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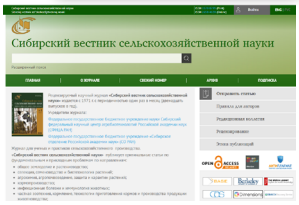

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Применение ResNet для идентификации сорных растений в посевах зерновых

(✉)**Риксен В.С., Шпак В.А.**
Сибирский федеральный научный центр агробиотехнологий Российской академии наук
Новосибирская область, р.п. Краснообск, Россия
(✉)e-mail: Riclog@mail.ru

С развитием технологий искусственного интеллекта и машинного обучения сверточные нейронные сети (CNN) стали одним из ключевых инструментов в области компьютерного зрения. Они активно применяются для решения задач, связанных с идентификацией и классификацией объектов. В сельском хозяйстве, где точность и скорость обработки данных имеют критическое значение, CNN нашли широкое применение, в частности для автоматического распознавания сорных растений в посевах сельскохозяйственных культур. Целью данного исследования является разработка классификаторов изображений на основе сверточных моделей ResNet-18, ResNet-34 и ResNet-50 для идентификации сорняков и классификации степени их распространения на участках, занятых зерновыми культурами. В качестве исходной информации используются результаты фитосанитарного мониторинга посевов пшеницы (*Triticum aestivum* L.) и ячменя (*Hordeum sativum* L.) и фотографии учетных площадок. В ходе мониторинга выявлено 19 видов сорных растений, которые присутствовали на участках с разной степенью интенсивности. Для количественной оценки засоренности каждому виду присваивался идентификатор: 0 – если вид превышал экономический порог вредоносности (ЭПВ), 1 – если не превышал. Основная задача классификатора заключалась в распознавании сорняков на фотографиях и определении одной из двух градаций засоренности участка. Предложенный подход продемонстрировал высокую эффективность. Точность классификаций на тестовом наборе изображений составила в среднем 95%. Для оценки надежности метода была построена матрица ошибок, которая также подтвердила высокую точность предсказаний. Полученные результаты свидетельствуют о высокой эффективности использования архитектур ResNet для задач автоматического распознавания сорняков. Практическое применение разработанных классификаторов позволяет оперативно проводить фитосанитарную диагностику посевов, что способствует своевременному и точному подбору гербицидов для борьбы с сорными растениями.

Ключевые слова: сорные растения, глубокое обучение, классификатор, ResNet, пшеница яровая, ячмень яровой

Application of ResNet for identification of weeds in grain crops

(✉)**Riksen V.S., Shpak V.A.**
Siberian Federal Research Centre of Agro-BioTechnologies of the Russian Academy of Sciences
Krasnoobsk, Novosibirsk region, Russia
(✉)e-mail: Riclog@mail.ru

With the development of artificial intelligence and machine learning technologies, convolutional neural networks (CNN) have become one of the key tools in the field of computer vision. They are actively used to solve problems related to the identification and classification of objects. In agriculture, where the accuracy and speed of data processing are of critical importance, CNNs have found wide application, in particular, for the automatic recognition of weeds in crops. The purpose of this study is to develop image classifiers based on ResNet-18, ResNet-34 and ResNet-50 convolutional models

to identify weeds and classify their prevalence in grain crop areas. The results of phytosanitary monitoring of wheat (*Triticum aestivum* L.) and barley (*Hordeum sativum* L.) crops and photographs of accounting sites are used as initial information. During the monitoring, 19 weed species were identified that were present in the areas with varying degrees of intensity. To quantify the weediness, each species was assigned an identifier: 0 – if the species exceeded the economic threshold of harmfulness (ETH), 1 - if it did not exceed it. The main task of the classifier was to recognize weeds in photographs and determine one of two gradations of weediness of the area. The proposed approach demonstrated high efficiency. The accuracy of classifications on the test set of images was 95% on average. An error matrix was constructed to assess the reliability of the method, which also confirmed the high accuracy of predictions. The obtained results indicate the high efficiency of using ResNet architectures for automatic weed recognition tasks. Practical application of the developed classifiers allows for prompt phytosanitary diagnostics of crops, which contributes to the timely and accurate selection of herbicides for weed control.

Keywords: weeds, deep learning, classifier, ResNet, spring wheat, spring barley

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INTRODUCTION

Agriculture faces growing challenges such as climate change, resource depletion, and the need to increase crop yields. One key concern is weed control, which reduces crop productivity by competing for nutrients, water, and light [1]. Traditional weed control methods, including mechanical tillage and herbicide application, are often ineffective and can negatively impact ecosystems [2]. Therefore, the implementation of modern technologies such as artificial intelligence (AI) and machine learning (ML) to automate weed identification and classification processes is becoming increasingly important [3].

Convolutional neural networks (CNNs) have proven to be a powerful tool for image processing and object recognition. Among the various CNN architectures, ResNet (Residual Network)[1] has attracted particular attention. ResNet uses residual connections to avoid the vanishing gradient problem and facilitate deeper network learning, making it effective for image classification tasks[2]. In agriculture, ResNet is used to analyze the data collected by drones, satellites, and stationary cameras, enabling real-time data collection and automating crop monitoring and management processes[3] [4–7].

Recent studies demonstrate the high effectiveness of ResNet in weed identification. The authors note that ResNet provides high accuracy even when working with large datasets, which is especially important for analyzing agricultural images, where variability in shooting conditions (illumination, angle, resolution) can be significant [8]. A key advantage of ResNet is its ability to be trained on deep architectures without the loss of accuracy, which is especially important for tasks requiring high detail, such as weed identification [9, 10].

The accuracy of ResNet convolutional models used for classifying weeds in agricultural

[1]*He K., Zhang X., Ren S., Sun J.* Deep residual learning for image recognition // Proceedings of the IEEE conference on computer vision and pattern recognition, 2016, pp. 770–778.

[2]*Zhang X., Zhou X., Lin M., Sun J.* Shufflenet: An extremely efficient convolutional neural network for mobile devices // Proceedings of the IEEE conference on computer vision and pattern recognition, 2018, pp. 6848–6856.

[3]*Mulla D.J.* Twenty five years of remote sensing in precision agriculture: Key advances and remaining knowledge gaps // Biosystems engineering, 2013, vol. 114 (4), pp. 358–371. DOI: 10.1016/j.biosystemseng.2012.08.009.

crops ranges from 83 to 94%, demonstrating the high efficiency of this architecture for solving agronomic problems related to the automation of plant identification processes [11, 12].

The purpose of the study is to develop image classifiers based on the ResNet-18, ResNet-34 and ResNet-50 convolutional models for identifying weeds and classifying the extent of their spread in the areas occupied by grain crops.

MATERIAL AND METHODS

To train the convolutional neural network, the initial dataset used included photographs with a resolution of 1340 × 1790 pixels taken with a mobile camera, as well as phytosanitary diagnostic results. The total dataset size which was used included 146 images.

The classifier's purpose is to identify weeds in the images of grain crops (wheat and barley) and assess the degree of weed infestation, assigning it to one of two categories: low or high. Preliminary data processing identified 32 weed infestation classes. Each class is assigned a unique numerical label (see Table 1).

The identifier "0" is assigned to the weeds whose numbers do not exceed the economic threshold of harmfulness (ETH), and "1" to those whose infestation exceeds the ETH. A single image may contain either one or several weeds with a given gradation. Therefore, the classification task is a multi-component recognition task, where each image may correspond to multiple labels.

To solve this problem, the research utilized the ResNet (Residual Network) architecture, known for its residual blocks and ready-made implementations in the PyTorch framework. To simplify work with PyTorch, the FastAi library was used, providing a convenient interface for managing network components [13]. The ResNet-18, ResNet-34, and ResNet-50 models presented in the study are based on pre-trained networks from PyTorch. The image processing algorithm includes the following steps (see Fig. 1):

1. The original image is sequentially passed through convolutional layers, activation functions, and pool layers.

2. The output of ResNet is a multidimensional vector, where each element corresponds to the probability of the presence of a certain class of an object.

Табл. 1. Выделенные типы засоренности с учетом градаций
Table 1. Selected types of contamination taking into account the gradations

Identifier	Label	Identifier	Label
Chenopodiumalbum0	0	Lappulasquarrosa1	16
Chenopodiumalbum1	1	Lathyrussylvestris0	17
Cirsiumarvense1	2	Panicumcapillare0	18
Convolvulusarvensis0	3	Panicumcapillare1	19
Convolvulusarvensis1	4	Pastinacasylvestris1	20
Echinochloacrus-galli0	5	Persicariahydropiper0	21
Echinochloacrus-galli1	6	Persicariahydropiper1	22
Equisetumarvense0	7	Sinapisarvensis0	23
Equisetumarvense1	8	Sinapisarvensis1	24
Erodiumcicutarium0	9	Sonchusarvensis0	25
Euphorbiavirgata0	10	Sonchusarvensis1	26
Euphorbiavirgata1	11	Stachyssylvatica0	27
Fallopiaconvolvulus0	12	Stachyssylvatica1	28
Fallopiaconvolvulus1	13	Taraxacumofficinale1	29
Galiumaparine0	14	Viciasativa0	30
Lappulasquarrosa0	15	Viciasativa1	31

The classifier supports detection of multiple objects in a single image. An object is considered recognized if its probability exceeds 50%. This allows for the detection of both multiple weeds and instances of their absence.

RESULTS AND DISCUSSION

Due to the limited number of source images, which was insufficient to train a neural network with high generalization capacity, a dataset augmentation strategy was employed using the aug_transforms tool from the PyTorch library. This method implements a set of transformations, including rotations, scaling, translation, and randomized operations such as cropping, brightness, and contrast adjustments. Each transformation generates unique image variations that preserve the semantics of the original data but are visually distinct due to artificially created artifacts.

The use of synthetic augmentation allowed us to increase the training data volume by a factor of 6, significantly improving the model's generalization ability. A visualization of the process of creating modified images is shown in Figure 2.

The standard input format for ResNet neural networks is a 224 × 224 pixel matrix. PyTorch's built-in transformation functions are used to process images of arbitrary sizes. The original images, with a resolution of 1340 × 1790 pixels, were first reduced to 512 × 512 pixels using the Resize operation and then scaled to the target size of 224 × 224.

The amount of computation directly depends on the dimensionality of the input data. Since the original color images were taken using a standard digital camera, their digital representation on the computer has three channels: red, green, and blue, whereas a regular grayscale image has only one channel.

The neural networks were trained on a test dataset to minimize overfitting and improve model reliability. Key hyperparameters were set at the start of training:

– number of epochs: 100;
– batch size: 16 images;
– learning speed (learning rate).

To optimize the process, the Adam algorithm, which has proven effective in computer vision tasks, was used. Each epoch included a full processing cycle of all images, divided into batches of 16 images. This approach ensured a balance between training speed and the stability of gradient descent.

The final accuracy (accuracy_multi) of all models exceeds 95%, demonstrating the high efficiency of the algorithms in correctly classifying images (see Table 2). This accuracy is

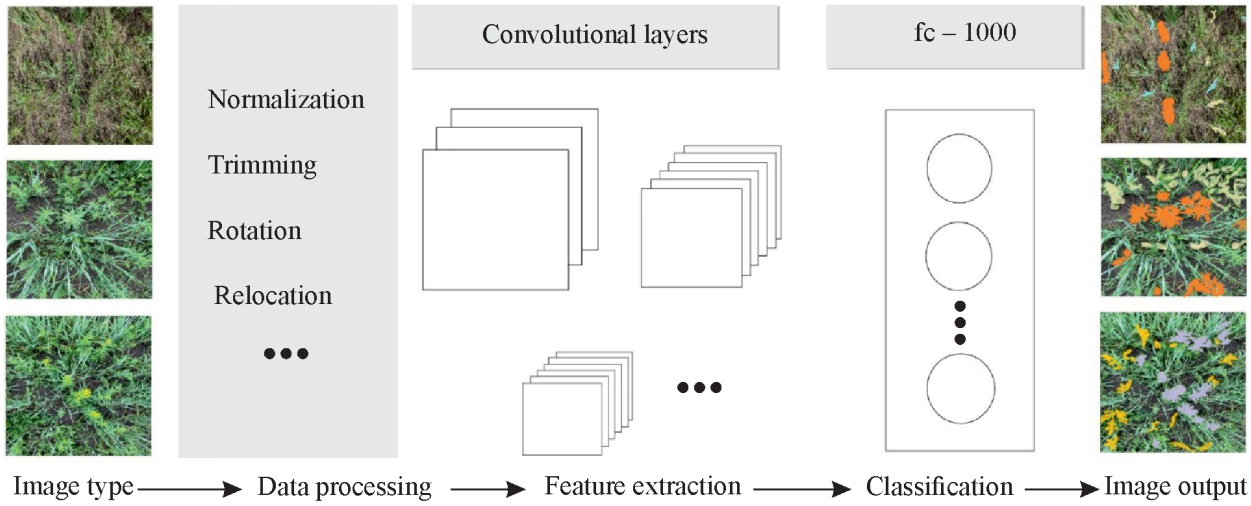

Рис. 1. Общее описание методологии классификации сорняков
Fig. 1. General description of the weed classification methodology

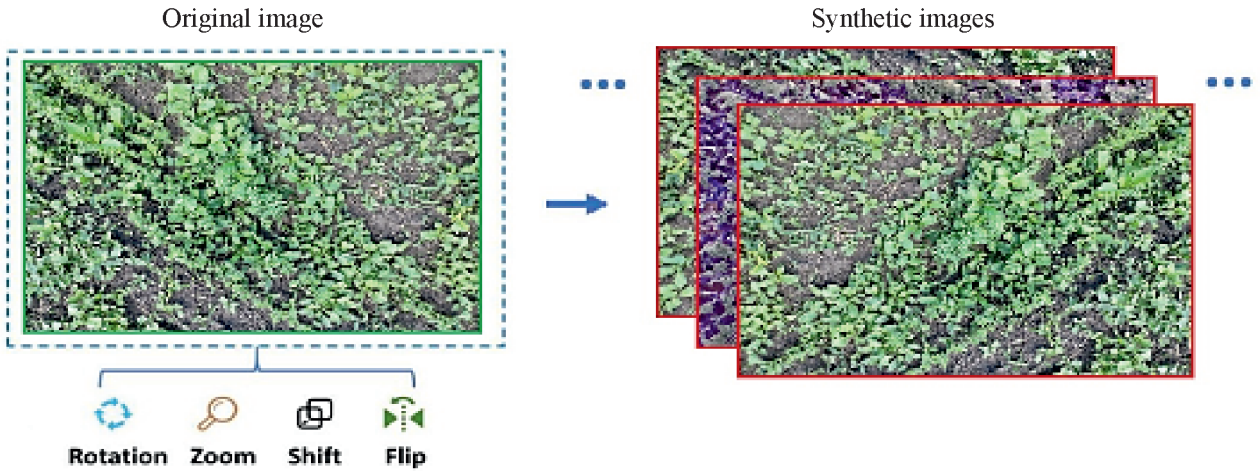

Рис. 2. Процесс генерации синтетических изображений
Fig. 2. The process of generating synthetic images

achieved through careful data preprocessing, including augmentation and normalization, as well as an optimized ResNet architecture adapted for working with agricultural images. However, given the specific nature of the task—identifying weeds under conditions of visual similarity to crop plants and variable lighting—the balanced F1 metric became a key aspect of the analysis.

Final F1-score values in the range of 0.60–0.68 indicate moderate task complexity due to high intra-class variability of weeds (e.g., differences in growth stages and shooting angles) and the need for further fine-tuning of the models. By comparison, in binary classification problems, the F1-score often exceeds 0.8, highlighting the multi-component nature of the current task.

Figure 3 shows the classification algorithm, including the following elements: the top block represents the true weed infestation labels, corresponding to the expert labeling; the bottom block represents the model's predicted labels, obtained after processing the image through ResNet convolutional layers. The example demonstrates how the model correctly identifies the dominant weed class.

Табл. 2. Результаты обучения нейросетей ResNet'-ов по эпохам
Table 2. Results of training ResNet neural networks by epochs

Epoch	train_loss	valid_loss	accuracy_multi	F1
		ResNet-18		
1	1.072530	0.726205	0.227371	0.182359
30	0.492774	0.235319	0.784483	0.378663
60	0.111596	0.138264	0.948276	0.577651
100	0.062181	0.131960	0.957974	0.596734
		ResNet-34		
1	1.064179	0.841298	0.252155	0.160588
30	0.481951	0.272955	0.719828	0.326994
60	0.100709	0.134861	0.949353	0.608854
100	0.048375	0.137498	0.951509	0.617053
		ResNet-50		
1	1.056238	0.754548	0.199353	0.181455
30	0.412729	0.216938	0.799569	0.442536
60	0.077629	0.166699	0.942888	0.650513
100	0.032208	0.179525	0.950431	0.675379

This visualization helps not only evaluate the overall performance of the model, but also identify the algorithm's "blind spots," such as difficulties in recognizing small or shaded weeds.

For in-depth error analysis, a confusion matrix metric was used, constructed using a "one versus all" strategy (see Figs. 4–6). The matrix's rows (2 × 2) display the distribution of images by their actual labels: Y – images belonging to the target contamination class (true positives); N – the set of images from all other classes (true negatives). The columns of the matrix correspond to the model's predictions: Y are the images classified as the target class; N are the images assigned to other categories. The matrix diagonal, from left to right, shows the images that were classified correctly (correct predictions); those outside the diagonal reflect incorrect classifications (incorrect predictions). This representation allows us to localize problem areas in classification and assess which classes the model most often confuses.

The obtained results demonstrate that ResNet-based models achieve high accuracy (95%) on real-world agricultural data. The high accuracy and stability of the F1 score make them suitable for implementation in automatic crop monitoring systems.

Classes 1, 3, 8, 16, 19, 28, 30, and 31 are predicted absolutely accurately with a total of 146 correct classifications (see Fig. 4). The smallest number of correct classifications (six incorrect classifications) was predicted for the class Convolvulusarvensis1 and Sonchusarvensis1.

The confusion matrix demonstrates a significant improvement in classification quality when switching to the ResNet-34 model (see Figure 5). Compared to ResNet-18, the number of absolutely correct predictions increased to 13, highlighting the effectiveness of the deeper architecture in processing complex features of agricultural images. The Euphorbiavirgata1 class was the source of six errors, which may be due to insufficient representativeness of the samples in the training set. The Euphorbiavirgata0 and Sonchusarvensis1 classes each resulted in five erroneous predictions. The likely cause is artifacts in the images (e.g., partially obscured leaves by cultivated plants).

The increase in the number of correct classifications in ResNet-34 confirms that the deeper network (34 layers) is better at recognizing subtle patterns such as leaf texture or inflorescence shape compared to ResNet-18.

The ResNet-50 model demonstrates an increase in the number of absolutely correct clas-

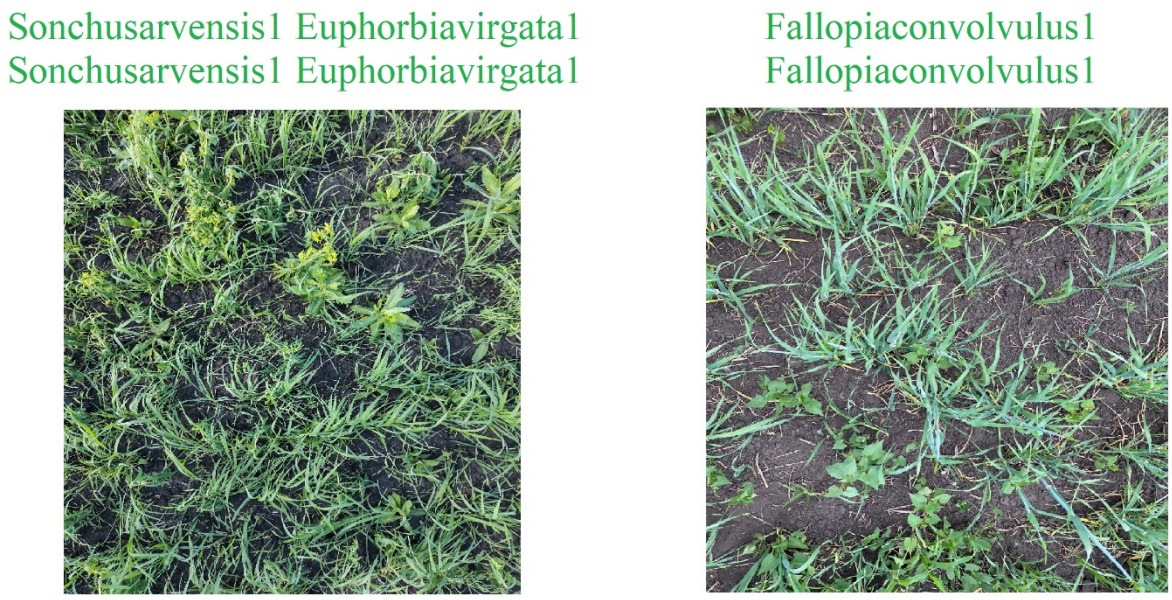

Рис. 3. Результат классификации двух снимков по нейросети ResNet
Fig. 3. The result of classification of 2 images by the neural network ResNet

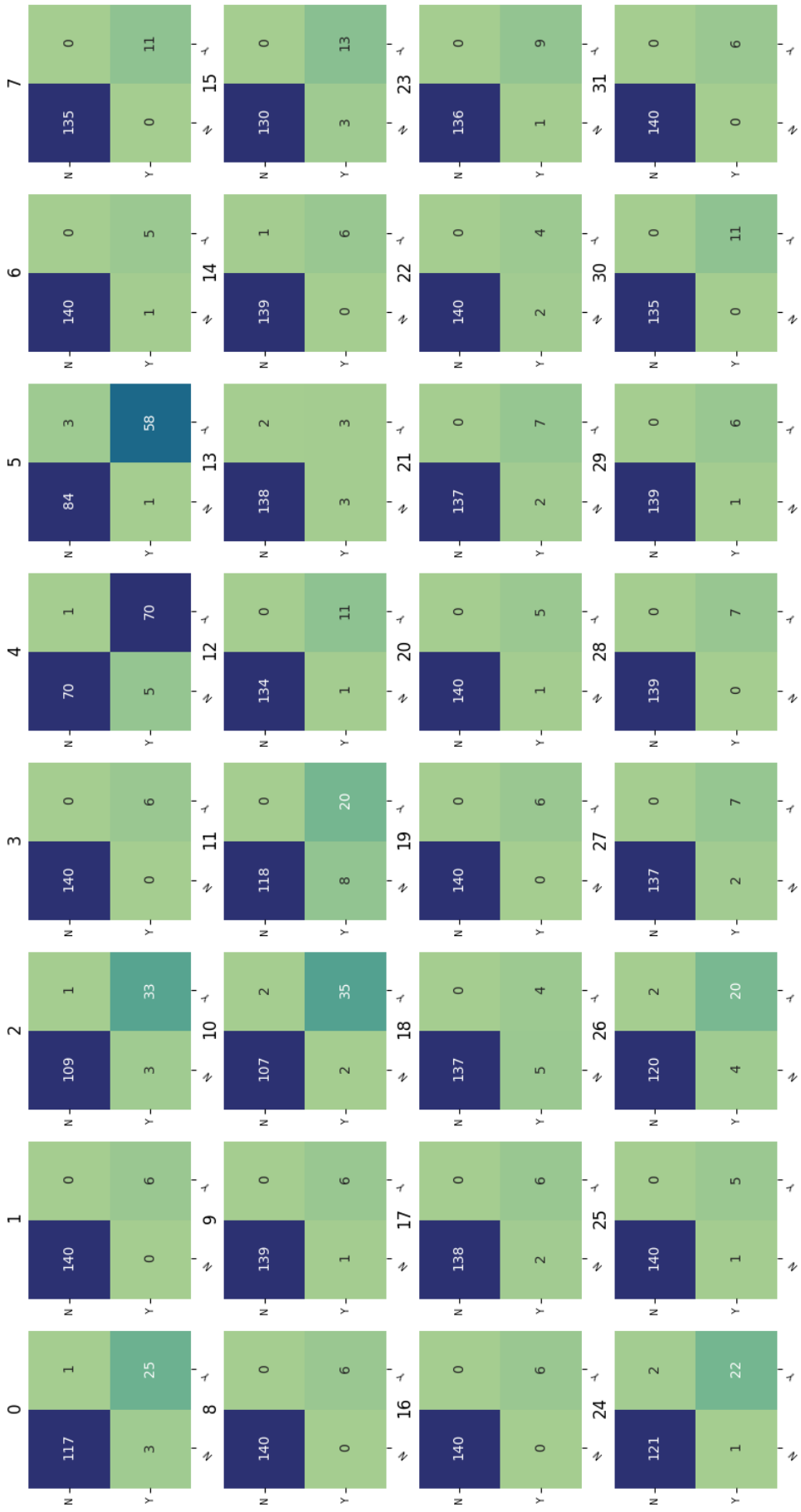

Рис. 4. Confusion matrix для классов засоренности для ResNet-18 (фрагмент матрицы)
Fig. 4. Confusion matrix for contamination classes for ResNet-18 (fragment of the matrix)

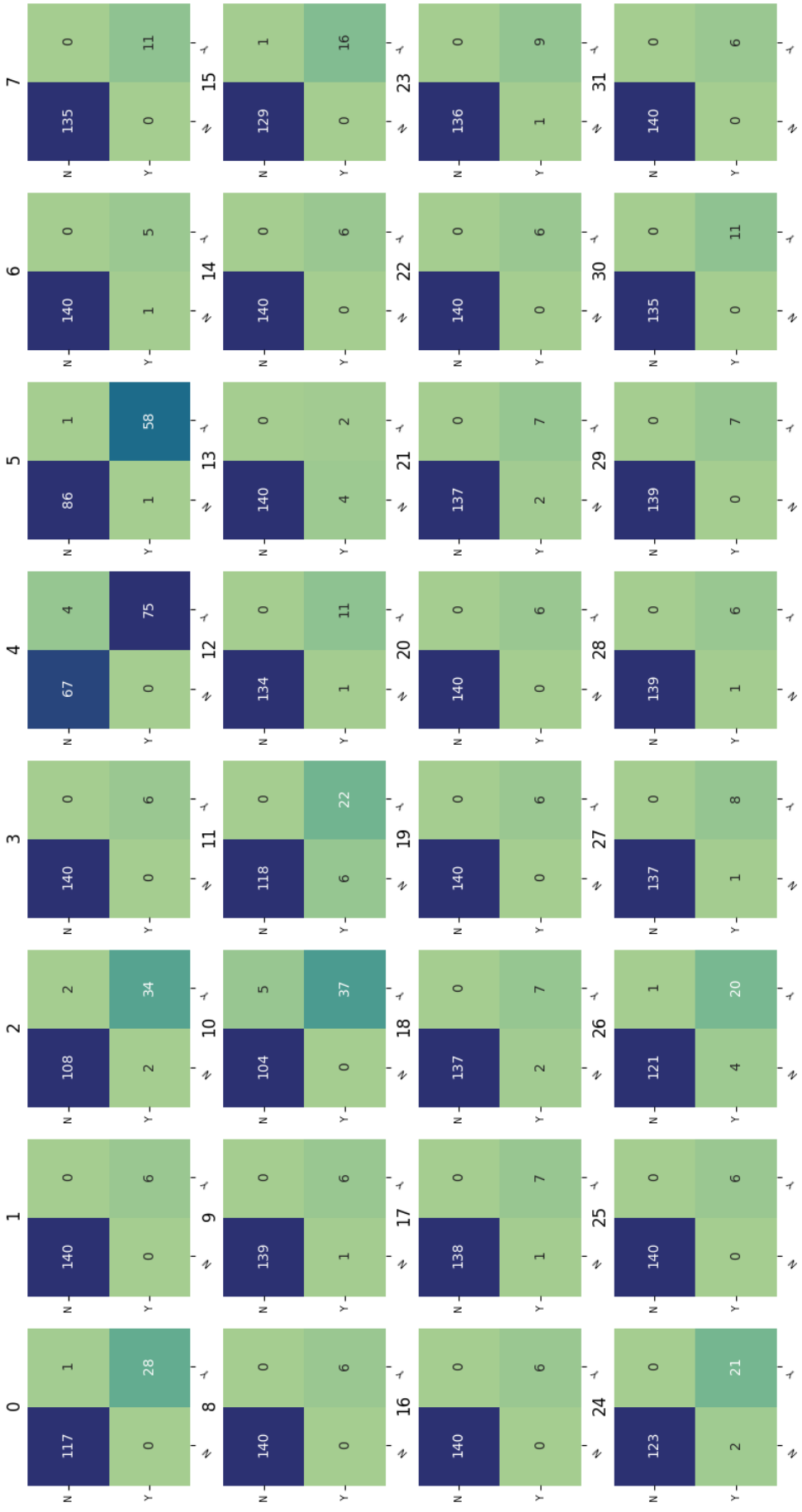

Рис. 5. Confusion matrix для классов засоренности для ResNet-34 (фрагмент матрицы)
Fig. 5. Confusion matrix for contamination classes for ResNet-34 (fragment of the matrix)

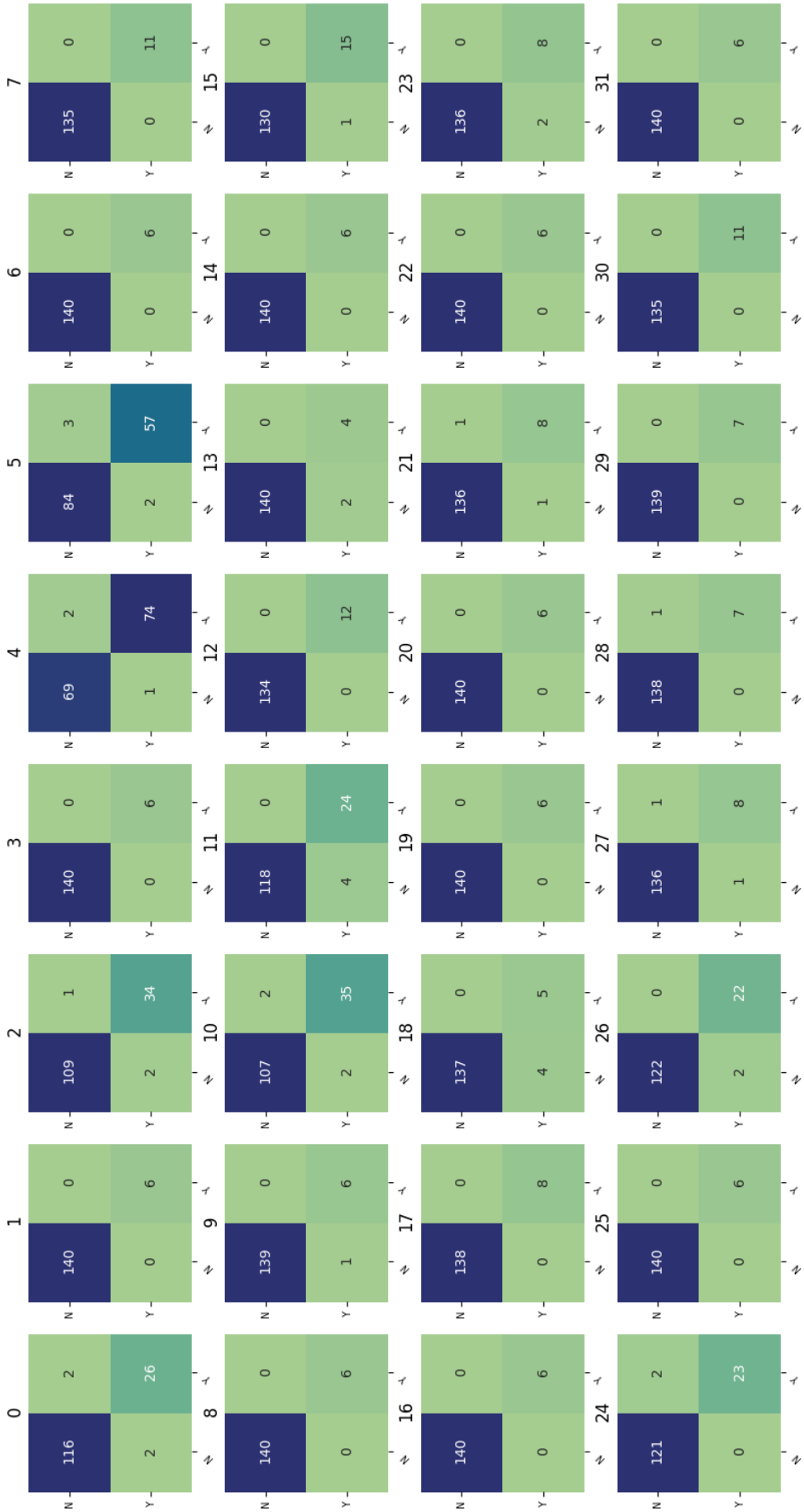

Рис. 6. Confusion matrix для классов засоренности для ResNet-50 (фрагмент матрицы)
Fig. 6. Confusion matrix for contamination classes for ResNet-50 (fragment of the matrix)

sifications to 16 cases (see Figure 6), which is three cases more than ResNet-34. However, this improvement is accompanied by a number of issues indicating a decrease in the predictive stability of the deeper architecture. Despite the increase in the number of correct predictions, ResNet-50 requires 40% more resources for training and inference, which is not justified by the slight increase in accuracy.

The conducted confusion matrix analysis allows us to convincingly recommend ResNet-34 as the optimal classifier for the task of identifying weeds in agricultural crops. Unlike ResNet-18 and ResNet-50, ResNet-34 demonstrates balanced performance, avoiding both the lack of expressiveness of shallow networks and the excessive complexity of deep architectures.

CONCLUSION

The study confirmed the effectiveness of using ResNet architectures for identifying weeds in agricultural crops. Key results:

1. Data augmentation using aug_transforms in PyTorch allowed us to increase the training sample size sixfold, significantly improving the generalization ability of the models. Transformations (rotations, scaling, and brightness changes) created variable yet semantically intact images, compensating for the initial data limitations.

2. Image preprocessing (compression to 512 × 512 pixels and subsequent scaling to 224 × 224) and the use of RGB channels ensured compatibility with the ResNet input format, preserving the information content of the data while reducing the computational load.

3. Training models with parameters (100 epochs, batch size = 16, Adam optimizer) demonstrated high accuracy (accuracy_multi >95%), but moderate F1-measure values (0.60–0.68) indicated the complexity of multi-class classification in the presence of intra-class variability of weeds and survey artifacts.

A comparison of ResNet architectures revealed that ResNet-34 is optimal: ResNet-18 demonstrated insufficient depth for recognizing fine details (leaf texture, inflorescence shape), leading to a 15% higher error rate in complex classes (e.g., Sonchusarvensis1). ResNet-50, despite increasing the correct prediction rate to 16, demonstrated a decrease in predictive stability due to overfitting to noise and a 40% increase in resource consumption.

Problematic classes such as Euphorbiavirgata1 (six errors) and Convolvulusarvensis1 (lowest accuracy) require dataset expansion and balancing techniques.

The study proved that the depth of the neural network should correspond to the complexity of the task: excessive parameterization (ResNet-50) worsens practical applicability, and insufficient (ResNet-18) limits the quality of the forecast.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

(✉)**Риксен Вера Сергеевна,** старший научный сотрудник, кандидат сельскохозяйственных наук; SPIN-код 7858-1352; **адрес для переписки:** Россия, 630501, Новосибирская область, р.п. Краснообск, а/я 463; e-mail: Riclog@mail.ru

Шпак Владимир Александрович, старший научный сотрудник, кандидат физико-математических наук; SPIN-код 7139-6571

AUTHOR INFORMATION

(✉)**Vera S. Riksen,** Senior Researcher, Candidate of Science in Agriculture; SPIN-code 7858-1352; **address:** PO Box 463, Krasnoobsk, Novosibirsk Region, 630501, Russia; e-mail: Riclog@mail.ru

Vladimir A. Shpak, Senior Researcher, Candidate of Science in Physics and Mathematics; SPIN-код 7139-6571

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Метод ранней диагностики устойчивости сортов пшеницы к совместному действию хлоридного засоления и инфицирования *B. sorokiniana*

(✉)**Гурова Т.А., Чесноченко Н.Е.**
Сибирский федеральный научный центр агробиотехнологий Российской академии наук
Новосибирская область, р.п. Краснообск, Россия
(✉)e-mail: guro-tamara@yandex.ru

Представлены результаты разработки метода ранней диагностики устойчивости сортов пшеницы к совместному действию хлоридного засоления и инфицирования возбудителем обыкновенной корневой гнили злаков *Bipolaris sorokiniana* Shoem (*B. sorokiniana*) по выходу электролитов из листовой ткани проростков. Исследования проводили в 2022, 2023 гг. на четырех сортах мягкой яровой пшеницы. Проростки выращивали из инфицированных патогеном трехсуточных проросших семян на растворе хлорида натрия (опыт) и на водопроводной воде (контроль) в заданных климатических условиях. Реакцию сортов на стресс определяли по относительному изменению удельной электропроводности (УЭП) водных вытяжек листьев на кондуктометрической установке edge EC, HANNA Instruments (Германия). Экспериментально определены условия подготовки образцов, обеспечивающих максимальные межсортовые различия: размер нарезки листовой ткани – 1,5 мм, соотношение листовой ткани и воды – 1 : 40, время экспозиции образцов в дистиллированной воде не менее 1,5 ч. Проведенная оценка устойчивости сортов пшеницы показала бóльшую дифференцирующую способность разработанного метода и наибольшие межсортовые различия по сравнению с морфофизиологическим методом. Выборочные средние для относительных изменений УЭП водных вытяжек листовой ткани сортов различались в 2,6–7,3 раза, показателей длины и биомассы ростков и корней у данных сортов – в 1,2–2,3 раза. Коэффициент корреляции рангов Спирмена между показателями устойчивости сортов составил $r_s = 0{,}88$ при $p \leq 0{,}01$. Результаты диагностики устойчивости исследованных сортов пшеницы по ростовым процессам и УЭП водных вытяжек листьев совпали. Выявлена сортоспецифичность: сорт Новосибирская 18 менее устойчив, Омская 18, Сибирская 21 и Новосибирская 44 более устойчивы к исследуемому совместному стрессу. Сделан вывод о возможности применения разработанного метода по выходу электролитов листьев для ранней диагностики (фенотипирования) сортов пшеницы по устойчивости к совместному действию *B. sorokiniana* и хлоридного засоления.

Ключевые слова: пшеница, сорт, устойчивость, совместный стресс, выход электролитов, удельная электропроводность

The method of early diagnostics of wheat varieties resistance to combined action of chloride salinity and *B. sorokiniana* infection

(✉)**Gurova T.A., Chesnochenko N.E.**
Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences
Krasnoobsk, Novosibirsk Region, Russia
(✉)e-mail: guro-tamara@yandex.ru

The article presents the results of developing a method for early diagnostics of wheat variety resistance to the combined effects of chloride salinity and infection with the causative agent of common root rot of cereals *Bipolaris sorokiniana* Shoem. (*B. sorokiniana*) based on the release of electrolytes

from the leaf tissue of seedlings. The studies were carried out in 2022 and 2023 on four varieties of soft spring wheat. The seedlings were grown from three-day-old germinated seeds infected with the pathogen in a sodium chloride solution (experiment) and in tap water (control) under given climatic conditions. The response of the varieties to stress was determined by the relative change in the specific electrical conductivity (SEC) of aqueous leaf extracts using an edge EC conductometric setup, HANNA Instruments (Germany). The conditions for preparing the samples that ensure maximum intervarietal differences were determined experimentally: the size of the leaf tissue cut is 1.5 mm, the ratio of leaf tissue to water is 1:40, the exposure time of the samples in distilled water is at least 1.5 hours. The conducted assessment of the resistance of wheat varieties showed a greater differentiating ability of the developed method and the greatest intervarietal differences compared to the morpho-physiological method. The sample means for relative changes in the SEC of the aqueous extracts of leaf tissue of the varieties differed by 2.6–7.3 times, and the length and biomass of the shoots and roots in these varieties differed by 1.2–2.3 times. The Spearman rank correlation coefficient between the resistance indicators of varieties was $r_s = 0.88$ at $p \leq 0.01$. The results of diagnostics of the resistance of the studied wheat varieties according to growth processes and the SEC of aqueous leaf extracts coincided. Variety specificity was revealed: the Novosibirskaya 18 variety is less resistant, Omskaya 18, Sibirskaya 21 and Novosibirskaya 44 are more resistant to the studied combined stress. A conclusion was made about the possibility of using the developed method for the release of leaf electrolytes for early diagnostics (phenotyping) of wheat varieties for resistance to the combined action of *B. sorokiniana* and chloride salinity.

Keywords: wheat, variety, resistance, combined stress, electrolyte leakage, electrical conductivity

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Conflict of interest
The authors declare no conflict of interest.

INTRODUCTION

Under natural conditions, plants, including soft spring wheat, are exposed to the combined effects of environmental stress factors (biotic and abiotic) in various combinations and doses, with sequential or simultaneous occurrences. The combined effects of damaging factors, such as common root rot of cereals (caused by *B. sorokiniana*), one of the most common and damaging diseases of soft spring wheat, as well as soil salinity, including chloride, reduce productivity and degrade grain quality, limiting agricultural production worldwide [1–3]. Stressful interactions between abiotic and biotic factors will become more common with observed and projected climate change [4].

A key economic factor in increasing soft spring wheat yields is the development and cultivation of the varieties resistant to the combined effects of the stressors, including *B. sorokiniana* and chloride salinity. Diagnosing the stress tolerance of the varieties, especially early ones, is a key component in selecting the breeding material for the development of new varieties and their application in agricultural technology[1] [5–7].

In field conditions, diagnosing crop stress resistance based on yield depression is a rather labor-intensive and time-consuming process

[1]*Gurova T.A., Osipova G.M.* The problem of combined stress resistance of plants under climate change in Siberia // Siberian Herald of Agricultural Science, 2018, vol. 48, N 2, pp. 81–92.

due to the impossibility of simulating the same annual stress load[2]. Therefore, stress resistance is often diagnosed in laboratory conditions using indirect methods that measure physiological, biochemical, and biophysical parameters of plants [8–10].

Laboratory diagnostic methods for the resistance of wheat varieties to *B. sorokiniana* and salt tolerance of grain crops are known: indirect biophysical methods based on optical and electrophysical parameters[3–5], as well as direct diagnostic methods based on morphophysiological and phytopathological indicators of seedlings[6, 7]. These methods can be used to evaluate the collection and breeding material of soft spring wheat to only one type of stressor—either *B. sorokiniana* or chloride salinity. The proposed method is based on our theoretically substantiated and experimentally established increase in the permeability of plant cell membranes under the combined effects of *B. sorokiniana* and chloride salinity. The relationship between changes in seedling cell membrane permeability and the resistance of soft spring wheat varieties was identified [11, 12]. Changes in the permeability of cell membranes are assessed using the conductometric method by measuring the electrical conductivity of electrolytes released through damaged cell membranes from plant tissues into distilled water, which can be an indicator of the stress resistance of the variety [13–15].

One of the most important requirements for a diagnostic method is its discriminatory power. This is determined by the reliability of assessing the resistance of closely related objects (varieties of the same crop, plants from the same varietal population). Therefore, in the experiment, it is necessary to select stress regimes (conditions) that ensure the greatest amplitude of varietal differences. We previously identified the pathogenic structure of the pathogen—the conidial suspension of *B. sorokiniana*—as an active factor, the level of stress load, the method and timing of its application, as well as the concentration of sodium chloride and the age of seedlings as methodological approaches for maximizing intervarietal differences [11].

The purpose of the work is to experimentally establish methodological techniques for sample preparation and SEC measurements and to develop a method for early diagnosis of the resistance of soft spring wheat varieties to the combined action of *B. sorokiniana* and chloride salinity.

MATERIAL AND METHODS

The experimental work was carried out at the Siberian Physical-Technical Institute of the Siberian Federal Scientific Center of Agro-Bio-Technologies of the Russian Academy of Sciences (SibPhTI SFSCA RAS) in the laboratory of physical processes in agrophytocenoses.

In order to develop methodological techniques for conductometric measurements that ensure maximum intervarietal differences in diagnosing stress resistance in spring wheat, laboratory experiments were conducted in a roll culture (see footnote 3) against the background of a simulated combined effect of sodium chloride and *B. sorokiniana* on seedlings of zoned spring wheat varieties Novosibirskaya 44, Novosibirskaya 18, Sibirskaya 21, bred by the Sib-

[2]*Goncharova E.A., Eremin G.V., Gasanova T.A.* Express-methods of evaluation of stress resistance of cultivated crops and strategy of its diagnostic for breeding // Russian Agricultural Sciences, 2015, N 5, pp. 21–24.

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NIISR - ICG SB RAS and Omskaya 18, bred by the Omsk Scientific Center.

The conditions for sample preparation and SEC measurements of aqueous leaf extracts were experimentally determined, including determining the leaf tissue cutting size, the leaf tissue-to-water ratio, and the exposure time of plant samples in distilled water. The studies were conducted on the Omskaya 18 and Novosibirskaya 18 cultivars, which contrast in resistance. To determine the dependence of the SEC on the size of the cuttings, cuttings of 1.5 mm and 1.0 cm in size were made from the middle part of the first leaf of wheat seedlings, and the SEC of the varieties was compared at different exposure times of the cuttings in distilled water (1.5 and 24 h). To establish the dependence of the SEC on the mass ratio of distilled water to leaf tissue, dilutions were prepared in the ratios of 1:40, 1:30, 1:20, 1:10. To establish the dependence of SEC on the duration of leaf tissue exposure to water, sample preparation times of 0.5; 1.5; 3.0 and 4.5 h were used.

Experimental options:

– control (without stress load);

– seed infection: *B. sorokiniana* + chloride salinity.

The stress load levels that allow us to differentiate wheat varieties when assessing their resistance to combined stress were as follows: conidial suspension of *B. sorokiniana* 5000 conidia per grain, sodium chloride (NaCl) concentration 1.3% [11]. The stress load levels were previously established by us in specially conducted pot experiments.

The plants were grown in a roll culture on tap water (control variant) and sodium chloride (variant – infection of seeds with *B. sorokiniana* + chloride salinization) in a Biotron-7 climate chamber (developed by SibPhTI SFSCA RAS) with a day-night photoperiod of 16 and 8 h, respectively, illumination of 253.0 and 0 μmol m^{-2} s^{-1} (day-night), temperature of 22 and 18 ºC (day-night), humidity of 60%.

Aqueous extracts were then prepared from the seedling leaves. The specific electric conductivity of the leaf water extracts was measured using the edge EC conductivity meter from HANNA Instruments (Germany).

A comparative assessment of the resistance of wheat varieties was carried out using the developed method and based on growth indicators and accumulation of wet and dry biomass of seedlings[8]. The response of the variety was determined by the relative change (K) in the measured parameters of seedlings after exposure of plants to combined stress conditions:

$$K = \frac{|A_O - A_K|}{A_K} \cdot 100\%, \qquad (1)$$

where A_K – control sample parameter; A_O – prototype model parameter.

The lower the indicator (K), the more resistant the variety is in the studied group of varieties.

The analytical experiments were repeated six times, and the biological experiments were repeated three times. A representative sample consisted of 200 seedlings in each experimental variant. The experimental data were processed mathematically using standard statistical software. The error of the mean did not exceed 1.5–2.0%.

To determine the significance of differences in mean values, Student's t-test was used. Mean values were calculated for the entire experimental data set. Three series of experiments were conducted.

RESULTS AND DISCUSSION

Development of methodological techniques for conductometric measurements

Changes in electrical conductivity of aqueous extracts of sprout leaves depending on the size of the cuttings. The role of crushing leaf cut-

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

tings must be taken into account, since with an increase in the number of injured cells, not only does the transition of the contents into water increase, but there is a long-term increase in permeability associated with irritation of the cells adjacent to the damaged ones[9, 10]. We found that increasing the cut size resulted in a significant ($p \leq 0.05$) decrease in SEC in the control variants of both varieties with exposure times of 1.5 and 24 hours, as well as in the experimental variants of the Omskaya 18 variety (see Table 1). In the experimental variants of the Novosibirskaya 18 variety, SEC increased by 13.4% with a cut size of 1.0 cm and an exposure time of 24 hours. The variability of the results increased significantly with a sample exposure time of 24 h and both cutting sizes ($p \leq 0.05$). In the variant with a sample exposure time of 1.5 h, the variability of the results was significantly ($p \leq 0.05$) higher with a cutting size of 1.0 cm. Intervarietal differences with a sample exposure time of 1.5 h were 1.7 and 1.3 times in the 1.5 mm and 1.0 cm variants, respectively. Based on these results, a cutting size of 1.5 mm was determined as a methodological approach for developing a method for assessing the stress resistance of varieties.

Changes in the specific electrical conductivity of aqueous extracts of the seedling leaves depending on the ratio of the test material and water. The mass ratio of leaf cuttings to distilled water affects the results of determining the exosmosis of electrolytes from leaf tissue into distilled water. Changes in the electrolyte concentration in the solution can lead to significant deviations in the measured parameters (see footnote 10). Therefore, one approach to setting up the method is to select a plant tissue-to-water ratio that minimizes intra-varietal variation and maximizes inter-varietal differences. The results of SEC changes depending on the mass ratio of leaf tissue to water are presented in Table 2.

It was found that with a decrease in the ratio

Табл. 1. Удельная электропроводность водных вытяжек листьев мягкой яровой пшеницы в зависимости от размера нарезок листьев и времени экспозиции образцов в контроле и при совместном действии хлоридного засоления и конидиальной суспензии *B. sorokiniana,* УЭП × 10^{-3} См/м ($M \pm m$)

Table 1. Specific electrical conductivity of aqueous extracts of soft spring wheat leaves depending on the size of leaf cuttings and the exposure time of the samples in the control and under the combined action of chloride salinity and conidial suspension of *B. sorokiniana*, EC × 10^{-3} Sm/m ($M \pm m$)

Option	Exposure time of samples			
	1,5 h		24 h	
	1,5 mm	1 cm	1,5 mm	1 cm
Novosibirskaya 18				
Control	4,20 ± 0,05	2,60 ± 0,04	11,70 ± 0,53	5,80 ± 0,07
Coefficient of variation, %	2,65	5,16	6,45	10,99
Conidial suspension *B. sorokiniana* + NaCl	31,90 ± 0,60*	25,10 ± 0,50*	59,2 ± 1,8*	67,1 ± 2,1*
Coefficient of variation, %	2,23	3,44	5,74	17,71
Omskaya 18				
Control	3,7 ± 0,08	1,50 ± 0,03	7,40 ± 0,36	2,44 ± 0,17
Coefficient of variation, %	3,36	5,33	11,9	17,62
Conidial suspension *B. sorokiniana* + NaCl	18,80 ± 0,14*	11,10 ± 0,20*	35,30 ± 0,30*	19,70 ± 0,90*
Coefficient of variation, %	1,86	6,50	2,07	11,65

Note. Here and in Tables 2 and 3: *differences from the control are significant at the 5% significance level. Mean data values and their standard errors are presented.

[9] *Koval S.F., Shamanin V.P.* Plant in an experiment. Omsk: ICG SB RAS, Omsk State Agrarian University, 1999, 204 p.

[10] *Grishenkova N.N., Lukatkin A.S.* Determination of plant tissue resistance to abiotic stressors using the conductometric method // Povolzhskiy Journal of Ecology, 2005, N 1, pp. 3–11.

of leaf tissue and water, the variability of the results increases, since the resulting extracts are highly diluted electrolyte solutions, therefore the ratio of distilled water, which is added to obtain extracts, and the mass of leaf tissue should be at least 1:40. Intravarietal differences at this ratio for the Novosibirskaya 18 and Omskaya 18 varieties were 6.8 and 5.0 times, respectively.

Changes in the specific electrical conductivity of leaf cuttings depending on the duration of exposure of the leaf tissue to water. The duration of exposure of the leaf tissue in water, as we have established experimentally, should be no less than 1.5 hours (see Table 3).

During this time, electrolytes are released from the headspace and cytoplasm of plant tissue cells into distilled water. With a shorter exposure period, electrolytes are released only from the headspace of the apoplast cell [11]. The lowest variability of results was also noted after 1.5-hour exposure of samples in distilled water. Intervarietal differences established at exposure times of 1.5; 3.0; 4.5 h were approximately the same and amounted to 1.5; 1.66 and 1.67 times, respectively. However, an increase in exposure time leads to an unjustified increase in the duration of the sample evaluation procedure and a decrease in productivity.

Development of a method for early diagnosis of resistance of soft spring wheat varieties to the combined action of B. sorokiniana and chloride salinity. The developed method is implemented as follows.

Preparation of *B. sorokiniana* infectious material. To prepare the infectious material, *B. sorokiniana* isolates are cultured on solid Czapek medium for 14–15 days. By this time, the fungal colonies have produced the largest number of viable conidia[11]. Then, 0.1% water agar is added to the test tube containing the fungal culture, shaken, and a conidial suspension is prepared for infecting wheat seeds. Infecting the seeds with the conidial suspension ensures direct contact between the pathogen and the host plant, which approximates natural experimental conditions and allows the fungus to exhibit its pathogenic properties, i.e., cause disease symptoms.

Табл. 2. Удельная электропроводность водных вытяжек листьев мягкой яровой пшеницы в зависимости от массового соотношения дистиллированной воды и образца листовой ткани в контроле и при совместном действии хлоридного засоления и конидиальной суспензии *B. sorokiniana,* УЭП × 10^{-3} См/м ($M \pm m$)

Table 2. Specific electrical conductivity of aqueous extracts of soft spring wheat leaves depending on the mass ratio of distilled water and leaf tissue sample in the control and under the combined action of chloride salinity and conidial suspension of *B. sorokiniana*, EC × 10^{-3} Sm/m ($M \pm m$)

Option	Water to leaf sample mass ratio			
	1 : 40	1 : 30	1 : 20	1 : 10
Novosibirskaya 18				
Control	4,14 ± 0,05	5,42 ± 0,12	6,85 ± 0,07	7,43 ± 0,02
Coefficient of variation, %	2,65	4,24	2,68	0,68
B. sorokiniana + NaCl	28,10 ± 0,26*	35,46 ±0,54*	37,75 ± 0,51*	42,70 ± 0,78*
Coefficient of variation, %	1,23	3,07	2,49	5,52
Omskaya 18				
Control	3,73 ± 0,08	3,83 ± 0,19	4,35 ± 0,03	5,45 ± 0,09
Coefficient of variation, %	5,36	10,97	1,38	6,92
B. sorokiniana + NaCl	18,82 ± 0,14*	23,34 ± 0,38*	27,64 ± 0,21*	30,62 ±0,33*
Coefficient of variation, %	1,86	3,64	1,52	3,84

[11] *Berezina V.Yu., Gurova T.A., Bereberdin N.A., Langolf E.I.* Evaluation of biological activity of the causative agent of common root rot of cereals: method. recommendations. Novosibirsk: SB RAS, 1994, 24 p.

Табл. 3. Удельная электропроводность водных вытяжек листьев мягкой яровой пшеницы изученных сортов в зависимости от времени подготовки образцов в контроле и при совместном действии хлоридного засоления и конидиальной суспензии *B. sorokiniana,* УЭП × 10^{-3} См/м ($M \pm m$)

Table 3. Specific electrical conductivity of aqueous extracts of soft spring wheat leaves of the studied varieties depending on the time of sample preparation in the control and under the combined action of chloride salinity and conidial suspension of *B. sorokiniana*, EC × 10^{-3} Sm/m ($M \pm m$)

Option	Sample preparation time, h			
	0,5	1,5	3,0	4,5
Novosibirskaya 18				
Control	3,20 ± 0,12	4,20 ± 0,24	3,20 ± 0,06	3,40 ± 0,11
Coefficient of variation, %	8,70	4,00	4,72	7,92
B. sorokiniana + NaCl	27,30 ± 0,20*	41,20 ± 0,22*	49,80 ± 0,28*	52,00 ± 1,30*
Coefficient of variation, %	6,10	1,29	2,48	1,98
Omskaya 18				
Control	2,30 ± 0,04	3,80 ± 0,08	3,20 ± 0,10	3,30 ± 0,05
Coefficient of variation, %	4,91	3,96	2,39	3,48
B. sorokiniana + NaCl	14,70 ± 0,33*	26,80 ± 0,57*	31,60 ± 0,48*	30,90 ± 0,49*
Coefficient of variation, %	4,97	4,22	3,99	4,78

A 1.3% sodium chloride solution is prepared in distilled water using the chemical reagent NaCl (sodium chloride) of analytical grade.

Growing experimental plants. Well-formed soft spring wheat seeds with no visible signs of disease, pests, or mechanical damage are selected. To eliminate surface saprophytic and pathogenic microflora, the seeds are sterilized with ethanol for 1 minute, then rinsed three times with sterile distilled water. Prepared seeds are germinated in a thermostat in Petri dishes on filter paper moistened with water. On the third day of cultivation, the germinated seeds are spread on filter paper strips and inoculated with a conidial suspension of a mixture of moderately pathogenic *B. sorokiniana* isolates prepared on 0.1% water agar. The infectious load—the titer of pathogen conidia in one drop of suspension—is 5,000 conidia per wheat kernel, determined in a Thom-Goryaev chamber.

Next, the infected plants are grown in a roll culture, first for 1.0–1.5 days on tap water until shoots 15–20 mm long appear above the roll surface, then on a 1.3% sodium chloride solution. The shoots are grown under controlled climate conditions: a day–night photoperiod of 16 and 8 h, a temperature of 22 and 18 ºC (day–night), illumination of 253.0 and 0 μmol m–2 s–1 (day–night), and humidity of 60% until the first leaf phase for 10 days. Control plants are grown in a roll culture under the same conditions on tap water. The samples are then prepared from the control and experimental plants for measuring specific electrical conductivity.

Measurement of specific electrical conductivity. The first leaves of 10-day-old control and experimental seedling samples are washed with tap water and dried with filter paper. A medium sample is prepared for each treatment. For this, the plant material is cut into 1.5 mm-long pieces from the middle part of the leaves using a blade.

The chopped plant material is placed in a nylon bag and washed under running tap water for 15 minutes, then rinsed with distilled water. The chopped tissue is dried with filter paper, and 200 mg portions are prepared and placed in a glass beaker with 5 ml of distilled water under ambient light and temperature conditions (e.g., E = 253.0 μmol m^{-2} s^{-1} at t = 22 °C). In our case, this was done in a Biotron-7 chamber. After at least 1.5 hours, the infusion is filtered through a nylon cloth, the filtered extracts are placed in the measuring cell of a conductometer, and the

SEC of the control and experimental samples is measured.

The resistance of a variety is judged by the relative change in the SEC (K) of the aqueous extracts of leaf tissue of seedlings after exposure to stressors:

$$K = \frac{|G_O - G_K|}{G_K} \cdot 100\%, \quad (2)$$

where G_K – specific electrical conductivity of aqueous extracts of the control sample leaves; G_O – specific electrical conductivity of aqueous extracts of the experimental sample leaves.

The obtained results were processed statistically using standard statistical programs.

The results of diagnostics of resistance of the four varieties of spring wheat to combined stress according to SEC and growth indicators and biomass are presented in Tables 4 and 5.

the evidence presented suggests that, the Novosibirskaya 18 wheat variety exhibits a more pronounced suppression of growth processes and a decrease in biomass accumulation than the Omskaya 18, Sibirskaya 21, and Novosibirskaya 44 varieties. This indicates that the Novosibirskaya 18 variety is less tolerant to the combined effects of *B. sorokiniana* and chloride salinity. Moreover, the sample means for relative changes in shoot and root length and biomass for these varieties differ by a factor of 1.2–2.3, while the sample means for relative changes in the SEC of aqueous leaf tissue extracts differ by a factor of 2.6–7.3.

Thus, the proposed method has a greater differentiation capacity and establishes the greatest intervarietal differences in the early diagnosis of resistance of soft spring wheat varieties to the combined action of *B. sorokiniana* and chloride

Табл. 4. Показатели проростков сортов мягкой яровой пшеницы в контроле и при совместном действии хлоридного засоления и конидиальной суспензии *B. sorokiniana* ($M \pm m$)

Table 4. Indicators of sprouts of soft spring wheat varieties in control and under the combined action of chloride salinity and conidial suspension of *B. sorokiniana* ($M \pm m$)

Option	Length, mm		Crude biomass, mg		Dry biomass, mg		Specific electrical conductivity, cm/m
	sprouts	roots	sprouts	roots	sprouts	roots	
Novosibirskaya 44							
Control	15,3 ± 0,6	9,3 ± 0,3	90,2 ± 1,2	86,2 ± 3,9	13,3 ± 1,0	10,7 ± 0,6	4,3 ± 0,1
B. sorokiniana + NaCl	13,2 ± 0,2*	4,6 ± 0,2*	79,4 ± 1,9*	57,8 ± 2,8*	12,2 ± 0,2	7,9 ± 0,4*	16,2 ± 0,3**
Novosibirskaya 18							
Control	19,6 ± 0,4	13,8 ± 0	100,1 ± 2,9	85,0 ± 2,5	15,8 ± 0,8	9,2 ± 0,1	3,5 ± 0,1
B. sorokiniana + NaCl	12,7 ± 0,2*	6,1 ± 0,4*	63,2 ± 0,9*	51,9 ± 0,9*	10,1 ± 0,1*	7,6 ± 0,1*	25,5 ± 0,9**
Omskaya 18							
Control	16,4 ± 0,6	10,9 ± 0,2	93,0 ± 1,2	97,0 ± 3,9	13,9 ± 0,1	10,0 ± 0,3	4,4 ± 0,2
B. sorokiniana + NaCl	12,7 ± 0*	6,1 ± 0*	71,5 ± 1,2*	72,2 ± 1,9*	11,9 ± 0,1*	9,2 ± 0	11,6 ± 0,2**
Sibirskaya 21							
Control	15,6 ± 0,4	10,3 ± 0,4	74,0 ± 3,6	91,9 ± 2,8	12,3 ± 0,6	10,8 ± 0,2	4,8 ± 0
B. sorokiniana + NaCl	12,7 ± 0,2*	6,7 ± 0,2*	60,4 ± 0,7*	73,6 ± 2,5*	9,9 ± 0,2*	8,6 ± 0,3*	15,6 ± 0,4**

Note. Differences with the control are significant at the level of: *5%; **1%. The mean data values and their standard errors are presented.

Табл. 5. Диагностика устойчивости сортов мягкой яровой пшеницы по ростовым параметрам и удельной электропроводности при совместном действии хлоридного засоления и конидиальной суспензии *B. sorokiniana*

Table 5. Diagnostics of resistance of soft spring wheat varieties by growth parameters and specific electrical conductivity under the combined action of chloride salinity and conidial suspension of *B. sorokiniana*

Option	Relative changes in indicators to the control, %						
	Length		Crude biomass		Dry biomass		Specific electrical conductivity
	sprouts	roots	sprouts	roots	sprouts	roots	
Novosibirskaya 44							
B. sorokiniana + NaCl	–13,7*	–50,5*	–11,9*	–32,9*	–22,2*	–8,9	+276,7**
Novosibirskaya 18							
B. sorokiniana + NaCl	–35,2*	–55,8*	–36,9*	–38,9*	–36,1*	–17,4*	+628,5**
Omskaya 18							
B. sorokiniana + NaCl	–22,6*	–44,0*	–23,1*	–25,6*	–14,4*	–8,0	+163,6**
Sibirskaya 21							
B. sorokiniana + NaCl	–18,6*	–34,9*	–18,4*	–19,9*	–19,5*	–20,4*	+225,0**

Note. Differences with the control are significant at the level of significance: *5%; **1%.

salinity. Moreover, the results of diagnosing resistance to the combined action of *B. sorokiniana* and chloride salinity of the studied wheat varieties based on growth processes and on the SEC of aqueous extracts of leaf tissue coincided. The Spearman rank correlation coefficient between the resistance indicators based on growth processes and on the SEC of aqueous extracts of leaf tissue of seedlings is $r_s = 0.88$ at a significance level of ≤ 0.01 according to the Student's test.

CONCLUSIONS

1. A method has been developed for early diagnostics of the resistance of soft spring wheat varieties to the combined action of the pathogen of common root rot of cereals *B. sorokiniana* and chloride salinity based on the release of electrolytes from the plant tissue of seedlings.

2. The conditions for sample preparation and measurement of the SEC of aqueous leaf extracts were experimentally established, ensuring maximum intervarietal differences: the size of leaf tissue cuttings is 1.5 mm, the ratio of leaf tissue to water is 1:40, the exposure time of plant samples in distilled water is at least 1.5 hours.

3. The developed method has greater discrimination power and identifies the greatest intervarietal differences. Moreover, the sample means for relative changes in the SEC index of aqueous extracts of leaf tissue of the varieties differ by 2.6–7.3 times, and the sample means for relative changes in the length and biomass of shoots and roots for these varieties differ by 1.2–2.3 times.

4. The results of diagnostics of the studied wheat varieties' resistance to the combined action of *B. sorokiniana* and chloride salinity, based on growth processes and the SEC of aqueous leaf tissue extracts, were consistent. Variety specificity was revealed: the Novosibirskaya 18 variety was less resistant, while the Omskaya 18, Sibirskaya 21, and Novosibirskaya 44 varieties were more resistant.

5. The obtained results allow us to conclude that the proposed method can be used for early diagnostics (phenotyping) of wheat varieties for resistance to the combined action of *B. sorokiniana* and chloride salinity during variety testing.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

(✉)**Гурова Тамара Алексеевна,** ведущий научный сотрудник, кандидат сельскохозяйственных наук; SPIN-код 5471-9138; **адрес для переписки:** Россия, 630501, Новосибирская область, р.п. Краснообск; а/я 463; e-mail: guro-tamara@yandex.ru

Чесноченко Наталья Евгеньевна, научный сотрудник; SPIN-код 8670-7247

AUTHOR INFORMATION

(✉)**Tamara A. Gurova,** Lead Researcher, Candidate of Science in Agriculture; SPIN-code 5471-9138; **address:** PO Box 463, Krasnoobsk, Novosibirsk Region, 630501, Russia; e-mail: guro-tamara@yandex.ru

Natalia E. Chesnochenko, Researcher; SPIN-code 8670-7247

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Технология производства мини-клубней картофеля в условиях защищенного грунта

(✉)**Попова Н.М., Чураков А.А.**
Красноярский государственный аграрный университет
Красноярск, Россия
(✉)e-mail: NMPopova@yandex.ru

Производство посадочного материала картофеля на основе оздоровленного исходного материала широко используют в картофелеводстве. Дальнейшее совершенствование приемов выращивания мини-клубней позволит увеличить урожайность в товарном секторе. Разработаны элементы сортовой технологии картофеля на этапе производства мини-клубней в условиях защищенного грунта. Для оценки отзывчивости распространенных в России сортов картофеля на элементы агротехники при выращивании мини-клубней проведен опыт в летней теплице в черте города Красноярска. Объектами исследования выбраны очень ранний сорт Метеор, ранние (раннеспелые) Фелокс, Розара, Гулливер, Ред Скарлетт, Вега. Установлена сортовая специфика отзывчивости сортов картофеля на приемы выращивания в закрытом грунте: объем горшков, перевалку. Использование двухлитровых емкостей обеспечивает максимальную выручку и выход мини-клубней у сортов Розара, Ред Скарлетт, Фелокс, литровых – Метеор, Гулливер. Минимальная эффективность получена при использовании пятилитровых горшков. Сила влияния фактора «размер горшка» была максимальной, составив 47,2% по числу клубней, и 63% – по массе клубня. На долю генотипа пришлось соответственно 15 и 17% изменчивости признаков. Сорт Розара положительно реагировал на перевалку из рассадной емкости 0,2 л в горшок 2 л. Наибольшая продуктивность сорта Вега получена при посадке в горшки 1 л с последующей декапитацией. Среднее число клубней в горшке составило 6,2 шт., масса клубня 8 г. Полученные данные возможно использовать в оригинальном семеноводстве изученных сортов. С учетом степени влияния генотипа на других образцах потребуется уточнение результатов.

Ключевые слова: теплица, сорт, размер посадочного горшка, безвирусное семеноводство, *Solanum tuberosum* L.

Technology of production of potato mini tubers in protected soil conditions

(✉)**Popova N.M., Churakov A.A.**
Krasnoyarsk State Agrarian University
Krasnoyarsk, Russia
(✉)e-mail: NMPopova@yandex.ru

The production of potato planting material based on improved source material is widely used in potato growing. Further improvement of mini tuber growing techniques will increase crop yields in the commercial sector. Elements of potato varietal technology have been developed at the stage of mini tubers production in protected soil conditions. An experiment was conducted in a summer greenhouse within the city of Krasnoyarsk to assess the responsiveness of potato varieties common in Russia to the elements of agricultural technology when growing mini tubers. The objects of the study were the very early variety Meteor, early (early ripening) Felox, Rosara, Gulliver, Red Scarlett, Vega. Varietal specificity of potato varieties' responsiveness to indoor cultivation techniques has been established: pot volume, transshipment. The use of two-liter containers ensures maximum revenue and output of mini tubers for the varieties Rosara, Red Scarlett, Felox, and liter containers – Meteor, Gulliver. Minimum efficiency was achieved using five-liter pots. The influence of the “pot size” factor was maximum, amounting to 47.2% for the number of tubers and 63% for the weight of the tuber. The genotype accounted for 15 and 17% of the variability of traits, respectively. The Rosara variety responded positively to being transferred from a 0.2 l seedling container to a 2 l pot. The highest productivity of the Vega variety was obtained when planted in 1 l pots with subsequent decapitation. The average number of tubers in a pot was 6.2 pcs., tuber weight was 8 g. The obtained data can be used in the original seed production of the studied varieties. Taking into account the degree of influence of the genotype on other samples, it will be necessary to clarify the results.

Keywords: greenhouse, variety, planting pot size, virus-free seed production, *Solanum tuberosum* L.

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INTRODUCTION

The importance of potatoes as a food, feed, and industrial crop is enormous. Among the general requirements for all potato breeding programs is the development of high-yielding varieties. One of the main reasons for the decline in potato yields in production is the use of seed material infected with viruses, viroids, and bacteria.

Therefore, to increase crop yields, the planting material that has been treated to meet regulatory requirements is currently being used. This is achieved through modern clonal propagation techniques of microplants obtained from regenerated apical meristems. The primary objective of this method is to increase the amount of source material, which can be achieved by achieving the maximum propagation rate, while preventing viral re-infection. Following the tissue culture-based recovery stage and subsequent selection of the best infection-free lines, potato seed production begins with the cultivation of mini-tubers in protected soil conditions. Hydroponic systems and other accelerated propagation methods are also used for this purpose.

In the field, healthy parent plants and clones are selected primarily by visual assessment, followed by laboratory testing for latent infections using polymerase chain reaction (PCR). When selecting a mini-tuber production technology for specific conditions, a number of factors must be considered. These include the analysis of production indicators, a comparison of investment costs, production costs, and profitability.

Active vegetative growth and intensive tuber formation are the primary goals when growing in greenhouses. To increase tuber production, optimal conditions for plant growth and development must be created: controlling temperature, humidity, and daylight hours, as well as ensuring adequate nutrition. Additionally, special preparations can be used to stimulate tuber formation and limit plant growth. In recent years, numerous attempts have been made to improve cultivation technologies. The main goal of these improvements is to obtain as many minitubers as possible from a single plant per unit of the greenhouse area. Various crop production methods are used to achieve this goal, but not all hypotheses proposed by scientific research prove effective in industrial production[1] [1–17].

The purpose of the study is to develop the elements of potato varietal technology at the stage of mini-tubers production in protected soil conditions.

MATERIAL AND METHODS

To validate the mini-tuber cultivation technology elements, experiments were conducted in a plastic greenhouse in 2021 and 2022. Test-tube plants and mini-tubers were grown at the breeding and seed production center at the Krasnoyarsk Agrarian University. The study subjects

[1]*Gavrilova A.Yu., Pavlovskaya N.E., Gagarina I.N., et al.* Technology of production of improved source material in elite potato seed production: recommendations for production. Oryol: Oryol State Agrarian University named after N.V. Parakhin, 2022, 60 p.

were the very early Meteor variety, and the early (early maturing) varieties Felox, Rosara, Gulliver, Red Scarlett, and Vega. The first experiment involved planting the Rosara, Meteor, Red Scarlett, Felox, and Gulliver varieties in 1-, 2-, and 5-liter pots.

In the second experiment, the Rosara and Meteor varieties were planted in 0.2-liter seedling cups, followed by transplanting the adapted and grown plants (12–15 cm tall) into 1-, 2-, and 5-liter pots. A comparison was made with planting without the transplanting stage.

To determine the effect of decapitation on the productivity of the potted Vega potato cultivar, a factorial experiment was conducted. Mechanical decapitation and a plant growth regulator based on chlormequat chloride were studied as experimental treatments. Plants without linear growth inhibition served as a comparison treatment. Plants were planted in 1- and 2-liter pots. This treatment was hypothesized to help remove apical dominance, which, in turn, would promote additional branching and stolon formation. Twenty microplants were planted in five replicates for each treatment.

An optimal microclimate in the greenhouse was maintained using ventilation and a shading net. Average air humidity was 68% in June, 71–73% in July, and 76–79% in August. Air temperatures were 20.8°C in June, 25.8°C in July, and 21.7°C in August.

The experimental data were processed using the STATISTICA 13.3 program[2].

RESULTS AND DISCUSSION

Potato productivity was assessed based on the pot volume for five genotypes (see Fig. 1). Using discriminant analysis, it was found that the variety has a significant effect on the number of tubers formed per pot. The partial lambda for this trait is 0.84, with the coefficient significance at $p = 0.04$. Pot volume is not a significant factor influencing the number, total weight, or average weight of a single tuber.

The maximum number of the tubers formed in 5-liter containers, regardless of the variety. The Rosara and Gulliver varieties showed the greatest responsiveness. For the three studied samples, growing in 2-liter containers showed a slight advantage. For the Gulliver variety, the number of tubers tended to decrease as container size increased from one to two liters. The number of formed tubers decreased by 22 percentage points, to 3.2.

The pot size factor had the greatest influence on the number of tubers – 47.2%. The influence of the genotype and the interaction of the factors amounted to 22 and 15%, respectively.

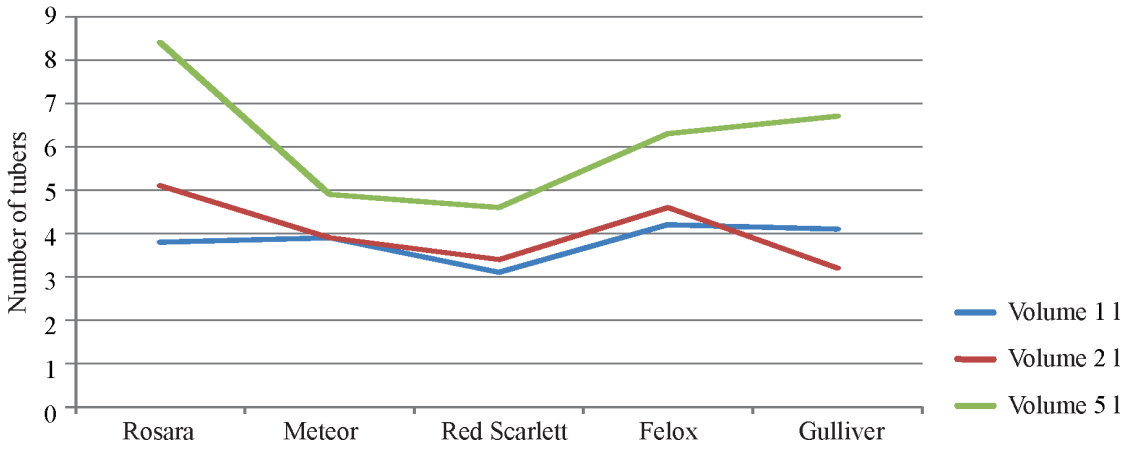

Рис. 1. Среднее число клубней у сортов картофеля в зависимости от объема горшка
Fig. 1. Average number of tubers in potato varieties depending on the volume of the pot

[2]*Khizhnyak S.V., Puchkova E.P.* Mathematical methods in agroecology and biology. Krasnoyarsk: Publishing house of KrasSAU, 2019, 240 p.

The size of the tubers also depended on the volume of the cultivation vessels (see Fig. 2).

Large tubers weighing 15–25 g were obtained when growing samples in 5-liter pots. The choice of 1- or 2-liter pots had a minor effect on the tuber weight. This factor had the greatest influence, reaching 63%. The variety accounted for 17.1% of the effect, and the interaction of the factors accounted for 9.2%.

All experimental variants yielded minitubers larger than 9 mm, which meets standard requirements. In this case, the yield of tubers per unit area of greenhouse soil will be the determining factor in choosing the planting option. Fifty-two 1- and 2-liter pots, and 19 5-liter pots, were placed per square meter in the greenhouse. The Red Scarlett variety yielded the fewest tubers (87) using 5-liter pots. The varieties are ranked as follows in the order of increasing tuber yield: Meteor (93), Felox (120), Gulliver (127), and Rosara (160).

The Meteor, Red Scarlett, and Felox varieties showed an insignificant advantage in tuber harvesting from two-liter containers compared to the liter ones. A significant difference ($p = 0.01$) was obtained for the Rosara variety, the number of the tubers was 265, which is 67 more than in the variant with a liter pot. The cost of peat substrate and minitubers is also a factor determining the economic performance of production. With a selling price of 30 rubles per a minituber and a cost of 1 liter of substrate of 7.06 rubles, the revenue per unit of greenhouse area was highest when planting the Rosara (7,273 rubles), Red Scarlett (4,632 rubles), and Felox (6,441 rubles) varieties in two-liter containers. Higher revenue for the Gulliver and Meteor varieties (5,998 and 5,657 rubles, respectively) was ensured by planting in liter pots.

An experiment to assess the effect of transshipment on the minituber yield was conducted on the Rosara and Meteor varieties. A significant ($p = 0.00$) negative effect for the "average tuber weight" trait was obtained for the Rosara variety. Transshipment resulted in a decrease in tuber weight by an average of 11 g across the variants (see Fig. 3). The number of the tubers obtained from one clone, on the other hand, increased by 1.3 pieces, or 24 percentage points (see Fig. 4). The average number of standard tubers per variety in one pot was 4.8. Taking into account the planting density, the advantage was in favor of planting in 2-liter pots when transshipping micro-plants.

The Meteor variety showed weak response to the studied technology element ($p = 0.22$) in terms of the "average number of tubers per pot" parameter. Transshipment resulted in a significant ($p = 0.02$) reduction in the tuber weight to 12 g, compared to an average value of 20 g in the control.

Pot size had the greatest impact on the tuber yield (49% of the total). The impact of the "variety – pot size" and "variety – transshipment"

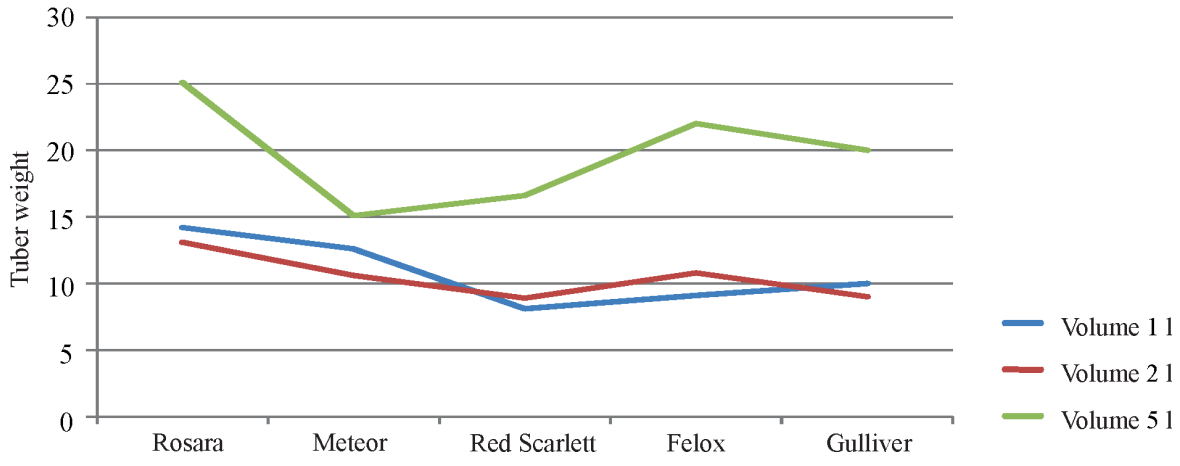

Рис. 2. Средняя масса клубня картофеля по вариантам эксперимента, г
Fig. 2. Average weight of potato tuber according to experimental variants, g

factors was 12% each. The least significant role was played by transshipment (7%), variety (3%) and the interaction of the three factors (2.5%). Tuber weight was affected by pot volume (the strength of the factor influence was 45%), transshipment (23%), variety (13%), the interaction of pot volume and transshipment (7%) and variety – pot volume – transshipment (3%). For the Rosara variety, the average tuber yield was 177, 234, 125 pcs/m^2, respectively, for the planting options of 1, 2 and 5 liters. For the Meteor variety, 156, 177, 86 pcs/m^2 were obtained, respectively.

Therefore, it is advisable to grow Meteor without transshipment in one- or two-liter pots, and the Rosara variety – with transshipment, choosing 2-liter pots for cultivation.

Decapitation is a well-known technique for increasing yield or disease resistance in field potato crops. When producing minitubers, this

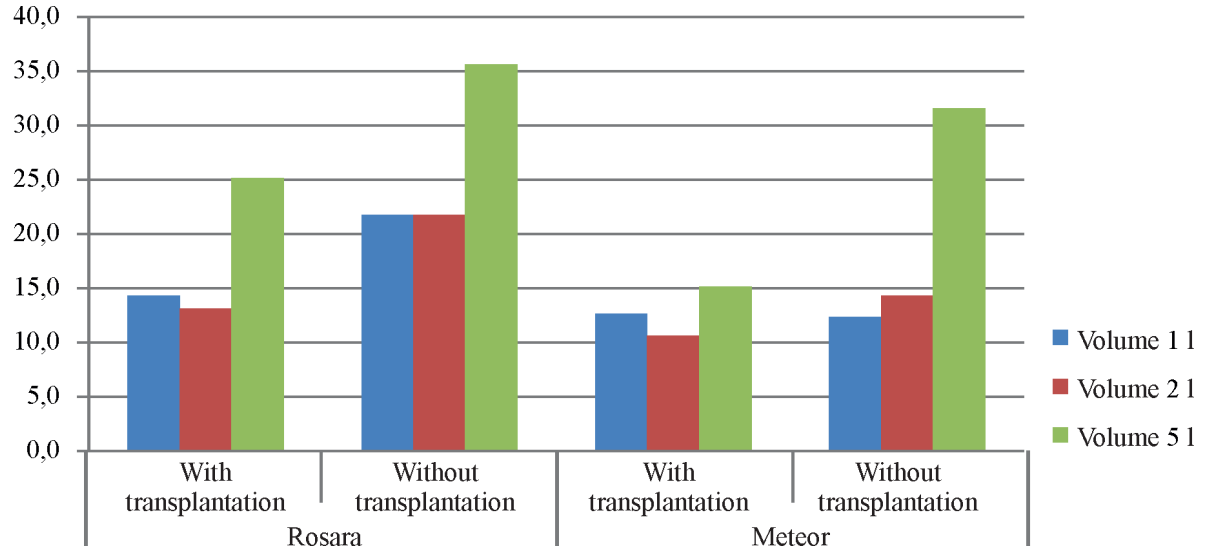

Рис. 3. Средняя масса клубня у сортов картофеля по вариантам посадки, г
Fig. 3. Average tuber weight for potato varieties by planting options, g

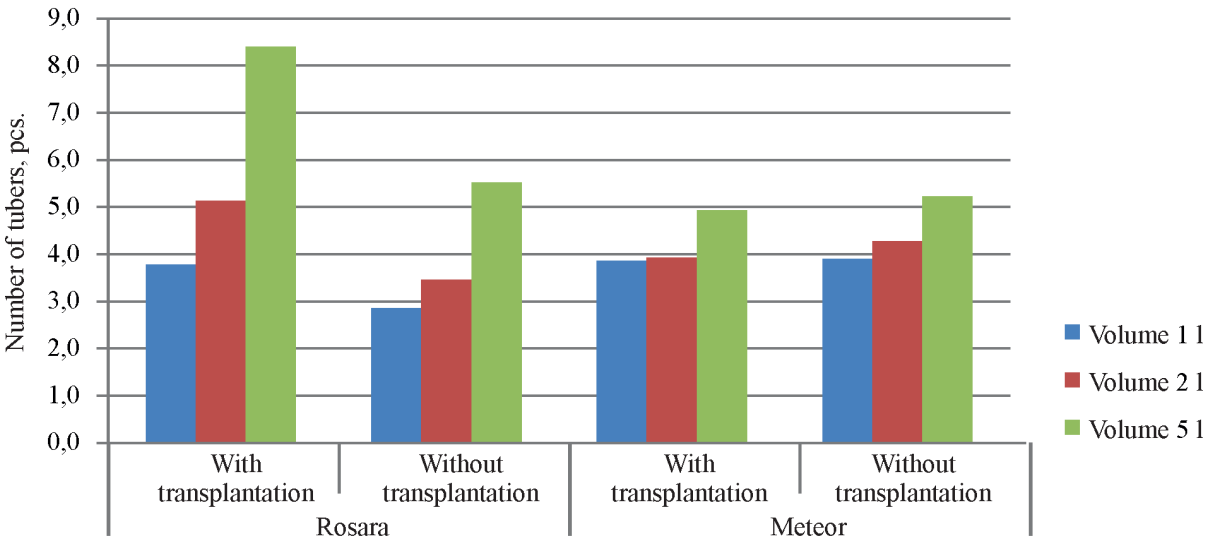

Рис. 4. Среднее число клубней у сортов картофеля по вариантам посадки
Fig. 4. Average number of tubers in potato varieties by planting options

method can increase stem count and, consequently, plant productivity[3, 4]. According to our data, top removal had a positive effect on tuber count. On average, 5.7 tubers were produced per treatment. In the control, the average tuber count was 3.96 tubers per plant.

Along with mechanical decapitation, a plant growth regulator containing the active ingredient chlormequat chloride was evaluated for yield and minituber quality. In the chemical decapitation trial, plants planted in two-liter pots showed an advantage (see Fig. 5).

When planted in liter containers, the number of the tubers decreased by 0.6 pieces and amounted to 3.7 pieces. In terms of tuber number, mechanical decapitation significantly (p = 0.00) outperformed the chemical preparation. In terms of the tuber weight, the variant with spraying with a growth regulator showed a clear advantage (p = 0.00). In addition, a clear trend of increasing average tuber weight with a decrease in their number per plant was evident. A strong inverse correlation (r = –0.81 ± 0.43) was established between the number of the tubers in one pot and the tuber weight.

Thus, mechanical decapitation of the Vega variety is a significant tool for increasing productivity and yield in the production of minitubers.

CONCLUSIONS

1. Varietal specificity of potato varieties' response to indoor cultivation techniques, such as pot volume and transshipment, has been established.

2. Increasing the size of the planting pot promotes an increase in the number and size of minitubers. However, due to the rational use of greenhouse space, the maximum revenue was obtained when cultivating the varieties Rosara (7,273 rubles), Red Scarlett (4,632 rubles), Felox (6,441 rubles) in two-liter containers, and Gulliver (5,998 rubles), and Meteor (5,657 rubles) in liter containers.

3. The Rosara variety proved to be responsive to transplanting: microplants should be planted in 0.2-liter containers, and the grown seedlings should be transplanted into two-liter pots. In this case, the yield of the tubers was 234 per square meter, with an increase of 57 and 109 tubers for the one-liter and five-liter pots, respectively.

4. It is necessary to plant the Vega variety in liter-sized pots in order to maximize the yield of minitubers. Decapitation at the beginning of active growth helps to obtain the maximum number of tubers per plant—6.2.

5. The data obtained will contribute to im-

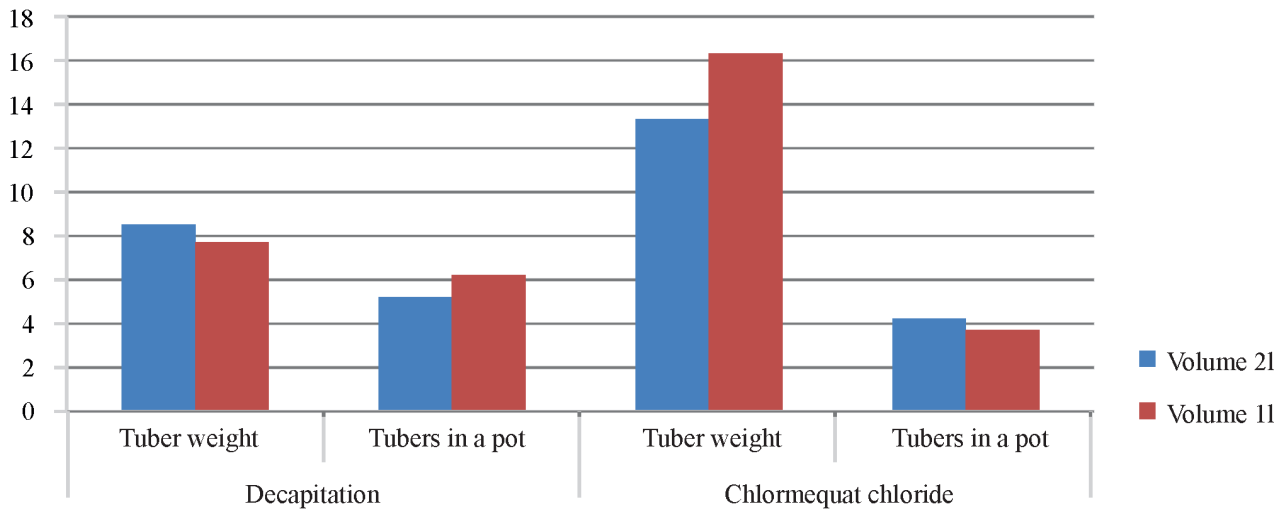

Рис. 5. Элементы продуктивности сорта Вега в связи с вариантами декапитации

Fig. 5. Productivity elements of the Vega variety in relation to decapitation options

[3]*Gasparyan I.N., Dyikanova M.E.* How to increase the yield of early potatoes // Potatoes and vegetables, 2018, N 2, pp. 29–31.

[4]*Evstratova L.P., Nikolaeva E.V.* Decapitation of potato plants in Karelia // Proceedings of Petrozavodsk state university, 2016, N 4 (157), pp. 38–41.

proving the efficiency of using greenhouses for minituber production. However, the different responses of the varieties to cultivation methods will require further research using other potato varieties.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

(✉)**Попова Наталья Михайловна,** научный сотрудник; SPIN-код 1592-6823; **адрес для переписки**: Россия, 660049, Красноярск, пр. Мира, 90; e-mail: NMPopova@yandex.ru

Чураков Андрей Андреевич, директор, кандидат сельскохозяйственных наук; SPIN-код 5184-9372

AUTHOR INFORMATION

(✉)**Natalia M. Popova,** Researcher; SPIN-code 1592-6823; **address:** 90, prospect Mira, Krasnoyarsk, 660049, Russia; e-mail: NMPopova@yandex.ru

Andrei A. Churakov, Director, Candidate of Science in Agriculture; SPIN-code 5184-9372

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Исследование жизнеспособности пыльцы малины ремонтантной в условиях Южного Прибайкалья

Киселева Е.Н., (✉)Раченко М.А., Раченко А.М.
Сибирский институт физиологии и биохимии растений Сибирского отделения Российской академии наук
Иркутск, Россия
(✉)e-mail: bigmks73@rambler.ru

Известно, что жизнеспособность пыльцы зависит не только от погодных условий, агротехники, но и от генотипа растения. В ходе исследования изучена взаимосвязь между жизнеспособностью пыльцы, отобранной в разные сроки, и сортом малины ремонтантной. Приведены результаты фенологических наблюдений за сортами и формами малины от начала вегетации до цветения, а также рассмотрена продолжительность данного периода в условиях Южного Прибайкалья. Принималась во внимание необходимость накопления сумм активных температур для прохождения фенологических фаз в условиях региона исследования. В итоге были выделены три группы сортов – ранне-, средне- и позднецветущие. Определена жизнеспособность пыльцы разных сортов и форм, выделены генотипы, характеризующиеся стабильно хорошей жизнеспособностью пыльцы (более 50%). Определен период, в котором жизнеспособность пыльцы практически всех генотипов оценена как хорошая. Исключение составили две формы, у которых за период исследования жизнеспособность была менее 50%. Статистический анализ показал, что наименьшее влияние на жизнеспособность пыльцы оказывает влажность воздуха (57%), наибольшее – температура воздуха (более 90%). Отмечено, что понижение ночных температур до отрицательных критически сказывается на жизнеспособности пыльцы. Так как жизнеспособность пыльцы у разных генотипов не была одинаковой, отбор родительских растений для опыления важно проводить и по данному признаку. Проведенное исследование может помочь в селекции малины ремонтантной: учитывая указанный признак при отборе родительских растений, можно повысить эффективность опыления и увеличить выход гибридных семян.

Ключевые слова: малина ремонтантная, пыльца, опыление, генотип, раннецветущие сорта, фенология

Study of remontant raspberry pollen viability in the conditions of the Southern Baikal region

Kiseleva E.N., (✉)Rachenko M.A., Rachenko A.M.
Siberian Institute of Plant Physiology and Biochemistry of the Siberian Branch of the Russian Academy of Sciences
Irkutsk, Russia
(✉)e-mail: bigmks73@rambler.ru

It is known that the viability of pollen depends not only on weather conditions and agricultural technology, but also on the genotype of the plant. The study examined the relationship between the viability of pollen collected at different times and the variety of remontant raspberry. The results of phenological observations of raspberry varieties and forms during the period from the beginning of vegetation to flowering are presented, and the duration of this period in the conditions of the Southern Baikal region is also considered. The need to accumulate sums of active temperatures for the passage of phenological phases in the conditions of the region under study was taken into account. As a result, three groups of varieties were identified: early, mid-season and late flowering. The viability of pollen of different varieties and forms was determined, and genotypes characterized by consistently good pollen viability (more than 50%) were identified. The period in which the viability of pollen of almost all genotypes was assessed as good was determined. The exceptions were two forms, for which viability was less than 50% during the study period. Statistical analysis showed that air humidity (57%) has the least influence on pollen viability, while air temperature has the greatest influence (more than 90%). It is noted that a decrease in night temperatures to negative critically affects the viability

of pollen. Since the viability of pollen in different genotypes was not the same, it is important to select parent plants for pollination based on this feature. Research can help in breeding remontant raspberries. It is possible to increase the efficiency of pollination and increase the yield of hybrid seeds taking this feature into account when selecting parent plants.

Keywords: remontant raspberry, pollen, pollination, genotype, early-flowering varieties, phenology

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INTRODUCTION

Everbearing raspberry varieties can bear fruit on both annual and biennial canes. These varieties can be grown in an annual cycle (harvesting from primocanes) or a combined cycle (harvesting from primocanes and floricanes). It is also important to note the duration of flowering and fruiting for these varieties. In the Southern Baikal region, everbearing raspberries begin bearing fruit in the second half of summer and continue until late autumn [1, 2].

The primary goal of breeding new raspberry varieties is to obtain the genotypes with high, high-quality productivity, adapted to specific environmental conditions [2–4]. One of the key productivity indicators is the fruit size. The development of large-fruited genotypes is a priority area of breeding [5]. When selecting parent plants, it is important to consider the characteristics of the reproductive organs, which are of great importance in the evolutionary process of plants[1] [6, 7]. The reproductive organs of plants are influenced by their own genetic factors and the external natural environment.

Weather conditions have a significant impact not only on plant growth and development during the growing season, but also on the morphological characteristics of its vegetative and generative organs [8–10]. Thus, the petal size, color, aroma, and nectar quantity can vary depending on the environmental conditions and ecology [7, 11, 12]. The influence of high or critically low air temperatures on the process of pollen grain formation can also be noted[2] [7, 11, 13]. In field conditions, temperature fluctuations of even 5°C have a significant impact on the rate of pollen tube growth [7].

[1]*Mochalova O.V.* History and promising directions of using polyploidy in breeding Siberian varieties of raspberries and blackberries // Siberian gardening of the XXI century – a vector of development: collection of materials of the All-Russian scientific and practical conference, dedicated to the 300th anniversary of the Russian Academy of Sciences and the 90th anniversary of the establishment of the M.A. Lisavenko Siberian Research Institute of Horticulture. Barnaul, 2023, pp. 66–71.

[2]*Vasekha V.V., Kozlovskaya Z.A., Vasilyeva M.N., Taranov A.A., Yakimovich O.A., Rudnitskaya N.L., Lugovtsova N.V.* Recommendations for the selection of pollinator varieties for the modern assortment of fruit crops and hazelnuts // Plodovodstvo, 2016, vol. 28, N 1, pp. 246–255.

The literature contains information on the influence of the environment on pollen maturation and viability. Extreme temperature and water conditions can cause pollen degeneration, leading to its sterilization or reduced viability[3]. Studying the quality of reproductive organs, including pollen viability, under different weather conditions will help identify the most adaptive varieties for pollination. Plant reproductive organs can be considered a link between plant adaptation and the mechanism of speciation, which is important for genetic diversity in breeding selection [6, 7, 14].

Pollen viability can become an additional criterion when selecting valuable genotypes in the breeding process. In berry crops, this indicator correlates with plant productivity[4].

The purpose of the study is to identify parental genotypes of remontant raspberries with a good level of pollen viability for effective pollination.

The research objectives are:

1) conducting phenological observations of the timing of the budding and flowering phases;

2) pollen collection and determination of its viability;

3) analysis of the data on pollen viability and the relationship of this indicator with the genotype and weather conditions of the year;

4) selection of the promising parental forms from the genotypes with high pollen viability for further use in the breeding process.

MATERIAL AND METHODS

The research was conducted in 2021–2023 at the collection site of the Bioresource Center of the Siberian Institute of Plant Physiology and Biochemistry, Siberian Branch of the Russian Academy of Sciences (SIPPB SB RAS). The objects of the study were raspberry varieties and forms with a remontant fruiting type.

The site is located on a southern slope at an angle of 8–10°. The soil is gray forest soil, with a medium loamy texture.

The 2021 growing season was characterized by low average air temperatures (13.6 °C) and sufficient precipitation (478.7 mm, HTC = 2.39). In 2022, the average air temperature during the growing season was 14.8 °C, with 263 mm of precipitation, and HTC = 1.08. In 2023, the highest average air temperature (15.1 °C) was recorded during the growing season, with 417 mm of precipitation, and HTC = 1.76 (see Table 1).

The first frosts were recorded in September. In 2021, the temperature dropped to –0.5°C on September 2, and to –2.4°C on the night of September 22–23. In 2022, the first temperature drop below zero was observed on September 3 (–1.9°C), and on the night of September 23, a drop to –1.2°C was noted. In 2023, the first temperature drop was recorded on September 10 (–1.8°C), and on the night of September 23, the temperature reached –1.9°C. The sum of active temperatures (SAT) above 0°C was 2395°C in 2023, 1843°C in 2022, and 2076°C in 2021. When cultivating everbearing raspberries, to achieve 80% of their potential productivity, the SAT should be 1700°C or higher. Growing season conditions during the study years met the crop's requirements. In terms of moisture, the 2021 and 2023 growing seasons and the first half of the 2022 growing season were characterized by high HTC.

Pollen was collected from buds close to blooming and as loose as possible (at least five flowers) at midday (12:00). On sunny days, anthers open in half-open buds; on cloudy days, anthers open when the petals are fully reflexed [15].

Pollen grains were sown on a drop of nutrient medium on the day of collection, in triplicate, and placed in a thermostat. The thermostat was maintained at 24°C. Pollen germination was achieved using a nutrient medium based on a 0.5% agar-agar solution containing 10% sucrose [13, 16]. Pollen tube growth was monitored daily. Pollen grains began to germinate on

[3]*Marakaeva T.V., Kazydub N.G.* Determination of the viability of pollen of common beans (*Phaseolus vulgaris*) at different times of the day in the conditions of the southern forest-steppe of Western Siberia // Advances in current natural sciences, 2016, N 6, pp. 96–99.

[4]*Gavrilova O.A., Tikhonova O.A.* Pollen quality of species and distant hybrids in the family Grossulariaceae DC // Advances in current natural sciences, 2016, N 12-1, pp. 68–73.

Табл. 1. Погодные условия вегетационного периода в годы проведения исследования
Table 1. Weather conditions of the growing season in the years of the study

Year	May	June	July	August	September
Amount of precipitation, mm					
2021	87	100	131	100	60
2022	8	82	110	38	25
2023	46	85	84	114	88
The sum of active temperatures above zero, °C					
2021	266,1	471,4	583,6	492,2	263,5
2022	412,4	521,4	548,7	473,7	299,5
2023	280,9	687,4	615,0	567,7	344,2
Hydrothermal coefficient					
2021	3,27	2,12	2,24	2,03	2,28
2022	0,19	1,57	2,00	0,80	0,83
2023	1,64	1,24	1,37	2,01	2,56
Average monthly air temperature, °C					
2021	8,60	15,40	18,80	16,60	8,80
2022	12,60	17,40	18,70	15,30	10,00
2023	9,10	16,80	19,80	18,30	11,50

the second day. Pollen viability was assessed on the third day by counting all germinating pollen grains. Grains were considered germinated if the pollen tube length was equal to or greater than the pollen diameter. The number of germinated pollen grains was counted under a microscope at 60x magnification in triplicate. The pollen viability percentage was determined based on the number of germinated pollen grains. The pollen viability level of at least 50% is considered good (see footnote 2).

Statistical processing of the results was carried out according to the standard method[5] using Microsoft Office Excel.

RESULTS AND DISCUSSION

In raspberry plants with remontant fruiting type, cultivated according to the annual cycle, the following phenological phases of development are distinguished: relative dormancy, beginning of vegetation, active growth, beginning of budding, flowering, fruiting (ovary), fruit ripening, leaf fall, relative dormancy [17]. The beginning of vegetation for remontant raspberry plants in annual culture is considered to be the beginning of growth of replacement shoots.

Phenological observations are the first and most important step in selecting parental forms for breeding. During selection, it is important to consider the flowering phases and their duration in order to establish a pollination schedule for the plants.

Due to climate conditions, the onset of vegetation for all varieties varies by year and occurs between the third ten-day period of April and the beginning of the second ten-day period of May. For everbearing raspberry shoots to begin growing in this region, the SAT above 0°C must reach approximately 190°C. Late-ripening varieties begin vegetation earlier than early-ripening ones. For budding to occur, different genotypes require SAT above 5°C to range from 610 to 1005°C. They enter the flowering phase when this indicator reaches 750–1260°C[6].

[5]*Ryazanova L.G., Provorchenko A.V., Gorbunov I.V.* Fundamentals of statistical analysis of research results in horticulture: teaching manual. Krasnodar, 2013, 62 p.

[6]*Kiseleva E.N., Rachenko M.A., Kamyshova L.E., Rachenko A.M.* Evaluation of the varieties and forms of remontant raspberries for early maturity in the conditions of the Cis-Baikal region // Proceedings of the II International Scientific and Practical Conference "Genomics and Modern Biotechnologies in Plant Reproduction, Breeding and Conservation" (Yalta, October 13–15, 2021). Simferopol, 2021, pp. 187–188.

The onset of flowering is considered to be the day when more than 10% of flowers have opened. Depending on the region, the flowering phase begins in early July and continues until frost. The duration of the phenological phases correlates with both genotype and weather conditions, ranging from 5 to 60 days. The longest phenological phase is the growing season (up to 60 days). The budding phase, depending on the plant genotype in the study region, can last up to 22 days.

The collection plot of the Bioresource Center of the Siberian Institute of Plant Physiology and Biochemistry of the Siberian Branch of the Russian Academy of Sciences (SIPPB) grows everbearing raspberry varieties with varying ripening times. Based on their flowering time, the studied varieties can be divided into early-, mid-, and late-blooming varieties. This is important to consider when selecting a pollinator plant, ensuring that the hybridization process involves not only plants with similar flowering times, but also those as close to them as possible. Despite the length of the flowering phase, earlier-blooming varieties may not have sufficiently developed buds to pollinate late-blooming varieties.

As a result of observations, varieties and forms that begin flowering in June were classified as early flowering. These include the Brilliantovaya, Penguin and Eurasia varieties, as well as the forms 37-15-4 and 1-220-1 (see Table 2). Mid-flowering varieties include those that begin flowering in the first half of July: the Gerakl, Zhar-ptitsa, Rubinovoe Ozherelie, Oranzhevoe Chudo varieties, and the form 32-151-1. The Bryanskoe Divo variety, whose earliest flowering over the years of observation was noted after July 20, was classified as late flowering.

In the July 12 selections, pollen viability of over 50% was observed for the Oranzhevoe Chudo variety over three years (65%, 66%, and 60%). For the Brilliantovaya variety, a high percentage of viable pollen was observed in 2021 and 2022 (July was warm and humid, with a hydrothermal coefficient (HTC) > 2). The same pattern was observed for form 1-220-1 (see Fig. 1, *а*). A one-way analysis of variance revealed a significant effect of pollen collection date on pollen viability. The calculated Fisher's exact test value exceeded the tabulated value ($F_ф$ = 7,34, $F_т$ = 2,35, $F_ф \geq F_т$). The least significant difference at the 5% significance level (LSD_{05}) is 14.9.

In the August 1 selections, high pollen viability was observed in almost all varieties (see Fig. 1, *б*). The exception was 2023, when viability exceeding 50% was recorded only for the Bryanskoe Divo (80%) and Gerakl (54%)

Табл. 2. Оценка генотипов малины ремонтантной по срокам цветения (2019–2023 гг.)
Table 2. Assessment of the remontant raspberry genotypes by flowering time (2019–2023)

Genotype (variety, form)	The minimum value of the sum of active temperatures above zero., ºC	Date of the flowering start	Characteristics of the flowering time
Bryanskoe Divo	900	July 20	Late flowering
32-151-1	600	July 6	
Hercules	680	July 12	
Zhar-ptitsa	810	July 14	Mid-flowering
Oranzhevoe Chudo	680	July 14	
Rubinovoe Ozherelie	620	July 12	
Brilliantovaya	680	June 12	
37-15-4	610	June 25	
1-220-1	660	June 12	Early flowering
Penguin	680	June 25	
Eurasia	660	June 12	

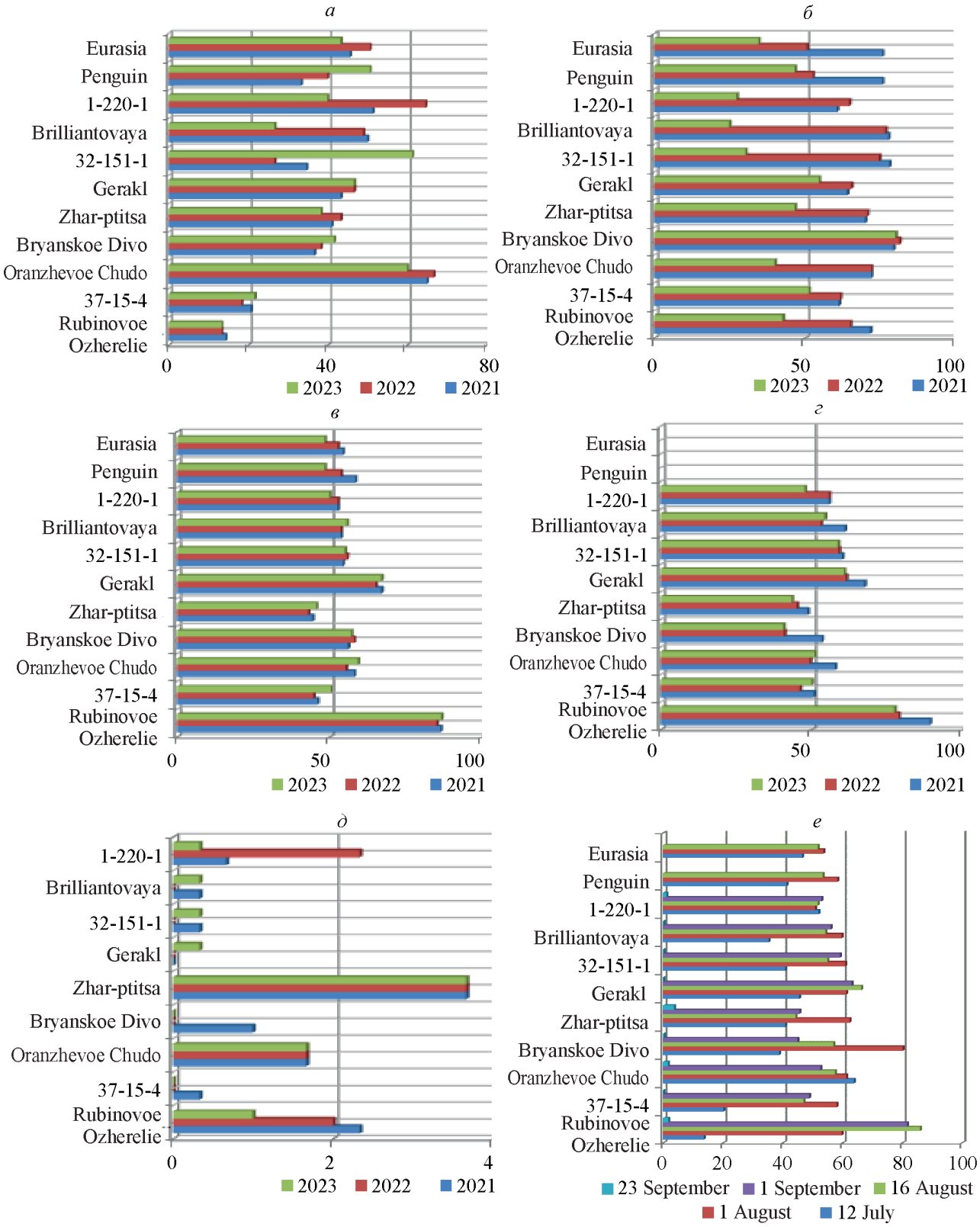

Рис. 1. Жизнеспособность пыльцы сортов и форм малины ремонтантной, отобранной в разные сроки, %:
а – 12 июля; *б* – 1 августа; *в* – 16 августа; *г* – 1 сентября; *д* – 23 сентября; *е* – среднее за весь период
Fig. 1. Viability of pollen of the varieties and forms of remontant raspberries selected at different times, %:
а – July 12; *б* – August 1; *в* – August 16; *г* – September 1; *д* – September 23; *е* – average for the entire period

varieties, as well as for the 37-15-4 form (51%) (the July HTC was 1.3). The results of one-way analysis of variance revealed a significant influence of the pollen collection date on its viability ($F_ф$ = 2,53, $F_т$ = 2,35, $F_ф \geq F_т$, LSD_{05} = 17,6).

In the August 16 samplings, pollen viability of slightly less than 50% was observed for the Zhar-ptitsa variety throughout the entire observation period (43–46%), and in 2023 for the Penguin and Eurasia varieties (48%) (see Fig. 1, *в*). By mid-August, the Penguin and Eurasia varieties had virtually no buds. Possibly, the low viability is due to the end of the budding phase. According to the results of a one-way analysis of variance: $F_ф$ = 7,11, $F_т$ = 2,35, $F_ф \geq F_т$, LSD_{05} = 3,93.

Pollen viability in the September 1 samplings was over 50% for most genotypes (see Fig. 1, *г*). The exceptions were the Oranzhevoe Chudo, Bryanskoe Divo, Zhar-ptitsa, Gerakl, and the form 37-15-4 varieties, for which pollen viability in 2022 was 41–49% (the HTC of September 2022 was 0.8). The anthers of the Penguin and Eurasia varieties were empty. A one-way analysis of variance revealed a significant effect of pollen collection date on pollen viability ($F_ф$ = 5,2, $F_т$ = 2,5, $F_ф \geq F_т$, LSD_{05} = 4,6).

In the September 23rd sampling, pollen viability was extremely low for all genotypes, which was influenced by subzero nighttime temperatures (see Fig. 1, *д*). A one-way analysis of variance revealed a significant effect of pollen collection date on pollen viability. The alternative hypothesis is accepted, since all mean values differ from each other (see Fig. 1, *е*). The calculated value of Fisher's exact test exceeded the table value ($F_ф$ = 18,03, $F_т$ = 2,5, $F_ф \geq F_т$). The least significant difference at the 5% significance level is 0.8.

The determination coefficient shows that air humidity (57%) has the least influence on pollen viability, while air temperature has the greatest influence (see Fig. 2). Air humidity and temperature were averaged over the two days prior to sampling and on the day of collection.

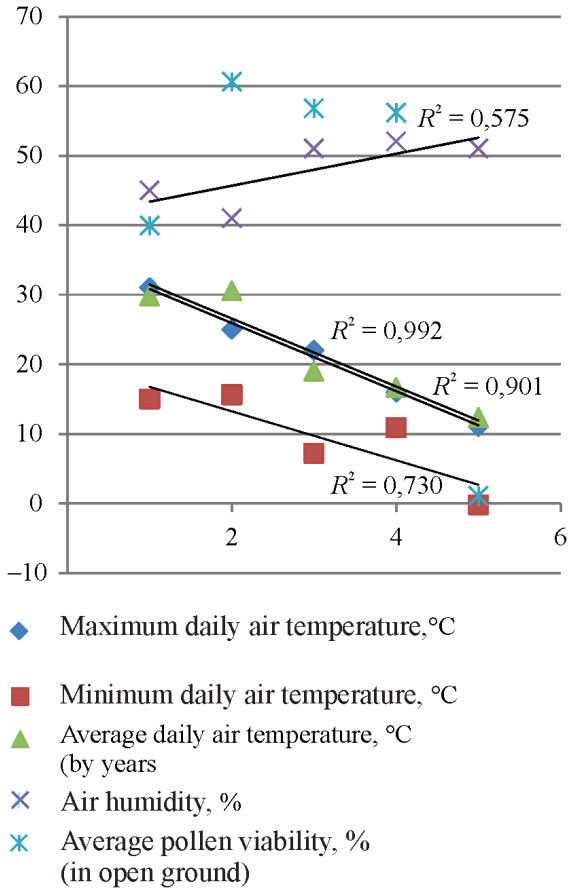

Рис. 2. График корреляции жизнеспособности пыльцы с условиями окружающей среды, %

Fig. 2. Graph of the correlation between pollen viability and environmental conditions, %

CONCLUSIONS

1. The following varieties are considered early flowering: Brilliantovaya, Penguin, Eurasia, the forms 37-15-4 и 1-220-1, to mid-flowering – the Gerakl, Zhar-ptitsa, Rubinovoe Ozherelie, Oranzhevoe Chudo varieties and the form 32-151-1, to late- flowering – the Bryanskoe Divo variety.

2. Over the entire observation period, it was noted that from August 1 to September 1, almost all varieties and forms demonstrated good pollen viability. The exceptions were the form 37-15-4, which only showed good pollen viability on August 1 (58%), and the Zhar-ptitsa variety, which had a pollen viability of 62% during the same period and less than 50% during the other periods.

3. Early-flowering varieties (even Eurasia and Penguin, which no longer had viable pollen by September 1) can be used to pollinate mid- and late-flowering varieties. Almost all of the

studied genotypes can be used as paternal forms, as they are characterized by consistently good pollen viability.

4. The coefficient of determination shows that air humidity (57%) has the minimal effect on pollen viability, while air temperature (more than 90%) has the maximal effect.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

Киселева Елена Николаевна, научный сотрудник, кандидат сельскохозяйственных наук; SPIN-код 2671-3418

(✉)**Раченко Максим Анатольевич,** заведующий отделом, главный научный сотрудник, доктор сельскохозяйственных наук; SPIN-код 1997-2163; **адрес для переписки:** Россия, 664032, Иркутск, ул. Лермонтова, 132; e-mail: bigmks73@rambler.ru

Раченко Анна Максимовна, ведущий инженер; SPIN-код 6033-5300

AUTHOR INFORMATION

Elena N. Kiseleva, Researcher, Candidate of Science in Agriculture; SPIN-code 2671-3418

(✉)**Maxim A. Rachenko,** Department Head, Head Researcher, Doctor of Science in Agriculture; SPIN-code 1997-2163; **address:** 132, Lermontova St., Irkutsk, 664032, Russia; e-mail: bigmks73@rambler.ru

Anna M. Rachenko, Lead Engineer; SPIN-code 6033-5300

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Вредоносность *Euzophera bigella* (Zell.) в садовых агроценозах

(✉)**Киек А.И., Подгорная М.Е.**
Северо-Кавказский федеральный научный центр садоводства, виноградарства, виноделия
Краснодар, Россия
(✉)e-mail: anastasyakovaleva97@mail.ru

Дан анализ зарубежных и отечественных работ, посвященных изучению вредоносности, распространения и биоэкологических особенностей развития *Euzophera bigella* (Zell.). Установлено, что данный вид встречается под несколькими латинскими названиями (*Ephestia bigella, Ephestia stenoptycha*, *Ephestia egeriella, Euzophera punicaella*), представленными в виде синонимов, но основным остается *Euzophera bigella*. Фитофаг широко распространен в 23 странах, включая Российскую Федерацию. Установлено, что вредитель является полифагом, его гусеницы питаются плодами и древесиной таких культур, как айва, яблоня, груша, слива, абрикос, вишня, персик, гранат, виноград, грецкий орех и оливковые деревья. В поврежденные фитофагом плоды попадают споры грибов, вирусы и бактерии, что приводит к загниванию, высыханию, растрескиванию, мумифицированию и опадению плодов. При повреждении древесины происходит набухание и растрескивание коры ветвей и перидермы стволов, что сопровождается обширным внутренним некрозом, вызывающим усыхание ветвей и гибель целых деревьев. В зависимости от страны, региона и условий возделывания культур у *E. bigella* наблюдается от двух до пяти поколений в год, повреждение плодов может достигать до 20–80%. Зимуют гусеницы в шелковистых коконах или в стадии гусениц третьего – пятого возрастов и куколок. Окукливание отмечено с конца апреля или с середины мая, когда среднесуточная температура устанавливается и составляет от 10 до 12 °С. Лёт наблюдается при сумме эффективных температур (выше 10 °С) и составляет от 185,9 до 210 °С (в среднем 187,5 °С).

Ключевые слова: *Euzophera bigella* (Zell.), распространение, вредоносность, биоэкологические особенности

Harmfulness of *Euzophera bigella* (Zell.) in horticultural agrocenoses

(✉)**Kiek A.I., Podgornaya M.E.**
North Caucasian Federal Scientific Center of Horticulture, Viticulture, Wine-making
Krasnodar, Russia
(✉)e-mail: anastasyakovaleva97@mail.ru

Analysis of foreign and domestic works devoted to the study of harmfulness, distribution and bioecological features of the development of *Euzophera bigella* (Zell.) is given. It has been found that this species occurs under several Latin names (*Ephestia bigella, Ephestia stenoptycha, Ephestia egeriella, Euzophera punicaella*), presented as synonyms, but the main remains *Euzophera bigella*. Phytophages are widespread in 23 countries, including the Russian Federation. It has been established that the pest is polyphagous; its caterpillars feed on the fruits and wood of crops such as quince, apple, pear, plum, apricot, cherry, peach, pomegranate, grape, walnut and olive trees. Fruits damaged by phytophages become contaminated with fungal spores, viruses and bacteria, which leads to rotting, drying out, cracking, mummification and falling off of the fruit. When wood is damaged, the bark of the branches and the periderm of trunks swell and crack, which is accompanied by extensive internal necrosis, causing the branches to dry out and the death of entire trees. Depending on the country, region and crop cultivation conditions, *E. bigella* has two to five generations per year, and fruit damage can reach 20–80%. Caterpillars overwinter in silky cocoons or as third to fifth instar caterpillars and

pupae. Pupation is observed from the end of April or from the middle of May, when the average daily temperature is established and is from 10 to 12 °C. Flight is observed when the sum of effective temperatures (above 10 °C) is from 185.9 to 210 °C (on average 187.5 °C).

Keywords: *Euzophera bella* (Zell.), distribution, harmfulness, bioecological features

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The composition of harmful entomofauna in fruit orchards varies and depends on the physiological state of the plant, its age, and the cultivation zone [1]. In the southern fruit-growing zone, one of the most dangerous pests remains a member of the Tortricidae family—the codling moth (*Cydia pomonella* L.). Its population dynamics and fruit damage are regularly monitored, and a basic defense system is being developed. However, in the last decade, significant crop damage has been noted from the double-banded pyralid codling moth (*Euzophera bigella* (Zell.)) [1].

The aim of the study is to analyze existing data on the biological characteristics, distribution, and harmfulness of *E. bigella* to identify and structure new knowledge about the phytophage, which will allow the development of an effective pest control system in fruit orchards in southern Russia.

The species was first described by the German entomologist Philipp Christoph Zeller and given the name *Ephestia bigella* (Zeller, 1848), described from the specimens collected in Sicily and Tuscany (Italy). Later, Herrich-Schäffer (1852–1855) described it under the name *E. stenoptycha*, Milliere (Milliere, 1873) – under the name *Ephestia egeriella.* Ragonot (Ragonot, 1901) reduced the last two names to synonyms *E. stenoptycha* Herrish-Schaffer = *Ephestia egeriella* Milliere = *Euzophera bigella* Zeller. In scientific works one can often find the mention of *Euzophera punicaella = bigella*[1] [2, 3].

Distribution and harmfulness of Euzophera bigella (Zell.)

The species, common in Western Europe and the Russian Federation, has not been studied in detail and is not noted as a pest. The harmfulness of *E. bigella* (Zell.) was first noted by A.M. Gerasimov in 1930, who described the codling moth as an insect that severely damages apple fruit in Central Asia (70–80%). However, he did not touch on the biological characteristics of this species. A.M. Gerasimov argued that the similarity of the damage caused by the phytophage to that caused by the pyralid codling moth led to the fact that when counting damaged fruit, the indicators were mixed up[2]. This suggests that the pyralid codling moth has been damaging fruit for a long time, simultaneously with the codling moth. In 1937, V.P. Nevsky identified *E. bigella* as the second most harmful pest to apple crops, second only to *C. pomonella* (see footnote 1). A.S. Kadamov (2017) noted that the pyralid codling moth population in Uzbekistan exceeded that of the codling moth by tens of times [4].

In Italy, *E. bigella* has been considered a pest of fruit crops for over three decades; in 2013, the species was observed on olive trees. In 2011, partial branch dieback and complete death of ol-

[1]*Kuznetsov V.I.* Biology and species affiliation of codling moths of the genus Euzophera that harm pomegranate, apple and quince // Entomological Review, 1957, vol. 36, N 1, pp. 59–71.

[2]*Gerasimov A.M.* The codling moth *Euzophera bigella* Zell. (Lepidoptera, Pyralidae), a new pest of apples in Central Asia // Russian Entomological Review, 1930, vol. 14 (1-4), pp. 179–181.

ive trees due to damage by the pyralid codling moth were reported in Greece[3].

In Hungary, the pest has caused damage to gooseberries and blackcurrants, but this information has not been confirmed by other sources [5]. In Iraq, the double-banded pyralid codling moth is one of the most dangerous pests of quince [6]. In Georgia, *E. bigella* damages walnuts and members of the Rosaceae family, including apples and apricots [7]. K. Kimsanboev et al. (2021) noted that the pest poses a serious threat to pomegranate plantations in Uzbekistan[4]. Later, N.G. Zagirov (2023) identified *E. bigella* as one of the main pests of pomegranate in the dry subtropics of Southern Dagestan [8].

According to information provided by the foreign website GBIF[5], the range of *E. bigella* is quite wide and includes Portugal, France, Austria, Denmark, Poland, Finland, Switzerland, Greece, Ukraine and Russia.

According to domestic and foreign literary sources, this pest is also found in Iran, North Africa, Belgium, Bulgaria, Great Britain, Hungary, Germany, Spain, Italy, Romania, the Czech Republic, Turkey and Albania [1, 8]. In the Russian Federation, *E. bigella* lives in the European Central Black Earth, Volga-Don, West Caucasian, East Caucasian, Crimean, Transbaikal regions, there is data on the South Ural and Krasnoyarsk regions, but the information is not confirmed [9].

In 1941, Yakhontov, based on the discovery of *E. bigella* caterpillars on grapevines, hypothesized that the species could cause damage to grapes. Later, in 1980, K.V. Deseo established that the pyralid codling moth causes moderate damage to ripe grapes and noted that *E. bigella* feeds on grapes in the same way as *Lobesia botrana* caterpillars (Denis & Schiff.), except that its caterpillars do not spin webs[6]. In a scientific paper published in 2018, A. Lucchi was among the authors who indicate that one of the main representatives of the Phycitinae family that damage grapevines in the Mediterranean was *E. bigella* (Zell.) [10].

In the Krasnodar Territory, the species was first discovered by I.A. Yarysheva in 2002 in peach orchards. In 2005, in the northern horticultural zone of the region, the pest caused serious damage in an apple orchard, and in 2012 it was noted on quince [1]. In her research, T.G. Popova (2017) indicates an increase in the harmfulness of the pest in the Krasnodar Territory: from 2012 to 2015, from 20 to 35% of fruits in fallen fruit were damaged by the phytophage [5].

It has been established that the pest's caterpillars can damage the wood of quince, apple, pear, plum, apricot, cherry, olive trees, and grapevines, as well as the fruits of quince, apple, pear, peach, walnut, pomegranate, and grapes. This is confirmed by research by domestic and international scientists, who classify *E. bigella* into two categories. The first category includes those that damage fruit and overwinter as late-instar caterpillars in cocoons in bark crevices on tree trunks and branches. The second category includes those that damage shoots and overwinter as caterpillars of various instars in habitats including the cambium and bark of trees (see footnote 3) [1, 5, 11]. Both of these groups develop on shoots in spring and early summer. During an early spring tree survey in fruit farms in the Krasnodar Territory, caterpillars were found under the bark of only living trees with bark damage no more than 1 m high, but not at the root collar. T.G. Popova (2017) noted that the caterpillars gnawed large cavities in the wood,

[3]*Simoglou K.B., Karataraki A., Roditakis N.E., Roditakis E. Euzophera bigella* (Zeller) (Lepidoptera: Pyralidae) and *Dasineura oleae* (F. Low) (Diptera: Cecidomyiidae): emerging olive crop pests in the Mediterranean // Journal of Pest Science, 2012, vol. 85, N 2, pp. 169–177. DOI: 10.1007/s10340-012-0418-1.

[4]*Kimsanboev K., Rustamov A., Jumaev R., Usmonov M. Euzophera punicaella* Mooze (Lepidoptera) bioecology and development of host entomophagic equilibrium in biocenosis // E3S Web of Conferences. EDP Sciences. 2021, vol. 244, p. 01003. DOI: 10.1051/e3sconf/202124401003.

[5]*Euzophera bigella* Zeller (1848) in GBIF Secretariat. GBIF Backbone Taxonomy. DOI: 10.15468/39omei (date of reference: 21.01.2025).

[6]*Kulieva H., Gasanova L.* Developmental biology and physiology of the Apsheron population of the pomegranate moth *Euzophera punicaella* Moore (Lepidoptera, Pyralididae) // Modern features of development of biological science as factors of solution of pressing problems of human survival and the natural environment: Research and Practice Conference (London, 19–25 November 2015). London, 2015, pp. 29–31.

filling them with excrement. They were present on trunks throughout the season, their numbers increasing toward the end of the growing season. The first pupae were found under the bark in the first ten days of March, but their greatest numbers (approximately 40% of the total) were recorded in early June and late July [5].

Silvestri (1943) reported that caterpillars bore tunnels in the bark of olive trees, reaching the cambium and wood [12]. De Andre's Cantero (1981) reported possible damage to the bark of olive trees, but did not provide information on the symptoms and status of the pest. In 2012, K.B. Simoglou reported symptoms on the branches and trunks of olive trees in commercial orchards in the municipality of Amphipolis (Northern Greece). The author noted severe damage caused by the lepidopteran pest, which was subsequently identified as *Euzophera bigella* (Zell.) by Dr. Ole Karsholt (see footnote 3).

Due to favorable climatic conditions and the abundance of olive trees, K.B. Simoglou (2012) considers *E. bigella* to be a potential major pest of olive trees in Greece. Furthermore, he refutes the findings of Zangheri et al. (1992) that bark damage by *E. bigella* does not pose a particular threat (see footnote 3).

K.B. Simoglou reports on the caterpillars' feeding behavior, which causes swelling and cracking of the bark of branches and the periderm of trunks, followed by extensive internal necrosis, leading to branch desiccation and tree death. In a tunnel formed under damaged bark, the author discovered caterpillars surrounded by large quantities of excrement and silk fibers (see footnote 3). According to M.E. Tzanakakes (2021), in Sicily, Tunisia, and Algeria, caterpillars were found exclusively on olive trees, in galls caused by the bacterium *Pseudomonas savastanoi* pv. Savastanoi.

On apple, pear, peach, pomegranate, walnut, and other fruit trees, *E. bigella* caterpillars damage ripening fruit. According to research by K.B. Simoglou et al. (2012), caterpillars damage fruit during its ripening period. Damage begins to appear no earlier than July, and becomes more intense in August and September (see footnote 6) [1]. M.E. Tzanakakes (2021) noted that young caterpillars can feed superficially on the peel, but do not penetrate or develop inside unripe fruit [12]. Maximum fruit damage was observed in July, August, and September, as well as in storage (see footnote 6).

As M.E. Tzanakakes notes in a 2021 paper, the symptoms resemble damage caused by the caterpillars *Grapholitha molesta* and *Cydia pomonella*. A distinctive difference between the pyralid codling moth and the codling moth is that the latter damages not only the seed chamber but also eats the fruit pulp. A single fruit can harbor five or more caterpillars, as noted by E.B. Balykina et al. in their 2020 paper [11].

In the Krasnodar region, a general trend was identified in the ratio of fruit damage by *C. pomonella* and *E. bigella* caterpillars. T.G. Popova in her 2017 work [5] indicates that from July, the codling moth was significantly predominant, in August the number of caterpillars almost equalized, and in September the proportion of the pyralid codling moth increased significantly.

According to international data, the *E. bigella* species is characterized by a change in food supply during the growing season. First-generation caterpillars can develop on the phloem of pomegranate, fig, and apple trees, and partially on peach blossoms and the ovaries of apple and pear trees in the absence of ripe fruit in the first half of summer. In pomegranates, caterpillars damage fruits at all stages of ripening (the first generation also feeds on flowers). In fruits of sufficient ripeness, they penetrate the calyxes and eat them from the inside, making the damage barely noticeable. V.I. Kuznetsov (1957) noted that the process of damage to the pomegranate fruit can be divided into three phases.

In the first phase, the caterpillars penetrate the fruit through the calyx, causing an inconspicuous wormhole. As the caterpillars grow, the affected area expands, feeding on seeds and membranes, primarily in the peel. In the second phase, a brown spot forms on the fruit surface at the base of the calyx, where a fungal disease develops, reducing the commercial value. In the third phase, the fruit splits into 2-4 pieces, allowing access for the next generation of pests. Analysis of the damaged fruit showed that split

fruits contained more caterpillars than undamaged ones (see footnote 1).

Research in Azerbaijan has shown that the first-generation caterpillar damages 5–6 flowers or developing fruits of pomegranates, the second generation – 1–2 fruits, and the third generation – 3–4 fruits (see footnote 6). For apple trees, caterpillars of the third and fourth generations are most dangerous. They damage the seed chamber and the pulp of apple fruits, introducing fungal spores, viruses, and bacteria, which leads to rotting, drying, cracking, mummification, and fruit drop. *E. bigella* caterpillars reach the seed chamber starting in the third instar, selecting fruits already damaged by the codling moth. Seed feeding leads to stunted growth and fruit drop, or to partial development of deformed fruits.

The analysis of the work revealed a trend towards a gradual increase in the harmful effects of *E. bigella* in different countries and on different crops.

Bioecological features of development of Euzophera bigella (Zell.)

Depending on the region and the growing conditions, the number and developmental characteristics of each generation of *E. bigella* can vary significantly. Thus, under natural conditions, the phytophage can produce from two to three generations in northern regions, and up to four or five in southern regions. According to the study by Zangheri et al. (1992), three generations per year are possible in Italy, while M.E. Tzanakakes (2021) reports two. T.G. Popova notes that under favorable climatic conditions, *E. bigella* is capable of producing up to four generations within the Krasnodar Territory [5].

During the winter, hibernating caterpillars are able to withstand extremely low temperatures, reaching minus 15°C. Part of the population constantly develops on the phloem, creating a natural reserve that allows for the maintenance of high numbers of the species during periods unfavorable for the development of subtropical and fruit crops [1]. V.I. Kuznetsov discovered that in the spring, in the cracks of the bark of the skeletal branches of the pomegranate bush, the pyralid codling moth, in the phase of overwintered adult caterpillars, begins to pupate without resuming feeding (see footnote 1).

Pupation of the pest begins from early April to mid-May, when average daily temperatures reach 10–12°C. Individuals that successfully survive the winter remain in the pupal state for 17–20 days.

S.R. Cherkezova (2017) notes that from mid-May to early June, butterfly flight is observed at a sum of effective temperatures (SET) from 185.9 to 210 °C (average 187.5 °C). M.E. Tzanakakes (2021) showed that adults emerge from late April to late May. The lifespan of butterflies ranges from 8 to 20 days [1]. The flight of butterflies lasts from 2 to 10 days, after which individuals begin to lay eggs. The female selects fruits damaged mechanically, by scab, codling moth, or by birds. The fecundity of the female ranges from 14 to 300 eggs, laying from 1 to 21 eggs per fruit.

The authors of scientific studies have found that butterfly fertility depends on the temperature and photoperiod conditions of the habitat. At an 8-hour photoperiod and temperatures below 18°C or above 40°C, egg-laying is suspended. Eggs are laid at night, from 10:00 PM to 4:00 AM, at temperatures of 20–24°C. As shown by the studies of T.G. Popova (2017) and A.K. Asoev (2013), oviposition depends on the sum of effective temperatures. In 2013, oviposition was noted at a SET of 227.7 °C from the second ten-day period of May, in 2014 – from the third ten-day period of April at a SET of 216.6 °C, in 2015 – in the second ten-day period of May at a SET of 237.4 °C. The oviposition process continues for 70–90 days, regardless of the generation. Although Kh.F. Kulieva previously noted that butterflies live a short period, it is possible that the author was referring to the total duration of oviposition of all generations (see footnote 6).

The process of egg development takes on average from 5 to 6 days, but can range from 3 to 4 and from 8 to 9 days. The caterpillar period, which includes five instars, lasts from 20 to 22 days, the pupal period – 12–14 days [1]. According to the research of E.K. Kayumova (see footnote 1), the duration of caterpillar development varies depending on seasonal fluctuations, de-

creasing from spring to summer and increasing towards autumn. This is confirmed by the data of H.F. Kuliyeva from Azerbaijan: in an individual living in the first half of summer, the egg period lasts 6–8 days, the caterpillar – 20–25 days, the pupa – 10–12. In an individual living in the second half of summer, the egg stage takes 5–7 days, the caterpillar – 10–12 days, the pupa – about half a month (see footnote 6).

According to research [12], with two generations per year, eggs of the first generation appear from mid-May to mid-June, and those of the second from early July to late September. Thus, caterpillars of both generations appear simultaneously and are present throughout the season, when stone and pome fruits, which can potentially serve as the pest's food source, begin to ripen or mature [12].

In the course of the study [5], the dynamics of the age composition of caterpillars found in damaged apple fruits was studied. At the beginning of September 2008, 33.3% of the caterpillars were in the first and second instars, 15.2% were in the third, 18.2% were in the fourth and fifth instars, and caterpillars were absent from 33.3% of damaged apples. During development (from the third instar), the caterpillars reached the seed chamber and created a fine web filled with excrement. The maximum number of caterpillars in one apple reached 7 individuals; the fruits rotted from the inside, but the life of the caterpillars continued. Pupae of the pest were found in the fruits near the exit hole [5].

Mass pupation of first-generation caterpillars (first and second ten-day periods of June) occurs at a set temperature of 416.7–555.8 °C. According to Kh. F. Kulieva, pupation of caterpillars continues for 15–20 days. Summer generation moths emerge from cocoons at the beginning of the third ten-day period of June, when the sum of effective temperatures reaches 550.6–570.5 °C. In the autumn, fully developed and fully formed caterpillars enter hibernation, where they overwinter in silky cocoons (see footnote 5). E. K. Kayumova and other researchers noted that in the conditions of the Fergana Valley, depending on meteorological factors, the pomegranate moth spends the winter as fourth generation caterpillars of the third to fifth instars and pupae [2].

In the process of analyzing information obtained from foreign and domestic scientific works, it became clear that it was necessary to conduct a detailed study of the biological characteristics of the species in question in order to obtain more accurate information about its development in the southern region of Russia.

CONCLUSION

This literature review summarizes data on the distribution and harmfulness of *E. bigella* (Zell.) in various countries worldwide. It has been established that the pest is polyphagous; its caterpillars feed on the wood of quince, apple, pear, plum, apricot, cherry, and olive trees, as well as the fruits of quince, apple, pear, peach, walnut, grape, and pomegranate. Fruit damage can vary from 20 to 80%. In different geographic zones and under various growing conditions, *E. bigella* is capable of producing two to five generations per year. Flight is observed when the sum of effective temperatures exceeds 10 °C and varies between 185 and 210 °C, averaging 187.5 °C.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

(✉)**Киек Анастасия Ивановна**, младший научный сотрудник, аспирант; SPIN-код 5476-6240; **адрес для переписки**: Россия, 350901, Краснодар, ул. им. 40-летия Победы, 39; e-mail: anastasyakovaleva97@mail.ru

Подгорная Марина Ефимовна, заведующая лабораторией, кандидат биологических наук; SPIN-код 6686-9037

AUTHOR INFORMATION

(✉)**Anastasia I. Kiek,** Junior Researcher, Post-graduate Student; SPIN-code 5476-6240; **address:** 39, 40-letiya Pobedy St., Krasnodar, 350901, Russia; e-mail: anastasyakovaleva97@mail.ru

Marina E. Podgornaya, Laboratory Head, Candidate of Science in Biology; SPIN-code 6686-9037

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Сравнительная оценка генетической структуры калмыцкой и герефордской пород скота по *SNP* и *STR* маркерам

Авадани Д.А.[1, 2], (✉)Гончаренко Г.М.[1], Халина О.Л.[1], Хорошилова Т.С.[1], Гришина Н.Б.[1], Заборских Е.Ю.[3], Луду Б.М.[4], Кан-оол Б.К.[4]

[1]*Сибирский федеральный научный центр агробиотехнологий Российской академии наук*
Новосибирская область, р.п. Краснообск, Россия

[2]*Новосибирский государственный аграрный университет*
Новосибирск, Россия

[3]*Горно-Алтайский научно-исследовательский институт сельского хозяйства – филиал Национального исследовательского Томского государственного университета*
Республика Алтай, Россия

[4]*Тувинский научно-исследовательский институт сельского хозяйства – филиал Сибирского федерального научного центра агробиотехнологий Российской академии наук*
Республика Тыва, г. Кызыл, Россия
(✉)e-mail: gal.goncharenko@mail.ru

Эффективное производство говядины от специализированных пород возможно как при промышленной технологии, так и при более дешевом длительным пастбищном содержании. Используемые при этом породы отличаются генетически обусловленным фенотипом, приспособленностью к разным условиям содержания. Цель исследования состояла в сравнительной оценке генотипической структуры интенсивного типа герефордской породы крупного рогатого скота и экстенсивного – калмыцкой. Изучение проводили с использованием *SNP* и *STR* маркеров. По частоте генотипов генов *LEP, TG5, SCD1, OLR1* различий не установлено, за исключением гена *TG5*, где в герефордской породе генотип $TG5^{CC}$ встречается выше на 24,8%, генотип $TG5^{CT}$ ниже на 27,5% по сравнению с калмыцкой. В полиморфных локусах *TGLA122* и *TGLA53* аллели 151 (локус *TGLA122*) и аллели 162 и 172 (локус *TGLA53*) в герефордской породе встречались чаще на 13,1; 19,0 и 15,2% соответственно по сравнению с калмыцкой. По основным селекционно-генетическим параметрам (*Ca, N_{aj}, V, PIC*), рассчитанных на основании частот аллелей и генотипов генов *LEP, TG5, SCD1, OLR1*, значимых различий не установлено: гетерозиготность составляет 0,618–0,648, число эффективных аллелей – 1,54–1,62, изменчивость – 35,9–39%, коэффициент полиморфности – 0,352–0,381. Генное равновесие в породах не нарушено и находится в пределах χ^2 = 0,008–4,198. Также не установлено различий между породами по популяционно-генетическим параметрам с использованием *STR*-маркеров. Среднее количество аллелей на локус в герефордской породе составляет 10,25, калмыцкой – 8,94, количество эффективных аллелей – 4,13–4,73. Гомозиготность находится на уровне 0,80–0,82. Инбридинга в стадах не выявлено, индекс *Fis* имеет отрицательное значение. Число приватных аллелей составляет в герефордской породе 32, в калмыцкой – 11. Полученные результаты можно считать промежуточными, для более убедительных данных необходимо увеличить исследуемое поголовье калмыцкой породы.

Ключевые слова: порода, микросателлиты, полиморфизм, аллель, генотип, гетерозиготность, индекс фиксации, генное равновесие

Comparative assessment of the genetic structure of the Kalmyk and Hereford cattle breeds by *SNP* and *STR* markers

Avadani D.A.[1,2], (✉)Goncharenko G.M.[1], Khalina O.L.[1], Khoroshilova T.S.[1], Grishina N.B.[1], Zaborskikh E.Yu.[3], Ludu B.M.[4], Kan-ool B.K.[4]

[1]*Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences*
Krasnoobsk, Novosibirsk region, Russia

[2]*Novosibirsk State Agrarian University*
Novosibirsk, Russia

[3]*Gorno-Altay Research Institute of Agriculture – Branch of the National Research Tomsk State University*
Altay Republic, Russia

[4]*Tuvan Research Institute of Agriculture – Branch of the Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences*
Tyva Republic, Kyzyl, Russia

(✉)e-mail: gal.goncharenko@mail.ru

Efficient production of beef from specialized breeds is possible both with industrial technology and with cheaper long-term pasture maintenance. The breeds used in this case differ in genetically determined phenotype, adaptability to different conditions of maintenance. The purpose of the research was to comparatively evaluate the genotypic structure of the intensive type of the Hereford breed and the extensive type of the Kalmyk breed. The study was carried out using *SNP* and *STR* markers. The differences were not found in the frequency of genotypes of the *LEP*, *TG5*, *SCD1*, *OLR1* genes, with the exception of the *TG5* gene, where in the Hereford breed the $TG5^{CC}$ genotype is found by 24.8% higher, the $TG5^{CT}$ genotype by 27.5% lower, compared to the Kalmyk breed. In the polymorphic loci *TGLA122* and *TGLA53*, alleles 151 (locus *TGLA122*) and alleles 162 and 172 (locus *TGLA53*) in the Hereford breed were found more often by 13.1%, 19.0% and 15.2%, respectively, compared to the Kalmyk breed. The significant differences were not found in the main selection and genetic parameters (*Ca*, *Naj*, *V*, *PIC*), calculated on the basis of the allele and genotype frequencies of the *LEP*, *TG5*, *SCD1*, *OLR1* genes: heterozygosity is 0.618–0.648; the number of effective alleles is 1.54-1.62; variability is 35.9-39 %; the polymorphism coefficient is 0.352–0.381. Gene equilibrium in the breeds is not disturbed and is within the range of χ^2=0.008–4.198. Also, no differences were found between the breeds in population genetic parameters using *STR* markers. The average number of alleles per locus in the Hereford breed is 10.25, in the Kalmyk breed 8.94, and the number of effective alleles is 4.13–4.73. Homozygosity is at the level of 0.80–0.82. Inbreeding in the herds was not detected, the *Fis* index has a negative value. The number of private alleles is 32 in the Hereford breed and 11 in the Kalmyk breed. The results obtained can be considered intermediate; for more convincing data, it is necessary to increase the studied population of the Kalmyk breed.

Keywords: breed, microsatellites, polymorphism, allele, genotype, heterozygosity, fixation index, gene equilibrium

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INTRODUCTION

One of the resources for increasing competitive meat production from specialized beef cattle breeds is the breeding of animals well adapted to local climatic conditions. The Kalmyk breed can be considered the most suitable for extensive beef production technology with extended grazing periods. Long-term natural selection and subsequent breeding have allowed the creation of animals that are genetically resistant to adverse factors and diseases. Thanks to the rapid accumulation of subcutaneous fat and downy undercoat, they are able to withstand harsh winters and periods of food shortage[1] [1].

Animals from the best lines of the Kalmyk breed have a fairly high slaughter yield, while the profitability is 6,52–8,29% [2, 3].

The Hereford breed is one of the best meat breeds for industrial beef production. Under intensive production conditions, animals of this breed can achieve an average daily weight gain of 900–1200 g by the age of 18 months, which is significantly higher than that of Kalmyk and Kazakh white-headed cattle, second only to the Belgian Blue [4]. According to research [5], the profitability of Hereford bulls for meat can reach 17%. Preference in breeding is given to tall animals born with a larger weight [6, 7].

Breed improvement is being directed toward increasing growth energy, reproductive performance, meat productivity, and meat quality, which can be achieved more successfully using marker-assisted selection. For comparative assessment of genetic similarities and differences between breeds and populations, and for monitoring population genetic parameters, two types of genetic markers—*SNP*s and *STR*s—can be used, which are informative and suitable for mass analysis. These markers complement each other when used simultaneously.

Given the functional role of SNPs in phenotype expression, their association with productive traits, as well as changes in genotype frequencies under rigorous selection or crossbreeding, are of interest. Among the marker genes tested in beef cattle breeding, genes associated with meat quality characteristics and animal resistance are of particular interest – *LEP, TG5, SCD1, OLR1*.

The *LEP* gene (leptin) influences the fat content in milk and the reproductive capacity of dairy cows [8, 9]. In breeds of meat productivity, the influence of the *LEP C528T* and *LEP C73T* genes on the formation of meat marbling and the yield of meat cuts has been established. [10].

The thyroglobulin gene (*TG5*) is a regulator of lipid metabolism, where the replacement of one of the nitrogenous bases leads to the synthesis of another amino acid, which changes the qualitative and quantitative composition of the animal's adipose tissue[2]. The *OLR1* (oxidized low-density lipoprotein receptor 1) gene is associated with lipid metabolism and influences fat deposition in the carcass and marbling of beef[3]. Также установлено его влияние на репродуктивные показатели животных[4], особенности роста и строения туши у мясного скота[5] [11].

[1] *Dorotyuk E.N.* Kalmyk cattle and ways of its improvement. Moscow: Rosselkhozizdat*, 1981*, 143 p.

[2] *Tyulkin S.V., Akhmatov T.M., Valliulina E.F., Vafin R.R.* Polymorphism of genes for somatotropin, prolactin, leptin and thyroglobulin in stud bulls // Vavilov Journal of Genetics and Breeding, 2012, vol. 16, N 4/2, pp. 1008–1011.

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[4] *Kowalewska-Luczak I., Czerniawska-Piatkowska E., Kowalewska-Luczak I.* Polymorphism in the OLR1 gene and functional traits of dairy cattle // Veterinarski Arhiv, 2018, vol. 88 (2), pp. 171–177. DOI: 10.24099/vet.arhiv.170228.

[5] *Fonseca S.D.P., Souza P.R.F., Camargo F.M. et al.* Association of ADIPOQ, OLR1 and PPARGC1A gene polymorphisms with growth and carcass traits in Nelore cattle // Meta Gene, 2015, vol. 4, pp. 1–7.

To solve other selection problems related to determining the degree of kinship, heterozygosity, controlling the transmission of genomic information from generation to generation, monitoring changes in the genetic structure of a particular breed/herd, microsatellite analysis can be used as the most informative and suitable for mass analysis in animal husbandry [12, 13].

Using *STR* markers, it is possible to determine with a high degree of certainty the variability of a breed's gene pool under the influence of improvement by another breed. As studies have shown [12], in the Black-and-White breed, when crossed with Holsteins over a period of 30–40 years, some alleles were lost, and new ones, not characteristic of the Black-and-White breed, appeared. The modern population of the Black-and-White breed turned out to be not identical to the Holstein. Furthermore, studies have appeared that refute the theory of the lack of a connection between microsatellites and selection processes. Long-term monitoring of allele frequencies in Holstein herds in Germany revealed a decrease in genetic diversity[6].

The purpose of the study is to present a comparative assessment of the genetic profiles of meat breeds that differ in production technologies: extensive (Kalmyk) and intensive (Hereford).

MATERIAL AND METHODS

Molecular genetic studies were conducted in the biotechnology laboratory of the Siberian Research and Design and Technological Institute of Animal Husbandry of the Siberian Federal Scientific Center of Agro-BioTechnologies of the Russian Academy of Sciences on Hereford (n = 200) and Kalmyk (n = 44) breeds. Genomic DNA was isolated from blood using the "Ampli Prime DNA-Sorb-V" clinical specimen extraction kit, as recommended by the OOO "NextBio" (Moscow). The quality and concentration of the isolated DNA were assessed in a 1% agarose gel by horizontal electrophoresis using the E-Box-CX5.TS-20.M gel documentation system.

LEP gene polymorphism was determined using the PCR-RFLP method described[7], *TG5*[8], *SCD1*[9], *OLR1*[10].

Amplification was performed in a C1000 "BioRad" thermocycler. The resulting gene amplification products were treated with restriction endonucleases HindIII, EcoICRI, HaeIII, and RsaI (SibENZIM, Novosibirsk) according to the manufacturer's instructions. Genotypes were visualized and identified by electrophoresis in a 3% agarose gel under UV light.

Microsatellite analysis was performed using the "GeneProfile Cattle" reagent kit, designed for genetic identification and relationship determination in cattle (Bos taurus). Genotyping was performed using multiplex amplification of 16 STR loci, followed by PCR product length analysis using capillary electrophoresis (*TGLA227*, *BM2113*, *TGLA53*, *ETH10*, *CSRM60*, *SPS115*, *TGLA122*, *BM1818*, *HAUT27*, *CSSM66*, *BM1824*, *ETH3*, *TGLA126*, *ETH225*, *INRA023*, *ILSTS006*). Twelve of these are recommended by the International Society of Animal Genetics (ISAG). Three loci are recommended by the Food and Agriculture Organization of the United Nations (FAO) for genetic studies of domestic animals: *CSSM66*, *CSRM60*, and *ILSTS006*. The highly polymorphic *HAUT27* locus is also included.

PCR primers were selected to allow amplification of all 16 *STR* loci in a single tube. The size of the amplified PCR products ranged from 64 to 236 base pairs (including all known al-

[6]*Brenig B., Schutz E.* Recent development of allele frequencies and exclusion probabilities of microsatellites used for parentage control in the German Holstein Friesian cattle population // BMC Genet., 2016, vol. 17, N 18. DOI: 10.1186/s12863-016-0327-z.

[7]*Kalashnikova L.A., Khabibrakhmanova A.Ya., Pavlova I.Yu., Ganchenkova T.B., Dunin N.M., Pridanovich I.E.* Recommendations for genomic evaluation of cattle. Lesnye Polyany, 2015, 33 p.

[8]*Surundaeva L.G.* Comparative analysis of the genetic structure of beef cattle populations based on polymorphic variants of the somatotropin and thyroglobulin hormone genes // Herald of Beef Cattle Breeding, 2016, vol. 96, N 4, pp. 21–29.

[9]*Yulmetyeva Yu.R., Safina N.Yu., Sharafutdinov G.S., Shakirov Sh.K.* Polymorphism of the stearylcoa desaturase gene *(SCD1)* for loci of quantitative traits of milk productivity *// Agrarian Scientific Journal*, 2018, N 11, pp. 43– 45. DOI: 10.28983/asj.v0i11.416.

[10]*Komisarek J., Dorynek Z.* Effect of ABCG2, PPARGC1A, OLR1 and SCD1 gene polymorphism on estimated breeding values for functional and production traits in Polish Holstein-Friesian bulls // J. Appl. Genet., 2009, vol. 2 (50), pp. 125–132. DOI: 10.1007/BF03195663.

leles). PCR results were analyzed by capillary electrophoresis using a Nanofor 05 automated genetic analyzer.

The frequency characteristics of the genotypes of the studied genes were estimated using the Hardy-Weinberg formula for two-allelic systems using the χ^2 criterion. The population homozygosity coefficient (*Ca*), polymorphism level (*Na*), degree of genetic variability (*V*), and polymorphism information content (*PIC*) were determined in accordance with recommendations[11]. Population genetic parameters were calculated using the GenAlEx and Past programs.

RESULTS AND DISCUSSION

Selection of specialized meat breeds is primarily focused on the vigor of young animals and the quantitative and qualitative characteristics of meat, which ensure the profitability of this industry. At the same time, specific feeding and housing conditions, as well as previous selection, may influence the genotypic structure of *SNP* and *STR* markers.

A comparative analysis of the genotypic structure was carried out on two breeds of beef cattle (see Table 1).

In terms of genotype frequencies of the genes studied, no significant differences were found, with the exception of the *TG5* gene. The Hereford breed is characterized by a higher (24.8%) frequency of the homozygous $TG5^{CC}$ genotype and a lower (27.5%) frequency of the heterozygous $TG5^{CT}$ genotype compared to the Kalmyk breed ($p \leq 0{,}01$, $p \leq 0{,}001$). A slight increase in the LEP^{CC} ratio (by 13.2%) was noted in the Hereford breed. It should be noted that the studied breeds have a fairly similar structure for the *SCD1* and *OLR1* genes, which is possibly due to traits not under selection pressure. Genetic balance in the breeds is not disturbed and is within $\chi^2 = 0{,}008–4{,}198$.

When analyzing the allelic profile of the compared breeds, no differences were found in the *LEP*, *SCD1*, and *OLR1* genes, with the exception of the *TG5* gene (see Table 2). The Kalmyk breed is characterized by a lower frequency of the $TG5^{C}$ allele (by 0.11) and, accordingly, a higher frequency of the $TG5^{T}$ allele compared to the Hereford breed ($p < 0{,}05$).

Similar to our studies, a similar frequency of genotypes and alleles of the *LEP* gene at different loci in the Hereford and Kalmyk breeds was established in [14]. A different ratio of TG5 gene genotypes in the Kalmyk breed was identified by the authors (see footnote 8), where the homozygous $TG5^{CC}$ genotype accounts for 62.5%, respectively, the frequency of the genotype $TG5^{CT}$ – 25,0%, $TG5^{TT}$ – 12,5%.

Based on the frequencies of genotypes and alleles, population genetic parameters were calculated, the analysis of which shows that homozygosity (*Ca*), the number of active alleles (N_{aj}),

Табл. 1. Генотипическая структура калмыцкой и герефордской пород по генам *LEP, TG5, SCD1, OLR1*

Table 1. Genotypic structure of the Kalmyk and Hereford breeds for the genes *LEP*, *TG5*, *SCD1*, *OLR1*

Genotype	Kalmyk breed (n = 44)	χ^2	Hereford breed (n = 200)	χ^2
LE^{PC}C	20,5 ± 6,1		33,5 ± 3,3	
LEP^{CT}	56,8 ± 7,5	0,882	46,0 ± 3,5	0,668
LEP^{TT}	22,7 ± 6,3		20,5 ± 2,9	
TG^{5C}C	43,2 ± 7,5		68,0 ± 3,3	
TG^{5C}T	54,5 ± 7,5	4,198	27,0 ± 3,1	2,190
TG^{5T}T	2,3 ± 2,2		5,0 ± 1,5	
$SCD1^{CC}$	56,8 ± 7,5		48,0 ± 7,1	
$SCD1^{CT}$	36,4 ± 7,3	0,008	44,0 ± 7,0	0,452
$SCD1^{TT}$	6,8 ± 3,8		8,0 ± 3,8	
OL^{R1}AA	2,3 ± 2,3		2,0 ± 2,0	
$OLR1^{AC}$	22,7 ± 6,3	0,045	18,0 ± 5,4	1,300
OLR^{1C}C	75,0 ± 6,5		80,0 ± 5,7	

[11] *Chesnokov Yu.V., Artemyeva A.M.* Evaluation of the measure of information polymorphism of genetic diversity // Agricultural biology, 2015, vol. 5, N 5, pp. 571–578. DOI: 10.15389/agrobiology.2015.5.571rus.

the degree of genetic variability (*V*) and the level of polymorphism (*PIC*) in herds of meat breeds are in the same range (see Table 3).

The homozygosity of individual genes in meat breeds is also close in its values (see Table 4). It should be noted that homozygosity for the *LEP* gene is 50.0%, while for the *OLR1* gene it is significantly higher - 76.5-80.4%. Homozygosity of the *TG5* and *SCD1* genes occupies an intermediate position.

Табл. 2. Частота аллелей генов *LEP, TG5, SCD1, OLR1* в породах молочного и мясного направления продуктивности

Table 2. Allele frequency of genes *LEP*, *TG5*, *SCD1*, *OLR1* in breeds of dairy and meat productivity

Genotype	Breed	
	Kalmyk (n = 44)	Hereford (n = 200)
LEP^C	0,489 ± 0,053	0,565 ± 0,024
LEP^T	0,511 ± 0,053	0,435 ± 0,024
TG^5C	0,705 ± 0,049	0,815 ± 0,019
$TG5^T$	0,295 ± 0,049	0,185 ± 0,019
$SCD1^C$	0,750 ± 0,046	0,700 ± 0,046
$SCD1^T$	0,250 ± 0,046	0,300 ± 0,046
$OLR1^A$	0,136 ± 0,037	0,110 ± 0,031
$OLR1^C$	0,864 ± 0,037	0,890 ± 0,031

The population genetic characteristics of the compared breeds using microsatellites show no significant differences (see Table 5). The average number of alleles per locus in the Hereford breed is 10.25, while in the Kalmyk breed it is 8.94. The number of effective alleles is 4.13 and 4.73, respectively, demonstrating their good genetic diversity and distinctness. No inbreeding was detected in the herds; the observed heterozygosity was higher than expected, and the *Fis* index was negative. Note that the *Fis* index value in the Kalmyk breed is close to zero, which may prompt the development of measures to increase genetic diversity by exchanging genetic material from unrelated populations.

Thus, analysis of the Hereford and Kalmyk breeds revealed a high degree of similarity in their gene pools, likely influenced by unidirectional selection. Moreover, higher genetic diversity was found in the Hereford breed.

The authors of [15] obtained population genetic characteristics of the Kalmyk breed that are closer to our values. These characteristics show that the average number of alleles is 8.13, the number of effective alleles is 4.79, the observed heterozygosity is 0.778, and the fixation index is (–0.007). Different information was obtained for the Hereford breed[12]. In this breed in the Tyumen region, the number of alleles per locus is signifi-

Табл. 3. Популяционно-генетические параметры мясных пород скота

Table 3. Population-genetic parameters of beef cattle breeds

Breed	*n*	*Ca*	N_{aj}	*V*	*PIC*
Kalmyk	44	0,618 ± 0,073	1,62 ± 1,90	39,0 ± 7,35	0,381
Hereford	50	0,648 ± 0,072	1,54 ± 1,74	35,9 ± 6,79	0,352

Табл. 4. Гомозиготность генов *LEP, TG5, SCD1, OLR1* калмыцкой и герефордской породах

Table 4. Homozygosity of *LEP*, *TG5*, *SCD1*, *OLR1* genes in the Kalmyk and Hereford breeds

Breed	*n*	C_a, %			
		LEP	*TG5*	*SCD1*	*OLR1*
Kalmyk	44	50,0	61,4	62,5	76,5
Hereford	50	50,0	65,7	58,0	80,4

[12]*Sheveleva O.M., Chasovshchikova M.A.* Characteristics of the genetic structure of the Hereford herd by STR loci // Animal husbandry and fodder production, 2018, vol. 101, N 4, pp. 71–78.

Табл. 5. Генетико-популяционная характеристика мясных пород скота

Table 5. Genetic and population characteristics of beef cattle breeds

Indicator	Breed	
	Hereford	Kalmyk
Na	10,25 ± 0,66	8,94 ± 0,65
Ne	4,13 ± 0,38	4,73 ± 0,37
I	1,58 ± 0,08	1,74 ± 0,07
No. Private Alleles	1,13 ± 0,22	0,50 ± 0,22
Ho	0,82 ± 0,03	0,80 ± 0,02
He	0,73 ± 0,02	0,77 ± 0,01
Fis	–0,12 ± 0,024	–0,04 ± 0,02

Note. *Na* – average number of alleles per locus, *Ne* – number of effective alleles, *I* – Shannon index, *No* Private Alleles – number of private alleles, *Ho* – observed heterozygosity, *He* – expected heterozygosity, *Fis* – fixation index.

cantly lower – 6.93, effective alleles – 4.96, observed heterozygosity – 0.783, the fixation index for individual loci is in the range of –0.288 … –0.032.

A comparative assessment of the population genetic parameters of the Hereford and Kalmyk breeds with the aboriginal Yakut breed shows that the average number per locus in the Yakut breed is 6.0, accordingly, the level of heterozygosity in the herds is also lower and is at the level of 0.596–0.631, the fixation index occupies an average position between the Hereford and Kalmyk breeds (–0,104) [16].

When analyzing the frequencies of individual loci characterized by the highest polymorphism, such as *TGLA122* and *TGLA53*, both rare and frequently occurring alleles were identified, which in most cases were the same in the breeds (see Table 6). Alleles 143, 151, and 161 can be considered frequently encountered in the

Табл. 6. Частота аллелей локусов *TGLA122* и *TGLA53* герефордской и калмыцкой породах

Table 6. Allele frequency of loci TGLA122 and TGLA53 in the Hereford and Kalmyk breeds

Locus *TGLA122*	Breed		Locus *TGLA53*	Breed	
	Hereford (*n* = 350)	Kalmyk (*n* = 43)		Hereford (*n* = 350)	Kalmyk (*n* = 43)
137	1,4 ± 0,6	0,0 ± 0,0	154	4,7 ± 1,1	4,7 ± 3,2
139	0,3 ± 0,3	3,5 ± 2,8	158	0,1 ± 0,2	2,3 ± 2,3
141	6,7 ± 1,3	4,7 ± 3,2	160	33,0 ± 2,5	24,4 ± 6,5
143	58,6 ± 2,6	46,5 ± 7,6	162	26,4 ± 2,4	7,0 ± 3,9
145	0,3 ± 0,3	1,2 ± 1,6	164	2,3 ± 0,8	4,7 ± 3,2
147	3,9 ± 1,0	1,2 ± 1,6	166	1,0 ± 0,2	3,5 ± 2,9
149	1,0 ± 1,0	0,0 ± 0,0	168	2,1 ± 0,8	7,0 ± 3,9
151	11,3 ± 1,7	24,4 ± 6,5	170	6,3 ± 1,29	17,4 ± 5,6
153	2,0 ± 2,0	5,8 ± 3,6	172	19,9 ± 2,1	4,7 ± 3,2
155	0,9 ± 0,5	0,0 ± 0,0	174	1,1 ± 0,6	0,0 ± 0,0
157	0,0 ± 0,0	3,5 ± 2,8	176	0,6 ± 0,0	3,5 ± 2,9
161	12,3 ± 1,8	5,8 ± 3,6	178	0,0 ± 0,0	11,6 ± 4,9
163	0,3 ± 0,3	1,2 ± 1,6	180	0,1 ± 0.2	5,8 ± 3,6
165	0,0 ± 0,0	1,2 ± 1,6	182	0,1 ± 0,2	2,3 ± 2,3
169	0,0 ± 0,0	1,2 ± 1,6	184	0,0 ± 0,0	0,0 ± 0,0
175	0,4 ± 0,6	0,0 ± 0,0	186	2,0 ± 0,7	0,0 ± 0,0
177	0,1 ± 0,1	0,0 ± 0,0	188	0,0 ± 0,0	1,2 ± 1,7
181	0,0 ± 0,0	0,0 ± 0,0	190	0,1 ± 0,2	0,0 ± 0,0

Табл. 7. Приватные аллели в герефордской и калмыцкой породах
Table 7. Private alleles in the Hereford and Kalmyk breeds

Breed	Locus	Allele	Frequency	Breed	Locus	Allele	Frequency
Hereford	*BM2113*	121	0,001	Hereford	*CSSM66*	205	0,001
Hereford	*BM2113*	123	0,007	Hereford	*BM1824*	184	0,004
Hereford	*TGLA53*	174	0,011	Hereford	*BM1824*	190	0,016
Hereford	*TGLA53*	186	0,020	Hereford	*BM1824*	192	0,006
Hereford	*TGLA53*	190	0,001	Hereford	*ETH3*	103	0,004
Hereford	*ETH10*	209	0,010	Hereford	*ETH3*	129	0,010
Hereford	*ETH10*	227	0,001	Hereford	*TGLA126*	113	0,044
Hereford	*CSRM60*	94	0,094	Hereford	*ETH225*	142	0,043
Hereford	*CSRM60*	112	0,003	Hereford	*ETH225*	152	0,003
Hereford	*SPS115*	258	0,009	Hereford	*INRA023*	204	0,031
Hereford	*SPS115*	262	0,003	Hereford	*INRA023*	220	0,014
Hereford	*TGLA122*	137	0,014	Kalmyk	*TGLA53*	178	0,116
Hereford	*TGLA122*	149	0,010	Kalmyk	*TGLA53*	188	0,012
Hereford	*TGLA122*	155	0,009	Kalmyk	*ETH10*	211	0,012
Hereford	*TGLA122*	175	0,004	Kalmyk	*CSRM60*	106	0,035
Hereford	*TGLA122*	177	0,001	Kalmyk	*CSRM60*	110	0,012
Hereford	*TGLA122*	183	0,006	Kalmyk	*TGLA122*	157	0,035
Hereford	*BM1818*	256	0,001	Kalmyk	*TGLA122*	165	0,012
Hereford	*BM1818*	260	0,087	Kalmyk	*TGLA122*	169	0,012
Hereford	*CSSM66*	201	0,001	Kalmyk	*BM1818*	274	0,012
Hereford	*CSSM66*	203	0,001	Kalmyk	*ETH3*	115	0,070
				Kalmyk	*INRA023*	216	0,023

TGLA122 locus. The remaining alleles are found in less than 10% of animals, among which are extremely rare ones – 155, 157, 163, 165, 169, 175, 177, 181. It should be noted that allele 151 in the Hereford breed was detected 13.1% lower compared to the Kalmyk breed ($p \leq 0,05$).

A similar pattern was found at the *TGLA53* locus. The highest allele frequency for this locus in both breeds was observed for allele 160 (33.0–24.4%). Significant breed differences were observed in the two alleles of this locus. In the Hereford breed, the frequency of allele 162 (by 19.0%) and allele 172 (by 15.2%) was higher than in the Kalmyk breed ($p \leq 0,05$, $p \leq 0,01$).

The list of private alleles is presented in Table 7. In the Hereford breed, 32 of them were identified, while in the Kalmyk breed – 11.

CONCLUSIONS

1. The Hereford and Kalmyk breeds have different genesis and differ in phenotype, quantitative, and qualitative traits. However, as our studies have shown, they have minor differences in the genotype and allele frequencies of structural genes and alleles of microsatellite loci. No differences were found between the breeds in the genotype and allele frequencies of the *LEP*, *TG5*, *SCD1*, and *OLR1* genes, with the exception of the *TG5* gene. In the Hereford breed, the $TG5^{CC}$ genotype and $TG5^{C}$ allele are more frequent – by 24.8% and 0.11%, respectively, compared to the Kalmyk breed. The population genetic characteristics of the compared breeds for *SNP* and *STR* markers are comparable in their significance.

2. In the polymorphic loci TGLA122 and TGLA53, both rare and frequently occurring alleles were identified, the values of which in the Hereford and Kalmyk breeds do not have significant differences, with the exception of allele 151 of the TGLA122 locus and alleles 162 and 172 of the TGLA53 locus, which may be a consequence of unidirectional selection or the species-specific structure of the loci.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

Авадани Диана Александровна, младший научный сотрудник, аспирант; SPIN-код 1051-7053

(✉)**Гончаренко Галина Моисеевна**, главный научный сотрудник, доктор биологических наук; SPIN-код 7129-7759; **адрес для переписки**: Россия, 630501, Новосибирская область, р.п. Краснообск, а/я 463; e-mail: gal.goncharenko@mail.ru

Халина Ольга Леонидовна, научный сотрудник; SPIN-код 2829-6687

Хорошилова Татьяна Сергеевна, старший научный сотрудник, кандидат биологических наук; SPIN-код 6629-8399

Гришина Наталья Борисовна, старший научный сотрудник, кандидат биологических наук; SPIN-код 4803-4208

Заборских Елена Юрьевна, старший научный сотрудник, кандидат сельскохозяйственных наук; SPIN-код 1844-8197

Луду Баир Маннаевна, заместитель директора, кандидат биологических наук; SPIN-код 5040-2866

Кан-оол Байлак Киимовна, научный сотрудник; SPIN-код 1137-9860

AUTHOR INFORMATION

Diana A. Avadani, Junior Researcher, Post-graduate Student; SPIN-code 1051-7053

(✉)**Galina M. Goncharenko,** Head Researcher, Doctor of Science in Biology; SPIN-code 7129-7759; **address:** PO Box 463, Krasnoobsk, Novosibirsk Region, 630501, Russia; e-mail: gal.goncharenko@mail.ru

Olga L. Khalina, Researcher; SPIN-code 2829-6687

Tatyana S. Khoroshilova, Senior Researcher, Candidate of Science in Biology; SPIN-code 6629-8399

Natalya B. Grishina, Senior Researcher, Candidate of Science in Biology; SPIN-code 4803-4208

Elena Yu. Zaborskikh, Senior Researcher, Candidate of Science in Agriculture; SPIN-code 1844-8197

Bair M. Ludu, Deputy Director, Candidate of Science in Biology; SPIN-code 5040-2866

Bailak K. Kan-ool, Researcher; SPIN-code 1137-9860

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Сап лошадей: оценка угроз возникновения и распространения (обзор)

(✉)**Беспалова Т.Ю.[1], Михалева Т.В.[1], Блохин А.А.[2]**
[1]*Самарский научно-исследовательский ветеринарный институт – филиал Федерального исследовательского центра вирусологии и микробиологии*
Самара, Россия
[2]*Нижегородский научно-исследовательский ветеринарный институт – филиал Федерального исследовательского центра вирусологии и микробиологии*
Нижний Новгород, Россия
(✉)e-mail: samara@ficvim.ru

Сап является заразным зоонозным и потенциально смертельным заболеванием, вызываемым бактерией *Burkholderia (B.) mallei.* К возбудителю сапа восприимчивы лошади, ослы, мулы, а также человек. В начале – середине XX в. сап был ликвидирован в Европе, Северной Америке, Японии, Австралии и в СССР. В странах Ближнего Востока, Азии, Южной Америки заболевание приобрело эндемичный характер. Развитие международной торговли лошадьми способствовало заносу возбудителя с латентными животными-носителями в свободные от инфекции регионы и в последние десятилетия стали регистрировать вспышки сапа в благополучных странах, в том числе в России, вследствие чего его стали относить к «возвращающимся» инфекциям. Способность возбудителя к заражению в низких дозах в форме аэрозоля обусловливает его причисление к потенциальным агентам биотерроризма. В настоящем обзоре показано современное распространение сапа с учетом увеличения поголовья лошадей в разрезе стран с развитым коневодством и рисков заноса инфекции в Россию с целью повышения информированности ветеринарных служб и усиления мер контроля в отношении данного заболевания.

Ключевые слова: *Burkholderia mallei*, поголовье лошадей, вспышка, эндемичность

Glanders in horses: assessment of threats and spread (review)

(✉)**Bespalova T.Yu.[1], Mikhaleva T.V.[1], Blokhin A.A.[2]**
[1]*Samara Research Veterinary Institute – Branch of the Federal Research Center for Virology and Microbiology*
Samara, Russia
[2]*Nizhny Novgorod Research Veterinary Institute – Branch of the Federal Research Center for Virology and Microbiology*
Nizhny Novgorod, Russia
(✉)e-mail: samara@ficvim.ru

Glanders, a contagious zoonotic and potentially fatal disease, is caused by the bacterium *Burkholderia (B.) mallei*. Horses, donkeys, mules and humans are susceptible to the pathogen. In the early to mid-20th century, glanders was eradicated in Europe, North America, Japan, Australia, and the USSR. In the countries of the Middle East, Asia, and South America, the disease became endemic. The development of international trade in horses contributed to the introduction of the pathogen with latent carrier equids to free regions, and in recent decades, outbreaks of glanders have been registered in disease-free countries, including Russia, as a result of which it began to be classified as a re-emerged disease. The ability of the pathogen to infect at low doses in aerosol form makes it a potential bioterrorism agent. This review shows the current distribution of glanders with regard to the increasing number of horses in countries with developed horse breeding and the risks of infection in Russia in order to increase the awareness of veterinary services and enhanced countermeasures against this disease.

Keywords: *Burkholderia mallei*, equine population, outbreak, endemicity

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INTRODUCTION

Glanders is a highly contagious zoonotic and potentially fatal disease of animals (primarily equids) and humans, subject to mandatory notification to the World Organization for Animal Health (WOAH) [1, 2]. Glanders is caused by the bacterium *Burkholderia* (B.) *mallei*, which belongs to pathogenicity group II. The disease manifests as both acute septicemia and chronic or subclinical forms, characterized by the formation of multiple pustules and granulomas on the mucous membranes and skin, which, when broken down, form ulcers with oily pus. The bacterium causes septic lesions of lungs and other internal organs, muscles, and joints, and often spreads through the lymphatic system to regional lymph nodes [3, 4].

Animals in latent (subclinical) form and asymptomatic carriers play a significant role in the spread of the pathogen, since due to the constant or periodic release of bacteria into the environment, they are a potential source of infection [1].

The disease has been known since the time of Hippocrates and was extremely widespread throughout the world until the end of the 19th century. In the 20th century, thanks to strict application of testing and national control programs, the prevalence of glanders worldwide has significantly decreased [5], and in developed countries of Europe, North America, Japan, Australia and the USSR glanders was eliminated [2, 4]. Currently, the disease persists in the regions of Africa, Asia, the Near and Middle East, Central and South America, mainly in endemic areas [6]. It is worth noting that in recent years, a number of Asian countries have adopted a policy of increasing their horse population, including through imports [7]. When horses are moved through international trade, the risk of spreading *B. mallei* from endemic to non-endemic areas with subclinically infected animals also increases [3]. It is alarming that over the past two decades, sporadic outbreaks of glanders have begun to be recorded in the areas where it was previously unknown or eradicated, as a result of which it has come to be classified as a re-emerging disease [2, 8]. Thus, after the eradication of glanders in the late 1950s, this disease has been repeatedly reported in Russia; in early 2023, an outbreak of equine glanders was recorded by the WOAH in the Trans-Baikal Territory[1] [4, 9].

Glanders causes significant economic damage to countries with developed horse breeding industries. When detected, restrictions on international trade are imposed. Many countries implement policies to test and destroy all infected horses, and quarantine them. Glanders impedes the development of the livestock sector and equestrian sports, hindering the trade of purebred horses and the holding of international competitions.

Research aimed at understanding the pathogenesis and host response to *B. mallei* infection remains limited. However, it has been established that a key feature of virulent *B. mallei* is its ability to persist in both extracellular and intracellular spaces, thereby evading the host immune response [3, 10].

Timely diagnosis of glanders is the key to managing the spread of the disease and is challenging due to latent carriage and the absence of specific clinical signs in the early stages of the

[1]Order of the State Veterinary Service of the Trans-Baikal Territory dated February 18, 2023, No. 24: URL: http://publication.pravo.gov.ru/document/7501202302200003 (date of reference: 15.10.2023).

disease [11].

Treatment of sick animals is complicated by the inherent multidrug resistance of *Burkholderia* to common antimicrobials. Specific prophylaxis for glanders in both horses and humans is not yet available [2], but active research into various types of vaccine candidates is underway worldwide [12].

The purpose of this study is to examine the current epizootic situation of glanders worldwide, taking into account the increasing horse population in countries with developed horse breeding and the risk of infection introduction into Russia. Data synthesis may contribute to increased awareness and professional vigilance among veterinary services to strengthen disease control measures.

MATERIAL AND METHODS

This study included a review of international and domestic scientific publications over the past 10 years, obtained using the following databases: PubMed, Scopus, Springer, Wiley Online Library, Google Scholar, and the Russian Science Citation Index (RSCI). The search was conducted using the following keywords: glanders, Burkholderia mallei, horse population, outbreak, and endemicity.

The horse population analysis in Russia was conducted using the data from the Federal State Statistics Service (Rosstat)[2] and the Information and Analytical Center of the Federal Service for Veterinary and Phytosanitary Surveillance[3]. To assess the prevalence of equine glanders, a literature review was conducted, and the data from the World Organization for Animal Health (WOAH) and the Russian Federation veterinary reporting were examined.

RESULTS AND DISCUSSION

Etiology and epizootology of glanders

Since ancient times, humanity has used biological weapons, which involve the mass destruction of living organisms or the contamination of inanimate objects using bacteria, viruses, or the toxins they produce. A potential biological threat is posed by pathogens that are highly virulent, have a low infectious dose, the ability to form aerosols, and are survivable in a variety of environmental conditions. The Biological Weapons Convention included the bacterium *Burkholderia mallei*, the causative agent of glanders, in its list of pathogens due to its high infectious doses from aerosols at low doses and is currently classified as a Category B potential bioterrorism agent [6, 13].

The causative agent of glanders is the bacterium *Burkholderia mallei* from the family Burkholderiaceae, which is closely related genetically to the causative agent of melioidosis, *Burkholderia pseudomallei* [6]. As an obligate intracellular pathogen of mammals, *B. mallei* does not survive long outside the host organism and, even under favorable conditions of heat and humidity, cannot survive for more than 100 days. Under most conditions, the probability of its survival in environmental objects does not exceed 2 weeks [2, 3].

In addition to members of the Equidae family, other animal species and humans are susceptible to *B. mallei*. Horses are considered the natural reservoir of infection, while humans are an accidental, but highly susceptible, host. Tigers, lions, and leopards (in zoo conditions) are sometimes susceptible. Susceptibility to glanders has been demonstrated in camels, bears, wolves, domestic cats, and dogs. Carnivores can become infected by consuming contaminated meat. Goats can become infected through close contact with infected horses. Cattle and pigs are resistant to this disease, but experimentally, glanders infection can be induced in them when infected with large doses of the pathogen. Birds, cold-blooded animals, and insects are immune to glanders; the pathogen persists in their bodies for 2–65 days, which indicates an additional or accidental habitat for *B. mallei* and does not

[2]Federal State Statistics Service Rosstat: URL: https://rosstat.gov.ru/storage/mediabank/Munst.htm (date of reference: 09.02.2024).

[3]Information and Analytical Center of the Federal Service for Veterinary and Phytosanitary Surveillance: URL: https://fsvps.gov.ru/sites/default/files/files/iac/atlas_ pogolove_2021.pdf (date of reference: 15.01.2024).

exclude the potential for infection transmission [3, 6, 14, 15]. In humans, glanders is rare among individuals in direct contact with sick animals or infected material (veterinarians, livestock specialists, grooms, slaughterhouse workers), and occupational infection is potentially considered a key risk factor. Less commonly, infection can occur through inhalation (laboratory cases), as well as through alimentary contact (as a result of consuming contaminated horse meat); human-to-human transmission of *B. mallei* is possible [3, 6, 16, 17].

The most common sources of infection are feed or water contaminated with respiratory secretions or ulcerated skin lesions from sick animals. In chronic and subclinical forms, the pathogen is periodically or continuously excreted in feces, saliva, and milk, contaminating the environment [18]. Transmission between the animals occurs through close contact, inhalation, and ingestion of contaminated material. Outbreaks most often occur where horses are housed together and share feeding and watering areas. Unsanitary conditions and overcrowded housing facilitate spread. The pathogen is easily spread through contaminated horse care items (harness and grooming tools). There are reports of *B. mallei* being transmitted sexually and in utero [6]. It is noted that in most cases the infection does not spread beyond the farm or group of animals raised together; spread to other areas occurs only through the movement of infected animals or fomites [2].

Course, clinical signs and laboratory diagnostics

The incubation period in horses is usually 2–6 weeks, but varies from 6 days to 6 months, which significantly complicates determining the source of infection during outbreak investigation [5, 19]. The course of the disease largely depends on the animal species, the pathogen dose, the route of infection, the state of the immune system (activation of innate and adaptive immunity), etc. [10]. One population of horses may have different levels of susceptibility to *B. mallei* [3]. In horses with chronic infection, immune studies have revealed strong humoral and proinflammatory cytokine responses [20].

Glanders can be classified into acute, chronic, and latent (asymptomatic carrier) forms. In horses, chronic (up to 12 weeks) and latent (up to several years) forms are more common. Stressful factors (changes in housing conditions, transporting animals to different environmental conditions, starvation, poor feeding, increased exploitation, etc.) can trigger the transition from latent glanders to an acute form. Depending on the primary sites of infection, the following clinical forms of glanders are distinguished: nasal, pulmonary, focal cutaneous, and septicemic. A combination of these forms is often observed simultaneously in a single animal; the pulmonary form can be a continuation of the nasal form and is observed in almost all clinical cases. Importantly, in chronic cases, symptoms are usually minimal and go unnoticed, most often manifesting as mild intermittent fever, nasal discharge, and intermittent shortness of breath [21]. As the disease (septicemia) progresses, animals experience deterioration, exhaustion, and general weakness. Under stress, lung lesions with signs of acute bronchopneumonia may develop. Swelling of the hind limbs, joint swelling, subsequent lameness, orchitis, hematuria, and diarrhea are possible. Exacerbation of nasal and cutaneous forms of glanders may occur [3].

It is important to note that characteristic symptoms of glanders in sick animals appear in the late stages of the disease, making early diagnosis difficult. Laboratory diagnostics of glanders in humans and animals utilizes a variety of methods, depending on the research objectives. For confirming clinical cases, the WOAH considers the isolation of B. mallei from clinical specimens (microbiological culture) and PCR (conventional or real-time PCR) to be the "gold standards" [5]. In monitoring studies and eradication programs, the serological complement fixation test (CFT) is the preferred method. This method can also be used to determine the infection status of animals, including before and after transport of horses in international trade, but is less suitable for confirming clinical cases of glanders [21]. However, the CFT can give false negative results, and since the method is

regulated in monitoring programs, when importing and exporting animals, this creates certain risks for the international movement of horses and can lead to the introduction of *B. mallei* into safe areas [3, 22]. Methods such as *B. mallei* – lipopolysaccharide (LPS) – Western blot, indirect I-enzyme-linked immunosorbent assay (ELISA) or C-ELISA (competitive ELISA) (based on monoclonal antibodies specific for *B. mallei*) have higher sensitivity and specificity, so they are recommended for confirmation of positive CFT results [6, 22]. The combined use of serological and molecular methods increases the detection rate of glanders and expands the possibilities for epizootological investigations [1, 23].

Distribution and risk assessment of equine glanders

An objective determination of the population size of susceptible animals in the infection risk zone is necessary for assessing the risk and predicting the potential spread of glanders in the equine population. The likelihood of new outbreaks is greatest in the regions with a high density of susceptible animals. As horse exports and imports increase, so does the risk of glanders outbreaks. In recent years, the world leaders in the number of horses have been countries such as the United States, Mexico, Brazil, Mongolia and China, with serologically positive animals being detected relatively frequently in Brazil and Mongolia, and outbreaks of glanders being periodically recorded in China [7, 23, 24]. The dynamics of the horse population in Russia demonstrate that horse breeding remains an important sector of the economy. In the Russian Federation, horse populations varied across farm categories from 2020 to 2022: agricultural organizations saw a decrease of 21,000 horses, while households and peasant farms saw increases of 16,000 and 13,000, respectively [25]. The horse population analysis in Russia based on data from the Federal State Statistics Service (Rosstat) for 2022, presented for farms of different categories at the level of administrative regions, showed that over the past seven years (from 2015 to 2022), there has been a trend towards an increase in the horse population in the Jewish Autonomous Region, Transbaikal Territory, and the Republics of Altai, Bashkortostan, Dagestan, and Tyva. At the beginning of 2022, according to Rosselkhoznadzor, the maximum number of horses (up to 183,000 heads) was registered in the Transbaikal Territory, the Republics of Altai, Bashkortostan, Sakha, and Tyva, and up to 55,500 heads in the Republics of Dagestan, Tatarstan, Khakassia, and Buryatia, Altai Krai, and Astrakhan, Irkutsk, Orenburg, and Omsk regions. Most of the named regions border neighboring countries in Asia and the Middle East, where outbreaks of glanders have been reported. Specifically, these are first- and second-tier neighboring countries such as Armenia, India, Iran, China, Turkey, Mongolia, and Nepal (see table). Iran and Brazil are officially recognized by the WOAH as endemic for glanders [21]. There are reports of endemicity in Mongolia, India, Iraq, and Pakistan [2, 24, 26].

Glanders was a global problem for several centuries, but by the early 20th century the disease had been eradicated in most European countries, Japan, North America, and Australia through mandatory testing and subsequent destruction of all positive animals, as well as through import control measures within national government programs [18]. In the USSR, glanders was eradicated in the 1940s–1950s [4]. Since the mid-20th century, the greatest prevalence of glanders has been observed in Asian countries; only isolated cases, usually associated with the import of imported horses, have been recorded in Europe, South America and Africa [4, 7, 18]. However, in the last 10–20 years, reports have begun to appear of sporadic outbreaks of glanders in disease-free countries. Since 2006, equine glanders have been registered in India, and since 2009 – in Brazil [26]. On the European continent, a single case of glanders was reported in Germany in 2014 and 2015 [27]. In Asia and the Middle East, outbreaks were registered in Bahrain in 2010–2012, Afghanistan in 2011, Turkey in 2017, and Kuwait in 2019. In China, the first outbreaks of glanders were recorded in 2018, Nepal in 2020, and Armenia in 2023 (see the figure) [18].

In Russia, six decades after its eradication,

Распространение сапа лошадей в мире (за 10 лет)
Distribution of glanders in horses in the world (over 10 years)

Name of the country	Year									
	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
Armenia										+
Brazil	+	+	+	+	+	+	+	+	+	
Ghana					+	+				
Germany	+	+								
India	+	+	+	+	+	+	+	+	+	
Iraq	+									
Iran	++	+	+	+	+	+	+	+	+	+
China					+	+				
Kuwait						+				
Mongolia					+					
Nepal							+	+	+	+
Pakistan	+	++								
Russia										+
Turkey				+		+	+			
Eritrea						+	+	+		

Note. Sources: World Organization for Animal Health Interface of the World Organization for Animal Health Database http://www.oie.int/wahis/public.php?page=home.

glanders has been reported repeatedly, according to various sources, but official data is virtually nonexistent. For example, there are reports of the disease in the Chita Region (now the Trans-Baikal Territory) in 2007, in the Amur Region and the Krasnodar Territory in 2013, and according to archival data, six horses with glanders were registered in the 2014 Trans-Baikal Territory statistical reporting form 1-vet. However, malleinization and serological testing yielded negative results (outbreaks have not been officially registered in the Russian Federation)[4] [4]. In 2020, due to the registration of equine glanders in the Republic of Buryatia, the region's status was changed to unfavorable for this disease [5].

In early 2023, the WOAH registered an outbreak of glanders at the Chita State Stud Farm (Trans-Baikal Territory). Two of the six horses infected died. To contain the outbreak, the state veterinary service seized and destroyed all affected and exposed livestock (up to 40 horses) and any animal products derived from them. Restrictive measures were introduced in accordance with current Veterinary Regulations[6]. In early June 2024, Rosselkhoznadzor (Federal Service for Veterinary and Phytosanitary Surveillance) reported a suspected case of equine glanders in the same Trans-Baikal Territory[7].

In general, when assessing the risk of introducing the glanders pathogen into Russia, taking into account endemicity, regularly occurring

[4]Information and Analytical Center of the Federal Service for Veterinary and Phytosanitary Surveillance: URL: https://fsvps.gov.ru/importexport/turciya/spisok-rossiyskih-predpriyatiy-104/ (date of reference 03.03.2024).

[5]Information and Analytical Center of the Federal Service for Veterinary and Phytosanitary Surveillance: URL: https://fsvps.gov.ru/sites/default/files/npa-files/2020/08/05/ regionalization_dif_2020-08-05.pdf (date of reference 03.03.2024).

[6]Veterinary rules for the implementation of preventive, diagnostic, restrictive and other measures, the establishment and cancellation of quarantine and other restrictions aimed at preventing the spread and eliminating glanders outbreaks (Approved by Order of the Ministry of Agriculture of Russia dated June 28, 2017 No. 311): URL: https://docs.cntd.ru/document/456078970 (date of reference: 10.02.2024).

[7]Information and Analytical Center of the Federal Service for Veterinary and Phytosanitary Surveillance: URL: https://fsvps.gov.ru/wp-content/uploads/2024/06/05.06.2024 г. pdf (date of reference: 07.06.2024).

outbreaks and in connection with international trade, the following countries, which have close economic ties with Russia, currently pose the greatest danger as potential sources: Brazil, Mongolia, China, India, Iran, Turkey.

Basic control measures. Treatment

Prevention and control of glanders epizootics depend on the program of early detection and humane destruction of test-positive animals, combined with strict animal movement controls, effective quarantine at the facilities, and thorough disinfection of the infected area where the outbreak has occurred [2, 6]. It is important that meat from the animals suspected of or infected with glanders is not fed to other animals. In free countries, measures to combat the introduction of glanders include screening the imported horses, which must be quarantined until all testing is completed and for at least 14 days, during which time any latent infection may become clinically apparent due to the stress of transport [3]. Other measures include clinical surveillance and laboratory testing of suspected cases [2].

Treatment of infected animals is used in endemic areas; without it, mortality can reach 95% [2]. It has been established that *B. mallei* has multiple genes for resistance to antibiotics (e.g., fluoroquinolones, macrolides, aminoglycosides) [2, 3]. A number of *B. mallei* strains showed different *in vitro* susceptibility to various antibiotics, including sulfadiazine, tetracyclines, neomycin and erythromycin, cephalosporins, carbapenems, etc. [28]. Antibiotic therapy typically results in a lengthy, aggressive, and not always effective treatment [10]. Treatment is not recommended during a glanders outbreak, as the use of antimicrobials, although it may reduce mortality, contributes to the survival of carrier animals, which does not allow for the eradication of the disease. Regarding the specific prevention of glanders in animals and humans, there are currently no commercial vaccines against *B. mallei*, and no ideal vaccine candidate has yet been identified [6]. Although significant progress has been made in their development in recent years, various vaccine candidates are being studied in the preclinical stage, ranging from heat-killed vaccines, live attenuated vaccines, subunits, and nanoparticle conjugates. Vector-based technologies and DNA vaccines are also worthy of consideration in the future [12].

CONCLUSION

Thus, given the increase in the horse population in regions of the Russian Federation located near first- and second-order neighboring countries, both endemic and with registered sporadic outbreaks of glanders, as well as in connection with the new emergence of cases of equine glanders in Russia, it is necessary to focus the attention of veterinary services to strengthen measures to control this disease.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

(✉)**Беспалова Татьяна Юрьевна,** заместитель руководителя группы; ORCID 0000-0002-0264-0218, SPIN-код 7891-9117; e-mail: samara@ficvim.ru; **адрес для переписки:** Россия, 443013, Самара, ул. Магнитогорская, 8

Михалева Татьяна Владимировна, ученый секретарь, кандидат ветеринарных наук; ORCID 0000-0001-6411-6027, SPIN-код 5920-2842

Блохин Андрей Александрович, ведущий научный сотрудник, кандидат ветеринарных наук; ORCID 0000-0001-5161-1184, SPIN-код 1178-6337

AUTHOR INFORMATION

(✉)**Tatiana Yu. Bespalova,** Deputy Head of the Group; ORCID 0000-0002-0264-0218, SPIN-code 7891-9117; **address:** 8, Magnitogorskaya St., Samara, 443013, Russia; e-mail: samara@ficvim.ru

Tatiana V. Mikhaleva, Academic Secretary, Candidate of Science in Veterinary Medicine; ORCID 0000-0001-6411-6027, SPIN-code 5920-2842

Andrey A. Blokhin, Lead Researcher, Candidate of Science in Veterinary Medicine; ORCID 0000-0001-5161-1184, SPIN-code 1178-6337

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Влияние фитобиотика на иммунологическую резистентность цыплят-бройлеров

(✉)**Лыско С.Б., Гофман А.А., Задорожная М.В., Сунцова О.А., Власенко В.С.**
Омский аграрный научный центр
Омск, Россия
(✉)e-mail: zamdir@sibniip.ru

Авторами изучено влияние фитобиотика на основе комплекса эфирных масел на клеточные и гуморальные звенья иммунной системы цыплят-бройлеров на фоне вакцинации поголовья против вируса Ньюкаслской болезни. Исследование проводили на цыплятах-бройлерах отечественного кросса «Смена 9» (опытная и контрольная группы по 70 гол.) на птицеводческом предприятии Омской области. Цыплята опытной группы с водой дополнительно получали российский фитопрепарат «Фитогеникс», состоящий из комплекса эфирных масел корицы, гвоздики, розмарина, тимьяна, кунжута индийского и органических кислот, в дозе 0,2 мл/л воды с 1-го по 7-й день и 0,3 мл/л воды с 8-го по 38-й день. В возрасте 9 дней проведена вакцинация птицы против вируса Ньюкаслской болезни. Через 6 и 18 дней после вакцинации осуществлен отбор проб крови для изучения показателей клеточного и гуморального иммунитета. Установлено, что большее влияние фитобиотик оказал на показатели клеточного иммунитета через 6 дней после вакцинации, что выражалось повышением активности катионных белков в 1,3 раза, уровня спонтанной НСТ-активности нейтрофилов – на 17,7%, общего количества лимфоцитов – в 2,1 раза, Т-лимфоцитов – в 3,0 раза, В-лимфоцитов – в 1,8 раза. На гуморальное звено иммунитета наибольшее влияние было оказано через 18 дней после вакцинации, что сопровождалось увеличением бактерицидной активности сыворотки крови на 23,4%, среднего титра поствакцинальных антител (иммуноглобулины G) – на 1,1 $\log^2$. Стимуляция естественной резистентности и адаптивного иммунитета при вакцинации на фоне применения комплекса эфирных масел положительно отразилась на жизнеспособности и продуктивности птицы, способствовала повышению сохранности цыплят-бройлеров на 4,3%, увеличению их живой массы на 3,4%. С учетом полученных результатов использованный фитопрепарат может быть рекомендован для повышения иммунологической резистентности, жизнеспособности и продуктивности цыплят-бройлеров.

Ключевые слова: фитобиотик, эфирные масла, цыплята-бройлеры, вакцинация, иммунитет, Т-лимфоциты

The effect of phytobiotics on the immunological resistance of broiler chickens

(✉)**Lysko S.B., Hoffman A.A., Zadorozhnaya M.V., Suntsova O.A., Vlasenko V.S.**
Omsk Agrarian Scientific Center
Omsk, Russia
(✉)e-mail: zamdir@sibniip.ru

The authors studied the effect of a phytobiotic based on a complex of essential oils on the cellular and humoral links of the immune system of broiler chickens against the background of vaccination against the Newcastle disease virus. The study was conducted on broiler chickens of the domestic cross "Smena 9" (experimental and control groups of 70 heads each) at a poultry enterprise in the Omsk region. Chickens of the experimental group with water additionally received a domestic phytopreparation "Phytogenics" consisting of a complex of essential oils of cinnamon, cloves, rosemary, thyme, Indian sesame and organic acids in a dose of 0.2 ml/l of water at the age of 1–7 days and 0.3 ml/l of water from day 8 to 38. At the age of 9 days, the poultry were vaccinated against the Newcastle disease virus. Blood samples were taken 6 and 18 days after the vaccination to study the indicators of cellular and humoral immunity. It was found that the phytobiotic had a greater effect on cellular immunity 6 days after the vaccination, which was expressed by an increase in the activity of cationic proteins 1.3 times, the level of spontaneous NBT activity of neutrophils by 17.7%, the total

number of lymphocytes 2.1 times, T lymphocytes 3.0 times, B lymphocytes 1.8 times. The greatest impact on the humoral link of immunity was exerted 18 days after vaccination, which was accompanied by an increase in the bactericidal activity of blood serum by 23.4%, the average titer of post-vaccination antibodies (immunoglobulins G) – by 1.1 $\log^2$. Stimulation of natural resistance and adaptive immunity during vaccination against the background of the use of a complex of essential oils had a positive effect on the viability and productivity of poultry, contributed to an increase in the safety of broiler chickens by 4.3%, live weight by 3.4%. Taking into account the obtained results, the used herbal preparation can be recommended for increasing the immunological resistance, viability and productivity of broiler chickens.

Keywords: phytobiotic, essential oils, broiler chickens, vaccination, immunity, T lymphocytes

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INTRODUCTION

Vaccination is one of the primary methods for ensuring the epizootic safety of industrial poultry farms. The effectiveness and intensity of adaptive immunity depend not only on the quality of the vaccines and the correct selection of the vaccination schedule, but also on the state of the bird's immune system [1]. It should be noted that the immune system of chickens is formed in the first two weeks of life, with vaccinations beginning from the first days of life, and by two weeks of age, a chicken receives an average of three to five live vaccines. Such an antigen load inevitably affects the bird's immunological resistance. Furthermore, the presence of numerous stress factors, which are essential to modern poultry farming, also has a negative impact on the immune system of birds [2–4].

In order to increase natural resistance, adaptive immunity and resistance to infectious diseases, researchers propose using various drugs as immunocorrective agents: chemical (synthetic immunostimulants, vitamins, amino acids, mineral complexes, etc.), microbiological (probiotics, symbiotics, metabiotics, etc.), plant (phytobiotics) [5–8].

The advantages of herbal preparations are their naturalness and environmental friendliness, as well as their complex effect on the body (antimicrobial, antiviral, immunomodulatory, anti-inflammatory, antifungal, etc.) due to the content of a large number of various biologically active substances [9]. Phytobiotics in poultry farming are most often recommended for use as feed additives to increase livestock productivity and improve product quality [10–12]. In addition, scientific literature contains information on the positive effects of herbal preparations, including essential oils, on the immune system of animals and poultry due to increased lymphocyte activity, cellular and humoral immunity, immunoglobulin production, and interferon release [13–15]. In this regard, the creation of herbal preparations and the scientific justification for

their use in order to increase the immunological resistance of poultry are pressing issues in modern poultry farming, and essential oils and preparations based on them can be an effective solution.

The purpose of the study was to investigate the effect of a phytobiotic based on a complex of essential oils on the immunological resistance of broiler chickens when vaccinated against the Newcastle disease (ND) virus.

The research objectives consist in:

1) determining the effect of a preparation from a complex of essential oils on the cellular and humoral links of the immune system of broiler chickens against the background of vaccination;

2) determining on which immunity indicators the herbal preparation had the greatest impact during the period of the beginning and end of the immunological restructuring of the body after the introduction of an antigen (vaccine);

3) studying the effect of the herbal preparation on the viability and productivity of poultry.

MATERIAL AND METHODS

The study was conducted in 2023 at the Veterinary Department of the Siberian Research Institute of Poultry Farming and at a poultry farm in the Omsk Region. Day-old broiler chickens of the domestic "Smena 9" cross were selected based on the live weight, and were divided into control and experimental groups of 70 birds each. The poultry rearing period was 38 days. Feeding and housing conditions complied with the guidelines for handling this crossbred bird. The experimental group chickens were additionally treated with the Russian herbal preparation "Phytogenics," which consists of organic acids and a complex of essential oils from the following plants: *Cinnamomum zeylanicum* (cinnamon), *Syzygium aromaticum* (clove), *Rosmarinus officinalis* (rosemary), *Thymus vulgaris* (thyme), *Sesamum indicum* (Indian sesame). The phytopreparation was used at a dose of 0.2 ml/l of water at the age of 1–7 days and 0.3 ml/l of water at the age of 8–38 days.

All birds were vaccinated against Newcastle disease virus at 9 days of age using a live vaccine from the La Sota strain produced by the RPE "Avivak" (Russia). Six and 18 days after vaccination, which corresponded to the onset and end of the body's immunological restructuring following antigen administration, blood samples were collected from the axillary vein to study cellular and humoral immunity. For immunological studies, blood was collected in heparinized tubes, and serum was collected in tubes moistened with saline.

Oxygen-independent leukocyte activity was assessed by the amount of lysosomal cationic proteins (CP) using the method of M.G. Shubich, and neutrophil oxidase activity was assessed using the nitroblue tetrazolium test (NBT test) in both spontaneous and stimulated variants. Total lymphocyte counts were calculated in blood smears stained according to Pappenheim. The number of T lymphocytes, cytotoxic T lymphocytes (CTL), and B lymphocytes was determined using the spontaneous, indirect globulin, and complement rosette formation tests*. The blood serum bactericidal activity (BSBA) was studied according to the method of O.V. Smirnov and T.A. Kuzmin using an ELx800 photometer. The titer of post-vaccination antibodies (immunoglobulins G) to the ND virus was determined in the hemagglutination inhibition reaction using a test system developed by the Federal Centre for Animal Health (ARRIAH).

Statistical processing of the experimental data was performed using IBM SPSS Statistics. v23.1. Analysis of variance (ANOVA) was used to assess the effect of the phytobiotic (η^2) on cellular and humoral immunity. A difference was considered statistically significant at $p < 0,05–0,001$.

RESULTS AND DISCUSSION

The increase in lysosomal phagocytic activity of blood leukocytes in broiler chickens of the experimental group was indicated by a statistically significant increase in the number of CP

*Guidelines for biochemical and immunological methods for studying cells, their components and other biological substrates / prepared by: G.F. Koromyslov et al. Moscow: VIEV, 1980, 40 p.

6 and 18 days after vaccination by 1.3 and 1.9 times, respectively, compared to the control (see Table 1).

An increase in the oxidase activity of neutrophils was also noted 6 days after vaccination, with an increase in the level of spontaneous NBT activity by 17.7%, which can be associated with the body's response to the introduction of the antigen (vaccine) and the activation of antimicrobial systems.

Immunological changes in broiler chickens in the experimental group 6 days after antigen administration were accompanied by an increase in the total lymphocyte count by 2.1 times, T-lymphocytes by 3.0 times, CTLs by 1.2 times, and B-lymphocytes by 1.8 times compared to the control group. Eighteen days after vaccination, no significant differences in the content of immunocompetent cells were found between the experimental and control groups.

Thus, the use of a herbal preparation based on a complex of essential oils contributed to the activation of cellular immunity in broiler chickens in the initial period of formation of a post-vaccination immune response.

The positive effect of the herbal preparation on the humoral component of immunity was indicated by an increase in the bactericidal activity of the blood serum in chickens of the experimental group 6 and 18 days after vaccination by 5.3 and 23.4%, respectively, an increase in the average titer of post-vaccination immunoglobulins G by 0.4 and 1.1 $\log^2$ with a statistically significant difference 18 days after vaccination.

It was found that during the initial immune response to the vaccine (6 days after vaccination), the herbal preparation had a greater effect on cellular immunity, primarily on the total lymphocyte count, T- and B-lymphocytes, and to a lesser extent on the CP, as measured by the spontaneous and stimulated NBT test. Eighteen days after vaccination, a greater effect was observed

Табл. 1. Влияние фитопрепарата на показатели клеточного и гуморального иммунитета цыплят-бройлеров при вакцинации против вируса Ньюкаслской болезни

Table 1. The effect of phytopreparation on the cellular and humoral immunity of broiler chickens during vaccination against Newcastle disease virus

Indicator	Post-vaccination period, days	Group	
		control	experimental
CP, c.u.	6	0,96 ± 0,07	1,23 ± 0,09*
	18	1,33 ± 0,09	2,00 ± 0,08***
Spontaneous NBT-test, c.u. rd. pl.	6	0,79 ± 0,03	0,93 ± 0,03*
	18	0,49 ± 0,02	0,43 ± 0,03
Stimulated NBT-test, c.u. rd. pl.	6	0,79 ± 0,05	1,04 ± 0,06*
	18	0,56 ± 0,02	0,47 ± 0,02*
Lymphocytes, thous./μl	6	3,27 ± 0,34	7,01 ± 0,49***
	18	6,03 ± 0,33	6,18 ± 0,29
T-lymphocytes, thous./μl	6	0,56 ± 0,13	1,70 ± 0,15***
	18	1,08 ± 0,12	1,12 ± 0,08
CTL, thous./μl	6	1,27 ± 0,17	1,48 ± 0,09
	18	1,05 ± 0,12	0,95 ± 0,22
B- lymphocytes, thous./μl	6	0,74 ± 0,07	1,30 ± 0,10**
	18	1,18 ± 0,15	1,24 ± 0,21
BSBA, %	6	27,10 ± 5,30	32,40 ± 3,30
	18	34,20 ± 5,30	57,60 ± 3,00**
Average titer of immunoglobulins G to ND virus, $\log^2$	6	3,31 ± 0,39	3,75 ± 0,38
	18	4,50 ± 0,28	5,56 ± 0,40*

*$p < 0,05$.
**$p < 0,01$.
***$p < 0,001$.

on humoral immunity parameters (BSBA, mean immunoglobulin G titer), and a significant effect on the CP was also maintained (see Table 2).

Our findings, demonstrating the stimulating effect of phytobiotics on the immune system, are consistent with the data from other researchers. For example, feeding poultry the phytobiotic "Farmatan" increased the natural resistance of broiler chickens, as evidenced by an increase in serum bactericidal and lysozyme activity by 14.0% and 6.9%, respectively, and in leukocyte phagocytic activity by 4.73% [10]. The use of a pine needle-based supplement increased the natural resistance of broiler chickens: bactericidal activity of blood serum by 20.9–27.7%, lymphocytes by 1.4–1.5 times, T-lymphocytes by 2.1 times, B-lymphocytes by 2.2 times, as well as the adaptive immunity to the ND virus [13]. The use of the feed additive based on essential oils "Intebio®" contributed to an increase in the expression of interleukin genes in broiler chickens both in the intact (3.5–5.0 times) and in the *Salmonella enteritidis*-infected groups (4–6 times), which indicates an increase in the immune status of the bird [14].

Stimulation of natural resistance and adaptive immunity during vaccination against the ND virus, combined with the use of a complex of essential oils, had a positive effect on the viability and productivity of poultry. The survival rate of broiler chickens in the experimental group during the growing period exceeded that of the control group by 4.3%. Live weight of broiler chickens in the experimental group exceeded that of the control group by 0.4–3.7% throughout the study (see Table 3).

A statistically significant increase in the live weight of the experimental group representatives was recorded at 21 and 28 days of age (+ 3.5 and 3.7%, respectively), which coincided with the period of formation of adaptive immunity, as well as at the end of the growing period at the age of 38 days (+ 3.4%).

The average daily feed consumption and feed costs per kilogram of product in the experimental group were lower than in the control group by 2.17 g/head and 0.08 kg and amounted to 107.61 g/head and 1.52 kg, respectively. The European productivity index in the experimental group was 57 units higher than in the control group and amounted to 467 units.

CONCLUSION

The obtained data indicate a stimulating effect of the herbal preparation " Phytogenics" based on a complex of essential oils on the immunological resistance of broiler chickens against the background of vaccination against the ND virus.

Табл. 2. Доля влияния фитопрепарата на показатели клеточного и гуморального иммунитета цыплят-бройлеров при вакцинации против вируса Ньюкаслской болезни (η^2)

Table 2. The proportion of the effect of phytopreparation on the cellular and humoral immunity of broiler chickens during vaccination against Newcastle disease virus (η^2)

Indicator	Post-vaccination period, days			
	6		18	
	η^2	*p*	η^2	*p*
CP, c.u.	0,405	0,048*	0,792	0,001*
Spontaneous NBT-test, c.u. rd. pl.	0,560	0,013*	0,251	0,140
Stimulated NBT-test, c.u. rd. pl.	0,506	0,020*	0,530	0,017*
Lymphocytes, thous./μl	0,830	0,001*	0,140	0,742
T-lymphocytes, thous./μl	0,811	0,001*	0,009	0,793
CTL, thous./μl	0,130	0,306	0,020	0,698
B- lymphocytes, thous./μl	0,723	0,002*	0,006	0,837
BSBA, %	0,510	0,532	0,699	0,003*
Average titer of immunoglobulins G to ND virus, $\log^2$	0,064	0,163	0,335	0,001*

*$p < 0,05–0,001$.

Табл. 3. Изменение живой массы цыплят-бройлеров на фоне применения фитопрепарата, г
Table 3. Live weight of broiler chickens against the background of the use of phytopreparation, g

Age of broiler chickens, days	Group	
	control	experimental
1	43,2 ± 0,4	43,4 ± 0,4
7	166,7 ± 2,1	168,1 ± 2,1
14	486,1 ± 14,1	489,3 ± 2,1
21	911,7 ± 14,1	943,2 ± 6,3*
28	1519,8 ± 24,6	1575,0 ± 13,3*
35	2270,8 ± 34,3	2289,0 ± 27,4
38	2607,1 ± 38,3	2695,5 ± 23,5*

*$p < 0,05$.

The phytobiotic had a greater effect on the indices of cellular immunity ($\eta^2 = 0.405–0.830$, $p < 0.05–0.001$) in the initial period of formation of adaptive immunity (6 days after vaccination), which was expressed by an increase in the activity of cationic proteins by 1.3 times, the level of spontaneous NBT activity by 17.7%, the total number of lymphocytes by 2.1 times, T-lymphocytes by 3.0 times, B-lymphocytes by 1.8 times. A significant effect on the humoral component of immunity was also recorded 18 days after the vaccination ($\eta^2 = 0,335– 0,699$, $p < 0,05–0,001$), which was accompanied by an increase in the bactericidal activity of blood serum by 23.4% and the average titer of post-vaccination antibodies (immunoglobulin G) by 1.1 $\log^2$.

The use of the herbal preparation contributed to an increase in the survival rate of broiler chickens by 4.3%, live weight by 3.4%, and the European productivity index by 57 units.

Thus, a herbal preparation based on essential oils can be recommended for increasing the immunological resistance, viability and productivity of broiler chickens.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

(✉)**Лыско Светлана Борисовна,** главный научный сотрудник, доктор ветеринарных наук; SPIN-код 2247-1909; **адрес для переписки:** Россия, 644555, Омская область, с. Морозовка, ул. 60 лет Победы, 1; e-mail: zamdir@sibniip.ru

Гофман Алёна Андреевна, старший научный сотрудник, кандидат ветеринарных наук; SPIN-код 8768-6208

Задорожная Марина Валерьевна, ведущий научный сотрудник, кандидат ветеринарных наук; SPIN-код 1185-4997

Сунцова Ольга Александровна, ведущий научный сотрудник, кандидат ветеринарных наук; SPIN-код 5246-0463

Власенко Василий Сергеевич, главный научный сотрудник, доктор биологических наук; SPIN-код 8354-6130

AUTHOR INFORMATION

(✉)**Svetlana B. Lysko,** Head Researcher, Doctor of Science in Veterinary Medicine; SPIN-code 2247-1909; **address:** 1, 60 Let Pobedy St., Morozovka, Omsk Region, 644555, Russia; e-mail: zamdir@sibniip.ru

Alyona A. Hoffman, Senior Researcher, Candidate of Science in Veterinary Medicine; SPIN-code 8768-6208

Marina V. Zadorozhnaya, Lead Researcher, Candidate of Science in Veterinary Medicine; SPIN-code 1185-4997

Olga A. Suntsova, Lead Researcher, Candidate of Science in Veterinary Medicine; SPIN-code 5246-0463

Vasily S. Vlasenko, Head Researcher, Doctor of Science in Biology; SPIN-code 8354-6130

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Рапсовое масло и жмых в рационах цыплят-бройлеров в условиях Томской области

(✉)**Фахрутдинова Р.Ш.[1], Сыманович О.В.[1], Каракчиева Н.И.[1], Афонина И.А.[2], Кинсфатор О.А.[2], Сошникова Т.А.[3]**
[1]*Национальный исследовательский Томский государственный университет*
Томск, Россия
[2]*Новосибирский государственный аграрный университет*
Новосибирск, Россия
[3]*Томский государственный университет систем управления и радиоэлектроники*
Томск, Россия
(✉)e-mail: zootehnik_tshi@mail.ru

Поиск новых видов кормов для повышения конечной живой массы и снижения затрат комбикорма в процессе выращивания остается главной задачей на современном этапе развития бройлерного производства. В статье представлены результаты исследования химического состава и питательной ценности рапсового жмыха, которому уделяется особое внимание как основному компоненту комбикорма в финишный период откорма птицы. Цель данной работы заключалась в изучении откормочных и мясных качеств цыплят-бройлеров при замене в рационе мясной муки, подсолнечных шрота и масла на соевый шрот, рапсовые жмых и масло в тех же количествах. Качество корма, поедаемого птицей, оказывает большое влияние на показатели роста и затраты кормов. По результатам химических исследований установлено, что по энергетической и протеиновой питательности рапсовый жмых близок к соевому. Наилучшие показатели при этом были получены при включении в состав комбикорма жмыха и масла из рапса. Повышение сохранности цыплят-бройлеров составило 5,0%, увеличение живой массы – 290 г. Удалось достичь снижения затрат кормов на 1 кг прироста на 0,34 ед. и повышения индекса продуктивности на 2,1%. Важным показателем качества мясной продуктивности цыплят-бройлеров являются масса и выход съедобной части тушки. В опытной группе масса потрошеной тушки была выше на 8,58%, масса ее съедобной части – на 122 г. Использование в составе комбикорма рапсовых жмыха и масла способствовало снижению себестоимости 1 кг прироста живой массы, повышению прибыли на 16,02% и увеличению рентабельности производства мяса цыплят-бройлеров на 4,49%.

Ключевые слова: жмых и масло рапса, соевый шрот, живая масса, экономическая эффективность

Rapeseed oil and cake in the rations of broiler chickens in the conditions of the Tomsk region

(✉)**Fakhrutdinova R.Sh.[1], Symanovich O.V.[1], Karakchieva N.I.[1], Afonina I.A.[2], Kinsfator O.A.[2], Soshnikova T.A.[3]**
[1]*National Research Tomsk State University*
Tomsk, Russia
[2]*Novosibirsk State Agrarian University*
Novosibirsk, Russia
[3]*Tomsk State University of Control Systems and Radioelectronics*
Tomsk, Russia
(✉)e-mail: zootehnik_tshi@mail.ru

The search for new types of feed to increase the final live weight and reduce the cost of feed for growing remains the main issue at the current stage of development of broiler production. The article presents the results of a study of the chemical composition and nutritional value of rapeseed cake, which receives special attention as the main component of compound feed in the finishing period of poultry fattening. The purpose of this work was to study the fattening and meat qualities of poultry when replacing sunflower meal, sunflower oil and meat meal in the diet with rapeseed cake, rapeseed oil and soybean meal in the same quantities. The quality of the feed eaten by the poultry has a great impact on the growth and feed costs indicators. According to the results of chemical studies it was

found that the energy and protein nutritional value of rapeseed cake is close to soybean cake. The best results were obtained with the inclusion of rapeseed cake and oil in the compound feed. Thus, the viability of broiler chickens increased by 5.0%; live weight increased by 290 g. It was possible to achieve a reduction in feed costs per 1 kg of gain by 0.34 units and an increase in the productivity index by 2.1%. An important indicator of the quality of meat productivity of broiler chickens is the weight and yield of the edible part of the carcass. Thus, the mass of the gutted carcass was higher in the experimental group by 8.58% and the edible parts of the broiler carcass of the experimental group were higher by 122 g. The use of rapeseed cake and oil in the compound feed contributed to a decrease in the cost of 1 kg of live weight gain of broiler chickens and an increase in profit by 16.02% and an increase in the profitability of broiler meat production by 4.49%.

Keywords: rapeseed cake and oil, soybean meal, live weight, economic efficiency

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Conflict of interest
The authors declare no conflict of interest.

INTRODUCTION

One of the factors hindering poultry productivity growth is the lack of protein components in compound feed, which leads to excess feed consumption and increased production costs. Currently, legumes and oilseeds are the most widely used sources of plant protein. Therefore, the topic of our study is quite relevant and has both theoretical and practical significance.

Poultry production volumes are increasing annually due to rising demand for poultry meat and poultry-based products. According to forecasts, annual global broiler production in 2025 will be approximately 105.26 million tons [1–3].

Feeding regimens and feed composition are crucial, influencing growth and blood counts, carcass yield, and meat quality in broiler chickens, as well as the organoleptic, nutritional, and technological properties of the resulting products. Currently, there is a need to utilize alternative feed resources and evaluate them in terms of maintaining overall poultry health and optimizing production.

In industrial production in Western Siberia, broiler chickens are fed complete feed, which meets the birds' energy, nutrient, and vitamin requirements. This feed includes grain, mineral supplements, meat and fish meal, and liquid vegetable oils (sunflower and soybean), significantly increasing the cost of meat production.

Protein and fat are the most important components in a poultry diet. During digestion, protein is broken down into amino acids, which influence growth, productivity, and immunity[1-3]. Plant-based feed supplements derived from oilseeds are most often used as the primary source of protein. Fat, included in the feed, provides energy, which is important for rapidly growing birds.

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The problem of replenishing the deficiency of crude protein and fat in the broiler diet can be solved by introducing rapeseed cake and oil, which have a positive effect on the digestibility of nutrients, the composition of intestinal microflora, and muscle tissue growth [4–6]. Soybean meal is also used as a source of protein in the broiler diet. Moreover, it is recommended to use crushed meal with a particle size of 625 and 1406 μm to improve protein digestibility in the ileum due to a larger contact surface and a decrease in the rate of passage [7–9].

In the countries with developed poultry farming, the problem of vegetable oil shortages is being addressed by increasing rapeseed oil production. Global oilseed production in 2020–2022 amounted to 600.47 million tons, indicating large volumes of cake and oil extraction residues that can be incorporated into feed [10–12].

The purpose of this work was to study the fattening and meat qualities of poultry when replacing meat meal, sunflower meal and oil in the diet with soybean meal, rapeseed cake and oil in the same quantities.

The research objectives are:

1) to study the chemical composition and nutritional value of rapeseed cake;

2) to study the biochemical parameters of the blood of broiler chickens;

3) to determine the meat characteristics of poultry.

The article is the first to study the combined effect of rapeseed and soybean plant feeds on the productive performance of broiler chickens.

MATERIAL AND METHODS

The study involved 25- to 40-day-old broiler chickens of the ROSS-308 cross. During the experiment, the birds were divided into two groups – control and experimental (see Table 1). The total number of chickens was 70,000. The experiment was repeated three times.

Representatives of the control and experimental groups were raised in cages at a stocking density of 25 heads/m^2, room temperature of 16–18 °C, and 24-hour lighting with an intensity of 10–15 lux.

The control group was fed standard PK-6 finishing feed based on wheat grain (60%) with the addition of sunflower meal (10%), meat meal (8%), wheat bran (6%), and sunflower oil (2%). The experimental group's feed was supplemented with rapeseed cake (10%) and oil (2%) instead of sunflower seed meal, and soybean meal (8%) instead of meat meal.

During the experiment, the birds' survival was monitored by recording dead and culled young birds. For biochemical studies, fasting blood samples were collected from the axillary vein at 25 and 40 days of age (the blood was stabilized with 1% heparin solution, 2 drops/100 ml). Red blood cell and white blood cell counts were determined using a MEKOS-Ts2 automated microscope (Russia), and hemoglobin was determined using the hemoglobin cyanide method using a spectrophotometer. Biochemical parameters of broiler chicken blood serum (total protein concentration, glucose, phosphorus, and calcium content) were determined using a Stat Fax 1904 PLUS semiautomated biochemical analyzer (USA).

Табл. 1. Схема исследования
Table 1. Research scheme

Group	Number of heads	Conditions of the study
Control	35 000	Finishing compound feed brand PK-6: wheat grain – 60%; sunflower meal – 10%; meat meal – 8%; wheat bran – 6%; sunflower oil – 2% Dose: 250 g/head per day
Experimental	35 000	Finishing compound feed brand PK-60: wheat grain – 60%; rapeseed cake – 10%; soybean meal – 8%; wheat bran – 6%; rapeseed oil – 2% Dose: 250 g/head per day

The live weight of broiler chickens was determined by individual weighing in the morning before feeding at 25 days of age and before slaughter. Based on these weighing results, average daily, absolute, and relative weight gains were determined. In the slaughterhouse, the marked birds were stunned, bled, and eviscerated. The following parameters were then determined: carcass yield, edible and inedible parts yield, muscle yield, and the European Performance Index.

RESULTS AND DISCUSSION

According to chemical studies, compared to sunflower meal, the content of crude protein in rapeseed cake is 1.45% higher ($p \leq 0.05$), crude fat is 5.66% lower, and crude fiber is 28.58% lower ($p \leq 0.05$) (see Fig. 1).

According to the results obtained, the hematological and biochemical parameters of the blood of all broiler chickens were within physiological norms. Blood parameters of birds at the beginning of the experiment (25 days) did not differ significantly between the control and experimental groups (see Fig. 2).

According to Fig. 2, by the slaughter period (40 days), a positive effect on metabolic processes in the body of broiler chickens was observed due to the addition of soybean meal, rapeseed cake, and oil to the feed. Thus, in the representatives of the experimental group, the red blood cell count was 8.23% higher ($p \leq 0.05$), and the hemoglobin concentration increased by 11.21% ($p \leq 0.05$) relative to the control.

It was found that the total protein content in the blood of broiler chickens in the experimental group was 18.64% higher ($p \leq 0.05$) than that of the control birds. Increased carbohydrate metabolism in the body of the experimental group was evidenced by a 26% increase in blood glucose concentration compared to the control group.

The European Broiler Performance Index reflects live weight, survivability and feed costs (conversion).

According to the study, the inclusion of rapeseed cake and oil, as well as soybean meal, in the compound feed contributed to an increase in live weight by 161 g ($p \leq 0.05$) relative to the control, a decrease in feed costs per 1 kg of gain by 0.10 units ($p \leq 0.05$), and an increase in the produc-

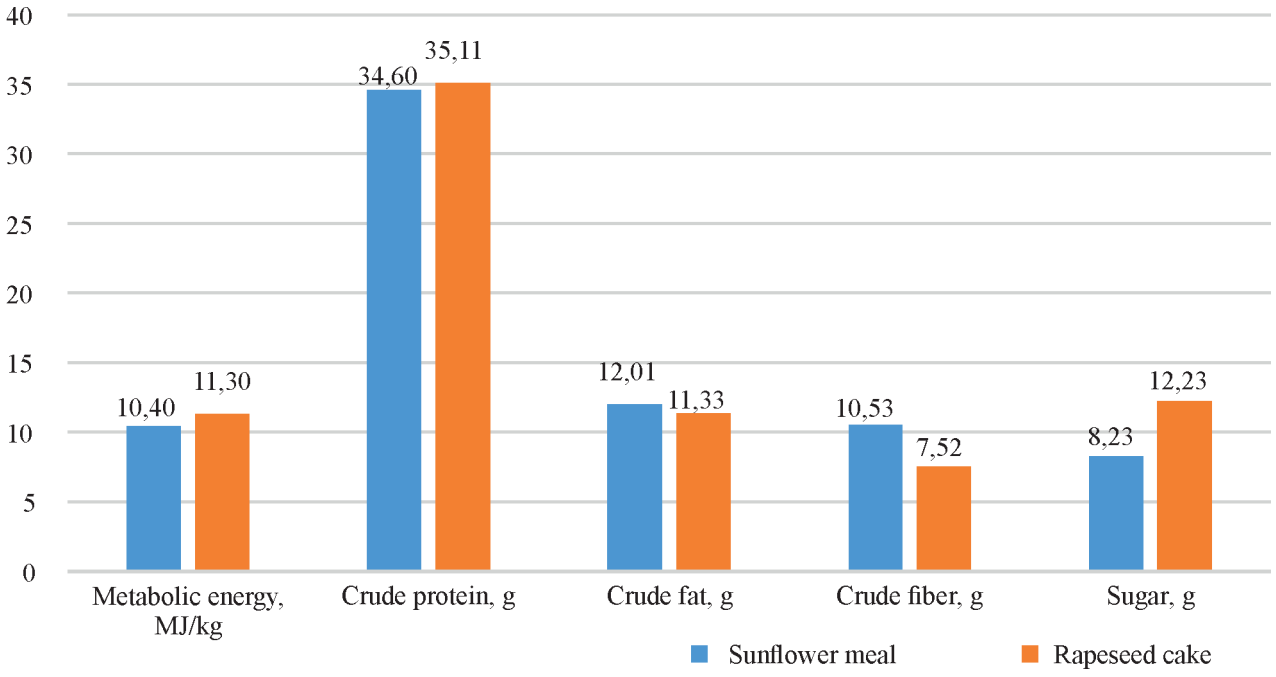

Рис. 1. Химический состав рапсового жмыха и подсолнечного шрота
Fig. 1. Chemical composition of rapeseed cake and sunflower meal

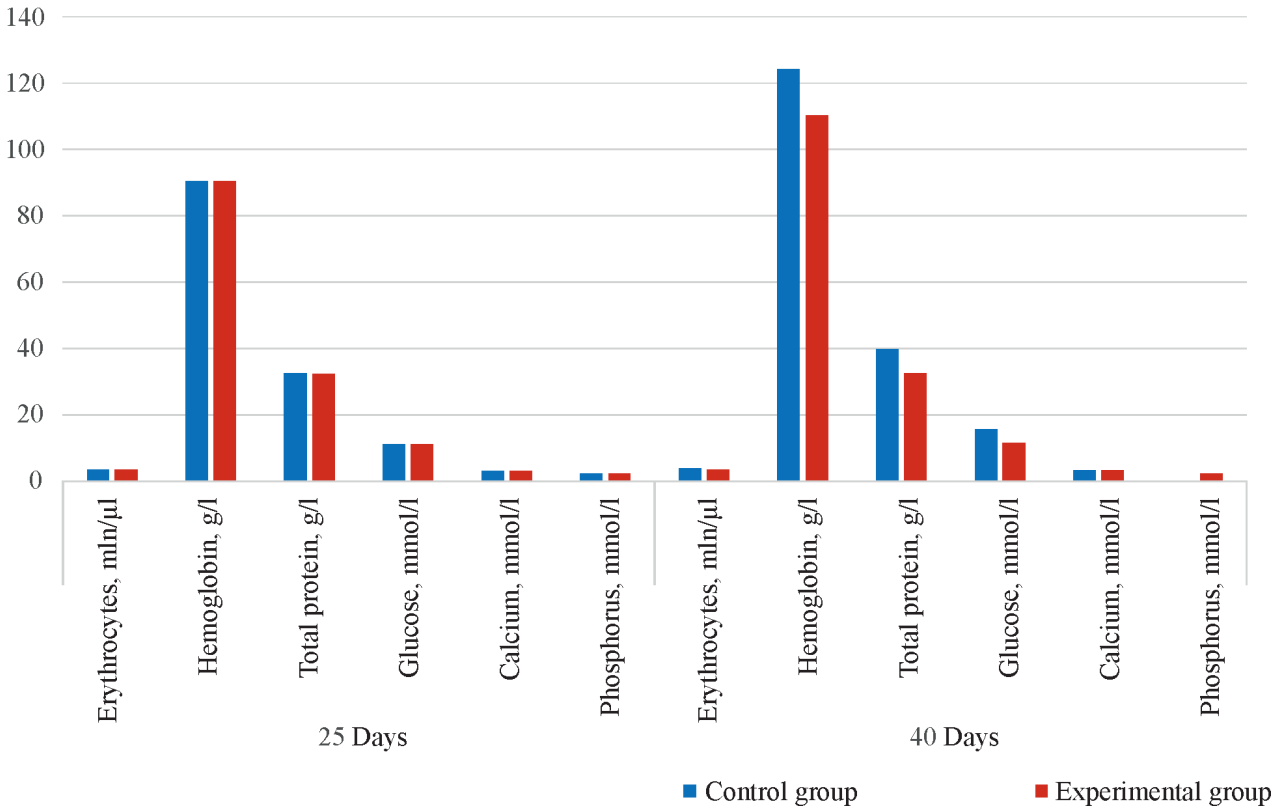

Рис. 2. Гематологические и биохимические показатели крови цыплят-бройлеров ($p \leq 0,05$)
Fig. 2. Hematological and biochemical parameters of blood of broiler chickens ($p \leq 0,05$)

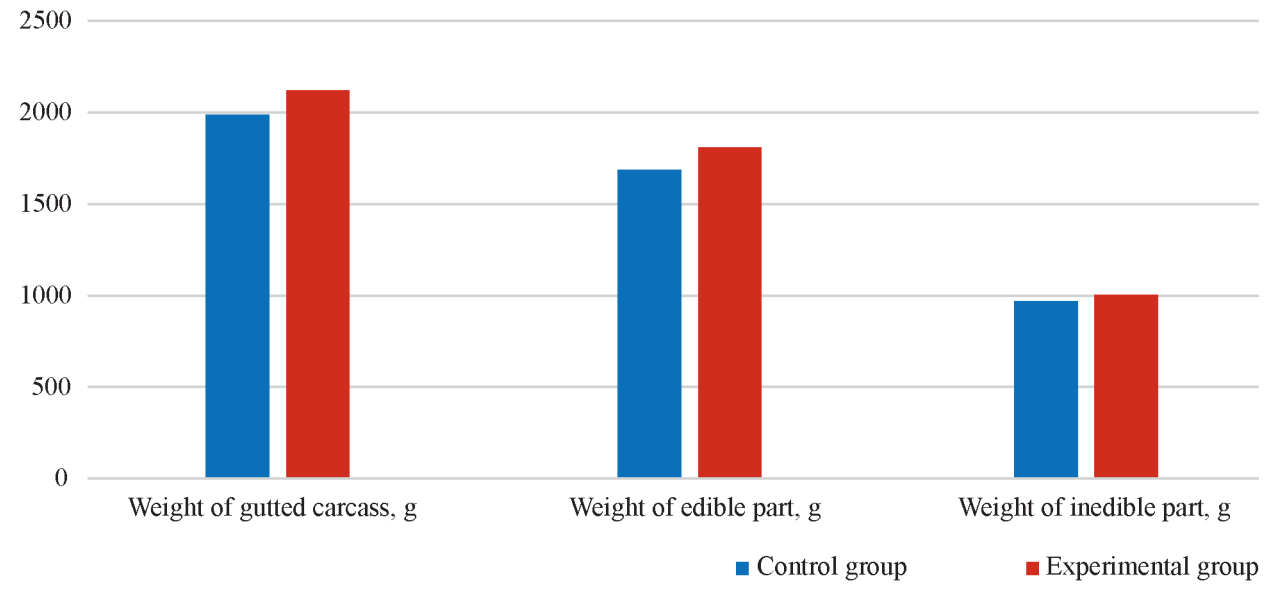

Рис. 3. Показатели убойного выхода ($p \leq 0,05$)
Fig. 3. Slaughter yield indicators ($p \leq 0,05$)

Табл. 2. Продуктивные показатели цыплят-бройлеров ($p \leq 0,05$)
Table 2. Productive indicators of broiler chickens ($p \leq 0,05$)

Group	Live weight, g		European Productivity Index	Feed conversion rate, kg/kg
	at the beginning of the period (25 days)	at the end of the period (40 days)		
Control	1254,3 ± 0,03	2654,0 ± 0,04	231 ± 0,03	2,50 ± 0,03
Experimental	1254,5 ± 0,03	2815,0 ± 0,04	265 ± 0,03	2,40 ± 0,03

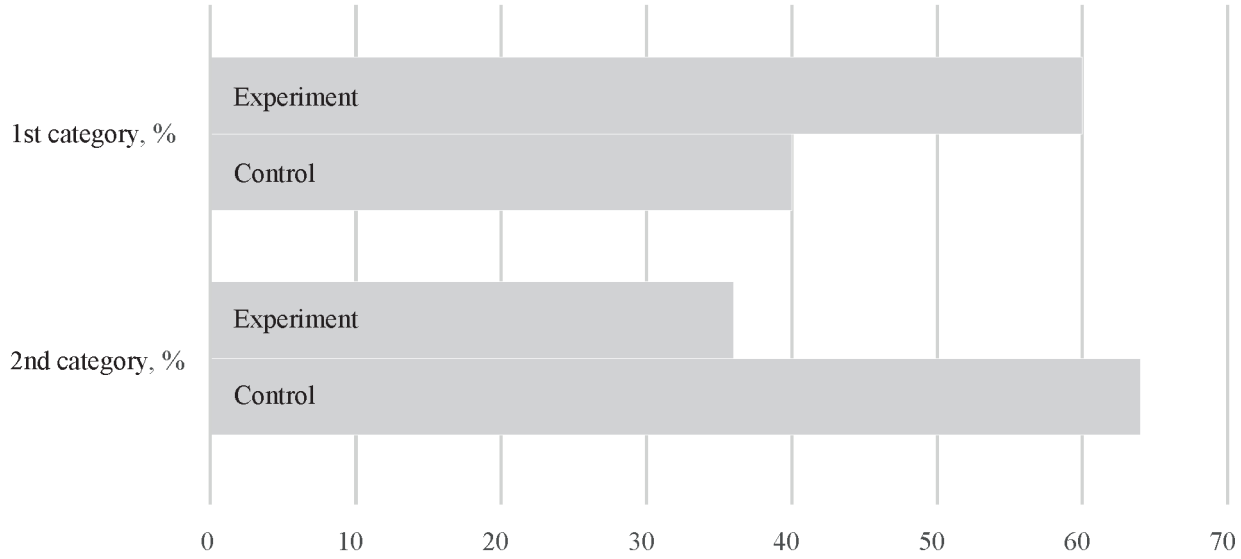

Рис. 4. Соотношение тушек 1-й и 2-й категорий качества ($p \leq 0,05$)
Fig. 4. Ratio of carcasses of the 1st and 2nd quality categories ($p \leq 0,05$)

tivity index by 34 units ($p \leq 0.05$) (see Table 2).

An important indicator of broiler chicken meat productivity is the yield of edible carcass meat. In the experimental group, the eviscerated carcass weight was 6.16% ($p \leq 0.05$) higher, and the edible carcass weight was 6.82% ($p \leq 0.05$) higher than in the control group (see Fig. 3). The experimental group produced more high-quality carcasses, as the proportion of Category 1 carcasses was 20% higher than in the control group (see Fig. 4).

According to available data, the reduction in the cost of 1 kg of live weight gain of broiler chickens in the experimental group was 3 rubles (2.6%) compared to the control group, which contributed to an increase in profits by 16.51% and an increase in the profitability of meat production by 4.72% (see Table 3).

CONCLUSIONS

1. 1. Rapeseed cake, compared to sunflower cake, is characterized by a higher content of crude protein (+ 1.45%, $p \leq 0.05$), exchange energy (+ 7.96%) and sugars (+ 32,71%).
2. 2. Sunflower cake contains more crude fat (+ 5.66%) and crude fiber (+ 28.58%) than rapeseed cake.
3. After introducing soybean meal, rapeseed cake, and oil into the broiler chicken diet, the total protein concentration in the blood of

Табл. 3. Показатели эффективности выращивания цыплят-бройлеров
Table 3. Performance indicators of broiler chicken growing

Indicator	Group	
	control	experimental
Number of heads	35 000	35 000
Number of heads taking into account the viability	30 590	31 675
Live weight, g:		
at the beginning of the period	1254,3 ± 0,03	1254,5 ± 0,03
at the end of the period	2654,0 ± 0,04	2815,0 ± 0,04
Duration of rearing, days	15	15
Average daily gain in live weight, g	93,31 ± 0,25	104,0 ± 0,25
Gross live weight during the fattening period, thous.kg	42,815	49,556
Cost price of 1 kg of live weight, rubles	114,26	111,26
Total cost, thous.rubles	4892,042	5513,600
Selling price, rubles	200,0	200,0
Total selling price of meat, thous.rubles	8563,00	9911,20
Profit, thous.rubles	3670,958	4397,600
Production profitability, %	75,04	79,76

the experimental group was 18.64% higher ($p \leq 0.01$) than that of the control group. Activation of carbohydrate metabolism in the experimental group is evidenced by an increase in blood glucose concentration (+26% compared to the control).

4. The inclusion of rapeseed cake and oil, as well as soybean meal, in the compound feed contributed to an increase in the live weight of broiler chickens in the experimental group by 161 g ($p \leq 0.05$), a decrease in feed costs per 1 kg of gain by 4% ($p \leq 0.05$) and an increase in the productivity index by 34 units ($p \leq 0.05$) compared to the control.

5. 5. The gutted carcass weight was 6.12% higher in the experimental group ($p \leq 0.05$) compared to the control. The edible carcass weight was also 6.81% higher in the experimental group ($p \leq 0,05$).

6. The experimental group produced more high-quality carcasses: the proportion of category 1 carcasses was 20% higher.

7. The reduction in the cost of 1 kg of live weight of broiler chickens in the experimental group was 3 rubles (2.6%) compared to the control, which contributed to an increase in profits by 16.52% and an increase in the profitability of meat production by 4.72%.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

(✉)**Фахрутдинова Раиса Шакирьяновна,** доцент кафедры, кандидат сельскохозяйственных наук; SPIN-код 5973-9801; **адрес для переписки:** Россия, 634050, Томск, пр. Ленина, 36; e-mail: zootehnik_tshi@mail.ru

Сыманович Оксана Викентьевна, доцент кафедры, кандидат сельскохозяйственных наук; SPIN-код 3096-0070

Каракчиева Наталья Ивановна, старший научный сотрудник, кандидат химических наук; SPIN-код 8210-4325

Афонина Ирина Анатольевна, заведующая кафедрой, кандидат биологических наук; SPIN-код 8503-7260

Кинсфатор Ольга Александровна, доцент кафедры, кандидат сельскохозяйственных наук; SPIN-код 9553-0847

Сошникова Татьяна Александровна, доцент кафедры, кандидат биологических наук, доцент; SPIN-код 5160-9677

AUTHOR INFORMATION

(✉)**Raisa Sh. Fakhrutdinova,** Assistant Professor, Candidate of Science in Agriculture; SPIN-code 5973-9801; **address:** 36, Lenina ave., Tomsk, 634050, Russia; e-mail: zootehnik_tshi@mail.ru

Oksana V. Symanovich, Assistant Professor, Candidate of Science in Agriculture; SPIN-code 3096-0070

Natalia I. Karakchieva, Senior Researcher, Candidate of Science in Chemistry; SPIN-code 8210-4325

Irina A. Afonina, Department Head, Candidate of Science in Biology; SPIN-code 8503-7260

Olga A. Kinsfator, Assistant Professor, Candidate of Science in Agriculture; SPIN-code 9553-0847

Tatiana A. Soshnikova, Assistant Professor, Candidate of Science in Biology, Associate Professor; SPIN-code 5160-9677

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Влияние пищевой фукозы на постантибиотическое восстановление микробиоты в условиях дефицита гликопротеина муцин-2

(✉)**Бец В.Д.[1], Магер С.Н.[2], Литвинова Е.А.[1]**
[1]*Новосибирский государственный технический университет*
Новосибирск, Россия
[2]*Сибирский федеральный научный центр агробиотехнологий Российской академии наук*
Новосибирская область, р.п. Краснообск, Россия
(✉)e-mail: betsvd.bio@gmail.com

Фукоза, являясь ключевым компонентом кишечных гликанов, играет очень важную роль в регуляции микробного сообщества и создании оптимальной микросреды для эпителиальных клеток хозяина. В данном исследовании изучено влияние пищевой фукозы на восстановление кишечной микробиоты у лабораторных мышей с дефицитом муцина-2 (*Muc2*$^{-/-}$) после проведения антибиотикотерапии. В качестве контрольных особей использованы мыши дикого типа (WT, C57BL/6). В ходе эксперимента животные обоих генотипов были разделены на четыре группы: входящие в состав 1-й группы мыши антибиотики не получали (негативный контроль), представители 2-й группы получали антибиотики (14 дней) и фукозу (позитивный контроль для фукозы), представители 3-й группы – антибиотики (14 дней) и стандартный корм (модель дисбиоза), 4-й – только фукозу (основная экспериментальная группа). Результаты исследования показали, что у *Muc2*$^{-/-}$ мышей уровень связанной фукозы в кишечнике был достоверно ниже, чем у контрольных животных (p = 0,0001), что сопровождалось дисбалансом микробиоты. Антибиотикотерапия усугубляла эти изменения, снижая количество *Bacteroides* spp. (p = 0,0003) и *Lactobacillus murinus* (p = 0,0268) и повышая уровень условно-патогенных *Escherichia coli* (p = 0,0004). Добавление фукозы в рацион способствовало избирательному восстановлению *Bacteroides* spp. у *Muc2*$^{-/-}$ мышей (p = 0,0015), но не влияло на численность *L. murinus*. Важным открытием стало опосредованное подавление *Enterococcus* spp. за счет стимуляции *Bacteroides*, что свидетельствует о сложных межмикробных взаимодействиях в рамках микробиома кишечника. Также установлено, что фукоза снижала уровень *E. coli* (p = 0,0149), демонстрируя свою защитную роль. У мышей C57BL/6 пищевая фукоза восстанавливала уровень связанной фукозы (p = 0,0249), тогда как у *Muc2*$^{-/-}$ особей этого не наблюдалось, что подчеркивает ключевую роль муцина-2 в поддержании микробного гомеостаза. Полученные данные раскрывают сложные взаимосвязи между муциновым барьером, составом микробиоты и ее устойчивостью к антибиотикам, открывая перспективы для разработки новых подходов к коррекции микробиоценоза при нарушениях кишечного барьера.

Ключевые слова: муцин-2, дисбиоз, микробиота, *Lactobacillus*, *Enterococcus*, фукоза, антибиотики

Effect of dietary fucose on post antibiotic microbiota recovery under conditions of mucin-2 glycoprotein deficiency

(✉)**Bets V.D.[1], Mager S.N.[2], Litvinova E.A.[1]**
[1]*Novosibirsk State Technical University*
Novosibirsk, Russia
[2]*Siberian Federal Scientific Centre of Agro-BioTechnologies of the Russian Academy of Sciences*
Krasnoobsk, Novosibirsk region, Russia
(✉)e-mail: betsvd.bio@gmail.com

Fucose, a key component of intestinal glycans, plays an important role in regulating the microbial community and creating an optimal microenvironment for host epithelial cells. In this study, the effect of dietary fucose on the restoration of intestinal microbiota in mucin-2-deficient (*Muc2*$^{-/-}$) laboratory mice after antibiotic therapy was investigated. Wild-type mice were used as controls (WT, C57BL/6). During the experiment, animals of both genotypes were divided into four groups: mice in group 1 did not receive antibiotics (negative control), representatives of group 2 received antibiotics (14 days)

and fucose (positive control for fucose), group 3 received antibiotics (14 days) and standard feed (dysbiosis model), and group 4 received only fucose (main experimental group). The results of the study showed that *Muc2*$^{-/-}$ mice had significantly lower levels of bound fucose in the intestine than the control animals ($p = 0.0001$), which was accompanied by an imbalance in the microbiota. Antibiotic therapy aggravated these changes, reducing the number of *Bacteroides* spp. ($p = 0.0003$) and *Lactobacillus murinus* ($p = 0.0268$), while increasing the level of opportunistic *Escherichia coli* ($p = 0.0004$). Addition of fucose to the diet resulted in selective restoration of *Bacteroides* spp. in *Muc2*$^{-/-}$ mice ($p = 0.0015$), but had no effect on the number of *L. murinus*. An important finding was the indirect suppression of *Enterococcus* spp. by stimulation of *Bacteroides*, indicating complex intermicrobial interactions within the gut microbiome. It was also established that fucose reduced the level of *E. coli* ($p = 0.0149$), demonstrating its protective role. In C57BL/6 mice, fucose restored the level of bound fucose ($p = 0.0249$), whereas this was not observed in *Muc2*$^{-/-}$ mice, highlighting the key role of mucin-2 in maintaining microbial homeostasis. The obtained data reveal complex relationships between the mucin barrier, the composition of the microbiota and its resistance to antibiotics, opening up prospects for the development of new approaches to correcting the microbiocenosis in cases of intestinal barrier disorders.

Keywords: mucin-2, dysbiosis, microbiota, *Lactobacillus*, *Enterococcus*, fucose, antibiotics

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INTRODUCTION

Intestinal mucin, predominantly represented by mucin-2 (*Muc2*), plays a key role in maintaining homeostasis in the animal digestive tract [1]. This highly glycosylated protein, secreted by goblet cells, forms a complex three-dimensional network that creates physical and biochemical barriers between intestinal epithelial cells and its contents [2].

Mucin performs key biological functions through its glycosylated domains. They simultaneously prevent pathogen adhesion to the epithelium and serve as a nutrient substrate for beneficial saccharolytic bacteria, including *Akkermansia muciniphila* and some strains of *Bacteroides* [3].

Mucin and saccharolytic bacteria interact symbiotically: bacteria break down mucin glycans, and the fatty acids they secrete, in turn, enhance mucin production by goblet cells [4, 5].

Mucin plays a crucial role in the development of immune tolerance to commensal microbiota. Acting as a platform for interaction with dendritic cells, it regulates the balance of proinflammatory and regulatory immune responses by preventing excessive reactions to autochthonous microorganisms [6].

The absence of mucin-2, a major structural component of the intestinal mucosal barrier, leads to profound alterations in the microbial community, which has serious consequences for the entire organism[1].

In the case of mucin-2 deficiency, glycan-utilizing bacteria, particularly *Bacteroides spp*., are forced to switch from mucin carbohydrates to alternative sources, including epithelial gly-

[1]*Johansson M.E.V., Larsson J.M.H., Hansson G.C.* The two mucus layers of colon are organized by the MUC2 mucin, whereas the outer layer is a legislator of host-microbial interactions // Proceedings of the National Academy of Sciences, 2011, vol. 108, Suppl. 1, pp. 4659–4665.

coproteins. This causes damage to the intestinal barrier, facilitating pathogen adhesion, and also reduces the population of *Lactobacillus murinus*, which normally produces bacteriocins and maintains an acidic environment in the epithelium [7]. Mucin-2 deficiency alters the intestinal environment, promoting the growth of resistant *Enterococcus spp*. and enhancing the pathogenic properties of *Escherichia coli* [8].

Disturbances in the microbial balance develop in a cascade: a reduction in lactobacilli weakens protection, and changes in pH and redox potential promote the growth of proteobacteria and the formation of a pathological community adapted to the damaged barrier[2].

Mucin-2 deficiency causes a fundamental restructuring of the intestinal ecosystem, disrupting its spatial organization and trophic connections. These systemic changes increase susceptibility to infections, contribute to the development of chronic inflammation and metabolic disorders [9].

The intestinal microbiota of mice is a complex ecosystem, where key representatives of the microbiome include *Enterococcus* spp., *Bacteroides* spp., *E. coli* and *L. murinus*, each of which plays a unique role in intestinal physiology [10].

The interactions between these bacteria in the intestine are competitive. For example, *Bacteroides spp*., using mucin glycans, can limit substrate availability for *L. murinus*, leading to a decrease in its protective functions. In turn, *L. murinus*, through acidification of the environment, inhibits the growth of *Enterococcus spp*. In dysbiosis caused by mucin deficiency, this balance is disrupted: a decrease in *L. murinus* and an increase in *Bacteroides spp*. create conditions for the expansion of opportunistic *E. coli* and *Enterococcus spp*., which contributes to the development of inflammation [11].

Thus, the intestinal microbiota of mice is a dynamic system in which *E. coli*, *Enterococcus spp*., *Bacteroides spp*., and *L. murinus* occupy strictly defined ecological niches, and their interaction determines not only normal intestinal physiology, but also pathological changes when homeostasis is disrupted [12].

Fucose (6-deoxy-L-galactose) is a key component of intestinal glycans, whose bacterial metabolism supports microbial balance, barrier function, and immune regulation. This complex process involves catabolism, signaling pathways, and microbe-host interactions [13].

Fucose plays an important role in the formation and maintenance of the intestinal microbial community. It serves as a selective substrate for bacteria responsible for the breakdown of mucins, such as *Akkermansia muciniphila* [14], providing them with an ecological advantage.

The immunomodulatory properties of fucose and its derivatives include the induction of regulatory T cell (Treg) differentiation through effects on dendritic cells, resulting in decreased levels of proinflammatory cytokines (TNF-α и IL-6) [15].

The therapeutic potential of fucose is associated with its ability to restore the microbial community after antibiotic therapy, promoting the growth of beneficial bacteria, in particular *Bacteroides*.

The molecular mechanisms of fucose utilization by bacteria highlight its central role in the interaction between the microbiota and the host. Studying these processes may open new perspectives for developing strategies to correct dysbiosis and treat diseases associated with intestinal barrier dysfunction.

In this regard, the purpose of this work was to determine the role of dietary fucose in the restoration of the microbial consortium under conditions of mucin-2 deficiency in the intestines of model mice *Muc2*$^{-/-}$.

MATERIAL AND METHODS

The study involved laboratory mice with a knockout for the Muc2 gene (Muc2$^{-/-}$), as well as wild-type (WT, C57BL/6) individuals used as a control. During the experiment, the animals were divided into four groups. Representatives

[2]*Di Cerbo A., Palmieri B., Aponte M., Iannitti T.* Mechanisms and therapeutic effectiveness of lactobacilli // Journal of Clinical Pathology, 2016, vol. 69, N 3, pp. 187–203.

of the first group did not receive antibiotics (negative control). Mice from the second group received antibiotics (14 days) and fucose (positive control for fucose). Animals of the third group received antibiotics (14 days) and standard feed (dysbiosis model). Representatives of the fourth group received only fucose (the main experimental group).

To prepare a combination antibiotic preparation, 0.5 g/L of gentamicin, ampicillin, and metronidazole, as well as 1 g/l of vancomycin, were added to drinking water. The antibiotic solution was prepared 24 hours before use and then stored at 4°C. During antibiotic therapy, the animals' body weight and clinical condition were regularly monitored at the intervals of 48–72 hours.

All manipulations with mice were performed in full compliance with international bioethical standards established by the European Convention for the Protection of Laboratory Animals. Ethical review was conducted by the Ethics Committee of the Research Institute of Neuroscience and Medicine (Protocol No. 28 dated June 19, 2015).

Samples were homogenized in liquid nitrogen. Tissue samples (5 mg) were extracted in 1 ml of 96% ethanol (5 min, stirring) and then centrifuged at 3000 rpm (10 min, 4°C). The supernatant was used for free fucose analysis. The pellet was treated with 0.1 N NaOH solution to determine the bound form.

H_2SO_4 (6:1) was added to the samples, heated to 100°C, then cooled and mixed with 3% L-cysteine hydrochloride (90 min, 20°C). Optical density was measured at 405/450 nm. Concentration was calculated using a calibration curve, normalizing to protein content (the Bradford method).

In the intestines of both $Muc2^{-/-}$ and C57BL/6 mice, the levels of free and bound fucose were determined using several therapeutic regimens: without antibiotics (free fucose: $n = 10$, $n = 10$; bound: $n = 10$, $n = 9$), only antibiotics (free: $n = 4$, $n = 5$; bound: $n = 4$, $n = 5$), antibiotics and fucose (free: $n = 3$, $n = 5$; bound: $n = 3$, $n = 5$), only fucose (free: $n = 7$, $n = 5$; bound: $n = 7$, $n = 5$).

Bacterial content in colon tissue and feces was assessed using quantitative real-time PCR, normalizing the amount of bacterial 16S rRNA to the level of 28S rRNA of *Mus musculus*. DNA was isolated using the DT-250 system (Biolabmix, Russia) according to the manufacturer's protocol.

The following were used for detection:

1) universal primers to the conserved region of 16S rRNA (total bacterial load);

2) specific primers;

3) primers to mouse 28S rRNA (normalization control).

Primers were developed using Primer-BLAST and Unipro UGENE software. PCR analysis was performed on a CFX96 Touch thermocycler (Bio-Rad Lab., USA) with the BioMaster HS-qPCR SYBR Blue reaction mixture (Biolabmix, Russia) according to the standard protocol: first 5 min at 95°C, then 40 cycles at different temperatures (95°C/15 s, 62°C/25 s, 72°C/25 s).

The quantitative assessment was carried out using the ΔCt method, using the following formulas:

1) 2^(Ct 28S - Ct 16S) – for the total bacterial load;

2) 2^(Ct 16S - Ct specific16S) – for individual taxa.

The data were normalized relative to the C57BL/6 control group, which was taken as 1 conventional unit. All primer sequences are given in the table.

PCR was performed with initial denaturation (95°C, 5 min), followed by 40 cycles (95°C, 15 s), primer annealing (30 s), and elongation (72°C, 30 s). The specificity of amplification was confirmed by melting curve analysis.

Statistical data processing was performed using IBM SPSS Statistics (version 23) software. Nonparametric PERMANOVA statistical methods were used to analyze samples that did not follow a normal distribution. Comparisons between two groups were performed using Student's t-test for samples with a normal distribution, and the Mann–Whitney test for samples without a normal distribution.

RESULTS AND DISCUSSION

To assess the role of mucin-2 in the formation of the intestinal microbiome, C57BL/6 mice and animals homozygous for a null mutation of the Muc2 gene, obtained from heterozygous parents (C57BL Muc2-/wt), were used. The use of siblings minimized the influence of maternal microflora on the formation of microbial communities in individuals with different genotypes.

Since fucose mediates the adhesion of commensal microorganisms and modulates the mucosal immune response, the concentrations of free and bound fucose in the intestine were studied. Figure 1, а and б schematically show the differences in intestinal epithelial structure between animals with mucin deficiency ($Muc2^{-/-}$) and without it (C57BL/6) (images created using Figma software).

The study showed that $Muc2^{-/-}$ mice had significantly lower levels of bound fucose than wild-type mice (one-way PERMANOVA, F = 23.37, p = 0.0001). However, no significant differences in free fucose content were observed between the two genotypes (one-way PERMANOVA, F = 2,189, p = 0,1573) (see Fig. 1, в).

After the use of antibiotics, a significant decrease in the level of bound fucose was recorded in both genotypes (one-way PERMANOVA, F = 4,746, p = 0,0498 for Muc2–/– and F = 6,984, p = 0,0161 for C57BL/6) (see Fig. 1, г). While the level of free fucose remained approximately the same and did not differ significantly between the two genotypes (one-way PERMANOVA, F = 0,166, p = 0,6965 for $Muc2^{-/-}$ and F = 1,925, p = 0,1859 for C57BL/6).

In C57BL/6 mice given antibiotics together with dietary fucose, the level of bound fucose was restored (one-way PERMANOVA, F = 9,29, p = 0,0249). In mice with a null mutation of the Muc2 gene, no significant regeneration occurred (one-way PERMANOVA, F = 1,508, p = 0,2479).

Fucose serves as a key energy substrate for commensal intestinal bacteria. However, mucin-2 deficiency, as well as mucin-2 deficiency combined with antibiotic therapy, leads to a sharp decrease in available fucose, which can lead to significant changes in the composition of the microbiota, with a predominance of op-

Последовательности праймеров
Primer sequences

Goal	Primer name	Primer sequence 5'-3'
Bacterial 16S	16S F	TCCTACGGGAGGCAGCAG
	16S R	ATTACCGCGGCTGCTGG
Mouse 28S	28S F	CCTGGCGCTAAACCATTCGT
	28S R	AAAGCCCGCAGAGACAAACC
Bacteroides spp. 16S	Bac.spp-F	GAGAGGAAGGTCCCCCAC
	Bac.spp-R	CGCTACTTGGCTGGTTCAG
Lactobacillus murinus 16S	Lac.mur-F	AGCAGTAGGGAATCTTCCA
	Lac.mur-R	CACCGCTACACATGGAG
Escherichia coli 16S	E.coli-F	GGCAGGCCTAACACATGCAA
	E.coli-R	CCCCTCTTTGGTCTTGCGAC
Enterococcus spp. 16S	tuf-Ent-F	GCAACGCTTGCAGCAATGG
	tuf-Ent-R	CGCCAGCAAGCAATACAGAT

Note. All primers are developed in the laboratory.

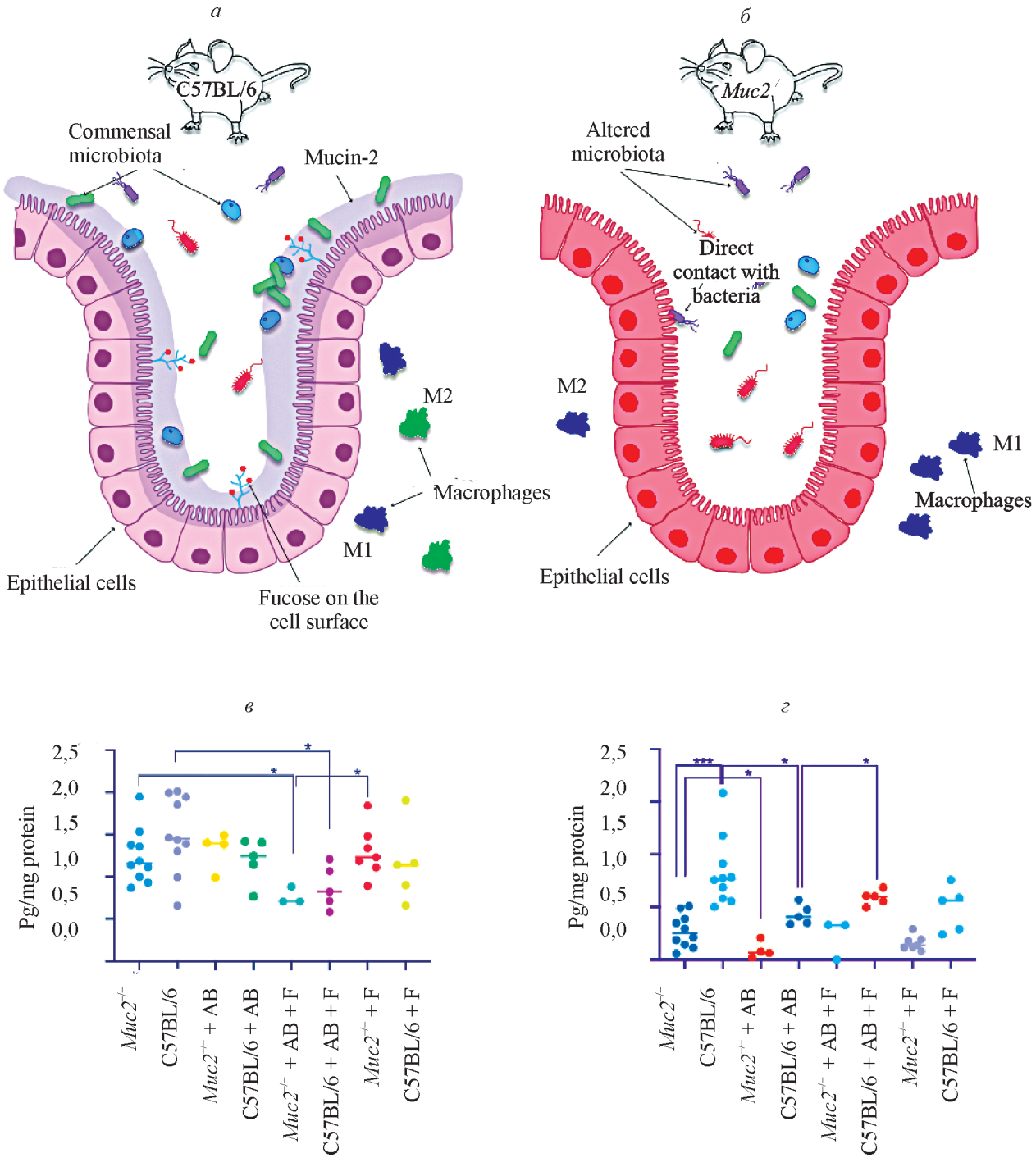

Рис. 1. Анализ содержания свободной и связанной фукозы в кишечнике мышей *Muc2*$^{-/-}$ и C57BL/6:
а, б – схематичное изображение строения кишечного эпителия у мышей без нарушения и с нарушением муцинового слоя; *в, г* – уровень свободной и связанной фукозы соответственно. АБ – антибиотики; Ф – фукоза.
*$p < 0,05$, ***$p < 0,001$, тест PERMANOVA. ND – не определено

Fig. 1. Analysis of free and bound fucose content in the intestines of *Muc2*$^{-/-}$ and C57BL/6 mice:
а, б – schematic representation of the structure of the intestinal epithelium in mice without and with a disruption of the mucin layer; *в, г* – the level of free and bound fucose, respectively. АБ – antibiotics; Ф – fucose.
*$p < 0.05$, ***$p < 0.001$, PERMANOVA test. ND – not defined

portunistic species.

In this study, we examined how the abundance of four main representatives of the intestinal microbiota in mice changes during antibacterial therapy and its subsequent restoration using dietary fucose. Using the method of single-strand conformational polymorphism (SSCP) of the variable (V4) region of the 16S rRNA gene, it was shown how the level of Bacteroides spp., E. coli, L. murinus and Enterococcus spp. changes. The amount of DNA of the indicated bacteria was determined according to the therapeutic regimens presented above: without antibiotics (n = 11, n = 9), antibiotics only (n = 6, n = 7), antibiotics and fucose (n = 5, n = 5), fucose only (n = 5, n = 4).

Bacteroides spp. in the mouse intestine are known to play a key role in mucin fermentation, hydrolyzing fucosylated glycans, including mucin-2, using bacterial fucosidases, which ensures their growth and maintains the homeostasis of the mucous layer, while simultaneously stimulating the production of short-chain fatty acids to nourish intestinal epithelial cells.

In Muc2$^{-/-}$ and C57BL/6 mice, a significant decrease in the number of Bacteroides spp. in the intestine was observed after antibacterial therapy (one-way PERMANOVA, F = 29,43, p = 0,0003 for Muc2$^{-/-}$ and F = 44,07, p = 0,0001 for C57BL/6) (see Fig. 2, а). Addition of dietary fucose to the drinking water of animals receiving antibiotics significantly increased the level of Bacteroides spp. in Muc2$^{-/-}$ mice (one-way PERMANOVA, F = 144,1, p = 0,0015), whereas no such changes were observed in C57BL/6 (one-way PERMANOVA, F = 4,361, p = 0,1306).

One of Bacteroides spp.'s competitors for mucin utilization niches is L. murinus. Both species exhibit complex relationships that combine elements of competition and metabolic cooperation. Thus, Bacteroides spp. actively utilize mucin fucose, reducing its availability to L. murinus, while L. murinus depends on simple sugars because it is unable to break down complex glycans, which limits its growth when Bacteroides spp. dominates.

It was found that the content of L. murinus did not differ significantly between the individuals with different genotypes (one-way PERMANOVA, F = 1,65, p = 0,2279), which can be explained by the low requirement of lactobacilli for mucin glycans. At the same time, antibiotic therapy had a detrimental effect on representatives of the genus Lactobacillus, reducing their levels to the point of non-detection (one-way PERMANOVA, F = 4,84, p = 0,0268 for Muc2–/– and F = 4,253, p = 0,0328 for C57BL/6) (see Fig. 2, б).

Moreover, the addition of fucose to drinking water did not significantly affect the restoration of L. murinus numbers, which may be logical in light of their low tropism to fucosylated glycans.

Enterococcus spp. showed different dynamics: their level significantly decreased under the influence of antibiotics (one-way PERMANOVA, F = 6,6, p = 0,0061) (see Fig. 2, в). However, fucose did not exert a direct stimulating effect. Its effect is likely mediated through stimulation of Bacteroides spp., which are natural antagonists of enterococci in the intestinal consortium.

In addition to commensals and opportunistic bacteria, changes in the levels of pathogenic E. coli were monitored. Although it utilizes mucins weakly, it can also consume these types of glycans. When mucin is deficient, E. coli activates alternative metabolic pathways (e.g., aerobic respiration), which enhances its pathogenicity.

It was noted that in Muc2$^{-/-}$ mice the level of E. coli was significantly lower than in the control C57BL/6 mice. (one-way PERMANOVA, F = 23,25, p = 0,0003) (see Fig. 2, г). Antibacterial therapy significantly increased the number of E. coli in the intestines of mucin-deficient mice (one-way PERMANOVA, F = 33,29, p = 0,0004). However, in C57BL/6 mice, the use of antibiotics, on the contrary, significantly reduced the number of E. coli (one-way PERMANOVA, F = 6,18, p = 0,0255).

In addition, in the group where the animals received only dietary fucose, the number of E. coli was significantly lower compared to the

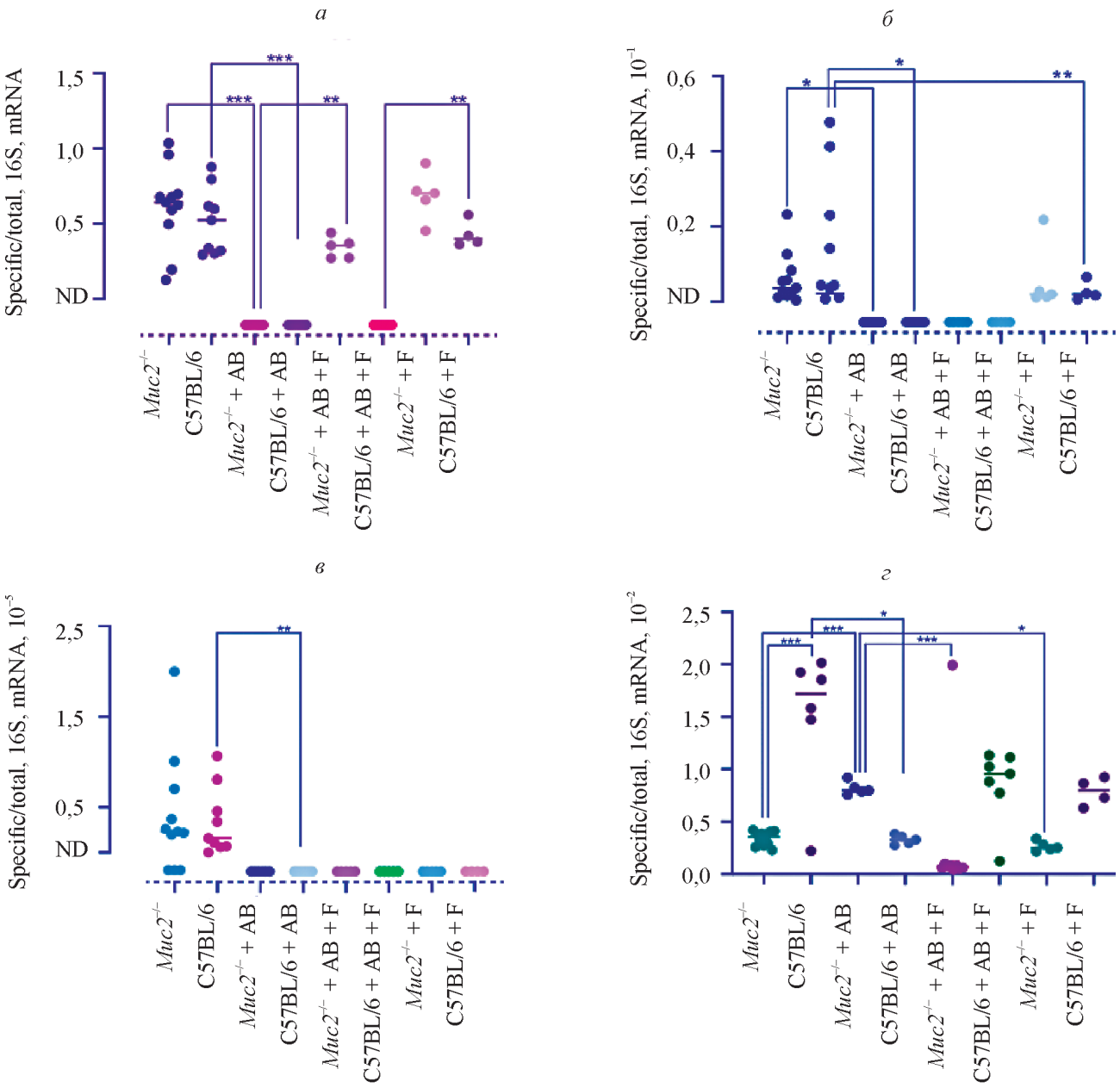

Рис. 2. Изменение количества ДНК четырех основных представителей микробиоты кишечника в фекалиях мышей *Muc2*$^{-/-}$ и C57BL/6:
а – Bacteroides spp.; *б – L. murinus*; *в – Enterococcus* spp.; *г – E. coli*. *$p < 0,05$, **$p < 0,01$, ***$p < 0,001$, тест PERMANOVA. ND – не определено

Fig. 2. Changes in DNA abundance of four major representatives of gut microbiota in feces of *Muc2*$^{-/-}$ and C57BL/6 mice:
a – Bacteroides spp.; *b – L. murinus*; *c – Enterococcus* spp.; *d – E. coli*. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, PERMANOVA test. ND – not defined

group that received only antibiotics (one-way PERMANOVA, F = 15,8, p = 0,0149).

Thus, our data confirm the key role of mucin-2 in regulating the intestinal microbiome through control of fucose availability. For example, in the work of C. Duan et al. [16], it was shown that fucose plays a critical role in maintaining intestinal homeostasis through modulation of the metabolism of commensal bacteria. In our study, a significant decrease in the level of bound fucose was observed in Muc2$^{-/-}$ mice, which is consistent with the findings of these authors.

Interestingly, antibiotic treatment reduced the level of bound fucose in both genotypes, but regeneration after dietary fucose supplementation occurred only in wild-type mice. Studies by Z. Wang et al. [17] confirmed that protein fucosylation, including mucins, is necessary for protecting the intestinal epithelium from damage. Our data complement these observations, showing that mucin-2 serves as a key mediator in this process.

Microbiota analysis revealed important patterns. For example, J.M. Garber et al. [18] emphasized the importance of fucose for the growth of Bacteroides spp., which are the main degraders of mucin. We found that in Muc2$^{-/-}$ animals, the addition of fucose more effectively restored the Bacteroides population than in the wild type, which may indicate compensatory mechanisms activated by mucin deficiency.

The paradoxical response of E. coli (its increase in Muc2$^{-/-}$ mice in the presence of antibiotics) also finds an explanation in the literature. A study by R. Wang et al. [19] showed that fucose can modulate the toxic effects on the microbiota, which is consistent with our observations indicating a decrease in the number of E. coli with the addition of fucose.

Thus, our results, consistent with those of other authors, confirm the central role of mucin-2 in maintaining microbial balance and demonstrate new aspects of the interaction between fucose metabolism and the intestinal microbiome under conditions of mucin deficiency.

CONCLUSION

The study confirmed the key role of mucin-2 in maintaining intestinal microbial homeostasis and antibiotic resistance. Muc2 deficiency was associated with a decrease in the numbers of protective symbionts (Bacteroides spp., L. murinus) and an increase in E. coli, accompanied by a decrease in the content of fucose, a key mucin component.

We were able to determine the protective effect of exogenous fucose, which restores Bacteroides spp. due to the ability of these bacteria to utilize this sugar. However, even after rederivation, complete restoration of the microbiota did not occur, highlighting the fundamental role of mucin-2 in the formation of the intestinal ecosystem.

The obtained data reveal the complex relationships between the mucin barrier, microbiota, and antibiotic resistance, opening up prospects for the development of new approaches to correcting the microbiome in cases of intestinal barrier disorders.

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ИНФОРМАЦИЯ ОБ АВТОРАХ

(✉)**Бец Виктория Дмитриевна,** старший преподаватель; SPIN-код 7210-1661; **адрес для переписки:** Россия, 630073, Новосибирск, пр. К. Маркса, 20; e-mail: betsvd.bio@gmail.com

Магер Сергей Николаевич, руководитель СибНИПТИЖ СФНЦА РАН, доктор биологических наук, профессор; SPIN-код 3317-9048

Литвинова Екатерина Анатольевна, доцент кафедры, кандидат биологических наук; SPIN-код 2995-8611

AUTHOR INFORMATION

(✉)**Victoria D. Bets,** Senior Lecturer; SPIN-code 7210-1661; **address:** 20, K. Marx Ave., Novosibirsk, 630073, Russia; e-mail: betsvd.bio@gmail.com

Sergey N. Mager, Head of the SibRDTIAH SFSCA RAS, Doctor of Science in Biology, Professor; SPIN-code 3317-9048

Ekaterina A. Litvinova, Assistant Professor, Candidate of Science in Biology; SPIN-code 2995-8611

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Алгоритм машинного обучения для распознавания плодов земляники садовой и классификации их степени зрелости

(✉)**Кутырёв А.И., Филиппов Р.А.**
Федеральный научный агроинженерный центр ВИМ
Москва, Россия
(✉)e-mail: alexeykutyrev@gmail.com

В статье представлены результаты исследования по разработке алгоритма на основе современной модели сверточной нейронной сети глубокого обучения, который позволил повысить точность распознавания плодов земляники садовой и классификации степени съемной спелости ягод. В исследовании применялся метод трансферного обучения, позволяющий адаптировать используемую модель YOLOv10-M (You Only Look Once version 10 medium), изначально обученную на датасете COCO, для решения задачи классификации степени зрелости ягод земляники. Для создания обучающего набора данных проведена аннотация (разметка) изображений. Использован сервис Supervisely, который позволил с помощью прямоугольных рамок присвоить выделенным областям соответствующие классы. По итогам разметки изображений выделено три класса ягод с учетом их степени зрелости: незрелая ягода (unripe_strawberry), зрелая ягода (ripe_strawberry), несозревшая ягода (half_ripe_strawberry). К классу «несозревшая ягода» отнесены плоды, у которых площадь красного цвета на изображении составляла менее 60%. Аугментация набора данных, включающая такие операции, как обрезка, изменение размера, поворот, вертикальное отражение, размытие, изменение контраста, добавление шума, случайная коррекция цвета и добавление эффектов погодных условий, позволила увеличить объем выборки до 4500 изображений. Проведено обучение модели YOLOv10-M на созданной выборке данных, использовано 500 эпох, размер пакета данных установлен на уровне 8. В качестве алгоритма оптимизации выбран стохастический градиентный спуск (SGD, Stochastic Gradient Descent) с начальной скоростью обучения 0.01. Анализ графиков и метрик бинарной и мультиклассовой классификаций для оценки качества модели позволил определить оптимальные настройки и выбрать порог уверенности (0,7), при котором достигается наилучший баланс между точностью (метрика precision 0,93) и полнотой (метрика recall 0,89). Средняя абсолютная процентная ошибка (MAPE) распознавания изображений тестовой выборки для всех классов составила 3,4%. Наибольшие трудности при распознавании возникли с классом «несозревшая ягода», для которого средняя абсолютная процентная ошибка составила 4,6%.

Ключевые слова: земляника, определение зрелости, распознавание ягод, машинное обучение, сверточная нейронная сеть, глубокое обучение, роботизированный сбор

Machine learning algorithm for strawberry fruit recognition and classification of their maturity level

(✉)**Kutyrev A.I., Filippov R.A.**
Federal Scientific Agroengineering Center VIM
Moscow, Russia
(✉)e-mail: alexeykutyrev@gmail.com

The paper presents the results of research on the development of an algorithm based on a modern deep learning Convolutional Neural Networks model, which improved the accuracy of strawberry fruit recognition and classification of the degree of removable ripeness. The research utilized transfer

learning method to adapt the used YOLOv10-M (You Only Look Once version 10 medium) model, originally trained on COCO dataset, to solve the task of strawberry berry ripeness degree classification. Image annotation (markup) was performed to create a training dataset. The Supervisely service was used, which allowed assigning appropriate classes to the selected areas of interest using rectangular frames. As a result of image annotation, 3 classes of garden strawberries of different maturity levels were identified: immature berry (class "unripe_strawberry"), mature berry (class "ripe_strawberry") and unripe berry (class "half_ripe_strawberry"). The unripe berry class included fruits that had less than 60% of the total red area in the image. Augmentation of the dataset, including operations such as cropping, resizing, rotation, vertical reflection, blurring, contrast variation, noise addition, random color correction, and addition of weather effects, increased the sample size to 4500 images. The YOLOv10-M model was trained on the created data sample, 500 epochs were used, the batch size was set to 8 (batch size). Stochastic Gradient Descent (SGD) with an initial learning rate of 0.01 (learning rate) was chosen as the optimization algorithm. Analysis of the graphs and metrics of binary and multiclass classification to evaluate the quality of the model allowed us to determine the optimal settings and to select a confidence threshold (0.7) that achieves the best balance between accuracy (precision metric 0.93) and completeness (recall metric 0.89). The mean absolute percentage error (MAPE) of recognition of the test sample images for all classes was 3.4%. The greatest difficulty in recognition occurred with the unripe berry class, for which the mean absolute percentage error (MAPE) was 4.6%.

Keywords: strawberries, maturity determination, berry recognition, machine learning, convolutional neural network, deep learning, robotic harvesting

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INTRODUCTION

Garden strawberries are one of the most profitable and widespread berry crops in the world. The most labor-intensive operation in their cultivation is harvesting, which accounts for 40–60% of labor costs.

Currently, this operation is primarily performed manually, as mechanizing or automating the harvest of fragile strawberries is quite difficult. Machine harvesting of garden strawberries is associated with a number of problems related to the biological development characteristics of this plant, such as overlapping berries, excessive foliage on the bushes, and fallen flower stalks. Machines for continuous berry harvesting did not gain widespread popularity, as the harvested fruit was mainly suitable for technical processing due to significant damage to the berries [1]. The next step in the development of harvesting machines was the emergence of robotic technology for selective harvesting of ripe berries [2–5].

To ensure high-quality robotic harvesting of garden strawberries in open ground, it's essential to maintain high row cultivation standards. Using black polyethylene film for row mulching creates a uniform background, significantly

facilitating berry recognition by machine vision systems. The uniform texture minimizes the effects of shadows and color variations, allowing algorithms to more accurately determine berry ripeness.

With the development of optics and microelectronics, computer vision systems have become more accessible, which has contributed to the intensification of research in the field of creating robotic systems with optical sensors for determining the ripeness of strawberries during automated harvesting [6–9]. The widespread adoption of these technologies in crop production is hampered by the lack of technical solutions capable of automated recognition of ripe strawberries in real time.

Scientists used computer vision systems based on color thresholding methods [10, 11]. These methods use color differences to differentiate ripe berries from unripe berries and leaves [12]. Machine learning algorithms were used for pixel-by-pixel recognition of strawberry stalks [7, 13]. RGB-D stereo depth cameras were used to recognize berries and determine their location coordinates [14]. However, most commercially grown strawberry varieties do not ripen uniformly enough for machine picking. Machine vision systems face the challenge of accurately classifying berry ripeness.

The development of an algorithm capable of increasing the accuracy of strawberry recognition and classification of harvest maturity will improve the quality of robotic harvesting.

The purpose of the study is transfer learning of a convolutional neural network model for recognizing garden strawberries and classifying their degree of ripeness.

MATERIAL AND METHODS

The research analysis on the use of machine learning algorithms for biological object recognition revealed that among a variety of modern deep neural network architectures (e.g., ResNet, DenseNet, EfficientNet, MobileNetV3, InceptionV4, Xception), the YOLOv10(n-x) (You Only Look Once version 10) model is the most performant at real-time object detection. YOLOv10 combines classification accuracy and image processing speed, making it particularly effective for tasks involving the detection and classification of small objects, such as strawberries. Unlike other neural network architectures (such as SSD (Single Shot Detector) and Faster R-CNN), the YOLOv10 model offers greater robustness to challenging imaging conditions, including uneven lighting, shadows, low resolution, and partial object occlusion. Models from the EfficientDet family are widely used for recognizing berries and other generative parts of strawberries, thanks to their optimized architecture, which achieves a balance between accuracy and computational complexity. Well-known models such as RetinaNet and Mask R-CNN demonstrate high accuracy in segmentation and object detection tasks, but their real-time performance is significantly lower than YOLOv10 [15].

The YOLOv10-M (Medium) model contains 15.4 million parameters. It achieves high object recognition accuracy by using a machine learning algorithm, such as Big Data learning. Compared to previous models (YOLOv5–YOLOv9), the modern YOLOv10 model does not use the NMS (Non-Maximum Suppression) module to eliminate overlapping objects. In YOLOv10, this operation is replaced by a Multiple Assignment system. This solution not only speeds up the inference process but also simplifies the model architecture without affecting its accuracy [16].

The model architecture includes a Dual-Head block, consisting of two parts. The first part uses multiple label assignments, which improves training quality by allowing for more efficient object distribution for accurate recognition. The second part is based on a one-to-one approach, which improves accuracy through optimized matching of predictions to ground truth. The Efficient Classification Head in YOLOv10 part reduces the computational load while maintaining high object classification accuracy, making the model lighter and faster [17, 18] (see Fig. 1).

The study utilized transfer learning, which allows the YOLOv10-M model, initially trained on the COCO (Common Objects in Context) dataset, to be adapted to work with new data. This not only accelerates the learning process

when recognizing objects in new contexts but also improves accuracy by leveraging the model's previously acquired knowledge. [19].

The study was conducted on third-year Selva garden strawberry plants. A GoProHero 12 RGB camera with a 27MP sensor and 3840 × 2160 Ultra HD video resolution was used to collect data (images of strawberry rows). Illumination during filming ranged from 50,000 to 120,000 lux.

To create a training dataset, images were annotated (labeled). The Supervisely service was used to assign the corresponding class to selected areas using rectangular frames (Rectangl) (see Fig. 2).

The ripening process of the Selva variety's berries can be divided into several stages, each with a distinct surface color. At the "green" stage, the entire surface is a uniform green color. At the "blanching" stage, the berries acquire the conical shape typical of the variety, and their surface takes on a whitish color. At the "beginning of coloring" stage, the berry surface takes on a pale pink color of varying intensity. At the "ripening" stage, the entire berry except the "tip" is light pink to light red. At this stage, the berry's coloring ranges from 60-90% of the total surface area. At the "full color" stage, the berries acquire a uniform red color of varying intensity. The surface color of ripe berries in different varieties of garden strawberries can vary from pink to dark red with various shades of burgundy, raspberry, or orange (see Fig. 3).

Image tagging resulted in three classes of berries based on ripeness: unripe berry (unripe_strawberry), ripe berry (ripe_strawberry), and half-ripe berry (half_ripe_strawberry). The "unripe berry" class included fruits in which the red area in the images accounted for less than 60% of the total berry surface area. JSON (JavaScript Object Notation) format was used to store the tagging data.

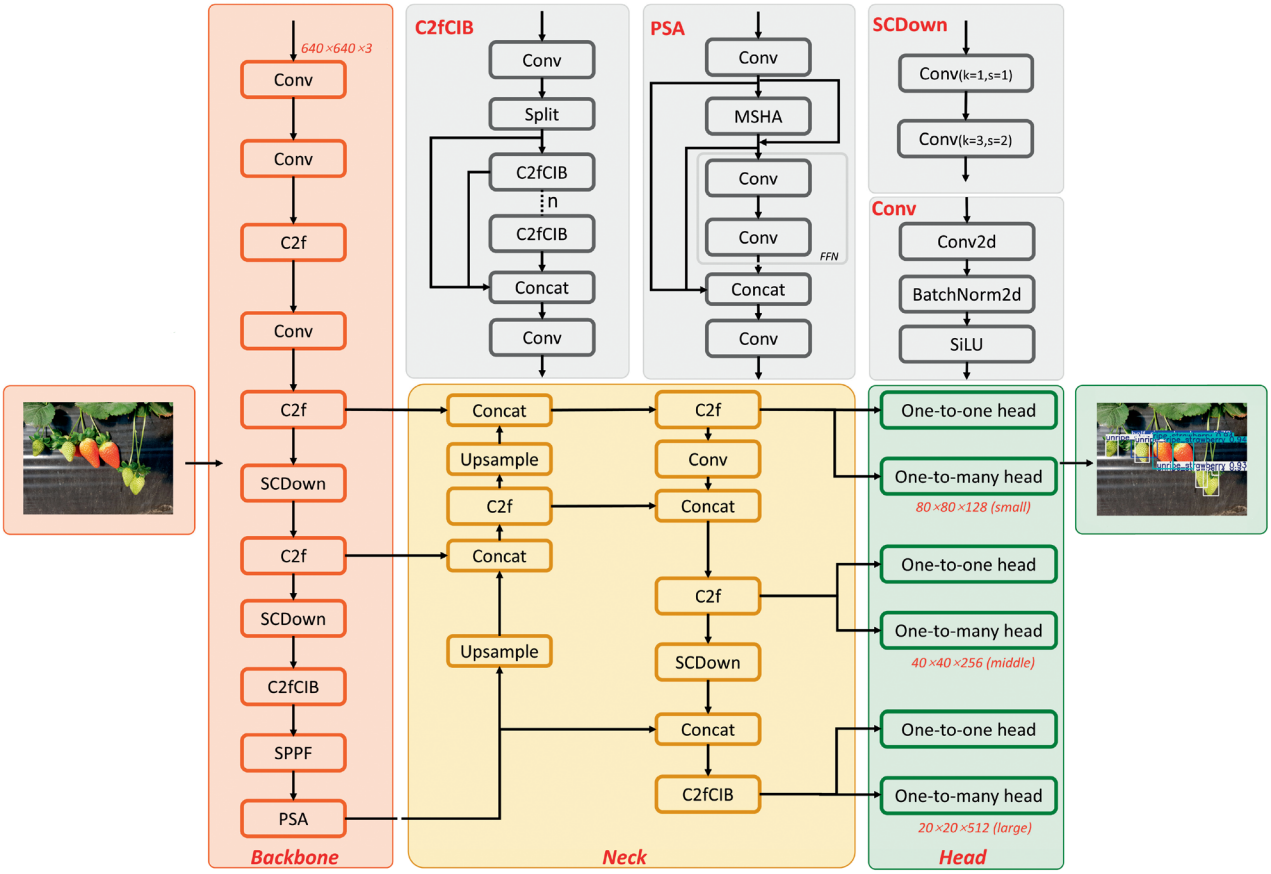

Рис. 1. Архитектура модели сверточной нейронной сети глубокого обучения YOLOv10

Fig. 1. Architecture of the YOLOv10 deep learning convolutional neural network model

To ensure class balance in the dataset, oversampling was used by creating new examples based on the existing data. A chain of image transformation functions (Pipeline) was constructed for this purpose (see Fig.).

Data augmentation included the following operations: cropping (crop: 1 px), resizing (resize), rotating by 45° (rotate: 45°), flipping vertically (flip: vertical), blurring (blur), changing contrast (contrast), adding noise (noise), random color correction (random color), and adding weather effects (fog and rain). As a result, the data size for model training was increased to 4,500 images.

To achieve an optimal balance of classes, the dataset was divided into three parts: training, validation, and test. The training set included 3,150 images (70% of the total) used to train the model. The validation set consisted of 900 images (20%) and served to evaluate the model's performance during training. The test set contained

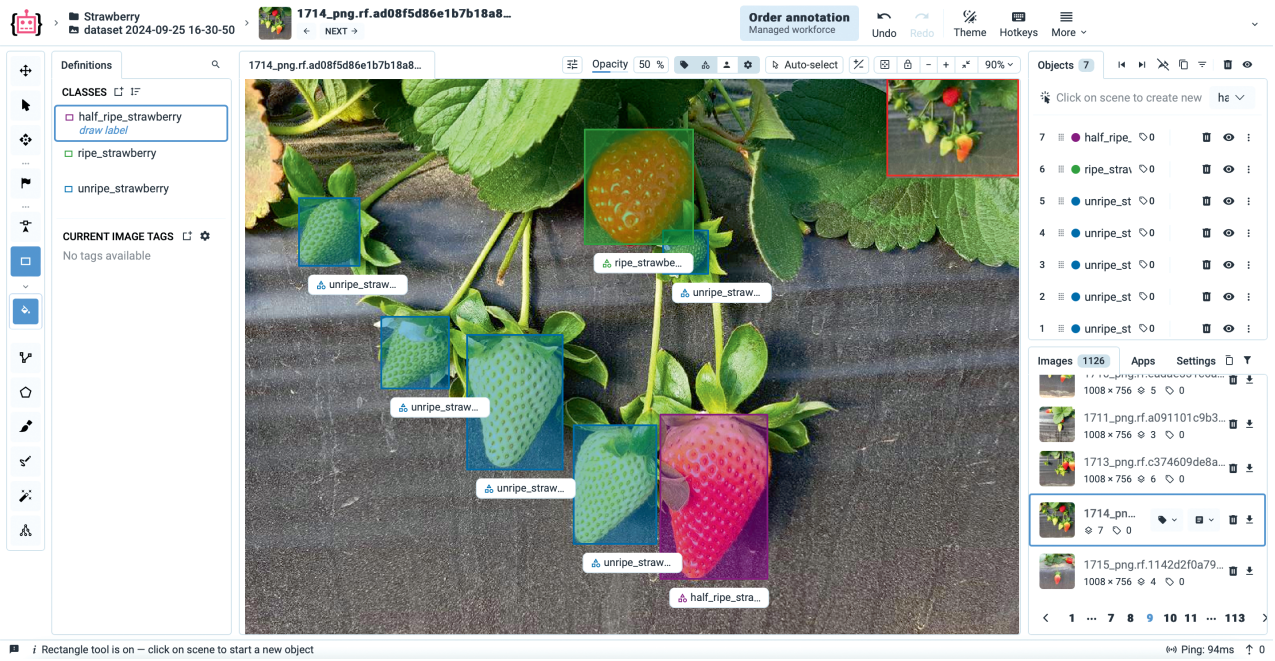

Рис. 2. Аннотация изображения в сервисе Supervisely
Fig. 2. Image annotation in the Supervisely service

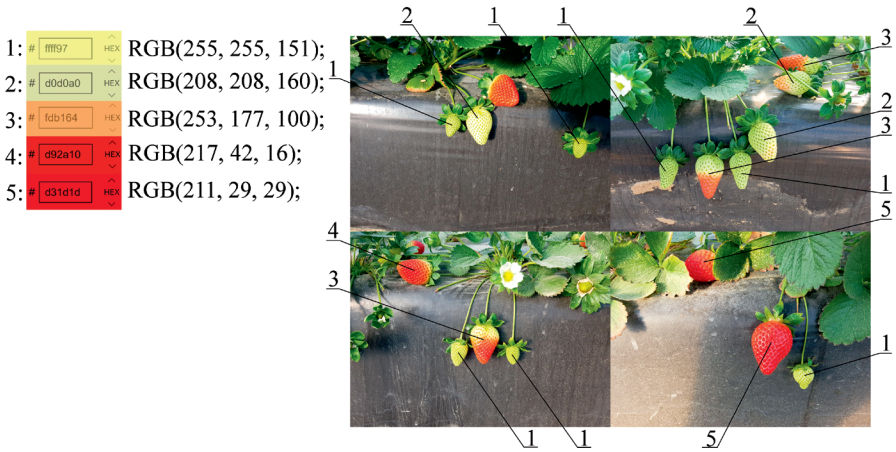

Рис. 3. Средние значения цветовых кодов RGB ягод земляники садовой и основные стадии созревания:
1 – зеленая; 2 – побеление; 3 – начало окрашивания; 4 – созревание; 5 – полный окрас
Fig. 3. Average RGB color codes of garden strawberries and main stages of ripening:
1 – green; 2 – whitening; 3 – beginning of coloring; 4 – ripening; 5 – full coloring

450 images (10%) and was intended for final validation of the model on independent data.

To train the YOLOv10-M model on the generated dataset, 500 epochs were used. The batch size was set to 8. Stochastic Gradient Descent (SGD) was chosen as the optimization algorithm with an initial learning rate of 0.01.

The YOLOv10-M model's performance was assessed using well-known metrics of precision (Precision) and recall (Recall). The mean average precision (mAP) metric was also used, providing a comprehensive assessment of model performance that takes into account both precision and recall, as well as correct and incorrect predictions at various classification thresholds. The analysis was conducted for both individual classes (binary classification) and all classes combined (multiclass classification) [20].

To train the neural network, we used a computing system with a 10-core Intel Core i9-10900X processor and two NVIDIA GeForce RTX 2080 Ti graphics cards for parallel data processing. The system has 64 GB of RAM, using Kingston DDR4 DIMM modules. A 1 TB Intel PCI-E SSD memory unit was used for data loading.

RESULTS AND DISCUSSION

During the study, the YOLOv10-M model was trained. The results of recognizing the given classes of garden strawberries in images are presented in Fig. 5.

The obtained graphs mAP50(Box)-Iterations, mAP95(Box)-Iterations, Precision (Box)-Iterations, Recall (Box)-Iterations, Box_loss-Iterations, Cls_loss-Iterations, Dfl_loss-Iterations show how the accuracy of the model (see Fig. 6) and the loss function (see Fig. 7) change depending on the iteration (epoch) of training. The graphs allow us to analyze how the metric values vary at different stages of model training at certain threshold values (confidence threshold 50 and 90–95%) in the recognition of bounding boxes and classification of garden strawberries.

Curve analysis allowed us to determine the number of training epochs (154) at which the model achieves the optimal balance between precision and recall. Training hyperparameters were determined to achieve the best model performance and maximum recognition accuracy for the given classes. The total training time for

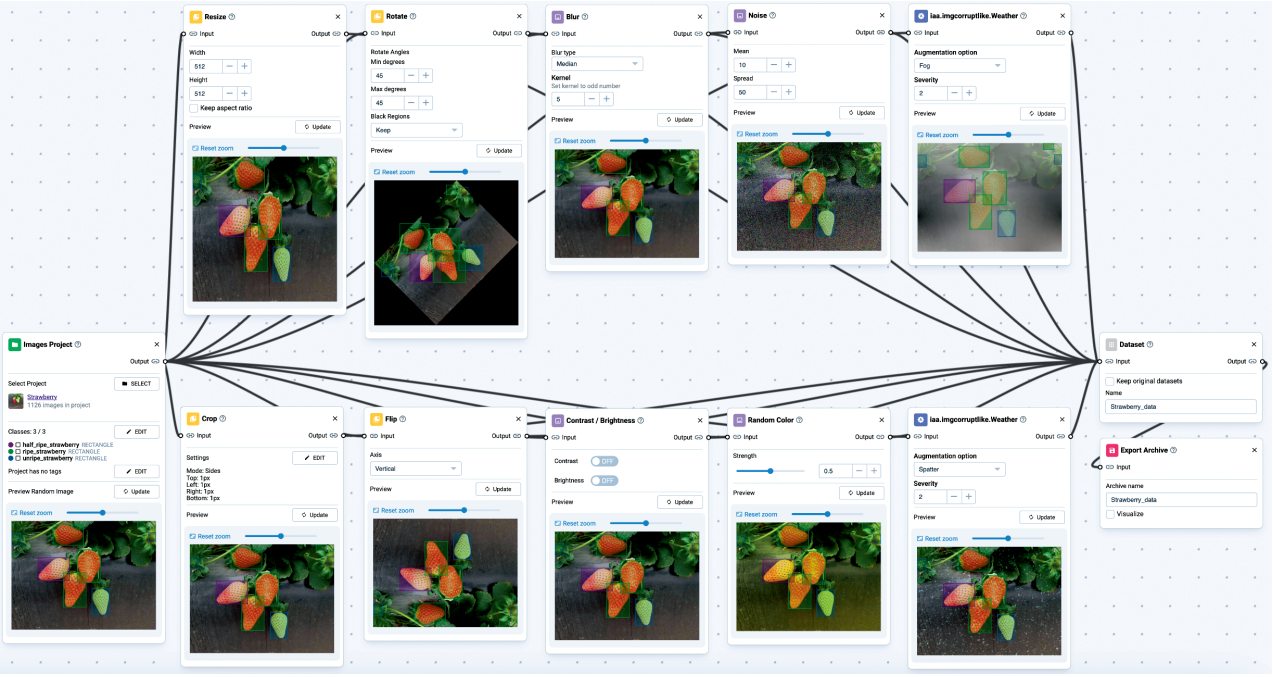

Рис. 4. Pipeline аугментации полученного набора данных
Fig. 4. Pipeline for augmenting the received dataset

the YOLOv10-M model using a GPU (Graphics Processing Unit) was 13 hours 54 minutes.

The results of calculating metrics for recognizing both individual classes and average values for all classes (multi-class classification) are presented in Table 1.

Analysis of the obtained results showed that the "half_ripe_strawberry" class was the most difficult to recognize, with an average precision (mAP50) of 0.712. The lowest recognition difficulty was observed for the "ripe_strawberry" class, with an average precision of 0.868 and a recall of 0.964.

Analysis of the mean absolute percentage error (MAPE) in recognizing the given classes in the images of the test data set was also carried out (see Table 2).

Analysis of the obtained results showed that the average absolute percentage error of image recognition in the test sample for all classes was 3.4%. The greatest difficulties in recognition arose with the class "half_ripe_strawberry", for which the MAPE was 4.6%. The average frame processing speed of the YOLOv10-M model was 25.3 frames/s.

CONCLUSION

The research resulted in the development of a machine learning algorithm for recognizing garden strawberries and classifying their ripeness. It was found that variations in berry color at different stages of ripening make it difficult to recognize unripe berries. Analysis of graphs and metrics for binary and multiclass classifications

Puc. 5. Результаты распознавания ягод земляники садовой на изображениях
Fig. 5. Results of recognition of garden strawberry berries in images

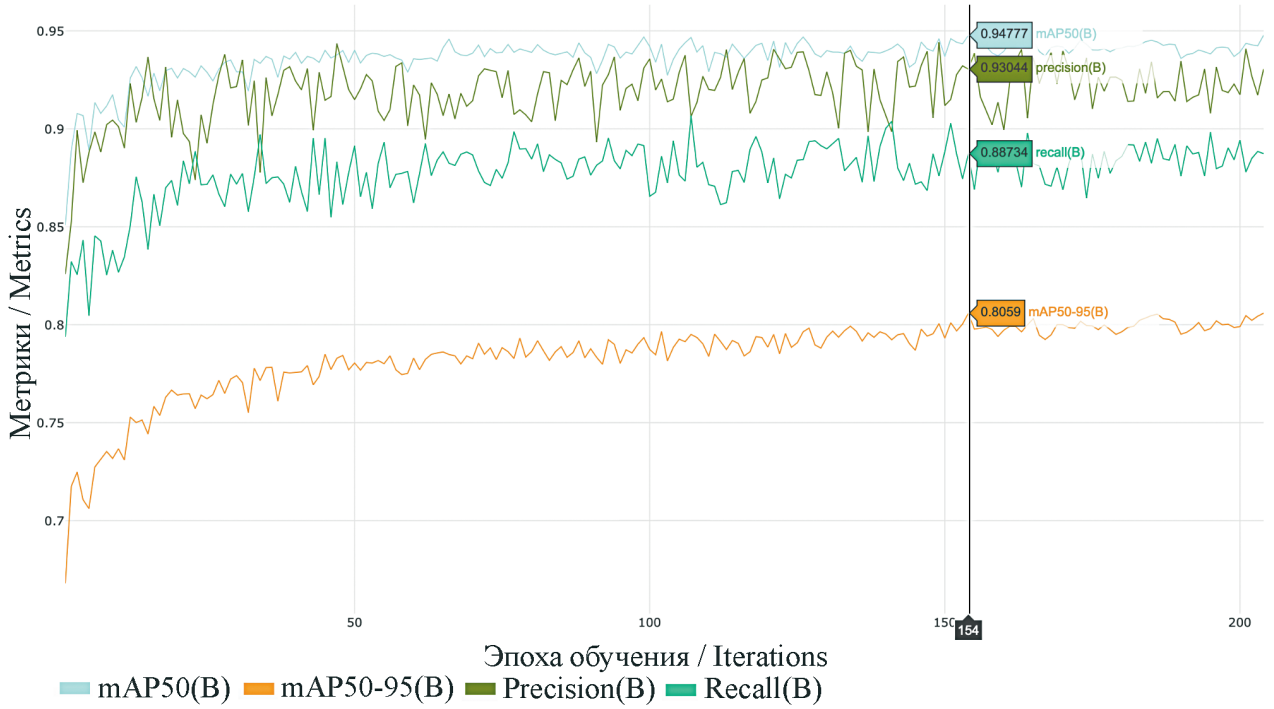

Рис. 6. Графики метрик точности и полноты

Fig. 6. Precision and Recall Metrics Graphs

Рис. 7. Графики метрик функции потерь

Fig. 7. Graphs of loss function metrics

to assess the quality of the YOLOv10-M model allowed us to determine the optimal settings and select a confidence threshold (0.7) that achieves the best balance between precision and recall.

Augmentation of the training set of berry images, including cropping, resizing, rotation, vertical flipping, blurring, contrast changes, adding noise, random color correction, and weather effects, increased the accuracy of the YOLOv10-M model by 7.2% compared to training without augmentation.

The developed algorithm will not only allow for the recognition and classification of garden strawberry fruits for the effective use of growth regulators (mineral nutrition, water regime, etc.), but also for calculating optimal trajectories for robotic devices during automated harvesting.

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Табл. 1. Результаты расчета метрик бинарной и мультиклассовой классификации
Table 1. Results of calculating binary and multiclass classification metrics

Classifier	Precision	Recall	mAP50	mAP50-95
Ripe_strawberry	0,911	0,919	0,964	0,868
Unripe_strawberry	0,935	0,866	0,942	0,837
Half_ripe_strawberry	0,945	0,877	0,937	0,712
Average	0,930	0,887	0,948	0,806

Табл. 2. Результаты расчета средней абсолютной процентной ошибки модели нейронной сети YOLOv10-M
Table 2. Results of calculating the average absolute percentage error of the YOLOv10-M neural network model

Classifier	Number of test sample images, pcs.	Number of the images correctly classified by the trained model., pcs.	Mean absolute error (MAPE), %
Ripe_strawberry	450	439	2,4
Unripe_strawberry		436	3,1
Half_ripe_strawberry		429	4,6
Average		435	3,4

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ИНФОРМАЦИЯ ОБ АВТОРАХ

(✉)**Кутырёв Алексей Игоревич,** заведующий лабораторией, ведущий научный сотрудник, кандидат технических наук; SPIN-код 9793-5074; **адрес для переписки:** Россия, 109428, Москва, 1-й Институтский проезд, 5; e-mail: alexeykutyrev@gmail.com

Филиппов Ростислав Александрович, ведущий научный сотрудник, кандидат сельскохозяйственных наук; SPIN-код 4742-7353

AUTHOR INFORMATION

(✉)**Alexey I. Kutyrev,** Laboratory Head, Lead Researcher, Candidate of Science in Engineering; SPIN-code 9793-5074; **address:** 5, 1st Institute passage, Moscow, 109428, Russia; e-mail: alexeykutyrev@gmail.com

Rostislav A. Filippov, Senior Researcher, Candidate of Science in Agriculture; SPIN-code 4742-7353

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Средства механизации для поверхностного улучшения горных кормовых угодий

Джибилов С.М., (✉)**Гулуева Л.Р.**
Северо-Кавказский научно-исследовательский институт горного и предгорного сельского хозяйства – филиал Федерального научного центра «Владикавказский научный центр Российской академии наук»
Республика Северная Осетия – Алания, с. Михайловское, Россия
(✉)e-mail: luda_gulueva@mail.ru

В статье представлен малогабаритный комбинированный агрегат, оснащенный орудиями и приспособлениями для восстановления природных кормовых угодий. Разработаны технология и конструкторская документация, изготовлен лабораторный образец агрегата. Техническую экспертизу и испытания проводили в горной зоне Республики Северная Осетия – Алания на высоте 1540 м н. у. м. Определены показатели условий испытаний и функциональные показатели работы агрегата: объем цистерны, коэффициент опорожнения емкости цистерны, фактическая доза внесения удобрений, необходимая норма высева, коэффициент прикатывания семян в почву. Предметом исследований являлись технология и нормы высева семян, конструкция агрегата и функциональные показатели рабочих органов (маятниковых высевающих аппаратов, емкостей для жидких удобрений). Главным отличием конструкции разработанного блок-модуля являются его малые габариты (ширина 2,4 м) и маневренность в условиях горных мелкоконтурных участков. Лабораторный образец агрегата соответствует агротехническим требованиям и техническому заданию. Согласно расчетам определены необходимый объем цистерны ($E_ц$ = 240 л), коэффициент опорожнения емкости цистерны ($K_о$ = 0,995), выявлено, что прикатывание семян необходимо производить двухрядными катками, масса высеваемых семян за один оборот катушки составляет 2 г. В результате изготовлен комбинированный малогабаритный агрегат, который способствует образованию более густого травостоя, препятствует водной и ветровой эрозии почв, способствует снижению деградационных процессов склоновых участков путем улучшения качества и количества многолетних трав в виде высококачественных кормов, обеспечивает повышение продуктивности агроландшафтов и рентабельности сельскохозяйственного производства на 15–20%.

Ключевые слова: горы, склоны, луга, пастбища, семена трав, жидкие удобрения, механизация

Mechanization tools for surface improvement of mountain forage lands

Dzhibilov S.M., (✉)**Gulueva L.R.**
North Caucasian Institute of Scientific Research of Mountain and Foothill Horticulture – Branch of the Federal Scientific Center "Vladikavkaz Scientific Center of the Russian Academy of Sciences"
Mikhailovskoe village, Republic of North Ossetia – Alania, Russia
(✉)e-mail: luda_gulueva@mail.ru

The article presents a small-sized combined unit equipped with tools and devices for restoring natural forage lands. Technology and design documentation have been developed, and a laboratory sample of the unit has been manufactured. Technical expertise and tests were carried out in the mountainous area of the Republic of North Ossetia – Alania at an altitude of 1,540 meters above sea level. The parameters of the test conditions and the functional parameters of the unit are determined: the tank volume, the tank capacity emptying coefficient, the actual dose of fertilizers, the required seeding rate, and the coefficient of seed compaction into the soil. The subject of the research was the technology and norms of seeding, design of the unit and the working bodies: pendulum seeding mechanisms, containers for liquid fertilizers. The main difference between the design of the block module is its small dimensions (a width of 2.4 m) and maneuverability in mountainous shallow-contoured areas. The laboratory sample of the unit complies with agrotechnical requirements and technical specifications. According to the calculations, the following were determined: the required tank volume E_c = 240 liters, tank emptying coefficient (K_o = 0.995), and it was found that it is necessary to do rolling of the seeds

with double-row rollers, the weight of the sown seeds per one revolution of the roller is 2 g As a result, a combined small-sized unit has been manufactured that promotes the formation of a thicker herbage, prevents water and wind erosion of soils, reduces the degradation processes of sloping areas by improving the quality and quantity of perennial grasses in the form of high-quality feed and increases the productivity of agricultural landscapes and profitability of agricultural production by 15–20%.

Keywords: mountains, slopes, meadows, pastures, grass seeds, liquid fertilizers, mechanization

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Conflict of interest
The authors declare no conflict of interest.

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INTRODUCTION

In the Russian Federation, mountainous landscapes occupy approximately 57% of the country's total area, including 48% of North Ossetia's territory. In conditions of North Ossetia, natural hayfields and pastures are generally the only feasible and reliable sources of high-quality, environmentally friendly, and affordable feed. However, the republic's mountainous forage lands are currently depleted, necessitating urgent measures to restore and improve their productivity.

Average crop yields in mountainous areas are 1.7–2.0 times lower than on the plains [1]. Furthermore, mountainous areas are characterized by a high degree of risk and dependence on natural and climatic conditions, as well as low social, industrial, and technical infrastructure. The lack of sufficient mechanization in the industry and, as a result, the outflow of population and producers from the mountainous zone to the plains entails a reduction in the area of hayfields and pastures.

The lack of basic pasture and hayfield maintenance has led to progressive soil erosion and a significant reduction in soil fertility. As a result, the topsoil has been partially or completely destroyed, and the humus and mineral content has decreased by 25–80%. This could lead to the complete degradation of mountain meadows and pastures [2].

Specific conditions of mountainous areas (small contours, complex configurations, slopes of varying steepness, and fragmented terrain) do not allow the use of standard equipment designed for flat terrain [3]. Equipment used in mountainous areas must be stable on slopes, maneuverable, compact, and designed to take into account all the characteristics of mountainous slopes.

The purpose of the research is to develop a laboratory prototype of a small-sized multifunctional unit block module capable of performing three operations in succession on meadows and pastures in the mountainous zone of the Central Caucasus: sowing grass seeds, applying liquid fertilizers and then compacting them with the soil.

The objectives of the research included:

1) study, justification and development of technology for overseeding grasses and applying liquid mineral fertilizers to mountain meadows and pastures, taking into account the characteristics of sloping lands;

2) development of a circuit diagram and production of a laboratory prototype of a multifunctional unit;

3) determination of the functional indicators of the working parts of the unit.

MATERIAL AND METHODS

Employees of the North Caucasian Institute of Scientific Research of Mountain and Foothill Horticulture have proposed a method for surface improvement of mountain meadow-pasture lands, including the scattering of grass seeds with the simultaneous application of liquid fertilizers to the surface of the seeded area and subsequent rolling of the seeds with special pressure rollers, as well as a basic diagram of a laboratory prototype of a multifunctional unit for carrying out the above operations.

This method is effective even during dry periods, since the application of liquid fertilizers and the use of pressure rollers prevent the seeds from being carried away by the wind.

Such small-sized and maneuverable units for sowing grass seeds with the simultaneous application of liquid mineral fertilizers and subsequent rolling are necessary for the restoration of forage lands in mountain meadows and pastures [4].

The North Caucasus Mountains are considered relatively "young," as evidenced by the large number of rocks in the soil and on its surface. Therefore, the design of the laboratory prototype incorporates special spring-loaded devices that prevent damage to the working parts upon impact with rocks and restore their working position after passing the obstacle.

The proposed method and unit create favorable conditions for the germination of grass seeds: they provide moisture and nutrition in the form of liquid mineral fertilizers and subsequent rolling, creating close contact between the seeds and the soil [5].

Tests of all three modules of the prototype unit were carried out in the mountainous zone of the Republic of North Ossetia-Alania (Dargavskaya basin, the NCISRMFH support point) at an altitude of 1540 m above sea level on sites with a steepness of up to 15° in accordance with OST (all-union standard) 10 6.1–20001 (liquid fertilizer application by spraying) and OST 10 5.1–20002 (seed sowing). When studying the seed rolling operation, a method for determining the seeding depth and compaction coefficient developed jointly with the NCISRMFH mountain meadow farming department for a sheep hoof "simulator" was used.

According to agrotechnical requirements (ATR):

1) the volume of work is 300-400 hectares per season, depending on the plan for improving slope lands;

2) the laboratory sample should ensure operation on the slopes on soils with a moisture content of 60–80% and a hardness of up to 3.5 MPa;

3) when working on slopes, the surface slopes should be no more than 16°;

4) before work, pastures must be cleared of livestock;

5) the stubble height before sowing grass and applying liquid fertilizers should be no more than 5–7 cm [6].

The calendar dates for overseeding grasses with the simultaneous application of liquid fertilizers are as follows: September 1st to 30th, August 15th to 30th, March 1st to April 30th, and any other time convenient for the farm, provided there is no risk of seed and fertilizer being washed away by meltwater and rainfall. Overseeding should be carried out when the soil moisture level is sufficient (60–80%), in accordance with the technological map for cultivating meadows and pastures [7].

[1]OST 10 6.1–2000. Industry Standard. Agricultural Machinery Testing. Sprayers and Machines for Preparing Working Fluids. Methods for Assessing Functional Indicators. Moscow, 2000.

[2]OST 10 5.1–2000. Industry standard. Agricultural machinery testing. Seeding machines. Methods for evaluating functional indicators. Moscow, 2000.

RESULTS AND DISCUSSION

Based on the technical specifications and in accordance with the ATR, NCISRMFH staff developed a basic diagram of a laboratory prototype of a multifunctional unit for sowing grass with pre-sowing application of liquid fertilizers on sloping areas of mountain meadows and pastures (see Fig. 1).

The KCHG-2.4 compact mountain cultivator with a working width of 2.4 m, designed by NCISRMFH, is used as the basis. A tank is mounted on the cultivator frame, and compressed air is supplied to the top of the tank from the tractor's compressor via an air duct and a receiver. The working solution is then pumped under pressure from the tank into the manifold, where it is sprayed onto the soil surface using nozzles. A solution flow control valve is installed in the tractor cab, allowing the driver to shut off the flow of the working solution when turning at the end of a field or when moving from one field to another in transport mode.

The air pressure in the tank is regulated by a pressure-reducing valve mounted on the receiver and monitored by a pressure gauge. When refueling and parking the unit, the spray system must be vented of the compressed air. A drain valve is installed on the receiver for this purpose.

The tank is filled with the working solution through the tank filler cap. The working solution level in the tank is monitored using a level gauge. To ensure complete emptying on a slope, the tank is equipped with a suction manifold, which ensures fluid intake when the unit tilts to the right or left [8].

Next, the seeds are sown onto the moistened soil using a grass seeding unit. The grass seeding unit is driven by a chain drive connected to a support and drive wheel. The seeding rate is adjusted using replaceable sprockets and the working length of the seeding unit roller.

To prevent seeds from spilling into the right or left side of the box when working on trans-

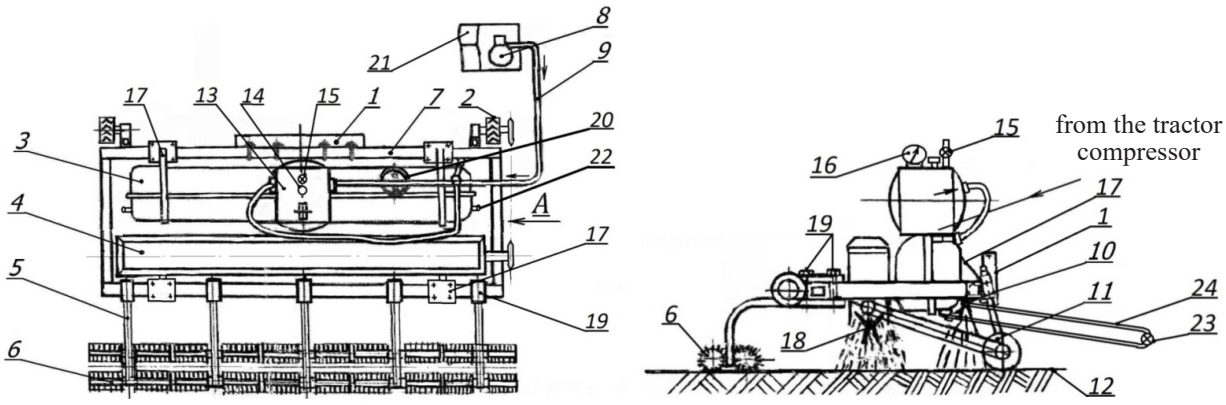

Рис. 1. Принципиальная схема конструкции опытного образца блок-модуля многофункционального агрегата:
1 – автосцепка СА-2; *2* – опорно-приводное колесо; *3* – цистерна; *4* – травяной высевающий аппарат; *5* – пружинистая стойка; *6* – прижимные катки; *7* – рама культиватора КЧГ-2,4; *8* – компрессор трактора; *9* – воздухопровод; *10* – коллектор; *11* – цепной привод; *12* – почва; *13* – ресивер; *14* – редукционный клапан; *15* – спускной клапан; *16* – манометр; *17* – кронштейн крепления; *18* – разбросная конусная трубка; *19* – хомуты крепления; *20* – крышка цистерны; *21* – трактор; *22* – уровнемер; *23* – кран управления потоком рабочего раствора; *24* – шланг для подачи рабочего раствора

Fig. 1. Schematic diagram of the design of a prototype block module of a multifunctional unit:
1 – automatic hitch SA-2; *2* – supportive driving wheel; *3* – tank car; *4* – grass-seed feed; *5* – flexible tine; *6* – pressing rolls; *7* – KChG-2,4 cultivator frame рама; *8* – tractor compressor; *9* – air pipe; *10* – collector; *11* – chain drive; *12* – soil; *13* – receiver; *14* – pressure-regulating valve; *15* – drain valve; *16* – manometer; *17* – mounting bracket; *18* – broadcast cone pipe; *19* – attachment clips; *20* – tank lid; *21* – tractor; *22* – level gauge; *23* – working solution flow control valve; *24* – hose for supplying working solution

verse slopes, the seed hopper is equipped with partitions between the reel-type seeding units [9, 10].

From the roller seeding unit, a spontaneous flow of seeds enters a cylindrical receiving tube, from which they reach the top of a ribbed spreading cone. From the top of the cone, the seeds are evenly distributed around the perimeter, then directed by gravity through the inter-rib channels to the base of the cone. Near the center of the cone, the seeds encounter an air flow, which disperses them into a uniform spray onto the soil. A pendulum suspension allows the spreading unit to remain vertical on both flat and sloped surfaces.

While previously sown grass seeds were traditionally trampled by the hooves of sheep, driven repeatedly by the flock, the newly developed unit is equipped with double-row ring rollers of the NCISRMFH design for this purpose. To prevent damage when encountering stones or rocky outcrops [11], the rollers are suspended on spring-loaded stands from Väderstad company (Sweden).

Two-cascade rollers cover a larger surface and increase the seed compaction coefficient over the treated area by 2 times compared to single-cascade rollers.

In the experimental workshop conditions, the NCISRMFH mechanization group manufactured a laboratory prototype of the machine in accordance with the design and technical documentation (see Fig. 2).

According to the proposed basic diagram, three functional units and components are installed on the frame of the KCHG-2.4 cultivator: a unit that ensures the process of applying liquid mineral fertilizers by spraying; a unit for sowing grass seeds by broadcasting; a unit for rolling seeds into pre-fertilized and moistened soil.

The process is as follows. The working solution is poured into the tank through a filler neck equipped with a filter mesh. The solution level is monitored using a level gauge. The solution should occupy 4/5 of the tank's volume, as the compressed air will occupy the remaining 1/5 of the solution above. Compressed air is then injected into the top of the tank, creating excess pressure inside (P = 0,25–1,50 kg/cm^2).

Рис. 2. Общий вид лабораторного образца агрегата
Fig. 2. General view of the laboratory sample of an aggregate

Under pressure, the solution from the tank flows into the spray manifold through a filter, preventing debris from clogging the sprayers. The application rate of liquid fertilizer is regulated by the pressure in the tank and replaceable sprayers with different outlet diameters [12, 13].

Seeds are poured into the hopper of the grass seeding unit. A chain drive transmits rotation to the seeding unit's rollers, which eject a spontaneous stream of seeds into the receiving tube. The receiving tube directs the flow of seeds to the apex of the spreading cone, from which they are evenly distributed across the soil surface. The seeding rate is adjusted by adjusting the chain drive ratio using replaceable sprockets and by varying the length of the working section of the seeding unit's roller [14]. To disengage the seeding unit, move the support-drive wheel away from the soil (transfer the unit to the transport position).

Next, the grass seeds scattered over the soil surface must be compacted to a depth of 3–4 mm. This operation is accomplished using NCISRM-FH ring-type compactor rollers, which imitate sheep hooves. The seed compaction depth is adjusted by adjusting the position of the support wheels and the choice of spacers between the tine and the cultivator frame.

The rollers are suspended from a spring-loaded shank to prevent damage when encountering rocky outcrops or stones. Furthermore, the spring-loaded roller suspension and their staggered arrangement across the machine's direction of travel ensure full contact with the soil and conform to its macro- and micro-unevenness [15].

Evaluation of the functional indicators of the liquid fertilizer application unit

Functionally, the tank must hold the amount of solution required for 1 hour of continuous operation of the unit without refueling. If the speed on the slopes is 5 km/h and the unit's working width is 2.4 m, then the volume of solution required for continuous operation of the block module for 1 hour can be determined using the formula

$$E_ч = S_ч \cdot N, \quad (1)$$

where $E_ч$ – the tank capacity, providing 1 hour of unit operation without refueling, l; $S_ч$ – field area treated by the unit in 1 hour, ha; N – application rate of the working solution, l/ha. In our case it is $N = 200$ l/ha.

The area of the field treated in 1 hour can be calculated using the formula

$$S_ч = Ш_р \cdot V_р \cdot k \cdot 0{,}1, \quad (2)$$

where $Ш_р$ – working width of the unit, m ($Ш_р$ = 2,4 m); $V_р$ – operating speed of the unit, km/h ($V_р$ = 5 km/h); k – coefficient of utilization of the working time taking into account the time lost on turns, idle runs, etc. (k = 0,8).

Transforming formulas (1) and (2), we receive

$$E_ч = Ш_р \cdot V_р \cdot k \cdot 0{,}1 \cdot N =$$
$$= 2{,}4 \cdot 5 \cdot 0{,}8 \cdot 0{,}1 \cdot 200 = 192 \sim 200 \text{ л.}$$

Therefore, we assume the working solution volume in the tank for continuous operation of the unit for 1 hour is 200 liters. However, it is important to keep in mind that there must be some space left in the upper part of the tank for compressed air. According to our calculations, this space should be 1/5 of the tank's volume. Consequently, the total capacity of the tank will be:

$$E_ц = E_ч + 1/5E_ч = 200 + 200/5 = 240 \text{ л.}$$

Based on the calculation results, a 240-liter tank was installed on the prototype.

The tank emptying coefficient was determined by the formula

$$K_о = \frac{M_3}{M_1}, \quad (3)$$

where M_1 – mass of liquid fertilizers initially loaded into the tank, kg (M_1 = 240 kg);

M_3 – mass of liquid fertilizers consumed after maximum unloading of the tank, kg.

At the same time

$$M_3 = M_1 - M_2,$$

where M_2 – mass of liquid fertilizers remaining in the container after its maximum unloading, kg (M_2 = 1,2 kg).

The moment of completion of emptying the container is considered to be the cessation of a steady flow of fertilizer.

By transforming formula (3) and substituting the digital values into it, we obtain

$$К_о = \frac{М_1 - М_2}{М_1} = \frac{240 - 1,2}{240} = 0,995.$$

$К_о = 0,995$ – this is a fairly high coefficient of emptying the tank of the liquid.

The actual fertilizer application rate ($Qф$, kg/m^2) was determined by weighing the fertilizer container before and after application to a given area. The weighing error was no more than ±2%. The experiment was repeated three times.

The number of teeth on the chain drive sprocket of the spread seeding unit was calculated based on the maximum sainfoin seeding rate of 40 kg/ha. The laboratory unit was equipped with one seed hopper, with 12 reel-type seeding units at the bottom. This means that 12 seeding units operate within a 2.4 m working width. The unit must sow seeds after travelling 1 linear meter:

$$N_{пм} = \frac{N}{S}, \quad (4)$$

where $N_{пм}$ – required seeding rate, kg/ linear meter; N – seeding rate, kg/ha; S – length of the path when processing 1 ha, m.

The length of the vehicle's path is calculated using the formula

$$S = \frac{10\,000}{Ш_р}, \quad (5)$$

where $Ш_р$ – working seeding width, m; 1 ha = 10 000 m^2.

Substituting formula (5) into (4), we receive

$$N_{пм} = \frac{N \cdot Ш_р}{10\,000}.$$

The standardized quantity of the granules ejected by one reel apparatus is determined by the formula

$$N_{пм} = \frac{N \cdot Ш_р}{10\,000}.$$

After calculations we get

$$q = V_о \cdot \gamma,$$

where q and Vo – mass and volume of granules/ fertilizers passing through the coil in one revolution, respectively g and cm^3; γ – bulk density of fertilizers, g/cm^3.

The volume of fertilizer passing through the rollers per revolution is always different from their working volume. The special feature of the rollers is that, as they rotate, they shift and displace the so-called active layer of granules (outside the grooves), and not just the granules directly in the grooves.

Taking this circumstance into account, the following condition must be met:

$$V_о = V_ж + V_{ак},$$

where $V_ж$ and $V_{ак}$ – volume of fertilizers in the grooves and outside the grooves (active layer), cm^3 (on average $V_{ак} = 0,3V_ж$).

The volume of the grooves can be calculated using the formula

$$V_ж = \frac{\pi r^2}{4} \cdot n \cdot l,$$

where r – radius of the grooves, cm ($r = 0,06$ cm); n – number of the grooves on the reel ($n = 12$); l – coil length, cm ($l = 4$ cm).

After the transformations, we receive the formula for determining the weight of fertilizers passing through the coil in one revolution:

$$q = 1,3\frac{\pi r^2}{4} \cdot n \cdot l \cdot \gamma.$$

Substituting the available values into the formula, we receive

$$q = 1,3\,\frac{3,14 \cdot 0,036}{4} \cdot 12 \cdot 4 \cdot 1,2 = 2,14 \text{ г.}$$

We take the weight of sown grass seeds per one revolution of the coil to be equal to 2 g.

The number of revolutions of the seeding roller when the unit passes 1 linear meter can be determined from the expression

$$n_{кат} = \frac{N\,пмк}{q}, \text{ об/пог. м,}$$

where $N_{пмк}$ – seeding rate per linear meter with one roller.

Substituting the numerical values into the formula, we receive

$$n_{кат} = \frac{0,8}{2} = 0,4 \text{ об/пог. м.}$$

The number of revolutions made by the support-drive wheel during the passage of 1 linear meter can be determined by the expression

$$n_{опк} = \frac{1 \text{ пог. м}}{\pi D},$$

where $D_{опк}$ – diameter of the support and drive wheel, m.

Let's calculate the number of revolutions:

$$n_{опк} = \frac{1 \text{ пог. м}}{3{,}14 \cdot 0{,}45} = 0{,}7 \text{ об/пог. м.}$$

Then the gear ratio of the drive from the support wheel to the seeding unit can be expressed as

$$i = \frac{n_{опк}}{n_{кат}} = \frac{0{,}7}{0{,}4} = 1{,}75.$$

We take the number of teeth of the sprocket on the support wheel Z1 = 30, then the number of teeth on the shaft of the seeding unit

$$Z_1 = \frac{Z_1}{i} = \frac{30}{1{,}75} = 17.$$

To determine the seed compaction coefficient into the soil after the test sample has passed through the plot, we select a site measuring 1 x 1 m and record the area compacted by the pressure rollers on it.

The rolling coefficient is determined by the formula

$$К_п = \frac{S_п}{S_{об}},$$

where $S_п$ – the area rolled by rollers, m^2; $S_{об}$ – total area of the treated plot, m^2.

Taking into account the dimensions of the ring rollers (length of two rollers L = 400 mm), the diameter of the roller ($D_к$ = 100 mm), the diameter of the sheep hoof "simulators" ($D_{им.к}$ = 25 mm), the height of the roller with "simulators" (H = 140 mm), the number of sheep hoof "simulators" on two ring rollers (n = 200), we will make the necessary calculations.

Let's calculate the area of one sheep hoof "simulator":

$$S = \pi r^2 = 3{,}14 \cdot 1{,}25^2 = 490{,}6 \text{ мм}^2.$$

The area rolled by 200 sheep hoof "simulators" is equal to

$$S_п = 490{,}6 \cdot 200 = 98\,125 \text{ мм}^2.$$

The distance traveled by one "simulator" in one revolution:

$$l = \pi H = 3{,}14 \cdot 140 = 439{,}6 \text{ мм.}$$

The area of the site covered by the "simulators" in one revolution:

$$S_{об} = L \cdot l = 400 \cdot 440 = 176\,000 \text{ мм}^2.$$

It follows that the rolling coefficient is equal to

$$К_п = \frac{98\,125}{176\,000} = 0{,}558.$$

The obtained results show that the developed design of the compacting rollers ensures compaction of just over half of the seeds. Therefore, to increase the compaction coefficient, repeated compaction can be performed or a second cascade of rollers can be installed.

The proposed method and unit promote the formation of denser grass, create favorable conditions for accelerated seed germination even during dry periods, and prevent water and wind soil erosion. Furthermore, the weight load of the unit on the meadow agrocenosis during movement is minimized to a safe level — from 5 to 10 kg/m^2.

CONCLUSION

During the research, NCISRMFH specialists developed, manufactured, and tested a laboratory prototype of a multifunctional unit based on the KCHG-2.4 for underseeding grass with simultaneous application of liquid fertilizers and subsequent seed rolling in the mountainous conditions of the Central Caucasus. The laboratory prototype complies with agronomic requirements and the technical specifications. The required tank volume was determined based on the calculations ($Е_ц$ = 240 l) and the coefficient of emptying the tank capacity ($К_о$ = 0,995). It was found that seed rolling must be done using double-row rollers.

The developed laboratory prototype of the multifunctional unit has increased the productivity of agricultural landscapes and the profitability of agricultural production by 15–20%, promotes the formation of denser grass stands, creates favorable conditions for accelerated seed germination even during dry periods, and pre-

vents water and wind soil erosion. Furthermore, the weight load of the unit on the meadow agrocenosis during movement is minimized to a safe level (5–10 kg/m^2).

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ИНФОРМАЦИЯ ОБ АВТОРАХ

Джибилов Сергей Майрамович, кандидат технических наук

(✉)**Гулуева Людмила Романовна,** научный сотрудник; SPIN-код 1293-1244; **адрес для переписки:** Россия, 363110, Республика Северная Осетия – Алания, с. Михайловское, ул. Вильямса, 1; e-mail: luda_gulueva@mail.ru

AUTHOR INFORMATION

Sergey M. Dzhibilov, Candidate of Science in Engineering

(✉)**Ludmila R. Gulueva,** Researcher; SPIN-code 1293-1244; **address:** 1, Williams St., Mikhailovskoe village, Republic of North Ossetia – Alania, 363110, Russia; e-mail: luda_gulueva@mail.ru

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AUTHOR GUIDELINES

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

The journal publishes original articles on fundamental and applied issues by the following directions:

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

Section name	Code and name of the scientific specialty in accordance with the Nomenclature of Scientific Specialties, for which academic degrees are awarded
Agriculture and chemicalization	4.1.1. General agriculture and crop production 4.1.3. Agrochemistry, soil science, plant protection and quarantine
Plant growing and breeding	4.1.1. General agriculture and crop production 4.1.2. Plant breeding, seed production and biotechnology
Plant protection	4.1.3. Agrochemistry, soil science, plant protection and quarantine
Fodder production	4.1.1. General agriculture and crop production 4.1.2. Plant breeding, seed production and biotechnology 4.1.3. Agrochemistry, soil science, plant protection and quarantine
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Mechanization, automation, modelling and dataware	4.3.1. Technologies, machinery and equipment for the agro-industrial complex
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Submission of an article to the journal "Siberian Herald of Agricultural Science" implies that:

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an article is not subject to review in any other journal;
all co-authors agree with the publication of the current version of the article.

Before submitting an article, it is necessary to make sure that the file (files) contains all the information required in Russian and English, tables and figures provide the source of the information presented, all references are written correctly.

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The text of the manuscript is printed in Times New Roman font, type size 14 with 1.5 spacing, all margins 2.0 cm, page numbering at the bottom. The size of a manuscript should not exceed 15 pages (including tables, illustrations and bibliography); the articles placed in the sections "From dissertations" and " Brief reports" should not exceed 7 pages.

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8. The main body of the article. When presenting original experimental data, it is recommended to use subheadings:

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Monograph

Klimova E.V. Field crops of Zabaikalya: monograph. Chita: Poisk, 2001. 392 p.

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, 1990. pp. 230-235.

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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Example: Author A.A., Author B.B., Author C.C. Title of article.

Transliteration of the authors. English title of the article.

Zaglavie jurnala = Title of Journal, 2012, vol. 10, no. 2, pp. 49–54.

Transliteration of the source = English name of the source

Monograph

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

Part of a book

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

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FOOTNOTES:

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