

The study of fermented compound feeds from agricultural production waste and processing industry

A.P. Karmazin ^{1,*}, O.A. Mironova ², A.A.Mironova ³

¹ Institute of environmental engineering of the Peoples' Friendship University of Russia, 6 Miklukho-Maklaya str., Moscow, 117198

² Peoples' Friendship University of Russia (RUDN University), 117198, 6 Miklukho-Maklaya str., Moscow

³ The North Caucasus Zonal Veterinary Research Institute is a branch of the Federal State Budgetary Scientific Institution "Federal Rostov Agrarian Scientific Center. Rostov region, Novocherkassk, Rostov highway, 0

Annotation. The article is devoted to the study of quality and safety indicators of compound feeds made from agricultural and processing industry waste under the influence of microbiological fermentation. Compound feeds of the following formulation were studied: recipe No. 1: beer pellet -40%; wheat bran – 20.0 %; sunflower cake - 20%; mushroom substrate – 20%; recipe No. 2: beer pellet - 40%; wheat bran – 10.0%; sunflower cake - 10 %; mushroom substrate – 30%; recipe No. 3: beer pellet – 40%; wheat bran – 10.0%; sunflower cake - 20%; mushroom substrate – 20 %; recipe No. 4: beer pellet - 29%; wheat bran – 54.0%; sunflower cake - 17%; recipe No. 5: beer pellet – 25%; soy – 75.0%. The purpose of our research was to study the quality and safety indicators of compound feeds made up of agricultural and processing industry waste after microbiological fermentation with Lesnov's starter culture. It has been established that recipes No. 5 and No. 4 are the most preferable in terms of the content of metabolic energy for cattle, sheep, pigs and poultry. The content of mycotoxins (aflatoxin B1, deoxynivalenol, zearalenone, ochratoxin A, T-2 toxin), microscopic mold fungi and yeast, pesticides (malathion, pyrimithophosmethyl, cypermethrin, diflubenzuron), nitrates and nitrites, lead, arsenic, cadmium and mercury in fermented compound feeds of various formulations did not exceed MDU; GMO not detected. When choosing recipes, it is necessary to take into account the physico-chemical parameters of fermented compound feeds and the peculiarities of animal digestion (single-chamber or multi-chamber stomachs).

1 Introduction

In order to increase the production of livestock products to volumes that meet the needs of the country's food security, it is necessary to fully provide animals and poultry with

* Corresponding author: Fumrostov@mail.ru

highly nutritious feed, which occupies the lion's share in the cost structure of products. For example, in the industrial fattening of pigs, up to 65% of the cost of pork is associated with feed costs, therefore, the task of increasing the profitability of production by reducing feed costs without compromising their quality is faced by almost every producer [1].

One of the tasks of animal husbandry in modern economic conditions is to expand the feed base through the introduction of innovative technologies and an increase in the proportion of cheap feed raw materials in diets.

In recent years, scientists have increasingly shown interest in unused secondary products of low-value plant raw materials, which are waste products of grain processing, sugar, food and other agricultural industries. One of the promising methods of processing cellulose-containing raw materials is its bioconversion [2].

In many countries of the world, they are searching for effective ways to obtain feed protein. Microbiological fermentation is one of the promising areas of research with encouraging results [3].

Microorganisms synthesize biomass, vitamins, amino acids, enzymes, bacteriocins with protective and preventive properties during cultivation. Microbiological fermentation makes it possible to obtain protein feed enriched with biologically active components, prebiotics, and protective substances that ensure long-term storage of feed in its raw form [4].

The use of enzyme preparations of microbial origin that break down high-molecular compounds to easily digestible forms can significantly increase the degree of hydrolysis of feed nutrients, and, consequently, the productivity of animals at the same feed consumption [5].

Publications on solid-phase biofermentation of low-value plant raw materials provide data on the effectiveness of such processing using the example of processing grain waste and bran: an increase in the protein content in the feed by 2-2.5 times (with an initial protein content of 10-12% in bran, an increase to 20-25%) [6]. A method is known for obtaining feed products from certain types of waste, for example, beer pellets [7-9].

A significant reserve for providing livestock with feed is the widespread use of grain production waste in diets, in particular straw. In its native form, straw is poor in protein (3-5%), contains a lot of fiber (35-45%), which explains its poor digestibility. Scientists have achieved an increase in the digestibility of straw by fermenting it with special microorganisms collected in a starter culture, using solid-phase fermentation, which is an alternative to the use of silage technology, yeast and acid and alkali treatment [10,11].

2 The purpose of the research

To study in a comparative aspect the physico-chemical properties and safety of compound feeds, which include waste from agriculture and the processing industry after microbiological fermentation with Lesnov's starter culture.

3 Materials and methods of research

Studies of fermented compound feeds were carried out in 2023-2024. Compound feeds were prepared and fermented with Lesnov's starter culture according to the method proposed by the authors: for 1 hour of raw materials, 0.000005 parts of Lesnov's starter culture were added at a raw material humidity of 45-55%, a temperature of 50-55°C for 24 hours in INBIOTECH-K LLC, Moscow. Recipe No. 1: beer pellet – 40%; wheat bran – 20.0%; sunflower cake – 20%; mushroom substrate – 20%. Recipe No. 2: beer pellet –

40%; wheat bran – 10.0%; sunflower cake - 10%; mushroom substrate – 30%. Recipe No. 3: beer pellet – 40%; wheat bran – 10.0%; sunflower cake -20%; mushroom substrate – 20%. Recipe No. 4: beer pellet – 29%; wheat bran – 54.0%; sunflower cake - 17%. Recipe No. 5: beer pellet – 25%; soy – 75.0%. The objects of research were 50 samples of compound feeds made according to the above recipes of 10 each. Physico-chemical quality indicators, mycotoxin content: aflatoxin B1, deoxynivalenol, zearalenone, ochratoxin A, T-2 toxin; pesticides, nitrates and nitrites, toxic elements and GMOs were studied in the Testing Laboratory of the Federal State Budgetary Institution "Grain Quality Assessment Center" in Moscow. Microbiological indicators: mold fungi and yeast - in the Voronezh branch of the Federal State Budgetary Institution "Grain Quality Assessment Center" according to the current regulatory documentation (ND) using methods and techniques of laboratory studies of the tested substrates: qualitative and quantitative chemical analysis; high-performance liquid chromatography (HPLC); gas chromatography (GC); atomic – absorption spectrometry, etc. Laboratory methods of quality research (GOST R 54951-2012; GOST 27979-88; GOST 13496.4-2019 p.8; GOST 32905-14; GOST 31675-2012 p.7; GOST 26226-95 p.1; GOST 26176-2019 p.9; GOST R 54078-2010 Appendix A; GOST ISO 6493-2015; GOST 26483), chemical elements (GOST 32343-2013) and feed safety: mycotoxins (GOST 30711-2001; GOST EN 15851-2013; GOST 31691-2012; GOSTMUK 4.1 2204-07; instruction P43/C); pesticides (DIN EN 15662 2018); nitrates (GOST 13496 19-2015), nitrites (GOST 13496 19-2015); toxic elements (GOST R 53100-2008; GOST 31 650-2012), GMOs (GOST R 53214-2008).

4 Research results and discussion

Changes in the physico-chemical quality indicators of fermented leaven of Lesnov compound feeds made according to five different recipes are presented in Table 1.

Table 1. Comparison of physico-chemical properties of various fermented feed recipes.

Indicators, units of measurement	Compound feed (n=10)				
	Recipe №1	Recipe №2	Recipe №3	Recipe №4	Recipe №5
Mass fraction of moisture, %	7.4±0.28***	7.2±0.22***	7.2±0.23***	3.7±0.18	5.6±0.24**
The mass fraction of crude fat, in terms of dry matter, is not less than %	6.9±0.36***	4.0±0.14	6.0±0.26**	5.80±0.24*	5.80±0.22**
The mass fraction of crude protein in terms of dry matter, not less than, %	17.76±0.55**	13.2±0.42	16.23±0.51	20.25±0.62**	38.52±1.13***
Mass fraction of crude ash, in terms of dry matter, no more, %	5.8±0.3	5.8±0.3	6.0±0.3	6.4±0.3	6.0±0.3
The mass fraction of crude fiber in terms of dry matter, no more, %	26.0±2.2***	35.7±2.7***	40.5±2.9**	13.8±1.6*	9.9±1.4

Mass fraction of soluble carbohydrates, %	2.8±0.3	4.8±0.6**	2.8±0.4	7.4±0.7***	6.5±0.6***
Starch content in terms of dry matter, g/kg	90.0±6.6	58.0±4.6	70.0±5.2	139.0±10.3	66.0±6.6
%	9.0 **	5.8	7.0	13.9***	6.6
pH, unit pH	5.3±0.2	5.0±0.2	5.48±0.12	4.8±0.2	4.9±0.2

* p<0.05; ** p <0.01; *** p <0.001 в сравнении с самым низким значением.

Analyzing Table 1, we see that the lowest mass fraction of moisture ($3.7 \pm 0.18\%$) was established after fermentation of compound feed made according to recipe No. 4. Compound feed according to recipe No. 5 after fermentation had a moisture content 1.5 times higher than the previous one; compound feed according to recipes No. 2 and No. 3 - 2.0 times higher; compound feed made according to recipe No. 1 is 1.9 times higher.

The lowest mass fraction of crude fat in terms of dry matter after fermentation is found in compound feed prepared according to recipe No.2. In fermented compound feeds made according to recipes No. 4 and No. 5, the mass fraction of fat was 1.45 times higher; according to recipe No. 3 – 1.5 times; according to recipe No. 1 – 1.7 times.

After fermentation, the lowest crude protein content was found in compound feed made according to recipe No. 2 ($13.2 \pm 0.42\%$), the highest in compound feed made according to recipe No. 5 ($38.52 \pm 1.13\%$), which exceeded that in compound feed recipe No. 2 by 2.9 times. Fermented compound feeds made according to recipes No. 1, No. 3 and No. 4 contained 1.4, 1.2 and 1.5 times more protein in comparison with compound feed No. 2, respectively.

The content of soluble carbohydrates in fermented compound feed, compiled according to recipe No. 4, was the highest $7.4 \pm 0.7\%$; according to recipe No. 5, it was 13.8% lower; according to recipe No. 2, it was 35.1% lower; according to recipes No. 1 and No. 3, it was 62.2%.

The highest starch content was found in fermented compound feed, compiled according to recipe No. 4 (139.0 ± 10.3), the lowest (2.4 times less) is according to recipe No. 2. Fermented compound feed according to recipe No. 1 contains 1.5 times less starch in comparison with compound feed according to recipe No. 4, respectively, compound feed according to recipe No. 3 – 2.0 times less, according to recipe No. 5 – 2.1 times.

The fermented compound feed according to recipe No. 5 has the lowest percentage of crude fiber (9.9 ± 1.4), the highest in compound feed according to recipe No. 3 (40.5 ± 2.9) is 4.1 times higher. The mass fraction of crude fiber in terms of dry matter in fermented compound feed prepared according to recipe No. 4 was 1.4 times higher than that in compound feed according to recipe No. 5; in compound feed prepared according to recipe No. 1, 2.6 times higher compared to recipe No. 5; in compound feed prepared according to recipe No. 2, higher in compared with recipe No. 5 by 3.6 times.

The lowest percentage of crude ash was found in fermented compound feeds according to recipes No. 1 and No. 2 (5.8 ± 0.3). In fermented compound feed, compiled according to recipes No. 3 and No. 5, it was 3.5% higher; according to recipe No. 4, it was 10.4%.

The Rn of compound feeds made according to all five recipes was slightly acidic: from $4.8 = 0.2$ (recipe No. 4) to $5.48 = 0.12$ (recipe No. 3).

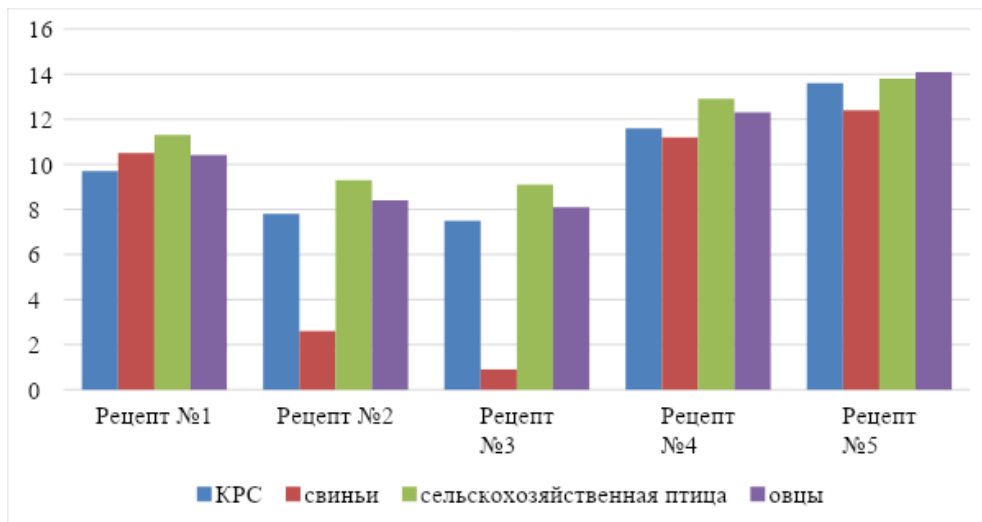


Fig. 1. Comparison of various fermented feed recipes by metabolic energy for different animal species, MJ/kg.

According to the diagram, fermented feed prepared according to recipe No. 5 (beer pellet – 25%; soy – 75.0%) contains the most metabolic energy and is equally suitable for cattle, pigs, sheep and poultry. Compound feeds prepared according to recipe No. 4 (beer pellet -29%; wheat bran – 54.0%; sunflower cake - 17%) and according to recipe No. 1 (beer pellet – 40%; wheat bran – 20.0%; sunflower cake - 20%; mushroom substrate – 20%) are also equally well suited for all animal species, although they contain less metabolic energy. Compound feed according to recipes No. 2 (beer pellet - 40%; wheat bran – 10.0 %; sunflower cake - 10%; mushroom substrate – 30 %) and No. 3 (beer pellet – 40%; wheat bran – 10.0 %; sunflower cake - 20 %; mushroom substrate – 20 %) according to the indicator metabolic energy is suitable for cattle, sheep and poultry, but it is not suitable for pigs.

Thus, taking into account the indicator of metabolic energy, fermented compound feed of prescription composition can be used for cattle according to preference: No. 5 (14.1 MJ/kg), No. 4 (12.3 MJ/kg), No. 1(9.7 MJ/kg), No. 2(7.8 MJ/kg), No. 3(7.5 MJ/kg); sheep: No.5 (13.6 MJ/kg), No.4 (11.6 MJ/kg), No. 1 (10.4 MJ/kg), No. 2(8.4 MJ/kg), No. 3(8.1 MJ/kg); pigs: No.5 (12.4 MJ/kg), No. 4 (11.2 MJ/kg), No. 1(10.5 MJ/kg), No.2(2.6 MJ/kg), No. 3(0.9 MJ/kg); for poultry: No. 5 (13.8 MJ/kg), No.4 (12.9 MJ/kg), No. 1 (11.3 MJ/kg), No. 2 (9.3 MJ/kg), No. 3 (9.1 MJ/kg). In addition, when choosing compound feed recipes, it is necessary to take into account the peculiarities of animal digestion (single-chamber or multi-chamber stomachs), taking into account the physico-chemical parameters of fermented compound feeds.

Since compound feeds include waste products from the production and processing of agricultural and food products and the biofermentation process takes place at a raw material humidity of 45-55% and a temperature of 50-55°C, that is, in conditions optimal for the development of microscopic mold fungi and yeast, fermented compound feeds according to current legislation must be examined for microbiological safety and mycotoxins (Table 2).

Table 2. Comparison of various fermented feed recipes by metabolic energy for different animal species, MJ/kg.

Indicators, units of measurement, MPC	Compound feed (n=10)				
	Recipe №1	Recipe №2	Recipe №3	Recipe №4	Recipe №5
Microbiological indicators					
Mold fungi, CFU/g from less 1.0*10 ² to 5.0*10 ²	3.0*10 ²	7.2*10 ¹	4.5*10 ²	9.1*10 ¹	4.4*10 ²
Yeast, CFU/g, from less 1.0*10 ² to 5.0*10 ²	3.3*10 ²	6.9*10 ¹	3.2*10 ²	7.7*10 ¹	3.9*10 ²

Mycotoxins					
Aflatoxin B1, mg/kg, MPC 0.025-0.1 mg/kg	<0.003	<0.003	<0.003	<0.003	<0.003
Deoxynivalenol, mg/kg, MPC 0.75-1.0 mg/kg	<0.058	<0.058	<0.058	<0.058	<0.058
Zearalenone, mg/kg, MPC not more than 1.0 mg/kg	<0.1	<0.1	<0.1	<0.1	<0.1
Ochratoxin A, mg/kg, MPC not more than 0.05 mg/kg	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005
T-2 toxin, mg/kg, MPC not more than 0.1 mg/kg	<0.05	<0.05	<0.05	<0.05	<0.05

Note: GOST 10444.12-2013; GOST 34108-2017; GOST 30711-2001; GOST EN 15851-2013; GOST 31691-2012; GOST MYK 4.1 2204-07; manual P43/B.

It was found that the number of microbial cells of mold fungi and yeast cells (CFU/g) after fermentation remained within the normative values. According to Table 2, the content of aflatoxin B1, deoxynivalenol, T-2 toxin, zearalenone, ochratoxin A in the studied samples of compound feeds made according to different recipes after fermentation did not exceed the limits of MDU.

Table 3. Chemical safety indicators and the presence of GMOs in fermented compound feeds

Indicators, units of measurement, MPC	Compound feed (n=10)				
	Recipe №1	Recipe №2	Recipe №3	Recipe №4	Recipe №5
Pesticides					
Malathion, mg/kg, MPC <0.01 mg/kg, DIN EN 15662:2018 (HPLC)	<0.01	<0.01	<0.01	<0.01	<0.01
Pyrimiphos- methyl, mg/kg, MPC <0.01 mg/kg, DIN EN 15662:2018 (GC)	<0.01	<0.01	<0.01	<0.01	<0.01
Cypermethrin, mg/kg, MPC <0.01 mg/kg, DIN EN 15662:2018 (GC)	<0.01	<0.01	<0.01	<0.01	<0.01
Diflubenzuron, mg/kg, MPC <0.01 mg/kg, DIN EN	<0.01	<0.01	<0.01	<0.01	<0.01

15662:2018 (GC)					
Toxic elements					
Lead, mg/kg, MPC<5.0 GOST P 53100-2008	<0.5	<0.5	<0.5	<0.5	<0.5
Arsenic, mg/kg, MPC<0.5 GOST P 53100-2008	<0.1	<0.1	<0.1	<0.1	<0.1
Cadmium, mg/kg, MPC<0.3 GOST P 53100-2008	<0.05	<0.05	<0.05	<0.05	<0.05
Mercury, mg/kg, MPC<0.1 GOST 31650-2012	<0.025	<0.025	<0.025	<0.025	<0.025
Nitrates and nitrites					
Nitrates, mg/kg, MPC <200.0 GOST 13496.19-2015	138.0±26.0	161.0± 25.15	164.0±28.02	144.0±22.0	128.0± 10.5
Nitrites, mg/kg, MPC <10.0 GOST 13496.19-2015	6.34±0.05	4.43±0.07	7.33±0.06	6.30± 0.09	5.24±0.10
GMO					
Qualitative determination of regulatory sequences in the genome of GM plants p-35S; t-NOS; p-FMV GOST P 53214-2008	The 35S promoter t-NOS p-FMV not detected	The 35S promoter t-NOS p-FMV not detected	The 35S promoter t-NOS p-FMV not detected	The 35S promoter t-NOS p-FMV not detected	The 35S promoter t-NOS p-FMV not detected

According to Table 3, the content of pesticides (malathion, pyrimiphosmethyl, cypermethrin, diflubenzuron) used in the cultivation and storage of crops, the processing waste of which is part of compound feeds, was below the MPC.

When examining samples of all fermented feed recipes, the content of nitrates, nitrites, lead, arsenic, cadmium and mercury were within the established standards.

The study of fermented compound feeds by the screening method of qualitative determination of regulatory sequences in the genome of GM plants p-35S; t-NOS; p-FMV was not detected.

Thus, the content of pesticides, toxic elements, nitrates and nitrites in fermented compound feeds does not exceed the legal norms of MPC; GMOs p-35S; t-NOS; p-FMV have not been detected.

5 Conclusion

1. In the course of research, it was found that, taking into account the indicator of metabolic energy, it is recommended to give preference to fermented compound feed of different formulations in the following sequence:

- cattle No. 5 (14.1 MJ/kg), No. 4 (12.3 MJ/kg), No. 1 (9.7 MJ/kg), No. 2 (7.8 MJ/kg), No. 3 (7.5 MJ/kg);

- sheep: No.5 (13.6 MJ/kg), No. 4 (11.6 MJ/kg), No. 1 (10.4 MJ/kg), No. 2 (8.4 MJ/kg), No. 3 (8.1 MJ/kg);

- pigs: No.5 (12.4 MJ/kg), No. 4 (11.2 MJ/kg), No. 1 (10.5 MJ/kg), No. 2 (2.6 MJ/kg), No. 3 (0.9 MJ/kg);

- agricultural poultry: No.5 (13.8 MJ/kg), No. 4 (12.9 MJ/kg), No.1 (11.3 MJ/kg), No. 2 (9.3 MJ/kg), No. 3 (9.1 MJ/kg)

2. When choosing recipes, it is necessary to take into account the physico-chemical parameters of fermented compound feeds and the peculiarities of animal digestion (single-chamber or multi-chamber stomachs).

3. The content of mycotoxins (aflatoxin B1, deoxynivalenol, zearalenone, ochratoxin A, T-2 toxin), microscopic mold fungi and yeast, pesticides (malathion, pyrimithophosmethyl, cypermethrin, diflubenzuron), nitrates and nitrites, lead, arsenic, cadmium and mercury in fermented compound feeds of various formulations did not exceed MDU; GMOs have not been detected.

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