sexing#:~:text=In%20poultry%20farming%2C%20in-ovo%20sexing,company%20Seleggt%20in%20November%202018 (accessed on 15.09.2022).

and it will tend to approach a sphere (to preserve future offspring) [13]. This will allow for a more even distribution of forces on the eggshell with the future chick under possible sudden mechanical loads and increase its resistance to shell damage compared to eggs of the opposite sex [3, 14].

The developed method includes the following techniques performed using a complex of computer programs [15].

Six basic parameters of the digital egg model in pixels (px) are determined: longitudinal size (*l*), transverse size (*b*), perimeter, area, volume, and shape index (the ratio of transverse *b* to longitudinal size *l*).

In the obtained digital egg model, the center of the ellipsoidal shape is established based on the values of longitudinal and transverse size.

The original images of ellipsoidal egg shapes are divided into four equal sectors, and from each sector, four new images are generated using mirror reflection. This determines the halves of the transverse and longitudinal sizes of the specific egg image and calculates their areas and perimeters. A circle is then inscribed from the center of the ellipsoidal shape model. The radius of the circle is equal to half of the transverse size of the specific egg image. The areas, perimeters, and their sums, differences, and ratios outside the inscribed circle are calculated both at the sharp and blunt ends of the egg image. This results in 45 variable features of the model.

Then, symmetrically from the center, 12 uniform sections perpendicular to the longitudinal axis l of the specific egg image are formed, and the values of all transverse sizes of the egg images, as well as all sizes of their intersections on the longitudinal axis of the specific egg image, are determined. Their ratios at all intersection points and the distribution of shape indices are calculated. Then, through uniform segments of ellipsoidal egg shapes (Ω = 10 degrees), they are divided into a system of 36 radius-vectors, and their modules from the chosen center of the shape to the contour boundary of the specific egg image are determined.

All the data obtained above, characterizing the shape of the digital egg model and its deviation from symmetry, are subjected to static

Рис. 1. Внешний вид роботизированного комплекса Ella

Fig. 1. External view of the Ella robotic complex

analysis. Only the data with the lowest level of significance in each selected dimensional group are highlighted. Then, the selected data are used in machine learning methods applicable when solving the problem of binary classification for small samples with a high dimensionality of the original feature set.

RESULTS AND DISCUSISION

For conducting experimental research, a viability assessment unit (hereafter referred to as VAU) and creating the necessary conditions for incubation and chick rearing was made. The VAU installation included an incubator *1*, a brooder *2*, a thermal imager *3*, a smartphone *4*, a laptop *5*, an electric heater *6*, a humidifier *7*, a device for measuring air temperature and humidity *8*, and a UV bactericidal air recirculator *9* (see Fig. 2). The VAU was placed in an isolated dark room with an area of 14 m^2, where the necessary temperature and relative humidity of the air were maintained using an oil-filled radiator POLARIS model PRE T 0915 and an air humidifier Ergopower ER 604, in accordance with the recommendations outlined in the incubator's operation manual.

A domestic Smart incubator "Intelligent Brood Hen" IB2NB-UI, TU4743-002-8610978–2008, designed for incubating six species of domestic birds, was selected as the incubator. It, like the setup for obtaining egg images using computer vision, was located in a dark room.

The main technical characteristics of the incubator, according to the accompanying documentation, are as follows: power supply voltage 12 V, current 8 A, maximum power 80 W, adjustable temperature range from 37.0 to 39.5 °C, temperature control accuracy ±0.1 °C, capacity for standard chicken eggs – 104 eggs; overall dimensions of the case (excluding the drive) 795 × 595 × 295 mm, weight in packaging not more than 4 kg.

To ensure the survival of the hatched chicks after incubation, they needed to be placed in a special insulated enclosed space where a higher thermal regime than in the heated room needed to be maintained. Additionally, this space had to provide all the conditions for the development of such a living organism. First and foremost, it was necessary to provide the chicks with drinking water with the possibility of adding stimulating and medicinal substances and food. It was also necessary to timely remove waste, eliminate the possibility of through-airflow from holes and gaps in the room, ensure the supply of clean and fresh air, etc. For these purposes, a dismountable brooder 77 with an infrared ceramic lamp and a drinker was purchased.

Before incubation, the batch of eggs was assessed for viability using an Alisa egg candler and a thermal imaging camera TE-Q1.

After that, precise images (digital models) of each incubation egg were obtained using the computer vision setup. To obtain the images, a digital camera Canon EOS 2000D EF-S 18-55 III Kit with a modern CMOS matrix (22.3 × 14.9 mm) and a powerful processor [3] was used. Its characteristics include a color depth of 42 bits per pixel, a matrix sensitivity range from 100 to 6400 ISO, a crop factor of 1.6, automatic exposure with priority for both aperture and shutter speed, a mirror (TTL) viewfinder with a field of view of 95%, and a fast-shooting speed of three frames per second.

A filter HSV (hue, saturation, value) was applied to each frame of the egg image, allowing for glare-free, high-resolution (1660 × 1900 px) black and white images. Thus, after these transformations, a single white object in the form of a closed oval geometric figure was formed in the frame, the shape of which corresponded to the object under investigation – a chicken egg.

Before incubation, all components of the VAU installation and the batch of eggs underwent disinfection in accordance with recommendations[6]. To maintain sanitary and preventive measures in the room, a bactericidal air recirculator DEFENDER 2-15C was used. After the procedure of determining and saving the parameters of the egg images on a personal computer, eight eggs

[6]GOST 10-85-87. Production of hatching eggs of agricultural poultry. Technology of preincubation processing. Basic parameters. Ministry of Agriculture of Russia, 1988.

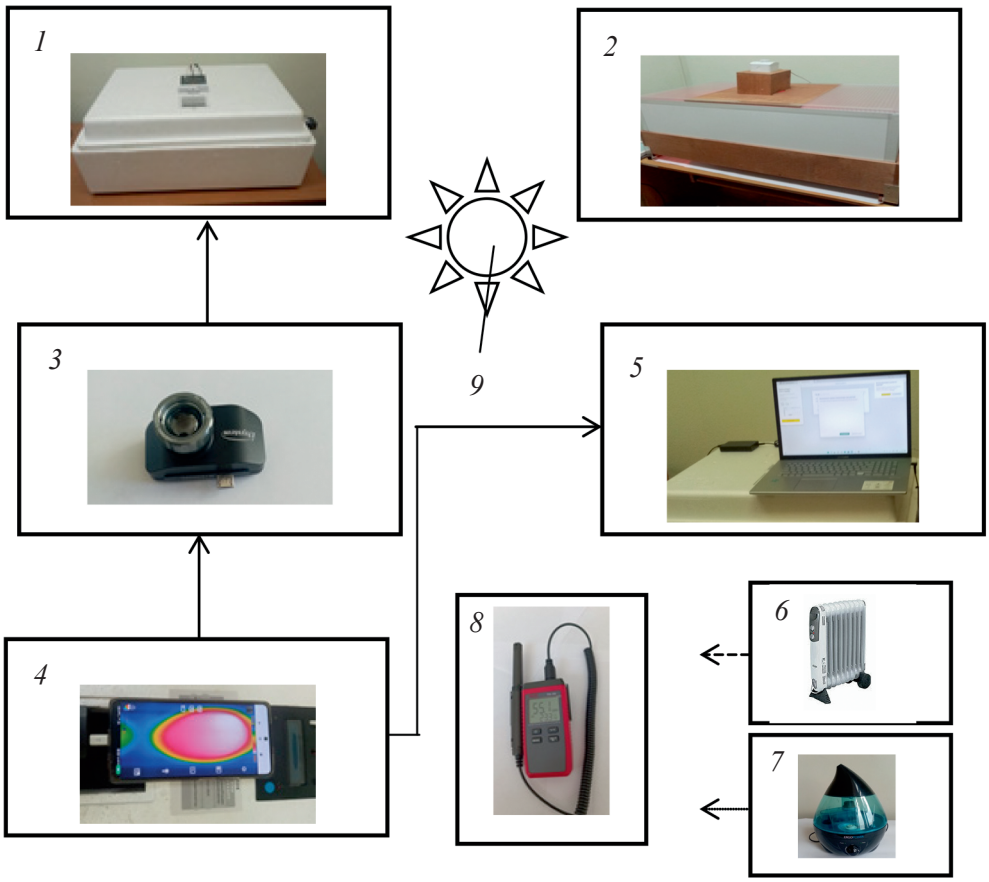

Рис. 2. Состав установки УПЖ
Fig. 2. Composition of the VAU (viability assessment unit)

with low embryo viability were culled from the entire batch of research eggs. A total of 72 chicken eggs (Dekalb White cross) were subjected to incubation for 21 days. During incubation, 69 live chicks hatched from the 72 eggs. Four chicks died in the brooder, and seven hatched with signs of rickets. Of the healthy chicks, only 38 (24 males and 14 females) could be reliably identified by sex, based on the coincidence of the external feather features of one-day-old chicks and their reflex reactions [15].

Thus, the initial dataset consisted of 38 samples of images, from which a set of numerical geometric characteristics was formed. Each set was described by 93 asymmetry features. Since the distribution of almost all asymmetry parameters differed significantly from normal, two criteria were used to test the hypothesis of differences in means: $p1$ - Mann-Whitney U criterion and $p2$ - Kolmogorov-Smirnov criterion [16].

The application of both criteria allowed for increased confidence in confirming the hypothesis of differences in means (mathematical expectations). Based on the results of statistical analysis, which did not yield significant differences for the two groups under consideration in terms of asymmetry parameters, 26 parameters were selected from all the indicators. Data with the lowest values of $p1$ and $p2$ criteria were chosen (see Table 2).

The obtained group of features included geometric characteristics of the egg: two features from basic parameters, four from shape index parameters based on cross-sectional and longitudinal dimensions, seven from radius vector module parameters, and 13 from asymmetry feature parameters based on quadrant divisions and inscribed circles.

However, the use of statistical analysis did not yield the expected results.

Therefore, it was decided to investigate the applicability of machine learning methods to obtain models capable of determining the sex of chicks based on geometric characteristics of the egg and identifying the most informative features.

When constructing models, the set of functions obtained using various image processing methods was grouped into several separate categories:

– G01 (6 features – perimeter, area, volume, longitudinal and transverse dimensions, shape indices);

– G02 (11 features – shape indices based on segmented cross-sectional and longitudinal dimensions);

– characteristics of radius vectors drawn from the center of the object to the contour boundary: G03 (36 features – vector length per segment); G04 (18 features – length ratio at 180 degrees); G05 (18 features – length ratio at 90 degrees);

– characteristics of the finished sample: G06 (16 features – Group 1: areas, perimeters); G07 (8 features – Group 2: using an inscribed circle); G08 (12 features – Group 3: averaged characteristics);

– G09 (4 functions – calculated volumes of elements of division);

– G10 (93 functions: combination of groups G01-G09);

– G11(53 functions: combination of groups G01-G03).

The following machine learning algorithms (ML) were used: the construction of models such as decision trees, random forests, AdaBoost, logistic regression, and support vector machines (SVM) for classification using the following configurations:

– M01: decision tree classifier with a maximum depth of 3;

– M02: decision tree classifier with a maximum depth of 5;

– M03: random forest classifier with 4 estimators and a maximum depth of 3;

– M04: random forest classifier with 10 estimators and a maximum depth of 5;

– M05: AdaBoost classifier with 4 decision tree estimators and a maximum depth of 3;

– M06: support vector machine classifier with an "RBF" kernel;

– M07: support vector machine classifier with a "linear" kernel;

– M08: logistic regression with L2 regularization.

The Python programming language was used for data processing and analysis. The scikit-learn library was chosen for implementing the listed machine learning algorithms, and the Orange 3 program with a graphical user interface was used for model construction. When evaluating model metrics, a cross-validation approach was applied in two different variants[7]. At the initial stage of work, the leave-one-out method was used, in which the test subset consisted of one sample (k = 1), and the number of divisions and models was equivalent to the number of samples (N = 38). During the model building and selection phase, a K-fold cross-validation method with K = 3 splits and result averaging was implemented.

The advantage of using cross-validation is that it allows for a more reliable assessment of the model's performance since it tests the model's ability to generalize new data that were not seen during training. This helps prevent overfitting, where the model performs well on the training data but poorly on new data. Thus, cross-validation can provide a more accurate assessment of model performance and make it more reliable for practical use. Among the numerous metrics available for evaluating classification models[8], this study used the AUC ROC (Area Under Curve Receiver Operating Characteristic) and F1-score. The F1-score is calculated as the harmonic mean of precision and recall, providing equal weight to both precision and recall.

The complexity of the research task required a series of experiments, each of which yielded the desired results.

[7]*Wong T.-T.* Performance evaluation of classification algorithms by k-fold and leave-one-out cross validation // Pattern Recognition, 2015, vol. 48, Issue 9, pp. 2839–2846.

[8]*Branco P., Torgo L., Ribeiro R.P.* A Survey of Predictive Modeling on Imbalanced Domains // ACM computing surveys (CSUR), 2016, vol. 49, Issue. 2, pp. 1–50.

Табл. 2. Список параметров, выбранных на этапе статистического анализа*
Table 2. List of parameters selected at the statistical analysis stage*

Number	Parameter code	Parameter description, px	Range (batches)	Hens	Cockerels	*p*1 (p-value)	*p*2 (p-value)
		Baseline parameters and cut asymmetry					
1	x_height	*l*	956,0 ÷ 1091	1053 ± 38	984 ± 28	0,44	0,64
2	x_perim	Perimeter (Per.)	2989,4 ÷ 3407,6	3234,7 ± 97,5	3218,9 ± 89,0	0,41	0,58
3	x_M1_shapeInd_01	*l/b* for the segment 1	2,2 ÷ 2,7	2,4 ± 0,1	2,4 ± 0,1	0,75	0,44
4	x_M1_shapeInd_09	*l/b* segment 9	36,2 ÷ 40,7	37,9 ± 1,2	37,8 ± 0,7	0,89	0,32
5	x_M1_shapeInd_10	*l/b* for the segment 10	34,8 ÷ 39.2	36,5 ± 1,2	36,3 ± 0,8	0,82	0,49
6	x_M1_heightPerN	Δ*l*	79,7 ÷ 92,6	87, 7 ± 3.2	87,7 ± 2,4	0,44	0,64
		Parameters of asymmetry by the modulus of radius vectors					
7	x_M3_a080	At Ω = 80 degrees	479 ÷ 558	527,0 ± 19,8	527,5 ± 14	0,42	0,70
8	x_M3_a090	At Ω = 90 degrees	479 ÷ 570	535,4 ± 21,8	534,5 ± 15	0,33	0,64
9	x_M3_a100	At Ω = 100 degrees	475 ÷ 557	527,0 ± 19,8	526,1 ± 15	0,41	0,64
10	x_M3_a140	At Ω = 140 degrees	423 ÷ 460	440 ± 10	439 ± 9	0,66	0,44
11	x_M3_a150	At Ω = 150 degrees	416 ÷ 434	425 ± 9	425 ± 8	0,86	0.44
12	x_M3_a250	At Ω = 250 degrees	490 ÷ 516	502 ± 14	502 ± 12	0,49	0.64
13	x_M3_a300	At Ω= 300 degrees	461 ÷ 510	489 ± 13	487 ± 9	0,59	0.49
		Parameters obtained from the reception of quarters and the inscribed circle					
14	x_M4_UL_height	*l*	958 ÷ 1116	1072 ± 44	1071 ± 30	0,34	0,49
15	x_M4_UL_perim-Down	Per. of the lower figure outside the inscribed circle	2149 ÷ 2716	2404 ± 136	2467,5 ± 100	*0,18*	*0,13*
16	x_M4_UL_perim-Upper	Per. of the upper figure outside the inscribed circle	2179 ÷ 2770	2418 ± 142	2484 ± 109	*0,14*	*0,13*
17	x_M4_UR_height	*l*	958 ÷ 1140	1072 ± 44	1070 ± 31	0,34	0,49
18	x_M4_DL_perim-Down	Per. of the lower figure outside the inscribed circle	2715 ÷ 3023	2914 ± 79	2895 ± 74	0,34	0,58
19	x_M4_DL_perim-Upper	Per. of the upper figure outside the inscribed circle	2715 ÷ 3023	2916 ± 81	2895 ± 74	0,30	0,39
20	x_M4_DR_perim-Down	Per. of the lower figure outside the inscribed circle	2711÷3065	2923 ± 80	2888 ± 84	0,31	0,64
21	x_M4_DR_perim-Upper	Per. of the upper figure outside the inscribed circle	2711 ÷ 3063	292 ± 82	2889 ± 84	0,23	0,49
23	x_M4_UL_perim_avg	Mean value (μ) Per. of the left upper segment outside the circle	2164 ÷ 2580	2411 ± 137	2476 ± 104	*0,16*	*0,13*
24	x_M4_DL_perim_avg	μ Per. of the lower right segments outside the circle	2715 ÷ 3023	2915 ± 80	2895 ± 74	0,30	0,39
25	x_M4_DR_perim_avg	μ Per. of both lower segments outside the circle	2711 ÷ 3009	2924 ± 85	2889 ± 84	0,27	0,64
26	x_M4_UR_volume	μ areas of both upper right segments outside the circle	332 384 976 ÷ 42 825 441	387 671 169 ± 28 014 778	385 584 292 ± 23 636 658	0,66	0,41

* The smallest values *p*1, *p*2 y of the analyzed parameters are marked in italics.

In the first experiment, the correctness of the identification of each sample using machine learning models was tested. The second experiment involved using different machine learning algorithms, leading to the creation of a preliminary set of models.

In the third and final experiment, the resulting feature set was formed, and the final machine learning model was obtained.

Let's take a closer look at the course of each of the conducted experiments.

In small-sized data sets, errors in data collection can significantly affect the final result. To study the identified object from the original sample using machine learning models, the Leave-One-Out method was used with ML algorithms M01-M06 for feature groups G01-G10. As a result, 38 × 10 × 6 models were built. Subsequently, machine learning algorithms M07 and M08 were added to the set of machine learning algorithms.

Based on the results of the first experiment, feature groups G01, G02, G03, and G11 were selected for model formation. For each feature group G01, G02, G03, G11, and machine learning algorithm M01-M08, models were built using K-fold cross-validation with three splits. Metrics for each shuffle were averaged to obtain the final classification metric scores. In total, 4 × 8 × 3 models were generated. The summarized results for AUC ROC and F1-score are presented in Table 3. Among the obtained models, those generated by algorithms M04 and M05 using features from groups G02 and G11 had the highest averaged metrics (AUC = 67-72%, F1 = 70-76%). Metrics for the feature group G02 (11 features) exceeded the performance of models created using the feature group G01 (53 features), so models with fewer features performed better than those with more features. This can be explained by the "curse of dimensionality," noise in the data, and increased entropy [16]. As the number of features increases, the amount of noise and randomness in the data also increases, making it more challenging to extract meaningful patterns and information from the data. Dimensionality reduction methods and feature selection methods are useful tools for addressing these issues and improving model performance.

The goal of the third experiment was to improve model performance by using more informative features. This was achieved by implementing SHAP values [17].

The data in Table 3 highlights three final models with accuracy indicators AUC = 73–72% and F1 = 69–72%: Random Forest classifier with 4 estimators and a maximum depth of 3, Random Forest classifier with 10 estimators and a maximum depth of 5, and AdaBoost classifier with 4 decision tree estimators and a maximum depth of 3 (highlighted in semi-bold).

CONCLUSIONS

1. The essential requirements for developing a non-invasive method for embryo selection in bird eggs during the first 6-7 days of incubation, when it is unlikely for embryos to experience perceptions and pain, have been substantiated.

2. The analysis of newly developed methods for determining and selecting embryos in the early days of incubation has shown that methods using computer vision and machine learning techniques are more aligned with this requirement.

3. A method for determining sexual dimorphism before incubation based on the analysis of asymmetry parameters of a precision information model of egg shape through machine learning for binary classification tasks with small sample sizes and high-dimensional original feature sets (logistic regression, single decision trees, random forests, random forests as part of adaptive boosting) has been proposed.

4. A setup for the experimental verification of the feasibility of the proposed method in practice has been developed and described.

5. On a small sample of incubated chicks consisting of 38 chicks, where gender identification was reliably possible, three final models with

Табл. 3. Результаты экспериментов (перекрестная проверка $K = 3$)
Table 3. Results of the experiments (Cross-validation, $K = 3$)

Experiment number	Group of functions	Metrics	M01	M02	M03	M04	M05	M06	M07	M08
2	G01	AUC	0,484	0,495	0,627	0,543	0,536	0,382	0,559	0,464
		F1	0,56	0,54	0,61	0,59	0,59	0,51	0,51	0,54
	G02	AUC	0,664	0,655	0,659	**0,673**	**0,718**	0,532	0,618	0,464
		F1	0,68	0,68	0,68	**0,76**	**0,74**	0,61	0,49	0,58
	G03	AUC	0,427	0,509	0,489	0,602	0,532	0,495	0,386	0,436
		F1	0,45	0,53	0,52	0,61	0,58	0,47	0,51	0,48
3	G11	AUC	0,614	0,584	0,698	**0,723**	**0,673**	0,591	0,309	0,432
		F1	0,58	0,59	0,65	0,68	**0,71**	0,54	0,51	0,58
	G12	AUC	0,668	0,634	0,734	**0,720**	**0,693**	0,600	0,591	0,364
		F1	0,68	0,62	0,73	**0,70**	**0,71**	0,51	0,58	0,49
	G13	AUC	0,602	0,627	0,668	0,702	0,602	0,577	0,386	0,368
		F1	0,63	0,65	0,67	0,61	0,64	0,51	0,51	0,54

accuracy values of AUC = 73–72% and F1 = 69–72% were obtained: Random Forest classifier with 4 estimators and a maximum depth of 3; Random Forest classifier with 10 estimators and a maximum depth of 5, and AdaBoost classifier with 4 decision tree estimators and a maximum depth of 3.

6. The results indicate that the proposed combination of machine learning algorithms allows the development of classification models for tasks where the feature space significantly exceeds the size of the sample with ambiguously identifiable samples.

7. Experimentally, the relationship between the asymmetry of egg shape and the determination of its sexual dimorphism before incubation has been identified, contributing to the solution of a complex problem - the accurate determination of the gender of the egg before incubation. This is an important step towards the implementation of an industrial method for gender determination in the near future. This also addresses a crucial commercial challenge - minimizing material (energy, heat, etc.) and time costs during incubation.

8. The proposed system of techniques for determining embryo sex in poultry eggs can be integrated with other computer vision-based quality detection systems. For example, in detecting mechanical defects, sorting, determining the viability and degree of embryo development in the egg before incubation, early diagnosis of disease development in the egg, etc.

9. Further research is planned to increase the sample size of incubated chicks and to obtain classification models with higher reliability for determining the sex of the embryo in the egg prior to incubation.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

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ИСПОЛЬЗОВАНИЕ НИЗКОТЕМПЕРАТУРНОЙ ПЛАЗМЫ ДЛЯ ОБЕЗЗАРАЖИВАНИЯ ОТКРЫТЫХ ПОВЕРХНОСТЕЙ ПРОИЗВОДСТВЕННЫХ ПОМЕЩЕНИЙ

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Приведены результаты исследований по инактивации микроорганизмов на открытых поверхностях птицеводческих помещений с использованием низкотемпературной неравновесной плазмы. В качестве ее источника использован электроискровой разряд переменного тока при атмосферном давлении. Типы разряда – стримерный, факельный. Рассмотрено одновременное воздействие электромагнитных полей, заряженных частиц и химически активных соединений, образующихся при электроискровом разряде, на эффективность инактивации патогенной микрофлоры для различных поверхностей (акриловый грунт, эпоксидная смола, лак яхтный, бетонно-графитовая смесь). Обрабатываемый материал (биологический макет подстилочной поверхности пола в птичнике с нанесенным защитным слоем) установлен после электроискровой разрядной камеры, продуваемой плазмообразующим газом (атмосферный воздух). Основными поражающими факторами являются активные химические соединения: озон; свободные радикалы (OH, O, O_2), ультрафиолетовое излучение в диапазоне 750–1600 ТГц, электромагнитное излучение от 50 Гц до 980 МГц, заряженные частицы и колебательно возбужденные молекулы азота и кислорода. Получены характеристики плотности потока электромагнитного излучения при электроискровом разряде. По результатам исследований максимальный эффект обработки открытых поверхностей низкотемпературной неравновесной плазмой достигается при использовании в качестве защитного материала поверхностей эпоксидной смолы. Количество инактивированных микроорганизмов при экспозиции 10–20 с достигает 100%. При инактивации микроорганизмов, находящихся на открытых поверхностях, длительность экспозиции экономически нецелесообразно принимать более 20 с. В исследованиях не выявлено существенного различия при использовании стримерного или факельного разрядов для обработки открытых поверхностей помещений.

Ключевые слова: низкотемпературная неравновесная плазма, электроискровой разряд, микроорганизмы, инактивация, патогенная микрофлора

THE USE OF LOW-TEMPERATURE PLASMA FOR DISINFECTION OF OPEN SURFACES OF INDUSTRIAL PREMISES

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The results of research on inactivation of microorganisms on open surfaces of poultry houses using low-temperature non-equilibrium plasma are presented. AC electrospark discharge at atmospheric pressure was used as its source. Discharge types are streamer, flare. The simultaneous effect of electromagnetic fields, charged particles and chemically active compounds formed by electrospark discharge on the efficiency of pathogenic microflora inactivation for various surfaces (acrylic primer, epoxy resin, yacht varnish, concrete-graphite mixture) is considered. The material to be treated (a biological model of the bedding surface of the floor in the poultry house with the applied protective layer) is installed after the electrospark discharge chamber blown with plasma-

forming gas (atmospheric air). The main affecting factors are active chemical compounds: ozone; free radicals (OH, O, O_2), ultraviolet radiation in the range of 750–1600 THz, electromagnetic radiation from 50 Hz to 980 MHz, charged particles and vibrationally excited nitrogen and oxygen molecules. Characterizations of electromagnetic radiation flux density at electrospark discharge are obtained. According to the research results, the maximum effect of treatment of exposed surfaces with low-temperature non-equilibrium plasma is achieved when epoxy resin is used as a surface protection material. The number of inactivated microorganisms at exposure of 10-20 s reaches 100%. When inactivating microorganisms on exposed surfaces, it is not economically feasible to take exposure time longer than 20 s. The studies found no significant difference when using streamer or flare discharges to treat outdoor facility surfaces.

Keywords: low-temperature nonequilibrium plasma, electrospark discharge, microorganisms, inactivation, pathogenic microflora

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The authors declare no conflict of interest.

INTRODUCTION

The transition from agricultural production to an industrial basis is primarily associated with an increase in the density of livestock and poultry in production facilities. This circumstance necessitates the creation of highly effective technical systems for disinfecting materials and media used in the production processes of goods.

Electrophysical methods for inactivating pathogenic microflora (direct and indirect), currently employed, typically utilize electromagnetic radiation (EMR) within a specific frequency range of varying intensity [1–3]. Comprehensive research results on electrophysical methods for microorganism inactivation are available in previous works [4–9]. The geometric dimensions of microorganisms and their electrophysical characteristics, which determine the degree of lethality when processed in electromagnetic fields, exhibit sufficiently large ranges of variation. It seems advisable to employ EMR across the entire spectrum - from radio frequencies to hard ultraviolet radiation (UVR) [10].

One possible way to implement such an approach is the use of non-equilibrium low-temperature plasma (NE LTP) generated during an electric spark discharge [6–8]. To assess the effectiveness of using NE LTP, it is necessary to determine the spectrum and radiation flux density generated during an electric spark discharge across the entire electromagnetic wave scale, while evaluating the degree of microorganism inactivation present in the air and on exposed surfaces (floor, walls) [11, 12].

The purpose of research is to determine the effectiveness of using low-temperature plasma in the inactivation of pathogenic microorganisms on various protective surfaces in indoor poultry facilities.

MATERIAL AND METHODS

The object of the study is the system for inactivating pathogenic microorganisms in a poultry facility. The microorganisms under investigation include *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus albus*, *E. coli*, and others. The microorganisms were applied to segments of standard ceramic tiles measuring 48 × 48 mm. Various protective surface layers are applied to the tiles as a substrate. The options for protective layers included acrylic primer, epoxy resin, yacht varnish, and concrete-graphite mix.

Total microbial count (TMC) was determined according to MG 4.2.734–99 "Microbiological

Monitoring of the Production Environment".

A plasma torch was used as the NE LTP source (see Fig. 1). The setup parameters are presented in Table 1.

Measured parameters:

– spectrum and flux density of electromagnetic radiation in the range from 3000 m to 100 nm;

– processing time of the test material;

– quantity of positive and negative air ions;

– ozone concentration and percentage of microorganism inactivation.

Controlled parameters:

– air temperature;

– air humidity;

– plasma-forming gas temperature.

Measurement instruments used:

– air ion counter MAC-01;

– multi-functional instrument Testo 435-2;

– universal gas analyzer GANK-4;

– multi-channel spectrometer "Kolibri";

– radiometer of UV-range (a, b, and c subranges) TKA-PKM;

– infrared radiation density measuring instrument "MK-meter";

– temperature measurement - infrared thermometer with switchable optics Testo 845, Kelvin Compact 1200/175 pyrometer.

Experimental Procedure

The treated material (a biological model of the floor bedding surface in a poultry house with an applied protective layer) was installed after the electric spark discharge chamber, which was purged with plasma-forming gas (ambient air). The main damaging factors include active chemical compounds: ozone, free radicals (OH, O, O2), ultraviolet radiation in the range of 750–1600 THz, electromagnetic radiation from 50 Hz to 980 MHz, charged particles, and vibrationally excited nitrogen and oxygen molecules.

Табл. 1. Параметры электроискровой установки для генерации плазмы

Table 1. Parameters of the electrospark unit for plasma generation

Parameter	Value
Voltage of high-voltage source, kV	12
Air velocity in the discharge chamber, m/s	1–4
Cross-sectional area of the discharge chamber, mm^2	80–110
Maximum power consumption of the installation, W	1500–3000
Calculated electric field strength in the discharge gap, V/m	450 000
AC frequency, kHz	20

The exposure to NE LTP during the discharge in gas was varied from 5 to 300 seconds. Upon removing the opaque screen from the surface of the quartz tube, the effectiveness of electromagnetic radiation exposure in the radio and ultraviolet ranges was additionally assessed.

The evaluation was based on the reduction in bacterial contamination of the bedding material in the experimental sample compared to the control. For this, 5 grams of the combined sample from each experimental exposure were weighed and added to 50 ml of sterile saline solution (dilution 1:10). The flask with the suspension was placed on a shaker at a frequency of 100 oscillations/minute and allowed to stand for 30 min-

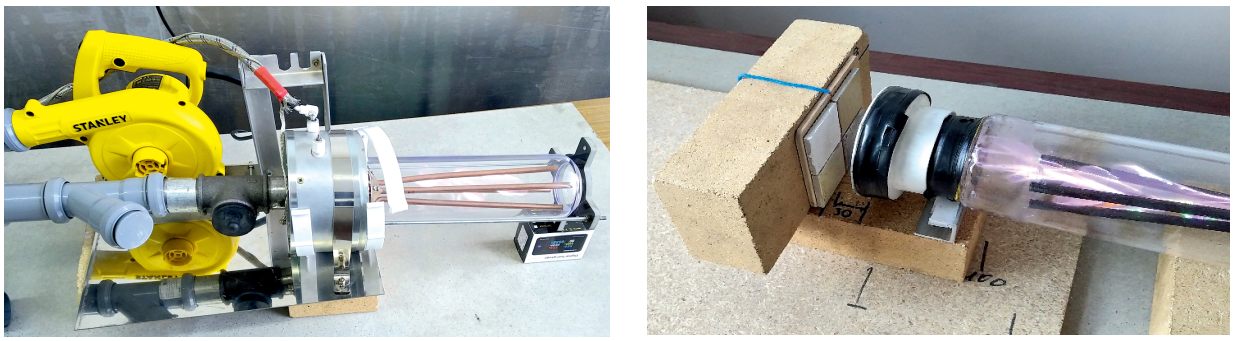

Рис. 1. Установка для генерации низкотемпературной плазмы (составлена авторами)

Fig. 1. Installation for low-temperature plasma generation (compiled by the authors)

utes at room temperature of 8 °C. Then, the suspension was centrifuged at 3000 rpm to separate large particles. Subsequently, successive dilutions were prepared from the obtained supernatant using the EasySpiral spiral seeding device. Each dilution was seeded in triplicate on Petri dishes with meat-peptone agar, with each dish receiving 100 µl. After 20 hours of incubation at 37 °C, the grown colonies were counted using the Scan 500 device. The arithmetic mean of the cups was taken as the final result. The microbial contamination of the investigated substrate was determined. The presence of growth of individual microorganism species was assessed on selective nutrient media.

The levels of electric and magnetic field intensity generated by low-temperature plasma, as well as radiation flux density, were measured at a distance of 20 cm from the surface of the glass tube of the plasma torch. Measurements were taken at fixed frequencies using the ATE-8507 device. The distance between the electromagnetic radiation flux density sensors and the electric spark chamber was 0.4 m.

RESULTS AND DISCUSSION

The parameters of electromagnetic radiation (EMR) generated during the electric spark discharge are presented in Table 2.

The flux density of EMR in the range of 190–400 nm is presented in Table 3.

The ozone concentration in the air stream was 7–8 mg/m^3.

The calculation of the electric field intensity in the interelectrode space was carried out using the ELCUT program. The maximum electric field intensity was 400,000–500,000 V/m. The general distribution of equipotential electric fields in the gas discharge chamber is shown in Fig.2.

To study the dynamic characteristics of the electric spark discharge, oscillography and high-speed video recording of the discharge current and voltage on the electrodes were performed in the modes of electric spark and streamer discharges (see Fig. 3, 4). The spectrum of NE LTP radiation in the range of 600–1500 THz is shown in Fig. 5.

Табл. 2. Значения напряженности электрического поля и плотности потока ЭМИ по диапазонам частот

Table 2. Values of electric field strength and electromagnetic radiation flux density by frequency ranges

EMI frequency	Electric field intensity, V/m	Flux density, W/m^2
100 kHz	270	148
200 kHz	149	62
500 kHz	128	45
1 MHz	174	84
10 MHz	192	98
13,56 MHz	158	67
100 MHz	138	60
900 MHz	0,93	0
1,8 GHz	0,7	0
2,4 GHz	0,5	0

Табл. 3. Плотность потока электромагнитного излучения в ультрафиолетовом спектре

Table 3. Electromagnetic radiation flux density in the UV spectrum

UV range	EMI flux density value, mW/m^2
UVA	48
UVB	50
UVC	130

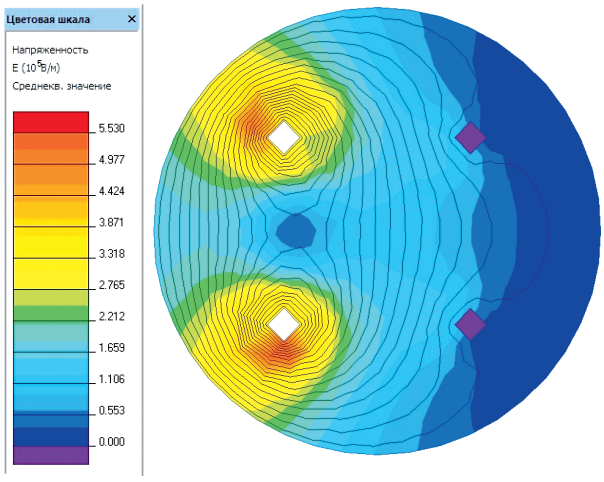

Рис. 2. Распределение напряженности электрического поля в приэлектродном пространстве плазмотрона

Fig. 2. Distribution of electric field strength in the near-electrode space of the plasmatron

The results of microorganism inactivation by electromagnetic radiation and chemically active compounds of NE LTP for various protective surfaces are presented in Table 4, and the results of the effectiveness of microorganism inactivation for streamer and torch discharges are in Table 5.

Based on the research results, the maximum effect when treating open surfaces with low-temperature non-equilibrium plasma was achieved when epoxy resin was used as the protective material. The assessment of the effectiveness of yacht varnish requires additional research.

The results presented in Table 5 allow the conclusion that streamer and torch discharges

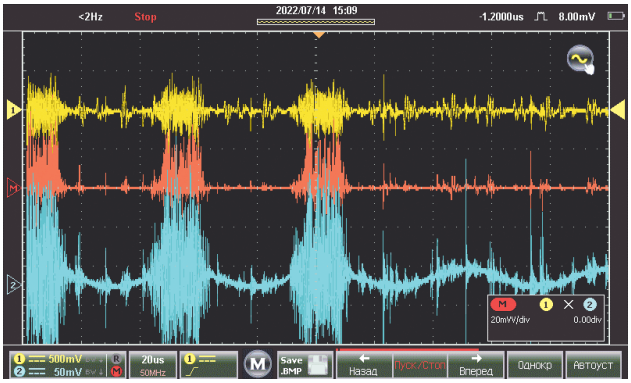

Рис. 3. Осциллограмма факельного разряда (ток, напряжение)

Fig. 3. Oscillogram of the electrospark discharge (current, voltage)

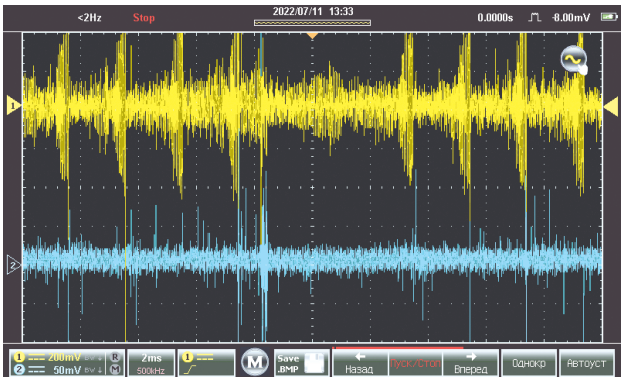

Рис. 4. Осциллограмма стримерного разряда (ток, напряжение)

Fig. 4. Oscillogram of the streamer discharge (current, voltage)

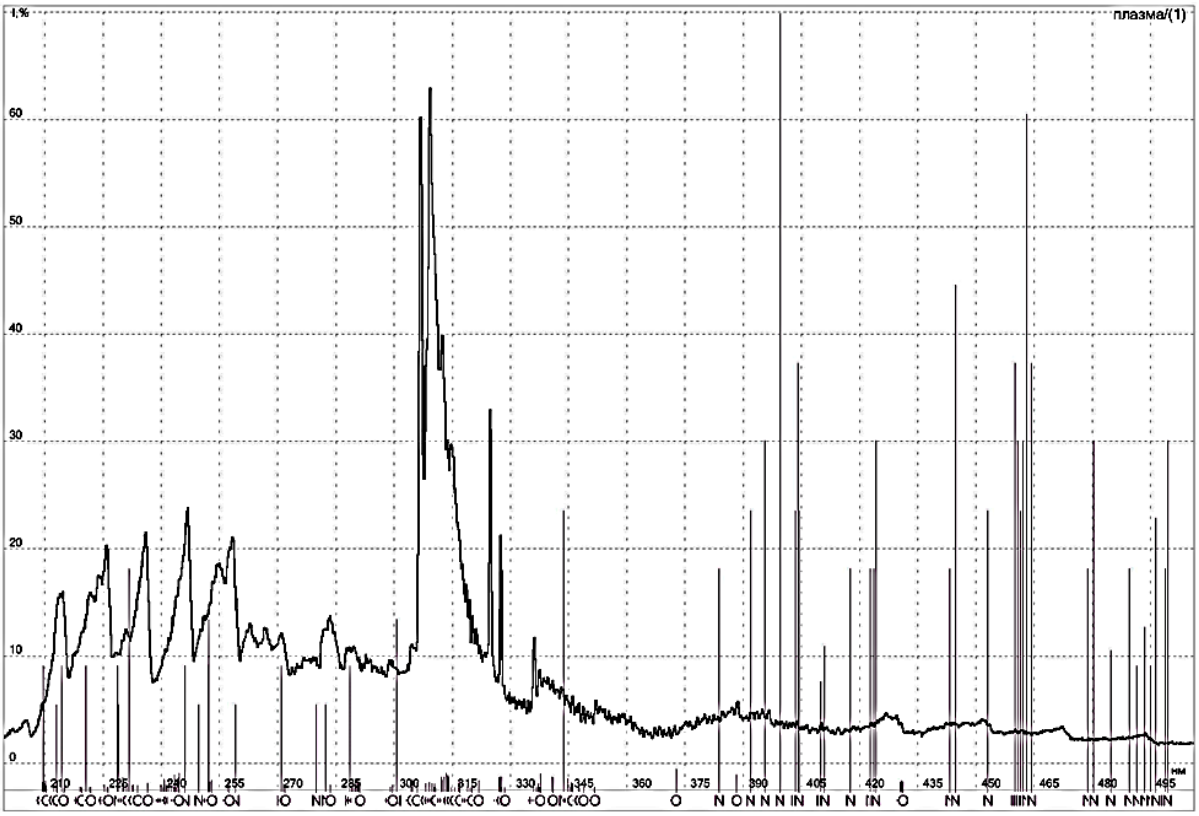

Рис. 5. Спектр излучения ННП в диапазоне 600–1500 ТГц

Fig. 5. Emission spectrum of the LNP in the range of 600–1500 THz

Табл. 4. Оценка инактивации микроорганизмов ННП на различных защитных поверхностях
Table 4. Evaluation of inactivation of LNP microorganisms on various protective surfaces

Sample number, substrate material (primer coat)	Exposure time, s	Number of bacteria, CFU/ml		Bacteria inactivation rate, relative units	Bacteria inactivation, %
		before treatment	after treatment		
Control (uncoated tiles)	Uncoated	1,30E + 06	1,30E + 06	1,00	0,00E + 00
Uncoated tiles	5	1,30E + 06	2,00E + 05	6,50	85
	10		2,00E + 05	6,50	85
Epoxy coating	5	1,30E + 06	3,00E + 05	4,33	77
	10		0,00E + 00	∞	100
Acrylic coating	5	1,30E + 06	2,00E + 05	6,50	85
	10		9,00E + 05	1,44	31
Tiles with flecks of metal	5	1,30E + 06	0,00E + 00	∞	100
	10		0,00E + 00	∞	100

Note. The distance to the plasma flare (*L*) was 30 mm.

Табл. 5. Результаты инактивации микроорганизмов ННП для различных видов электроискрового разряда
Table 5. Results of inactivation of LNP microorganisms for various types of electric spark discharge

Discharge characteristic	Substrate material	Exposure time, s	Number of bacteria		Bacteria inactivation rate	Bacteria inactivation
			before treatment	after treatment		
			CFU/ml		relative units	%
S	Uncoated	5	8,60E + 05	4,30E + 05	2,00	50,00
S		10		1,00E + 04	86,00	98,84
S		20		3,00E + 04	28,67	96,51
F		5		3,40E + 05	2,53	60,47
F		10		6,00E + 04	14,33	93,02
F		20		1,50E + 05	5,73	82,56
S	Yachting varnish	5	6,10E + 05	1,20E + 05	5,08	80,33
S		10		1,00E + 04	61,00	98,36
S		20		3,30E + 05	1,85	45,90
F		5		0,00E + 00	∞	100,00
F		10		3,60E + 05	1,69	40,98
F		20		6,10E + 05	1,00	0,00
S	Epoxy resin	5	7,10E + 05	2,00E + 04	35,50	97,18
S		10		0,00E + 00	∞	100,00
S		20		0,00E + 00	∞	100,00
F		5		1,00E + 04	71,00	98,59
F		10		0,00E + 00	∞	100,00
F		20		0,00E + 00	∞	100,00
S	Graphite, yacht varnish on top	5	6,60E + 05	1,00E + 05	6,60	84,85
S		10		9,00E + 04	7,33	86,36
S		20		3,00E + 05	2,20	54,55
F		5		7,00E + 04	9,43	89,39
F		10		1,00E + 04	66,00	98,48
F		20		1,70E + 05	3,88	74,24

Note. S - streamer, F - flare.

are approximately equivalent in terms of microorganism inactivation effectiveness on open surfaces.

The number of inactivated microorganisms with an exposure time of 10–60 s and the use of epoxy resin as a protective coating reached 100%.

When inactivating microorganisms on open surfaces, it is not economically advisable to use an exposure duration of more than 20 s.

Free radicals (hydroxyl group, etc.) were recorded in the plasma emission spectrum. The assessment of the impact of free radicals on microorganism inactivation requires further study.

In the future, it is necessary to assess the contribution of each of the damaging factors (electromagnetic radiation in the radio range, ultraviolet radiation, charged particles, ozone) to the degree of inactivation for different groups of microorganisms.

The obtained research results and a literature review of works by other authors [5–9] on this topic allow us to conclude the presence of a synergistic effect of the impact of damaging factors of low-temperature non-equilibrium plasma on the studied microorganisms.

Compared to the traditional method of surface treatment for air disinfection in a room (ozone treatment), the use of a low-temperature plasma generator allows achieving the required inactivation parameters and significantly reducing the exposure duration by simply changing the geometric dimensions of the electric spark chamber.

CONCLUSIONS

1. The maximum effect of treating open surfaces with low-temperature non-equilibrium plasma is achieved when epoxy resin is used as the protective material. The number of inactivated microorganisms with an exposure time of 10–20 s reaches 100%.

2. When inactivating microorganisms on open surfaces, it is not economically advisable to use an exposure duration of more than 20 s.

3. There is no significant difference observed when using streamer or torch discharges for treating open surfaces in rooms. Energy consumption is significantly lower for streamer discharge with comparable technological effects.

In further research on this topic, it is advisable to test the developed plasma torch prototype in production conditions with the presence of real microbial populations and assess the contribution of each of the damaging factors to the degree of inactivation for different groups of microorganisms.

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ПРОШЛОЕ И НАСТОЯЩЕЕ ПЛЕМЕННОГО ДЕЛА В МОЛОЧНОМ СКОТОВОДСТВЕ КРАЙНЕГО СЕВЕРО-ВОСТОКА

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Статья призвана с опорой на исторические факты и прошлые достижения актуализировать важность и доказать возможность успешного ведения племенной работы на уровне региона с целью повышения объемов производства качественных социально значимых продуктов питания для обеспечения продовольственной безопасности. В начале становления сельскохозяйственной науки в Магаданской области перед учеными стояла задача по подбору оптимальных для региона пород скота. За 1964–1967 гг. было обследовано более 4000 коров. Установлено, что в экстремальных природно-климатических условиях Магаданской области наилучшим образом себя зарекомендовала холмогорская порода. В 1967 г. с целью дальнейшего совершенствования холмогорской породы сделана первая попытка изучения генеалогической структуры стада, выявления и оценки высокопродуктивных линий. В 1974 г. для повышения жирномолочности помесных коров холмогорской породы применяли вводное скрещивание с быками-айрширами, проверенными по качеству потомства. С целью совершенствования дойных стад отбор коров по наследственным признакам сочетают с их проверкой по первой лактации. С 1976 г. в совхозах было начато изучение племенных и продуктивных качеств айрширского скота, который отличался большой обильномолочностью и высоким содержанием жира в молоке. С 1982 г. айрширская порода утверждена как плановая для разведения в хозяйствах Приохотской зоны. В этом же году начато поглотительное скрещивание животных холмогорской породы с производителями айрширской породы. В рамках повышения эффективности селекции по обильномолочности в хозяйствах вели работу по увеличению наследственной изменчивости этого признака за счет разведения по линиям и семействам. Вместе с тем продолжилось совершенствование холмогорского скота путем вводного скрещивания с чистопородными быками голштино-фризской породы. К 1990-м годам основным вектором развития стало создание высокопродуктивных стад молочного скота, но тяжелое финансовое положение хозяйств в 1990-е годы привело к резкому снижению поголовья крупного рогатого скота и продуктивности оставшихся животных.

Ключевые слова: Магаданская область, история, развитие, молочное скотоводство, селекционно-племенная работа, продуктивность

THE PAST AND PRESENT OF BREEDING IN DAIRY CATTLE BREEDING IN THE FAR NORTH-EAST

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Based on historical facts and past achievements, the work is designed to update the importance and prove the possibility of successful breeding work at the regional level in order to increase the production of high-quality, socially significant food products to ensure food security. At the beginning of the formation of agricultural science in the Magadan region, scientists were faced with the task of

selecting the optimal breeds for the region. More than 4000 cows were examined during 1964–1967. It was found that in extreme natural and climatic conditions of the Magadan region the Kholmogory breed proved to be the best one. In 1967, in order to further improve the Kholmogory breed, the first attempt was made to study the genealogical structure of the herd, to identify and evaluate highly productive lines. In 1974, in order to increase the milk fat content of crossbred cows of the Kholmogory breed, introductory crossing with the bulls of the Ayrshire breed, tested for the quality of the offspring, was used. In order to improve dairy herds, the selection of cows for hereditary traits is combined with checking them for the first lactation. Since 1976, state farms began to study the breeding and productive qualities of the Ayrshire cattle, which were distinguished by high milk production and high fat content in milk. Since 1982, the Ayrshire breed has been approved as a planned breed for breeding in the farms of the Okhotsk zone. In the same year, absorptive crossbreeding of the animals of the Kholmogory breed with the sires of the Ayrshire breed began. In order to increase the efficiency of breeding for abundant milk production, work began on the farms to increase the hereditary variability of this trait through breeding along lines and families. At the same time, the work on improving the Kholmogory cattle by introductory crossbreeding with the purebred bulls of the Holstein-Friesian breed continued. By the 1990s, the main vector of development was the creation of highly productive herds of dairy cattle, but the difficult financial situation at farms in the 1990s led to a sharp decrease in the number of cattle and the productivity of the remaining animals.

Keywords: Magadan region, history, development, selection and breeding work, dairy cattle breeding, productivity

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The intensive development of the Far Northeast, which is one of the most significant regions for the country in terms of resources and geopolitical characteristics, began in the second third of the 20th century. During geological expeditions to the upper Kolyma regions in the late 1920s, large gold deposits were discovered. As a result, the strategic tasks included comprehensive development and incorporation of previously uninhabited territories into the country's unified national economy as the main supplier of currency to implement large-scale industrialization plans. During the years of Soviet construction, the Far Northeast turned into a rapidly developing industrial region [1]. One of the priority tasks of that period was to establish a food base in areas where the creation of agricultural production seemed impractical. The need to provide the population with fresh food products contributed to the development of new agricultural sectors, the organization of state farms, large livestock farms, and poultry farms. One of the basic branches of agricultural production became dairy cattle breeding.

In the early 1930s, several agricultural enterprises were created by Dalstroy, for which large cattle of various breeds were imported from the Primorsky Territory without proper selection: Simmental, Kholmogory, Yaroslavl, East Friesian, Schwick, Bestuzhev, as well as Siberian and Red Steppe cattle. As of January 1, 1938, Dalstroy enterprises had 1,612 head of large cattle, including 729 cows. By the end of 1950, the size of the dairy herd reached 8,666 head,

including 3,680 cows. By the early 1960s, the public livestock industry in the region had 9,509 head, including 6,490 cows[1].

Until 1967, pedigree work was not carried out in the Magadan region. Improvement of the quality and productivity of cattle was done according to the scheme for commercial farms, where high-quality breeding bulls and highly productive breeding livestock were imported from the best breeding farms in the country. Artificial insemination of cattle was not widely used due to the lack of a station for breeding work and artificial insemination.

At the beginning of the formation of agricultural science in the Magadan Region, scientists were faced with the task of selecting optimal cattle breeds for the region. Based on the Magadan Regional State Agricultural Experimental Station, a comparative assessment of the productive and breed qualities of the Kholmogory, Simmental, and Kostroma breeds was carried out, and the Kholmogory breed was improved through planned selection (see Fig. 1). The research goals included identifying lines of purebred cattle through planned selection and developing a system for raising young stock from highly productive cows.

Based on the surveys, a comparative study of cattle breeds was conducted in nine farms producing over 50.0% of the milk in the region. Over the years 1964-1967, more than 4,000 cows were surveyed. As a result, it was established that the Kholmogory breed performed best in the extreme natural and climatic conditions of the region.

In 1967, scientific and economic experiments were conducted for the first time at the experimental production farm of the Magadan Regional Agricultural Experimental Station. Based on the principle of analogs, cows of the Kholmogory and Simmental breeds were selected. Research showed that the Kholmogory breed was more cost-effective in terms of labor and feed expenses, making it better adapted to the conditions of the Magadan Region. Under identical

Рис. 1. Проведение экстерьерных промеров сотрудниками Магаданской областной государственной сельскохозяйственной опытной станции

Fig. 1. Conducting exterior measurements by the employees of the Magadan Regional State Agricultural Experimental Station

feeding, care, and maintenance conditions, the productivity of the Kholmogory cows, in terms of 4% fat milk, was found to be different. During the lactation period, the milk yield of the Kholmogory cows was 321 kg higher than that of the Simmental cows, and the amount of milk per 100 kg of live weight was 66 kg higher. However, the feed cost for the Kholmogory cows was higher, requiring 6 feed units less to produce 1 ton of milk[2].

During the same period, the region developed a breeding plan for dairy cattle in state farms and collective farms for 1967-1970, in which the Kholmogory breed was designated as the first planned breed for the region. It was noted that "the problem of creating the first dairy cattle state farms in a still undeveloped region was solved by importing productive dairy cattle from the central regions of the country, as practical resolution of this issue through the local indigenous cattle population, due to its small numbers, was impossible." The plan provided character-

[1]*Shumilov M.F., Sysoev V.V.* Sketch of the development of veterinary service of Magadan region // Magadan reindeer-breeder, 1982, N 34, pp. 33-36.

[2]*Vaganova E.S.* Proceedings of the Magadan Regional State Agricultural Experimental Station. Magadan, 1968, Issue 3, pp. 69-72.

istics of productivity, breed, and class composition of the herds present in the Magadan Region. Out of 8,210 animals that underwent evaluation, 3,743 were selected for breeding, including 23 in the elite-record class, 560 in the elite class, 2,617 in Class I, and 543 in Class II. A brief description of the Kholmogory bull breeding was given. The main organizational, economic, and zootechnical activities were outlined within the framework of breeding work in farms for 1968-1970. The first attempt was made to study the genealogical structure of the herd, identify and evaluate high-productivity lines to further improve the Kholmogory breed.

To coordinate breeding work in dairy cattle farming in state farms and collective farms in the Magadan region, a plan was drawn up for the years 1971-1980. This plan determined the overall directions for work with the Kholmogory breed, planned industry indicators, and the plan for linear assignment of the Kholmogory stud bulls to regional farms. As of the beginning of 1971, the region had 16.1 thousand head of large cattle, including 9.5 thousand cows. In the period under consideration, the average annual milk yield per forage cow increased by 202 kg compared to 1961-1965, reaching 3,072 kg.

From March 1974, artificial insemination of cows with deeply chilled semen from bulls from the Central Artificial Insemination Station was introduced in the region. To improve the fat content of crossbred Kholmogory cows, admixture of new blood of the Ayrshire bulls, proven in terms of offspring quality, was used.

In the plan for 1976-1985, tasks and methods for further improvement of the livestock, principles of selection, and breeding by lines and families have been defined. The leading selection criterion, while maintaining satisfactory development of other indicators, remains milk productivity. Animals of the desired type should have a productivity of no less than 4000-5000 kg per year, an optimal weight of about 550 kg, a calm temperament, and a voluminous udder. To improve the dairy herds, the selection of cows based on hereditary traits should be combined with checking their performance based on the results of the first lactation. One of the most important conditions for the formation of high-productivity herds at that time was the acquisition and rearing of high-quality replacement young stock[3].

The Ayrshire breed, imported to the Magadan region from Karelian ASSR since 1969, began to attract increasing interest. Crossbred heifers of the first and second generations, aged 12-17 months, were imported, with crossbred cows of the Kholmogory, Brown Latvian, and Jersey breeds of various bloodlines as the maternal basis, and purebred Ayrshire bulls of Finnish origin as the male basis. Comparative study of economically valuable traits of the Kholmogory and Ayrshire cows showed that the milk yield for 305 days of lactation in the Ayrshire cows was 77 kg higher than in the group of their Kholmogory counterparts (see Fig. 2). These findings allowed concluding that the Ayrshire cattle in terms of productivity was not inferior to the Kholmogory and could be localized as a planned breed in the Priokhotsk zone. Starting from 1976, state farms began to study the breeding and productive qualities of the Ayrshire breed, which were characterized by significant milk yield and high fat content in milk. Under equal conditions, the milk yield of the Ayrshire cows was 493 kg higher than that of the Kholmogory cows. Moreover, the total fat content in the milk of the Ayrshire cows was 23.95 kg higher. In 1982, the Ayrshire breed was approved as planned for breeding in the farms of the Priokhotsk zone. In the same year, the absorptive crossbreeding of the Kholmogory animals with the Ayrshire producers began. For this purpose, the semen of purebred bulls was widely used, 30.0% of which were improvers[4].

In the formation of dairy-oriented herds, selection based on the productivity of first-calving heifers became particularly important. The

[3]Yelin G.Ya., Vaganova E.S., Avdeeva L.V. Proceedings of the Magadan Zonal Research Institute of Agriculture of the North-East. Magadan, 1978, Issue 7, pp. 78-81.

[4]*Vaganova E.S., Soskin A.A.* Selection and breeding in dairy cattle breeding on an industrial basis. Novosibirsk, 1981, pp. 68-77.

Рис. 2. Проведение экспериментальных исследований сотрудниками Магаданского зонального научно-исследовательского института сельского хозяйства Северо-Востока

Fig. 2. Conducting pilot studies by the employees of the Magadan Zonal Research Institute of North Eastern Agriculture

leading selection traits for productivity remain milk yield and fat content in milk. To increase the efficiency of selection for high milk production, work was carried out in farms to increase the hereditary variability of this trait through breeding by lines, families, and in-depth individual breeding work. At the same time, the improvement of the Kholmogory cattle continued through the use of crossbreeding with purebred Holstein-Friesian bulls[5,6].

Due to the extensive use of crossbreeding as a method of improving productive and breeding qualities, significant additions and changes were made to the breeding and breeding plan in the Magadan region for the 1980s-1990s in 1984. It was envisaged to maximize the use of existing breeding resources - increasing milk yields, fat content in milk, animal resistance to diseases, adaptability to industrial technology conditions, increasing growth energy, and reducing feed costs per unit of production.

Many years of experience in breeding Ayrshire cattle showed that these animals had high productivity and reproduction indicators in the region. In terms of udder quality, milk fat content, and feed payment with milk, they outperformed Kholmogory and their crosses with Holsteins, which were bred in the state farms of the region. As a result of purebred breeding and targeted selection, a population of the Ayrshires well adapted to the extreme conditions of the Far Eastern North was created [2]. According to breed records, by 1990, the Ayrshire population reached 17,070 head, accounting for 46.5% of the total cattle population. Based on the evaluation in the same year, the milk yield for 305 days of the last lactation of the Ayrshire cows in the region was 4016 kg of milk with a fat content of 3.65%, and on pedigree farms – 4979 kg with a fat content of 3.67% [3].

Starting from 1989, the Laboratory of Selection and Breeding of the Magadan Zonal Research Institute of Agriculture of the Northeast began developing plans for breeding and breeding work in dairy cattle farming for 1990-2000. The main direction of development was the creation of highly productive herds of dairy cattle adapted for use in industrial technology conditions, with a genetic potential for milk production of 5500-6000 kg of milk with 3.8% fat content, a live weight of 600 kg, and a milking speed of 1.7-2.0 kg/min.

Unfortunately, these plans were not implemented. In the 1990s, due to ill-conceived economic reforms and the underestimation of the strategic role of northern regions by the state, the socio-economic situation deteriorated sharply. A crisis situation developed in the agro-industrial complex. Local production of agricultural and industrial products decreased by 40.0-58.0%. At the same time, the import of food products to northern regions sharply decreased, putting the local population in a difficult situation[7].

The difficult financial situation of farms and the lack of feed led to a significant reduction

[5]*Vaganova E.S., Vorsanova G.A.* Breeding work with dairy cattle breeds // Intensification of animal breeding in the Magadan region, 1984, N 10, pp. 7-13.

[6]*Kanygin B.N., Mikhailov N.G.* State and prospects for the development of dairy cattle breeding // Intensification of animal breeding in the Magadan region, 1984, N 10, pp. 3-7.

[7]*Kashtanov A.N.* Development of northern agriculture in the light of the concept of N.I. Vavilov // Agriculture of the North at the turn of the millennium: a collection of scientific articles, Magadan, 2004, vol. 1, 253 p.

in the cattle population and its productivity. Already in 1995, according to evaluations, the cattle population was only half of what it was in 1990. The milk yield per cow of the Ayrshire breed decreased to 2417 kg of milk with a fat content of 3.72%. By the early 2000s, the regional Production Association for breeding work was liquidated, and control and coordination of breeding work in farms ceased. Breeding work with breeding cattle was not conducted, and the genetic potential built up over decades was lost. The main cattle population was concentrated in peasant farms, where they decided for themselves which breed of cows to breed. Almost all farms that did not use artificial insemination (which covered 40.0% of the breeding stock) had unsystematic natural mating without pedigree control. The bulls used for reproduction did not have certificates of origin[8].

In 2006, 100 Ayrshire heifers were imported to the region from the Vologda region. All animals were used to replenish the commercial herd in one of the farms. The cattle had difficulty adapting to the local conditions, most of the calvings had complications, and only 15 live calves were born from the heifers. In the Magadan region, by 2010, there were only about 400 heads of Ayrshire cattle whose origin was confirmed by pedigree certificates. In the last three years, Ayrshire cows and heifers were inseminated with semen from the Red-and-White Holstein bulls, conducting absorptive crossbreeding. As a result, the unique local population of the Ayrshire cattle, bred in the farms of the region for more than 40 years, was completely displaced by animals of other breeds. In Russia as a whole, the Ayrshire population remains stable. In terms of productivity, the Ayrshire cows rank third after the Holsteins and Black-and-Whites [4–8].

Currently, the main cattle population of the region is concentrated in peasant farms. The process of changing the breed composition of cattle is influenced by economic and subjective reasons [9, 10]. In most farms, artificial insemination is not carried out, and cattle are often imported from other regions that differ significantly from the Magadan Region in terms of climate and economic conditions. Breeds such as Holstein, Red-and-White Holstein, Simmental, and others were not previously grown or studied in the region, and their adaptation to the harsh conditions of the North was not explored[9].

In the current political climate, with a focus on reducing the country's dependence on external factors, increasing self-sufficiency in essential food products becomes strategically important. This necessitates the need to address the challenges of forming a food base at the regional level. Considering the remote location of the Magadan Region and the complex logistics in delivering goods, ensuring the population's access to quality food products is of practical significance [11]. Therefore, the study of the adaptive qualities of imported cattle, their productive and reproductive abilities, and the identification of breeds and genotypes best suited to the conditions of the region becomes particularly relevant. The revival of work in this direction should mark a new stage in the history of breeding and breeding work in dairy cattle farming in the extreme conditions of the Far Eastern Far North.

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МОНИТОРИНГ СЕЛЕКЦИОННОГО МАТЕРИАЛА ПОДСОЛНЕЧНИКА С ЦЕЛЬЮ ОПРЕДЕЛЕНИЯ УРОВНЯ УСТОЙЧИВОСТИ К ЛОЖНОЙ МУЧНИСТОЙ РОСЕ

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Увеличение производства маслосемян подсолнечника требует использования урожайных, адаптированных к условиям возделывания сортов и гибридов, устойчивых к вредоносным патогенам зоны выращивания. Снижению устойчивости культуры способствует целый ряд причин. Одна из них – возникновение условий для развития не только новых заболеваний, но и более агрессивных рас уже хорошо известных патогенов. Самым эффективным и экологически безопасным путем борьбы с болезнями сельскохозяйственных культур является создание устойчивого селекционного материала. В связи с этим в условиях Восточно-Казахстанской области были проведены исследования по оценке устойчивости к ложной мучнистой росе перспективного селекционного материала подсолнечника, созданного специалистами Восточно-Казахстанской сельскохозяйственной опытной станции. Определены количественные показатели пораженных растений и интенсивность поражения семядолей спороношением. Дана характеристика степени восприимчивости исследуемых образцов по 5-балльной шкале. Результаты опытов подтвердили возможность проведения отбора на разных этапах селекционной работы. Выделенный исходный материал может быть использован в качестве родительских форм при создании новых сортов и гибридов подсолнечника.

Ключевые слова: ложная мучнистая роса, подсолнечник, интенсивность поражения, тип реакции, устойчивость, патоген, гибрид, линия

MONITORING OF SUNFLOWER BREEDING MATERIAL TO IDENTIFY THE LEVEL OF RESISTANCE TO FALSE POWDERY MILDEW

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Increased production of sunflower oilseeds requires the use of yielding, adapted to the conditions of cultivation varieties and hybrids resistant to harmful pathogens of the growing area. A number of reasons contribute to the decline in crop resistance. One of them is the emergence of conditions for the development of not only new diseases, but also more aggressive races of already well-known pathogens. The most effective and environmentally friendly way to control crop diseases is to create sustainable breeding material. In this regard, in the conditions of the East Kazakhstan region studies were conducted to assess the resistance to false powdery mildew of the promising breeding material of sunflower created by the specialists of the East Kazakhstan Agricultural Experimental Station. Quantitative indices of affected plants and the intensity of sporoniferous lesions on seedlings were determined. Characterization of the degree of susceptibility of the tested samples on a 5-point scale was given. The results of the experiments confirmed the possibility of selection at different stages of the breeding work. The selected source material can be used as parental forms in the creation of new sunflower varieties and hybrids.

Keywords: Downy mildew, sunflower, lesion intensity, reaction type, resistance, pathogen, hybrid, line

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INTRODUCTION

In the East Kazakhstan region, there has been a stable trend of increasing sunflower cultivation areas over the past two decades. This trend is partly due to the high economic profitability of this crop. As the share of sunflowers in crop rotations grows, there is a natural need to provide agricultural producers with quality sunflower seeds. Undoubtedly, import and use of seeds of hybrids of foreign selection[1] partially solve the problem of meeting the needs of the region in sunflower seeds. However, due to the high cost of imported hybrid seeds, they are not accessible to all local farmers, and their quality does not always meet the claimed parameters. In this regard, the issue of establishing domestic sunflower seed production is urgent in East Kazakhstan.

[1]State program of development of agro-industrial complex of the Republic of Kazakhstan for 2017-2021, Astana, 2017, 150 p.

The expansion of sunflower cultivation areas in crop rotations and the return of this crop not after 5-8 years but after 2-3 years create favorable conditions for the accumulation of infection and the development of epiphytotics [1]. The agroclimatic conditions in East Kazakhstan are diverse, leading to the preservation and spread of a wide range of plant infectious diseases. The most harmful plant diseases in the region include false mildew, gray and white rot, Verticillium wilt, phomosis, and calathide dry rot[2].

One of the most common and harmful fungal diseases in sunflower crops in the mountain meadow-steppe zone of East Kazakhstan is false mildew (FM), caused by the obligate parasitic fungus *Plasmopara halstedii* (Farl.) Berl. et de Toni[3].

A characteristic feature of this disease is the variety of its manifestations. False mildew is known to have six forms of manifestation[4]. Until 2005, only one form, which exhibited moderate pathogenicity, was observed in sunflower crops in East Kazakhstan. In the past decade, all six forms have been spreading[5]. The disease has a latent course, which can make its identification based on morphological features difficult[6]. Infection of the calathides and seeds leads to the death of seedlings, significantly affecting the sowing qualities of the seed material [2].

In this situation, there is a need to provide the region with sunflower seeds free from this fungal infection. Therefore, it is necessary to study the harmfulness of downy mildew in sunflower crops in the region. To do this, an evaluation of sunflower seeds from the collection of the East Kazakhstan Agricultural Experimental Station (EKAES), one of the country's leading enterprises engaged in the breeding and seed production of oilseeds, was conducted. The research aims to determine the resistance level to downy mildew in sunflower selection material from the EKAES gene pool, with the identification of the most resistant initial forms.

MATERIAL AND METHODS

The research object includes 28 experimental hybrids and 30 self-pollinated lines from the EKAES gene pool. As a control, the Kruglik A/41 variety, which is susceptible to all races of the pathogen, was used. This variety was created by the specialists from the All-Union Research Institute of Oil Crops.

Laboratory research was conducted in 2020 at the National Research Laboratory of collective use of the S. Amanzholov East Kazakhstan University. The methodology for conducting such experiments was based on previous work by M.V. Ivebore, T.S. Antonova, and N.M. Araslanova[7-9] [3–5].

To prepare the inoculum, sunflower leaves with sporulation on the lower side of the leaves were collected from the field. Some plant material was placed in a humid chamber for pathogen identification. The false mildew inoculum was obtained from the surface of the selected samples by simple washing of zoospores with distilled water. The number of zoospores in 1 ml was counted in a Goryaev chamber and, if necessary, dilution was made to the required number of 10^6–10^9 cells/ml. The race of the pathogen

[2]*Gubareva N.S., Kuzmina G.N., Chursin A.S.* Species composition of registered diseases of sunflower in East Kazakhstan // Notes of the Ust-Kamenogorsk branch of the Kazakh Geographical Society: Proceedings of the international scientific and practical conference Ust-Kamenogorsk, 2014, Issue 8, pp. 104-112.

[3]*Gubareva N.S., Kuzmina G.N.* Training manual on determination of sunflower diseases in the East Kazakhstan region. Ust-Kamenogorsk, 2018, 59 p.

[4]*Vypritskaya A.A., Kuznetsov A.A., Puchin A.M.* Plasmopara halstedii (Farl.) Berl. et de Toni in the Tambov region // Tambov University Review. Series: Humanities, 2015, vol. 20, N 6, pp. 1595-1600.

[5]*Gubareva N.S.* Monitoring of sunflower diseases and field endurance of varieties and hybrids under cultivation in East Kazakhstan region. Ust-Kamenogorsk, 2018, pp. 6-7.

[6]*Ciftcigil T.H., Ozer N., Sabudak T.* A preliminary study on control of sunflower downy mildew (*Plasmopara halstedii*) with culture filtrates of antagonistic fungi // Procceedings of 19th International Sunflower Conference, Edirne, 2016, p. 1106.

[7]*Ivebor M.V.* Identification of races of sunflower false powdery mildew pathogen in the regions of the North Caucasus and selection of resistant to them source material for breeding: Extended abstract of candidate's thesis in agriculture, Krasnodar, 2009, 144 p.

[8]*Iwebor M.B., Antonova T.S., Araslanova N.M.* Features of the manifestation of false powdery mildew of sunflower under artificial infection with different races of the pathogen // Oilseed crops, 2009, N 1 (140), pp. 26-31.

[9]*Ekimova T.S., Kuzmina G.N.* Study of the methods of evaluation of sunflower breeding material for immunity to false powdery mildew // Global science and innovations 2019: Central Asia, Astana, 2019, vol. 2, pp. 315-319.

was not identified, and a population mixture was used for the inoculum.

For germination, 100 seeds were selected from a single calathide. The seeds were placed in a gauze filter and rinsed under a stream of distilled water for 15 minutes to reduce the number of saprophytic microorganisms. Then, the seeds were dried on filter paper and placed in Petri dishes. A filter paper was placed at the bottom of the dishes, moistened cotton was placed on top, and the Petri dishes were placed in a cultivation room under daylight lamps at a temperature of 25–27°C for 2–4 days (see Fig. 1).

After the formation of sprouts measuring 1.5–2.0 cm in length, visually healthy samples were selected, which had a healthy radicle, hypocotyl, and cotyledons. The selected seedlings were husked and planted in germinating cabinets, which were cells with coarse-filled filter paper[10] (see Fig. 2).

Infection of the sprouts was carried out by watering them with a suspension of zoospores. Each germinating cabinet received 150 ml of the suspension. The infection of the lines and hybrids with false mildew was carried out in a phytotron.

Over the first 7 days in the phytotron, the temperature was maintained at 23–25°C during the day and 18°C at night. In the following 7 days, the temperature was reduced to 16–18°C. During this period, watering with water was performed, and humidity was maintained at 90% by spraying 4 times a day with a sprayer[11].

On the 14th day, the infection was assessed and evaluated for the selection material (see Fig.3). The assessment involved determining the total number of affected plants and the intensity of disease manifestation in each sample. The assessment of the infection was based on the determination of the intensity of seed part damage, including the area of sporulation, chlorosis, and necrosis.

The degree of resistance to infection was assessed using a qualitative reaction scale of sunflower sprouts to pathogen penetration (see Table 1). The type of reaction to infection was determined on a 5-point scale.

RESULTS AND DISCUSSION

Low-quality seed material is, in part, a consequence of fungal infections. It is considered that losses due to infection by this pathogen constitute more than half of the gross yield. The prevalence of diseases is often linked by researchers to disease vectors, climatic conditions, and disruption of crop rotations. In addition to identifying and recording diseases, methods for preventing disease spread are being developed[12, 13]. Breeding for disease resistance has its peculiar-

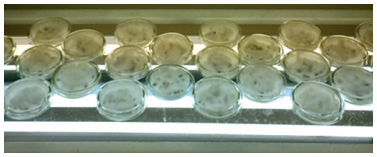

Рис. 1. Семена подсолнечника, помещенные в чашки Петри для проращивания

Fig. 1. Sunflower seeds placed in Petri dishes for germination

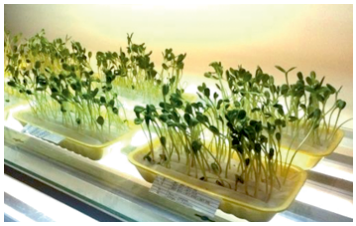

Рис. 2. Растильни с инокулированными проростками

Fig. 2. Planters with inoculated seedlings

Рис. 3. Проростки со спороношением на 14-й день после заражения

Fig. 3. Seedlings with sporulation on the 14th day after infection

[10]*Maslienko L.V., Araslanova N.M., Kovchigina M.A.* Search for the optimal method of artificial infection of sunflower with the causative agent of false powdery mildew to determine the effectiveness of prototype microbiopreparations // Oilseed crops, 2014, N 2, pp. 159-160.

[11]GOST 12044-93. Seeds of agricultural crops. Methods for determining disease infestation. Moscow: Standards Publishing House, 2011, 3 p.

[12]*Iwebor M., Antonova T.S.* Changes in the racial structure of *Plasmopara halstedii* (Farl.) Berl. et de Toni population in the South of the Russian Federation // Heliathis link is disabled, 2016, N 39 (64), pp. 113–121.

[13]*Kostina E.E., Lobachev Yu.V.* Breeding value and resistance to false powdery mildew and infestation of experimental hybrids of sunflower // Bulletin of Saratov state agrarian university in honor of N. I. Vavilov, 2012, N 5, pp. 26-27.

Табл. 1. Показатели оценки реакции проростков подсолнечника на искусственное заражение *Plasmopara halstedii* (Farl.)

Table 1. Indicators for assessing the reaction of sunflower seedlings to artificial infection with *Plasmopara halstedii* (Farl.)

Point	Characterization of the type of lesion	Reaction to infection
0	Affected plants are absent or single cases are noted, intensity of lesions on cotyledonary leaves – from 0,0 to 1,0%	Resistant
1	Affected seedlings – up to 50.0%, intensity of affected cotyledonary leaves – up to 10.0%	Weakly affected
2	Affected seedlings – up to 50.0%, with sporulation on seedlings – more than 10.0%, with weak sporulation – from 50.0 to 75.0%, intensity of cotyledonary leaves infection – up to 10.0%	Moderately affected
3	Affected seedlings with weak sporulation – from 50.0 to 75.0% with intensity of sporulation on cotyledonary leaves more than 10.0%, affected seedlings – from 75.0 to 100.0% with an average intensity of sporulation on cotyledons (from 10.0 to 30.0%)	Severely affected
4	Affected seedlings – from 75.0 to 100.0% with high level of sporulation on cotyledons (from 30.0 to 50.0%)	Susceptible
5	All seedlings with lesions, intensity of cotyledon lesions – 50.0% and more	Unstable

ities because the direction of work is related to the cultivated crop and the emergence of new pathogen variants [7]. Currently, the search for resistant breeding material appears to be a promising direction.

Significant variation in indicators for artificial infection of false mildew was identified in 28 hybrid combinations during the study. Table 2 reflects the average results of the experiment.

Based on the presented data, it can be seen that in different experimental variants, the proportion of infected seedlings ranged from 30.0% (HC 1/31) to 100.0% (HC 1/13, HC 1/42). In the control, this indicator reached an average of 90.0%. The intensity of seed part damage in the experiment ranged from 2.0% (HC 1/17) to 80.5% (HC 1/42). The maximum intensity of seed part damage was observed in two samples: HC 1/42 - 80.5%, HC 1/13 - 80.3%, with the disease spreading to all plants. Out of the 28 examined samples, six had the maximum degree of seedling damage, which allowed them to be classified as unstable (5 points).

Most hybrid combinations had a low degree of seedling damage (up to 22.0%). However, at the same time, the number of seedlings with sporulation accounted for more than half of all plants. Such samples were classified as moderately and heavily affected (see Fig. 4).

Based on the sum of characteristics, HC 1/39, HC 1/25, HC 1/57 were classified as weakly affected, moderately affected - HC 1/60, HC 1/59, HC 1/7, HC 1/45. The samples HC 1/17 and HC 1/31 showed a high degree of resistance. As a result, it was decided to use these nine hybrid combinations in further breeding work.

It should be noted that for heterozygous hybrids, an immune response to the false mildew pathogen is possible under the condition of the presence of horizontal and vertical resistance in the parental forms[14] [8–10]. Therefore, there was a need to study constant self-pollinated lines. In the course of the experiment, 30 constant self-pollinated lines were investigated for resistance to false mildew (see Table 3).

The assessment of the resistance of sunflower breeding material to false mildew showed that all the examined samples were affected to varying degrees by the infection. The percentage of seedlings with sporulation ranged from 40.0 (CL/21) to 100.0% (CL/32, CL/57). In the control variant, this indicator was 90.0% on average.

[14]*Markin N.V., Usatov A.V., Vasilenko V.N., Maidanyuk D.N., Getmantseva L.V.* SSR analysis of maternal and paternal lines selected in the don region (Russia) // American Journal of Agricultural and Biological Science, 2016, N 11 (1), pp. 13–18.

Табл. 2. Результаты заражения экспериментальных гибридных комбинаций

Table 2. Results of infection of experimental hybrid combinations

Selection index	Proportion of seedlings with sporulation, %	Intensity, %	Type of reaction, point
HC 1/31	30,0 ± 1,5	8,3 ± 0,4	0
HC 1/17	35,0 ± 1,8	2,0 ± 0,1	0
HC 1/25	45,0 ± 2,3	7,8 ± 0,4	1
HC 1/57	50,0 ± 2,5	6,3 ± 0,3	1
HC 1/39	37,5 ± 1,9	18,8 ± 0,9	1
HC 1/60	55,0 ± 2,8	4,0 ± 0,2	2
HC 1/59	55,0 ± 2,8	8,5 ± 0,4	2
HC 1/7	52,9 ± 2,6	16,8 ± 0,8	2
HC 1/45	73,7 ± 3,7	5,5 ± 0,3	2
HC 1/34	63,2 ± 3,2	21,8 ± 1,1	3
HC 1/58	65,0 ± 3,3	12,8 ± 0,6	3
HC 1/18	65,0 ± 3,3	15,3 ± 0,8	3
HC 1/23	65,0 ± 3,3	30,8 ± 1,5	3
HC 1/64	70,0 ± 3,5	14,3 ± 0,7	3
HC 1/33	70,6 ± 3,5	11,2 ± 0,6	3
HC 1/48	75,0 ± 3,8	13,5 ± 0,7	3
HC 1/53	75,0 ± 3,8	14,0 ± 0,7	3
HC 1/32	77,8 ± 3,9	18,9 ± 0,9	3
HC 1/16	80,0 ± 4,0	15,8 ± 0,8	3
HC 1/37	80,0 ± 4,0	20,5 ± 1,0	3
HC 1/55	80,0 ± 4,0	23,0 ± 1,2	3
HC 1/10	85,0 ± 4,3	38,3 ± 1,9	4
HC 1/9	88,2 ± 4,4	30,6 ± 1,5	4
HC 1/44	95,0 ± 4,8	14,8 ± 0,7	4
HC 1/43	95,0 ± 4,8	76,5 ± 3,8	4
HC 1/13	100,0 ± 5,0	80,3 ± 4,0	5
HC 1/42	100,0 ± 5,0	80,5 ± 4,0	5
Control	90,0 ± 4,5	56,0 ±2 ,8	5
$LSD_{0,05}$	± 3,5	± 1,1	

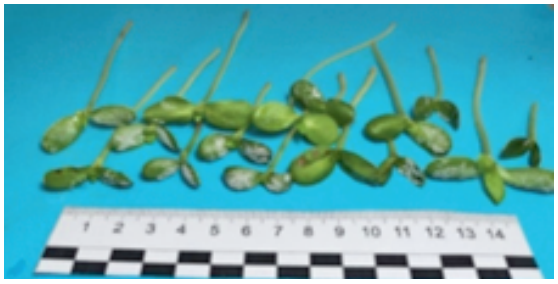

Рис. 4. Третий тип реакции

Fig. 4. Third type of reaction

The maximum intensity of seedling infection in constant lines ranged from 2.5 (CL/5) to 51.8% (CL/32), while in the control, it was 54.5%.

The research results allowed us to identify three lines with the third type of reaction, where the maximum number of affected plants was observed. The fourth type of reaction to infection, "susceptible," was found in ten samples. The intensity of seedling infection in this case ranged from 10.0 to 29.3%, and the number of affected plants reached 75.0%. Twelve samples were classified as moderately affected (see Fig. 5). In this group, more than half of the plants were infected, with slight seedling damage ranging from 2.8 to 9.5%.

Among the studied lines, samples with a small number of seedlings with weak infection (CL/5, CL/6, CL/12, CL/21) were identified as promising breeding material for resistance to false mildew. These lines can be characterized as weakly affected.

CONCLUSION

The conducted research not only identified the initial breeding material for creating new experimental hybrids resistant to false mildew but also weakly affected and resistant promising hybrid combinations.

It should be noted that the experiments were conducted in 2020, and at present, the selected breeding material based on resistance to false mildew may have lost its effectiveness due to the emergence of a more aggressive race within the pathogen population.

In summary, to enhance resistance to false mildew in constant lines and hybrids, further research is needed to identify stable forms that

Табл. 3. Результаты заражения самоопыленных линий

Table 3. Results of infection of self-pollinated lines

Selection index	Proportion of seedlings with sporulation, %	Intensity, %	Type of reaction, point
CL /21	40,0 ± 2,0	2,8 ± 0,1	1
CL /5	45,0 ± 2,3	2,5 ± 0,1	1
CL /6	50,0 ± 2,5	4,0 ± 0,2	1
CL /12	50,0 ± 2,5	2,8 ± 0,1	1
CL /4	55,0 ± 2,8	3,3 ± 0,2	2
CL /18	55,0 ± 2,8	2,8 ± 0,1	2
CL /33	55,0 ± 2,8	5,8 ± 0,3	2
CL /2	60,0 ± 30,0	4,3 ± 0,2	2
CL /23	65,0 ± 3,3	8,3 ± 0,4	2
CL /31	65,0 ± 3,3	7,3 ± 0,4	2
CL /61	65,0 ± 3,3	5,0 ± 0,3	2
CL 3	75,0 ± 3,8	6,0 ± 0,3	2
CL /15	75,0 ± 3,8	7,3 ± 0,4	2
CL /22	75,0 ± 3,8	9,5 ± 0,5	2
CL /26	75,0 ± 3,8	5,0 ± 0,3	2
CL /35	75,0 ± 3,8	8,8 ± 0,4	2
CL /24	60,0 ± 3,0	14,3 ± 0,7	3
CL /25	70,0 ± 3,5	16,3 ± 0,8	3
CL /30	70,0 ± 3,5	29,3 ± 1,5	3
CL /58	70,0 ± 3,5	10,5 ± 0,5	3
CL /60	70,0 ± 3,5	23,3 ± 1,2	3
CL /62	70,0 ± 3,5	18,8 ± 0,9	3
CL /29	75,0 ± 3,8	15,8 ± 0,8	3
CL /34	75,0 ± 3,8	16,0 ± 0,8	3
CL /56	75,0 ± 3,8	15,0 ± 0,8	3
CL /63	75,0 ± 3,8	10,0 ± 0,5	3
CL /59	95,0 ± 4,8	43,8 ± 2,2	4
CL /32	100,0 ± 5,0	51,8 ± 2,6	5
CL /57	100,0 ± 5,0	37,5 ± 1,9	5
Control	90,0 ± 4,5	54,5 ± 2,7	5
$LSD_{0,05}$	± 3,5	± 0,7	

Рис. 5. Второй тип реакции

Fig. 5. Second type of reaction

can be used in breeding and cell biotechnology. Simultaneously, the biology of the fungus and its racial composition in the conditions of the East Kazakhstan region should be studied.

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Periodical publication

Pakul A.L., Lapshinov N.A., Bozhanova G.V., Pakul V.N. Technological grain qualities of spring common wheat depending on the system of soil tillage // Siberian Herald of Agricultural Science. 2018. vol. 48. № 4. pp. 27-35. DOI: 10.26898/0370-8799-2018-4-4.

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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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