Protein feedstuff production based on microbial biomass

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Abstract. Until 2020, Russia has a state program for the development of agriculture, therefore the organization of livestock complexes is actively initiated and supported. In this regard, the arises the question of inexpensive feed for farm animals availability. More recently, the demand for protein feed products largely was covered by imports. However, at present, there is an economically determined decrease in demand for imported goods, which naturally increases interest in domestic analogs of protein feed products. One of these products is fodder yeast. Currently, this kind of feedstuffs was undeservedly forgotten and most production facilities in Russia curtailed. In the production of fodder yeast, for example from post-alcohol stillage, the profitability of grain processing is significantly increased. In addition, the processing of carbohydrate-containing wastes from grain processing enterprises reduces the negative impact on the environment. According to the results of the studies, the most effective protein producing strains and nutrient media were selected based on secondary raw materials from grain processing, as well as the optimal mode and conditions for their cultivation. It was found that the most effective strain was Rhodosporidium diobovatum 115 using post-alcohol stillage as a nutrient medium.

1. Introduction
The cost price of fodder yeast, as well as all commercial products, is made up of a plurality of production costs: the cost of raw materials and auxiliary materials, energy, water supply, wages, and other expenses. When using various grain crops as the main raw material, the share of costs for it reaches 60-70% of the prime cost and amounts to 10-12 thousand rubles per ton of product, which makes such production insufficiently profitable. However, in recent years, a number of enterprises have been built in Russia for the deep grain processing to produce gluten, starch, syrups, amino acids, alcohol, and other products. At such enterprises, carbohydrate-containing secondary raw materials (SRM) are formed in large quantities. These SRMs have almost zero cost since they are actually production wastes. Given this fact, they should be disposed of following waste management regulations. This task is not the easiest and cheapest. At the same time, it is possible to produce protein feed products on their basis, due to the synthesis of microbial biomass on carbohydrates contained in SRM. This will increase the profitability of the entire enterprise, reduce harmful emissions into the environment, and will allow to get an additional product with high demand and low cost. An analysis of secondary raw materials at existing grain processing enterprises has shown the feasibility of using starch B fraction (starch milk), pentosan fraction, bran, post-alcohol stillage, as well as their mixtures in various proportions as raw materials for
research. Some of the proposed SRM can be used as independent fodder products (post-alcohol stillage) or even food products (bran). However, the cost of such products on the market is low. At the same time, the cultivation of fodder yeast based on it allows to enrich them with protein, a wide range of vitamins and amino acids, which significantly increases its feed value and cost. [1,2]

The effectiveness of cultivation can be composed of three fundamental factors. It is a selection of the most effective strain of microorganism, the selection of the most comfortable nutrient medium for its vital activity (concentration of sugars, composition, mineral nutrition, rheological properties) and the conditions under which the cultivation is carried out (temperature, duration, pH, aeration conditions, etc.). The purpose of this research was to study and analyze the above aspects of protein feed production based on the synthesis of microbial biomass. In the first stage a number of strains has been selected which most fully meet the requirements of SRM processing from grain enterprises. Next, studies were conducted to develop modes of water-thermal and enzymatic processing of raw materials to obtain a nutrient medium. In conclusion, optimal culture conditions were selected on medium obtained.

2. Materials and methods
For research, a number of existing grain processing enterprises provided samples of the following secondary raw materials:

- a fraction of starch B (starch milk);
- a fraction of pentosans;
- wheat bran;
- bard from the joint processing of starch milk (a fraction of starch B) and bran for ethyl alcohol [3].

Qualitative indicators of secondary raw materials are given in table 1.

| Table 1. Qualitative indicators of feedstock. |
|-----------------------------------------------|
| Index                                      | A fraction of starch B | A fraction of pentosans | Wheat bran | Bard |
| Solids content - are common, %              | 31.7                   | 2.5                     | 86.8       | 6.5  |
| Solids content - soluble, %                 | 6.2                    | 1.95                    | -          | 3.3  |
| Solids content - weighted, %                | 25.5                   | 0.55                    | -          | 3.1  |
| Mass fraction of crude protein, % of dry substance | 9.4                    | 27.4                    | 16.0       | 22.5 |
| Mass fraction of protein according to Barnstein, % of dry substance | 9.3                    | 23.6                    | 15.6       | 21.1 |
| Conditional starchy, %                      | 23.5                   | 0.9                     | 22.8       | 0.3  |
| pH                                          | 4.45                   | 6.5                     | -          | 4.1  |
| Acidity, °D                                  | 0.2                    | 0.0                     | -          | 0.5  |

As a culture medium for the cultivation of fodder yeast following media were selected:

- option 1: a fraction of pentosans (in pure form);
- option 2: a mixture of the fraction of pentosans (80%) and the fraction of starch B (20%);
- option 3: a mixture of the fraction of pentosans (90%) and the wheat bran (10%);
- option 4: a post-alcohol stillage;
- option 5: a mixture of the post-alcohol stillage (80%) and the fraction of starch B (20%).

The limitation of the amount of starch fraction B in options 2 and 5 is due to the quantity of soluble solids in the nutrient medium at the level of 5-6% since the productivity of the strains at a sugar
concentration in the nutrient medium above 6% significantly decreases. The limitation of wheat bran dosage in option 3 is due to the high viscosity of the resulting medium [4].

Because carbohydrates in SRM are mostly in the form of starch and other polysaccharides, to increase the availability of sugars was carried out its water-thermal and enzymatic treatment. For the hydrolysis of polysaccharides, Novozymes company enzymes were used - Viscoferm (xylanase, beta-glucanase, cellulase) at a dosage of 0.3 liters/tonne dry matter, LpHerra (Thermostable alpha-amylase) at a dosage of 0.25 liters/tonne conditional starch and Saczyme Plus 2X (Glucoamylase) in a dosage of 0.5 liters/tonne conditional starch. The pH level of the culture media was adjusted to the values of 5.0-5.5, which corresponds to the optimal cultivation value of the majority of strains producing feed protein.

In the selection of microorganisms suitable for the efficient cultivation of protein feed product on selected raw materials, a wide list of microorganisms was considered, such as Saccharomyces cerevisiae of various races (Y-1218; YD-53; Y-3439; Y-3168), Candida tropicalis (SK-4, Y143, Y190, Y40), Candida utilis ATCC 9950, Torulopsis species and pinus (L-30, Y259, Y276, Y800), Rhodosporidium diobovatum (115, Y1002, Y2419). [5,6,7] As a result, the following strains were selected:

- Candida tropicalis SK-4;
- Saccharomyces cerevisiae YD-53;
- Rhodosporidium diobovatum 115.

The selected strains have optimal operational characteristics, in comparison with others, they are characterized by high assimilation of carbohydrates of raw materials, suitable cultivation conditions, high biomass productivity, etc. [8, 9]

These strains are stored in the collection of microorganisms All-Russian Research Institute of Food Biotechnology. Immediately prior to cultivation, with all strains, tests were carried out to assess their condition by plating on sterile culture media, as a result they all showed readiness for research.

The main method of the research was the modeling of the feed protein strains cultivation on the prepared media under laboratory conditions. Cultivation was carried out starting from the cultivation of a clean seed culture in a thermostat for 24 hours at a temperature of 30 °C and then on a shaker for 24 hours at a temperature of 32-34 °C. After that, the inoculum was introduced into the substrate in the amount of 10% of the volume of the initial medium. Along with seeding the culture, nitrogen nutrition (urea - CH₂N₂O) was added to the medium in an amount of 0.4% of the volume of medium in the flask. Culturing was performed on a shaker at a temperature of 34-36 °C for 12 hours.

The effectiveness of the cultivation process was judged by the content of the true protein (according to the Barnstein method) in the media before and after cultivation, as well as by the content of reducing sugars in samples (anthrone method). Amino acid and vitamin composition in the samples of fodder yeast was determined by high-performance liquid chromatography.

3. Results and discussion

Technological indicators of the culture media after cultivation are presented in table 2.

The presented data shows that in all samples there is no undissolved starch, which allows to consider the high efficiency of water-thermal and enzymatic raw materials treatment. In samples 2-5, the pH level is optimal for the selected strains and does not need to be adjusted immediately before cultivation. The pH level in the first option must be adjusted by adding sulfuric acid to optimal values. In samples 2 and 5, the content of fermentable sugars is at the level of 5.1-5.5%, which is much higher than in the rest. Samples 1, 3, and 4 are not sufficiently saturated with fermentable sugars. This may indicate a knowingly low efficiency of fodder protein cultivation.

The results of microorganisms growing on a nutrient medium are shown in table 3.

From the data obtained, it can be seen that all the variants well assimilated the sugars contained in the prepared nutrient media, which is evidenced by a concentration of residual sugars close to zero. The best result in protein content was shown in the second and fifth samples of culture media.
Table 2. Technological indicators of nutrient media.

| Indicators   | Options                                                                 |
|-------------|-------------------------------------------------------------------------|
| Raw materials | A 100% fraction of pentosans                                                |
|             | A mixture of the fraction of pentosans (80%) and the fraction of starch B (20%) |
|             | A mixture of the fraction of pentosans (90%) and the wheat bran (10%)    |
|             | A post-alcohol stillage                                                  |
|             | A mixture of the post-alcohol stillage (80%) and the fraction of starch B (20%). |

The concentration of solids (actual),%:
- are common: 2.3 8.3 10.8 6.5 8.3
- soluble: 2.2 7.3 2.5 5.7 7.1
- weighted: 0.1 1.0 8.3 0.8 1.2

The concentration of fermentable sugars, g / 100 cm³: 1.05 5.5 1.89 0.55 5.15

Undissolved starch, g / 100 cm³: 0.0 0.0 0.05 0.0 0.02

pH: 6.1 5.24 5.69 5.0 5.2

Mass fraction of protein according to Barnstein
- % of dry substance: 21.3 10.7 15.8 21.1 19.1
- g / 100 g: 0.49 0.89 1.66 0.72 0.75

Table 3. Indicators of culture fluid.

| Options | Strains | The concentration of solids (actual),% | Concentration of fermentable sugars, g / 100 cm³ | pH | Mass fraction of protein according to Barnstein, % of dry substance |
|---------|---------|---------------------------------------|-------------------------------------------------|-----|---------------------------------------------------------------|
| 1       | 1       | 2.2                                   | 0.2                                             | 6.5 | 27.0                                                          |
|         | 2       | 1.9                                   | 0.2                                             | 5.3 | 32.3                                                          |
|         | 3       | 1.8                                   | 0.2                                             | 7.6 | 31.6                                                          |
| 2       | 1       | 3.4                                   | 0.3                                             | 4.1 | 35.4                                                          |
|         | 2       | 3.3                                   | 0.2                                             | 4.6 | 37.3                                                          |
|         | 3       | 3.4                                   | 0.2                                             | 5.7 | 40.2                                                          |
| 3       | 1       | 8.8                                   | 0.3                                             | 5.9 | 19.8                                                          |
|         | 2       | 9.0                                   | 0.3                                             | 5.5 | 20.1                                                          |
|         | 3       | 8.7                                   | 0.4                                             | 5.7 | 20.3                                                          |
| 4       | 1       | 3.5                                   | 0.15                                            | 4.3 | 28.5                                                          |
|         | 2       | 3.3                                   | 0.2                                             | 4.2 | 27.7                                                          |
|         | 3       | 3.2                                   | 0.18                                            | 4.2 | 28.1                                                          |
| 5       | 1       | 3.45                                  | 0.25                                            | 4.3 | 38.3                                                          |
|         | 2       | 3.4                                   | 0.48                                            | 4.6 | 34.8                                                          |
|         | 3       | 3.27                                  | 0.2                                             | 4.2 | 38.6                                                          |
The rest of the media showed a low protein content, which can be justified by the initially low content of digestible carbohydrates. In this regard, to conduct further research in relation to samples 1, 3, and 4 is inappropriate and to recommend them for use, in the current composition, for production is impossible. In samples 2 and 5, the third strain, Rhodosporidium diobovatum 115, showed itself best of all, having true protein mass fraction of more than 40% per absolutely dry substance. The remaining strains also showed significant efficiency in the accumulation of protein up to 38% on absolutely dry matter, which allows to classify the resulting products as protein feed products.

The amino acid composition of second sample product with the strain Rhodosporidium diobovatum 115 is shown in table 4.

| Amino acids     | Amount, % on absolutely dry matter |
|-----------------|-----------------------------------|
| Lysine          | 1.26                              |
| Tryptophan      | 4.05                              |
| Histidine       | 2.59                              |
| Arginine        | 1.64                              |
| Aspartic acid   | 1.71                              |
| Threonine       | 1.23                              |
| Serine          | 1.59                              |
| Glutamic acid   | 8.13                              |
| Proline         | 2.67                              |
| Glycine         | 1.23                              |
| Alanine         | 1.33                              |
| Cystine         | 0.29                              |
| Valine          | 0.99                              |
| Methionine      | 0.31                              |
| Isoleucine      | 0.70                              |
| Leucine         | 1.72                              |
| Tyrosine        | 0.98                              |
| Phenylalanine   | 1.40                              |

The data in table 4 indicate a high content of almost all amino acids in the resulting product, especially lysine (1.26% on the absolutely dry matter), histidine (2.59% on the absolutely dry matter), arginine (1.64% on the absolutely dry matter) and Serine (1.59% on the absolutely dry matter). An analysis of the vitamin composition showed a high content in the test sample of B vitamins, in particular vitamin B1 (4.8 mg/kg absolutely dry matter), B2 (30.5 mg/kg absolutely dry matter), B6 (33.5 mg/kg absolutely dry matter), vitamin E (200 mg/kg absolutely dry matter), as well as beta-carotene (2.5 mg/kg absolutely dry matter), which significantly increases the feed value of the resulting product and increases its market value.

4. Conclusion
The results of the study showed the effectiveness of Rhodosporidium diobovatum 115, Candida tropicalis SK-4, Saccharomyces cerevisiae YD-53 in protein feedstuff production from secondary raw materials of enterprises for the deep grain processing. The results give a real opportunity to increase the profitability of grain processing enterprises by producing an additional product with high demand and added value, as well as reduce the cost of waste disposal, reduce the burden on wastewater treatment plants and improve the environmental situation around the enterprise.
References

[1] Anupamaa P R 2000 Value-added food: Single cell protein Biotechnology Advances 18(6) 459-79

[2] Halmemies-Beauchet-Filleau A, Rinne M, Laamminen M, Mapato C, Ampapon T, Wanapat M and Vanhatalo A 2018 Alternative and novel feeds for ruminants Nutritive value, product quality and environmental aspects Animal 12(82) 295-309

[3] Turshatov M V, Ledenev V P, Kononenko V V, Krivchenko V A, Soloviev A O, Moiseeva N D, Korzhenko L G, Lozanskaya T I and Khudyakova N M 2016 Technological aspects of obtaining bioethanol and feed from starch milk and bran formed during the deep processing of grain crops Promising enzyme preparations and biotechnological processes in the technology of food and feed ed V A Polyakov and L V Rimareva (Moscow: State Scientific Institution All-Russian Research Institute of Food Biotechnology of the RAAS) pp 413-9

[4] Rimareva L V, Lozanskaya T I and Khudyakova N M 2016 Biotechnological processing of post-alcohol stillage in dry fodder yeast Biotechnologies in the integrated development of regions (Moscow: Closed Joint-Stock Company Expo-Biochem-Technologies) p 95

[5] Artyukhova S I and Bondareva G I 2013 Biotechnology of new forms of carotenoid preparations based on microbial synthesis Young Russia: advanced technologies in industry 3 4-6

[6] Kurcz A Blazejak S, Kot A M, Bzducha-Wrobie A and Kieliszek M 2018 Application of industrial wastes for the production of microbial single-sell protein by fodder yeast candida Waste and Biomass Valorization 9(1) 57-64

[7] Chirkova, A I and Litvinov P V 2018 Use of yeast for the processing of alcohol stillage Young scientist 20(206) 210-3

[8] Valeeva R T 2007 Intensification of the production of fodder yeast based on alcohol stillage (Kazan) p 136

[9] Kolodina E N, Artemyeva O A, Kotkovskaya E N, Pavlyuchenkova O V and Pereselkova D A 2016 Study of the biological safety of Candida yeast as a potential source of feed protein Bulletin of the Orel State Agrarian University 5(62) 72-8