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USE OF ANIMAL PRODUCT PROCESSING WASTE IN OYSTER MUSHROOM PRODUCTION TECHNOLOGY

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The article presents a study of the possibility of using animal husbandry processing waste as a nutrient additive to compositions of synthetic fermented substrate for the cultivation of oyster mushrooms. Wastes that are of low value for use in other branches of agriculture and industry and are mainly disposed of as solid waste by burial were investigated.

In modern economic conditions, it is important to reduce the cost of the substrate, one of the possible ways to achieve this goal is the use of cheaper raw materials. Meals and bran are traditionally used to balance the substrate in terms of nitrogen content. These products are waste products from the processing of agricultural crops and are also classically used to balance the rations of farm animals and poultry. This creates competition between industries and increases the price of raw materials, which ultimately causes an increase in product prices. Therefore, the search and research of alternative balancing components for the preparation of oyster mushroom substrate, which are not competitive for animal husbandry, is relevant. The analysis of the obtained results made it possible to form the following conclusions: their use as additives for the preparation of the substrate will allow them to be used in further processing, with the obtaining of additional products of mushroom production, the study of the growth of colonies of oyster mushrooms using the selected additives made it possible to determine the best options: yes, the addition of skin shavings and the addition of pores. It does not inhibit the colonization of these additives, but, on the contrary, increases the growth rate of colonies compared to colonies developed without additional nutrition; the obtained data of the laboratory experiment can be considered primary and those that require further research, including when using other methods of preparation of additives (sterilization, solid-state fermentation), as well as verification in scientific and economic experiments on the determination of technological properties in substrate compositions and the calculation of biological effectiveness and productivity of oyster mushrooms and other saprophytes.

Key words: waste, leather, feathers, animal husbandry, technology, mushrooms, oyster mushroom.

ВИКОРИСТАННЯ ВІДХОДІВ ПЕРЕРОБКИ ПРОДУКЦІЇ ТВАРИННИЦТВА В ТЕХНОЛОГІЇ ВИРОБНИЦТВА ГЛИВИ

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В статті наведено дослідження можливості використання відходів переробки продукції тваринництва в якості поживної добавки до композицій синтетичного ферментованого субстрату для вирощування гливи звичайної. Досліджувались відходи, що представляють низьку цінність для використання в інших



галузях сільського господарства та промисловості і в основному утилізуються як тверді відходи шляхом поховання.

В сучасних економічних умовах важливим є зменшення собівартості субстрату, одним з можливих шляхів досягнення цієї мети є використання більш дешевої сировини. Для балансування субстрату за вмістом азоту традиційно використовують шроту та висівки. Ці продукти є відходами переробки сільськогосподарських культур і також класично використовуються для балансування раціонів сільськогосподарських тварин і птиці. Це створює конкуренцію між галузями виробництва і підвищує ціну на сировину, що в кінцевому результаті спричиняє ріст цін на продукцію. Тому пошук і дослідження альтернативних балансуєчих компонентів для приготування субстрату гливи, таких, що не є конкурентними для тваринництва є актуальним. Аналіз отриманих результатів дозволив сформулювати наступні висновки: використання в якості добавок для приготування субстрату дозволить використати їх в подальшій переробці, з отриманням додаткової продукції грибовиробництва, дослідження росту колоній гливи звичайної з використанням обраних добавок дозволив визначити найкращі варіанти: так, додавання стружки шкіри та додавання пір'я не гальмує колонізацію цих добавок, а, навпаки, збільшує швидкість росту колоній в порівнянні з колоніями, що розвивались без додаткового живлення; отримані дані лабораторного дослідження можна вважати за первинні та такі, що потребують подальшого дослідження, в тому числі при використанні інших способів підготовки добавок (стерилізації, твердотільної ферментації), а також перевірки в науково-господарських дослідженнях з визначення технологічних властивостей в композиціях субстрату та розрахунку біологічної результативності і урожайності гливи та інших сапрофітів.

Ключові слова: відходи, шкіра, пір'я, тваринництво, технологія, гриби, глива.

The processing of agricultural products involves the complex use of raw materials without the generation of production waste and the introduction of technologies for the use of generated waste to obtain additional products (Kirylenko I.V., 2020; Kovtunuk Z.I., 2021).

Today, oyster mushroom production is one of the most intensively developing industries. The consumption of artificially grown mushrooms is constantly increasing. This is connected, on the one hand, with the increase in the production of mushrooms and their transformation into an independent branch of agriculture, on the other hand, with the collection of mushrooms, which annually decreases in the places of their natural growth (Simakhina, 2023; Bandura I.I., 2020, 2021)

According to I.I. Bandura (2020, 2019), the intensive development of industrial cultivation of edible mushrooms is due to a number of factors:

- High productivity of mushrooms, which is the highest yielding agricultural crop (the average yield of oyster mushrooms is 20-25% of the weight of the substrate).
- Mushrooms are a source of protein, vitamins, trace elements and medicinal substances.
- To grow edible mushrooms, agricultural, forestry and processing industry wastes are used, which are transformed by mushrooms into food and medicinal substances; that is, the problem of production of food and medicinal substances and the ecological problem of waste disposal are simultaneously solved.
- Mushroom cultivation technology is environmentally friendly, it can be fully mechanized, and in some cases automated.



• Mushroom cultivation is a zero-waste production, as the substrates after harvesting the mushrooms can be used as a protein-fortified additive for livestock feed or as fertilizers. Thus, today oyster mushroom production is a promising and profitable branch of agriculture (Bandura, I.I., Kulyk, A.S., et al., 2020; Shcherbak O.V., 2022; Shovdra V., 2023)

The oyster mushroom is very technological, has a high growth rate and significant competitiveness in relation to strange microflora. The mushroom grows on various cellulose- and lignin-rich plant wastes from agriculture, food and wood processing industries. In general, the oyster mushroom has no equal in terms of the number of substrates on which it is cultivated. The simplest substrate for intensive cultivation is wheat straw (Mykhaylova O. et al., 2011; Vdovenko S., 2020).

In our country, the following lignin- and cellulose-rich wastes of agricultural production are the main components of substrates for growing boletus fruit bodies: straw of cereal crops: wheat, rye, barley, oats, millet, rice; shredded leaves, corn stalks and cobs, waste from the cotton processing industry, sunflower and buckwheat husks, sawdust and shavings of deciduous trees (Yuskov D.S., 2020; Vlasenko K.M. et al., 2022). These wastes contain the main components of the energy supply of the oyster mushroom, as a wood-destroying saprophytic fungus: cellulose and lignin.

To achieve the optimal physical and chemical composition of the substrate, different compositions of the initial components are used. Thus, it is necessary to take into account the technology of thermal preparation of the substrate, since the physical and chemical properties of the components and the finished composition may change with different technologies.

In modern economic conditions, it is important to reduce the cost of the substrate, one of the possible ways to achieve this goal is the use of cheaper raw materials. Meals and bran are classically used to balance the substrate in terms of nitrogen content. These products are waste products from the processing of agricultural crops and are also classically used to balance the rations of farm animals and poultry. This creates competition between the branches of production and increases the price of raw materials, which ultimately causes an increase in product prices (Kovalyov M.M. et al., 2020). Therefore, the search and research of alternative balancing components for the preparation of oyster mushroom substrate, which are not competitive for animal husbandry, is relevant.

The analysis of numerous literary sources and Internet publications indicated the main requirements for the components of the substrate:

- the component should not impair the physical composition of the substrate: air permeability, absorption and retention of moisture, should not emit harmful substances that impair the growth and development of mycelium;
- the component must not contain harmful substances, which can deteriorate the quality and affect the safety of food products (commercial oyster mushroom);
- the component should be easily accessible for procurement (purchase) and use, not requiring technological complications when using it;
- the component should easily and evenly mix with other components of the substrate, without forming lumps and without interfering with the operation of the equipment for forming the substrate;
- the component must be cheap and distributed throughout the country, not requiring special and expensive storage conditions;
- the use of the component should improve the ecological situation and prevent environmental pollution (principle 5R). It is desirable that this is one of the production



wastes that can be used for further processing production (Recycle principle) (Chernyshov I., 2022).

Taking into account the specified requirements, among a large number of possible options, we chose the following possible alternative components:

- leather shavings - leather production waste,
- waste of the fur industry - substandard hides, furs and trimmings,
- remnants of feathers - waste from poultry slaughter.

The use of the selected components is used in the production technology of protein supplements to balance the rations of farm animals and poultry, but, taking into account the complexity of processing and the energy intensity of the technology, it is advisable to find other areas of use, in particular, as components of substrates in industrial mushroom cultivation.

The purpose of the study. The purpose of the work is the study of animal husbandry waste (leather shavings, fur industry waste, feather remnants) as substrate components for the cultivation of saprophytic mushrooms (oyster mushrooms).

To fulfil the set goal, the following tasks were expected to be performed:

- to investigate the nutritional and technological value of livestock waste as additives to the substrate;
- calculate the biochemical and technological indicators of waste as components of the substrate;
- to investigate the features of oyster mushroom mycelium growth on the proposed supplements.

The scientific novelty of the obtained results: new data were obtained on the possibility of using livestock waste for balancing substrates for growing oyster mushrooms.

The practical significance of the work lies in the possibility of introducing new balancing additives based on livestock waste into the production of substrates for saprophytic mushrooms.

Research materials and methods. The scientific work was carried out in the conditions of the laboratory complex of the department of technologies of processing and storage of agricultural products of the Kherson State Agrarian and Economic University.

The work was an integral part of the scientific and research work of the Department of Technologies for Processing and Storage of Agricultural Products of the Biological and Technological Faculty "Development and improvement of technologies for the production, processing, examination and quality control of livestock products using the best domestic and global gene pool in the farms of the Southern region of Ukraine" during 2021- 2022.

The selection and preparation of substrate samples was carried out according to the method of agrochemical examination of greenhouse soils and substrates

Samples were weighed on VLTK-500 quadrant laboratory scales with an accuracy of 0.01 g.

The preparation of the nutrient medium (potato agar) and the actual technique of oyster mushroom mycelium sowing were performed according to generally accepted methods (V.V. Yevlash et al., 2020).

To evaluate the growth of the mushroom culture, a method based on the study and analysis of the dynamics of the increase in the radius of the colonies from the time of cultivation was used. The radial growth rate (V_r) was calculated according to formula (1):



$$Vr = \frac{a-b}{t_1-t_0} \quad (1)$$

where: **a** is colony radius at the end of growth, mm;

b is radius of the colony at the beginning of the phase of linear growth, mm;

t₁-t₀ is duration of linear growth, days.

The modified growth coefficient (GC_j) was calculated according to formula (2) (Bukhalo A.S., 1988):

$$PKj = d \cdot h \cdot g \cdot j / t \quad (2)$$

where, **d** is the diameter of the colony, mm;

h is colony height, mm;

g is colony density in points;

j is homogeneity of the colony in points;

t is the age of the colony, days.

The study of morphological and cultural features on different substrates was carried out using the criteria described by A.S. Bukhalo (1988).

The effect of the additive on the growth rate of mycelium was determined by sowing two growth points on one Petri dish, one of which remained without additional nutrition until the end of the experiment, and one of the selected additives was placed next to the other. The repetition of samples is threefold.

The average samples for conducting experiments were taken:

- leather shavings – from industrial waste dumps of PE "Novik V.P", Kherson, in the form of shavings after milling of leather;

- fur industry waste - from industrial waste of PE "Plishko T.O.", Kherson, in the form of fur flaps of rabbit leather after cutting;

- feathers - from industrial waste dumps of "Avangard" LLC, Kherson, in the form of a wet mixture of cover feathers after processing chicken carcasses on beating machines.

The average sample of each sample was dried to a constant weight of the sample, after which it was crushed in a laboratory disintegrator to a fine fibrous mass. The obtained mass was pasteurized at 80 °C for 15 min, imitating the temperature regime of substrate pasteurization according to the author's hydrothermal treatment technology¹.

Research results. After forming the experiment, Petri dishes with nutrient medium, mycelium and additives were placed in a thermostat with a constant temperature of 18 °C. During the first two days, the mycelium adapted to the changed conditions of growth and nutrition. At the end of the second day (after 40 hours from seeding), microscopy of the experimental samples was carried out. The growth rate of oyster mushroom mycelium at this, initial, stage was the same, since the conditions of nutrition and growth were the same, which was required by the conditions of the experiment. During the further development of the mycelium, the hyphae approached the dosage of the additive, penetrated inside and colonized with assimilation of nutrients. The development of mycelium on the opposite side of the Petri dish occurred with a lack of nutrients on the depleted potato agar medium.

¹ Pat. 138577 Ukraina, MPC (2006) B09B 3/00 B01J 2/22 (2006.01) Sposib utylizatsii vidkhodiv silskohospodarskoho vyrobnytstva [The method of disposal of agricultural production waste] Zayavl. 10.12.2019 ; Opubl. 13.03.2019, Bul. № 23. 4 c. (in Ukrainian).



By changing the intensity of hyphae growth, it is possible to draw conclusions about the ability of the oyster mushroom enzyme system to break down the nutrients of additives; or, in the case of the same intensity of growth with the control point of growth, the impossibility of splitting and consumption of additional nutrients; or the slowing down of hyphal growth compared to the control point in the case of toxic effects of additive substances.

At the initial stages of the experiment, the mycelium of the mushrooms adapted and began active growth on the supplements of leather shavings and feathers. So, in the variant with fur production waste, mycelial growth stopped at the border with the additive and did not progress further. Obviously, the decomposition products of the raw leather had a toxic effect on the growth and development of the mycelium. This assumption was proven on the 5th day, when we observed the death of two out of three repetitions of this version of the experiment. The mycelial samples died at both growth points, and the agar had a distinct smell of hydrogen sulphide and ammonia. This proves that the use of fur processing waste in its raw form as additives to the oyster mushroom substrate is impossible with this method of processing the substrate (pasteurization).

The detailed analysis of the stages of colonization of the selected supplements with oyster mushroom mycelium at the initial stages of growth is given in previous publications (Chernyshov I.V., 2021, 2022).

In the course of research, we found that the densest mycelium of the mushroom with high aerial hyphae was observed in the variant with the addition of leather shavings. On this supplement, the oyster mushroom mycelium had a fluffy white colony with a clear and even edge, a dense opaque layer. The height of the aerial mycelium was at the level of 3–5 mm with well-developed hyphae. The smell is pleasant, mushroomy (Fig. 1).

On the feather supplement, the mycelium formed loose white colonies with weak radial zonation. The density of the colonies was somewhat lower, but the height of the aerial mycelium was higher (5–6 mm). The growth rate was the highest, at the end of the experiment the culture colonized most of the area of the nutrient medium. The smell is pleasant, mushroomy (Fig. 2).

The culture colony in the version of the experiment with fur waste had a clear demarcation line from the additive, predominant growth in the opposite direction, reduced growth intensity, dense hyphae. Two of the three repetitions of the experiment at the end of the experiment were affected by bacterial colonies, a sharp smell of ammonia and hydrogen sulphide was observed (Fig. 3).

The photos (Figs. 1, 2, 3) show bacterial colonies that developed parallel to the inoculated oyster mushroom, but this is explained by the fact that the experiment was not conducted in sterile conditions, the development of mycelium was close to production conditions.

To analyze the growth intensity of oyster mushroom mycelium in different versions of the experiment, according to the methodology, the size of the colonies was measured and compared with control points of growth. Measurements were performed on the 7th day from the beginning of the experiment.



Fig. 1. Colony size of oyster mushroom with the addition of leather shavings (top) and growth control points on the 7th day of growth



Fig. 2. Colony size of the oyster mushroom with the addition of feathers (top) and growth control points on the 7th day of growth



Fig. 3. Colony size of oyster mushroom with addition of fur processing waste (top) and control point on the 7th day of growth

The experiment was considered complete after colonization of half of the area of the nutrient medium of the Petri dish on the 7th day after inoculation. We measured the diameter of the colonies in the experimental variants and calculated the radial growth rate and growth coefficients. The appropriate indicators of the growth of colonies in



dishes with cultures were taken as the control variant. The calculations are shown in Table 1.

Table 1

The speed of radial growth of colonies and growth coefficients of the oyster mushroom using different animal husbandry and aquaculture wastes

Type of supplement	Rate of radial growth of colonies Vr, mm/day	Growth coefficient GCj,
Control (no additive)	0.8±0.1	28.4±1.5
Leather shavings	5.0±0.7	57.7±2.1*
Fur production waste	0.7±0.1	27.3±1.4
Feathers	6.4±1.2	156.0±12.2*

Note: * statistically significant difference compared to control, $P < 0.05$

Therefore, the growth rate of oyster mushroom mycelium clearly reflects the ability of the mushroom enzyme system to break down complex organic compounds, using the breakdown products as additional nutrients. The highest growth rate was found for the variant of the experiment with bird feathers. The growth rate was 1.4 mm/day higher than in the variant with the addition of leather shavings and 5.7 mm/day higher than with the addition of fur industry waste.

The calculation of the growth rate of the mycelium colonies proved the results of the observation of the growth and development of the colonies and the characterization of the morphological signs of the growth of the colonies. The colonies grew almost as well as the no supplemented controls, but the photographs indicated that the growth was directed along the fur supplement and in the opposite direction, proving the toxic effect of this supplement.

Calculations of the growth coefficient, which additionally takes into account the growth of colonies in height, generally confirm the data obtained during observation and calculations of the growth rate. Thus, colonies with additional nutrition from leather shavings occupied an intermediate value, more than the control option and less than the option with the addition of feathers. Colonies with added fur waste had slightly lower growth rates, but the difference was not significant.

Colonies supplemented with feather supplements not only had greater radial colony growth, occupying a larger area of the Petri dishes, but also had larger colonies in height. Despite the lower density of mycelial hyphae growth, the significant area of capture of the nutrient medium in recalculation gave the value of the growth coefficient by 127.6 points more than in the control variant.

Discussion. The results of the conducted experiment as a whole prove the data obtained in the studies (Bandura I.I. et al., 2021, 2020).

The biological disposal of leather waste with the production of additional food products is undoubtedly the best option compared to the burial of these wastes, as it was outlined in the research of M.K. Kolyada et al. (2014). The use of enzymatic methods of processing has a number of advantages over chemical ones. Considering that the assimilation of nutrients from leather shavings in our experiments occurs under the influence of the enzyme system of the mycelium of mushrooms, it can be assumed that this is an enzymatic method of disposal.

The use of feathers in their natural form as a feed component of animal and poultry diets is limited by the low digestibility of keratin. According to V.S. Bomko et al. (2023), the structural protein of feathers (keratin) can be digested in the digestive



tract of animals only after hydrothermal hydrolysis. After that, the digestibility of raw protein increases significantly (75-80%). Meanwhile, the amino acid composition of feather protein is rather unbalanced, due to the low content of lysine, histidine and methionine and high content of cystine. This drawback significantly limits the use of feather meal in animal feeding. In addition, the technology for the production of feather flour is energy- and material-intensive. The use of native feathers without complex hydrolysis technology as a balancing additive in substrates for growing mushrooms can have a greater economic effect.

Therefore, higher mushrooms have an enzyme system that allows them to break down and consume the proteins of processed leather (leather shavings) and the biologically stable keratin of bird feathers. The use of such additives without additional industrial splitting, in their natural form, will theoretically increase the yield of mushrooms, but this requires additional scientific and economic experiments.

Conclusions. Thus, analyzing the development of oyster mushroom colonies grown with additives based on livestock waste, the following conclusions can be drawn:

- the study of the nutritional and technological value of livestock waste as additives to the substrate indicates a great potential in their use as balancing additives in the composition of substrate compositions for growing mushrooms, especially to supplement the lack of nitrogen in the main components of the composition;

- the analysis of the requirements for the substrate components made it possible to identify promising alternative additives, the use of which can reduce the cost of the substrate composition, since their use is not competitive with the animal feed base, and the selected additives are livestock waste;

- the use of selected additives will reduce the amount of waste that is not used or is used to a limited extent and is usually disposed of as solid industrial waste. The use as additives for the preparation of the substrate will allow to use them in further processing, with obtaining additional oyster mushroom production, which corresponds to the concept of waste management "5R";

- the most essentials growth and manifestation of cultural signs was observed in variants with the addition of leather shavings and feathers. Colonies that received additional nutrition from the specified additives in the first hours after contact with the additive colonized it and, as a result, had the largest sizes compared to the growth control points;

- the worst variant of development was observed with fur production waste. The biological availability of the protein of unwashed leather and the possibility of reproduction of microorganisms led to the rotting and decomposition of proteins with the release of hydrogen sulphide and ammonia, which inhibited the growth of the mycelium, and subsequently caused its death. Therefore, it can be concluded that with the selected method of preparation of additives (pasteurization), inoculation and incubation of mycelium, it is impractical to use these processing wastes as additives. But the use of these additives during sterile cultivation remains relevant and requires further research;

- the obtained data of the laboratory experiment can be considered primary and those that require further research, including when using other methods of preparation of additives (sterilization, solid-state fermentation), as well as verification in scientific and economic experiments on the determination of technological properties in substrate compositions and calculation of biological effectiveness and productivity of oyster mushrooms and other saprophytes.



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