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Recycling of Animal and Bird Waste Helped by Black Lion Fly Larvae

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Abstract

Due to the ability to process organic waste on an industrial scale with the help of larvae of the phytosaprophage - the Black soldier fly (Hermetia illucens), there is increased attention not only from scientists but also from agricultural producers. The resulting biohumus after the separation of larvae can be a good organic fertilizer. Studies were carried out to obtain a substrate using bacteria - Bacillus subtilis, microbiological studies of manure and droppings were carried out, the concentration of ammonia in the air was determined. The chemical composition of chicken droppings and pig manure before and after the introduction of bacteria was determined.

Disciplinary: Agriculture Science, Waste Management (Agriculture Waste), Sustainability & Green Technology.

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1 Introduction

Processing of organic agricultural waste, such as poultry droppings and pig manure is one of the global problems today. There are many methods and ways of processing, but the most acceptable, affordable, and inexpensive method, and even more effective, has not yet been found. More than 20 manure and droppings processing technologies have been developed and implemented in the world.

Collection, utilization, and processing of organic industrial, household, and agricultural waste is one of the modern global environmental problems that lead to environmental pollution (water, air, soil), which negatively affects the health of humans, animals, and the planet as a whole [5, 8].

It is possible to save the environment from agricultural waste due to the use of Hermetia illucens larvae, which will increase the biological value of manure and manure [2].

Having considered all this, we decided to thoroughly study the technology of pig manure and chicken droppings processing with the help of Hermetia illucens larvae, to preserve the environment from agricultural waste, to increase the biological usefulness of the metabolic byproducts of farm animals and poultry, and for its further use as feed protein, and the resulting zoohumus as fertilizer for crops.

Hay bacterium (lat. Bacillus subtilis) is an aerobic soil gram-positive spore-forming bacterium, which has the form of a straight rod, size 2-5 microns in length and 0.4-0.6 microns in width, colorless. The spores are oval in shape, not exceeding the size of the cell. Bacillus subtilis is often found in water, in the air, in the soil, which spread to plants by contact, and then enter the body of animals and humans when eating plant food. Reproduction of the Hay bacterium occurs in two ways by fission and sporulation. They are often connected in threads during transverse fission [1-4].

Bacillus subtilis produces enzymes that can remove the cellular and tissue ichorization debris, as well as synthesize amino acids, vitamins, and antibiotics. The hay bacterium has the ability to acidify the habitat where it is located, producing hyaluronic acid [6-7].

Hay bacterium has the ability to reduce the impact of various opportunistic, as well as pathogenic microorganisms such as yeast fungi, staphylococcus, salmonella, proteus, and others. Hay bacterium helps humans and animals to break down proteins, fats, and carbohydrates by digesting food.

We took this particular culture for work since it has been studied for a long time and sufficiently, the first laboratory experiments in which scientists described it were carried out back in the 19th century. It does not require high technology to work, it is quite simple to work with it.

Currently, the technology of processing solid agricultural waste has undergone significant changes and, thanks to innovative methods, has allowed to reduce the volume of waste produced, and to reuse them after processing. The use of Black soldier fly larvae allows processing organic agricultural waste from farm animals, waste from animal products processing, as well as fruits and vegetables. The larva of the Black soldier fly has a high rate of bioconversion, one adult individual per day is able to process about 25 mg of waste. The technology of bio-processing of organic residues by larvae of Black soldier flies in Russia is very relevant and has great prospects, but has not yet found much distribution [8-11].

The purpose of our study is to prepare the metabolic byproducts of pigs and birds for processing by Hermetia illucens larvae.

Based on the purpose of this study, the following tasks were set:

1. to study the effect of the bacterium Bacillus subtilis on chicken droppings and pig manure;

2. to determine and compare the chemical composition of chicken droppings and pig manure before and after the introduction of bacteria;

3. to evaluate the processing of the substrate enriched with larvae.

2 Material and Methods.

The research was funded by RFBR and Chelyabinsk Region, project number 20-416-740008\20 "Development of a method for increasing the degree of biological usefulness of the processing waste products of monogastric animals and birds using Hermetia illucens larvae" and conducted in the conditions of the Department of Animal Husbandry and Poultry Breeding of the Institute of Veterinary Medicine of the South Ural State Agrarian University.

The studies were carried out on specially prepared substrates with the addition of a probiotic preparation culture, namely a strain of soil spore bacteria Bacillus subtilis with content of living microorganisms of at least 10^5 CFU in 1 gram of the preparation (group 1 - chicken droppings with probiotic, group 2 - pig manure with the addition of the same culture and in the same amount).

Bacillus subtilis are bacteria (popularly called "hay bacterium"), they perform the function of "orderlies" - they have the ability to suppress the growth and development of pathogenic, opportunistic, putrefactive microflora in any ecosystem. We used Bacillus subtilis bacteria to reduce the content of harmful gases, reduce the decomposition time of droppings and manure, as well as for disinfection from pathogenic microorganisms.

The viability of bacterial cells was determined by the method of serial dilutions in accordance with GOST 10444.11-2013.

After thermostat, smear samples were stained from each dilution under study in accordance with GOST 7218-2011.

The species identity of the bacteria was established using the bacterial determinant of D. Bergi.

The resulting substrate was placed in a dark place and stored for 30 days at a temperature of 22°C. After the expiration of time, the experimental substrates (droppings + Bacillus subtilis) and (manure + Bacillus subtilis) were brought to optimal humidity and 3-day-old grown larvae were placed, which were introduced on the substrate surface, at the rate of 150 g of larvae per 5 kg of substrate.

The acidity of the substrates was determined and monitored daily, since Hay bacterium is actively growing at pH 7.0 and this is crucial when growing bacteria. During the cultivation of microorganisms, the acidity is constantly changing, most often this is due to uneven consumption of individual components of the nutrient medium and the formation of metabolic products during waste processing.

To measure the pH, we used paper indicators.

The analysis of droppings, manure, and biohumus was carried out in an interdepartmental laboratory according to generally accepted methods.

It should be noted that the optimal temperature for the development of larvae in the room is 27°C, which was monitored daily during the experiment. Natural lighting was mainly used with additional lighting during laboratory work.

3 Results and Discussion

The bacterium Bacillus subtilis had a positive effect on the ammonia contamination of the air on the surface of containers during the processing of pig manure and droppings.

Table 1 Dynamics of ammonia concentration on the surface, n = 15

Group	Ammonia concentration in the air, mcg/l	
	1st day	30th day
Control (pig manure)	14.2±1.22	15.7±1.51
Control (chicken dropping)	14.6±1.31	16.2±1.23
Experiment (pig manure)	14.7±0.91	6.3±1.23*
Experiment (chicken droppings)	14.8±0.91	6.9±1.13*

*P<0.05

From Table 1, after 30 days of the experiment, the concentration of ammonia on the surface of containers decreased by 2.3 times.

During the microbiological study of the microflora of manure and droppings, a variety of biocenosis was noted, which was mainly represented by conditionally pathogenic microflora, such as E. coli, staphylococci, Pseudomonas, mold fungi, data on the changed microbiocenosis of the litter material are presented in Table 2.

Table 2 The single application effect of a strain of soil spore bacteria Bacillus subtilis on microbiocenosis (M ± m; n = 15)

Microflora, CFU/gr	Group (pig manure)					
	Experimental group			Control group		
	before introduction	15th day	30th day	1st day	15th day	30th day
E.coli. 10^5	1.7±0.8	1.7±0.96	1.5±1.11	2.1±0.15	2.3±0.63	3.2±0.45
Staph.aureus. 10^5	1.9±0.13	1.4±0.26	1.3±0.25	1.1±0.17	1.3±0.15	1.5±0.44
Ps.aeruginosa. 10^5	1.5±0.25	1.3±0.11	1.2±0.98*	1.2±0.18	1.6±0.26	1.8±0.14
Aspergillus. 10^5	2.6±0.5	2.1±0.26	1.5±0.21	2.5±0.8	2.7±0.26	2.7±0.25
Mucor. 10^5	2.6±0.7	2.5±0.56	1.4±0.11	2.4±0.08	2.5±0.42	2.6±0.14
Tr.viride. 10^5	-	1.6±0.42	1.6±0.25	-	-	1.2±0.13
Lactobacterium. 10^5	-	1.7±0.42	1.9±0.98	-	-	-
Bac.subtilis. 10^5	-	1.7±0.17	2.6±0.37	-	-	-
Saccharomyces. 10^5	-	1.5±0.42	1.7±0.37	-	-	-
Microflora. CFU/gr	Group (chicken droppings)					
	Experimental group			Control group		
	before introduction	15th day	30th day	1st day	15th day	30th day
E.coli. 10^5	2.7±0.1	1.5±0.6	1.5±0.3	2.2±0.2	2.9±0.6	4.2±0.45
Staph.aureus. 10^5	1.5±0.1	1.4±0.2	1.6±0.25	1.4±0.1	1.3±0.1	1.5±0.6
Ps.aeruginosa. 10^5	1.5±0.25	1.3±0.1	1.7±0.2	1.5±0.18	1.4±0.26	2.3±0.14
Aspergillus. 10^5	2.68±0.5	2.71±0.6	1.5±0.2	2.58±0.8	2.77±0.16	2.79±0.2
Mucor. 10^5	1.4±0.3	2.5±0.51	1.4±0.11	2.4±0.08	2.5±0.42	2.6±0.14
Tr.viride. 10^5	-	1.6±0.42	1.6±0.25	-	-	-
Lactobacterium. 10^5	-	1.1±0.42	1.5±0.68	-	-	-
Bac.subtilis. 10^5	-	1.7±0.77	3.6±0.37	-	-	-
Saccharomyces. 10^5	-	1.6±0.1	1.7±0.7	-	-	-

*P<0.05

From Table 2 data, we noted that in the manure and droppings of the experimental group. in both experiments. a decrease in the number of micromycetes of mold fungi was observed. *Aspergillus* and *Mucor*. as well as the concentration of opportunistic microflora decreased.

By the 15th day of the experiment. *Ps.aeruginosa* in pig manure decreased by 87.0%. in chicken droppings by 80.0%.

The total content of micromycetes of the fungus of the genus *Trichoderma* in the litter material in both groups increased after a single application of Bacillus subtilis spore bacteria by an average of 30.0%. *Bacillus subtilis* probiotic culture - by 2 times. *Saccharomyces* yeast - by an average of 25.5%.

The work of Bacillus subtilis bacteria for the processing of manure and droppings was based on the utilization of nitrogen-containing components from manure for the synthesis of own protein. which was accompanied by fermentation processes. As a result. we received a substrate enriched with nitrogenous matter and other hazard classes in a short time. This was due to the fact that nitrogen was not disposed of. but transferred from one of its forms to another.

In the studies. poultry droppings from poultry farms and pig manure from the Agrofarm Ariant LLC in the Chelyabinsk region were used. in which its chemical composition was determined according to generally accepted methods (Table 1).

Table 3: Chemical composition of chicken droppings. pig manure in natural humidity

Indicator	Chicken droppings	Pig manure
Dry matter. %	25.32±1.21	18.35±0.53
N. %	1.85±0.13	0.58±0.38
P.%	1.0±0.25	0.32±0.38
Ca.%	1.72±1.18	0.51±0.20
pH	8.0±0.58	7.8±0.23
Fe. mg/kg	234.75±7.35	287.43±6.48
Cu. mg/kg	21.0±9.26	13.00±0.56
Zn. mg/kg	238.6±4.25	258.48±8.63
Co. mg/kg	1.43±0.05	1.53±4.45

The level of dry matter in the studied droppings was 25.32%, in manure 18.35%; nitrogen in chicken droppings 1.85%, in pig manure 0.58%; calcium and phosphorus in droppings 1.72 and 1.0% respectively, in pig manure 0.51% and 0.32, respectively. According to our research. we see a high content of iron (234.75 and 287.43. respectively). zinc (238.6 and 258.48. respectively) in droppings and manure. To a lesser extent, copper (21.0 and 13.0), cobalt (1.43 and 1.53). These elements are in a water-soluble form, being released gradually during the mineralization of organic matter.

Table 4: Composition of larvae grown on pig and chicken manure in natural humidity. %

Indicator	Percentage of matter. %	
	Chicken droppings + Bacillus subtilis	Pig manure + Bacillus subtilis
Dry matter. %	38.09±2.42	23.14±1.35
Crude protein. %	16.26±0.25	10.59±1.02
Crude ash. %	4.71±0.12	3.98±0.26
Phosphorus. %	0.28±0.05	0.19±0.10
Calcium. %	1.15±0.12	0.98±0.39
Iron. mg/kg	141.0±2.42	101.74±4.02
Copper. mg/kg	5.88±0.22	1.49±0.50
Zinc. mg/kg	72.61±4.70	15.93±2.00
Cobalt. mg/kg	0.25±0.02	0.15±0.01
Manganese. mg/kg	62.41±8.56	83.1±6.42

The protein content in the larvae was at the level of 10.59-16.26%. On the substrate of chicken droppings with the addition of Bacillus subtilis in the biomass of larvae. the level of crude protein was 53% higher relative to the level in pig manure.

The observed tendency of iron. copper. zinc increase in larvae obtained on a substrate from chicken droppings with the addition of Bacillus subtilis was several times higher than in larvae obtained on a substrate from pig manure with the addition of Bacillus subtilis.

Duration of droppings and manure processing into bio humus is of great importance in the poultry and pig waste disposal technology. Table 5 clearly shows the data of bioconversion of substrates by Hermetia illucens larvae.

Hermetia illucens larvae are an economic way of converting the litter nutrients into a full-value biological source of proteins. lipids. as well as obtaining biohumus.

Comparing the two groups by the chemical composition of biohumus. from Table 5 we see that the content of dry matter. nitrogen. phosphorus. copper. and cobalt is higher in the first group. The calcium content is almost the same in the groups. The first group surpasses the second group in terms of the content of iron and manganese. The zinc content was higher in the second group and amounted to 124.8 mg/kg.

Table 5 Chemical composition of zoohumus in natural humidity. %

Indicator	Group	
	I	II
Dry matter. %	41.26±1.13	22.18±2.42
N. %	0.74±0.08	0.39±0.12
P.%	0.48±0.12	0.33±0.08
Ca.%	0.069±0.01	0.060±0.01
Fe. mg/kg	143.8±23.20	117.0±14.70
Cu. mg/kg	12.6±6.23	8.32±3.85
Zn. mg/kg	99.33±5.00	124.8±10.80
Co. mg/kg	0.94±0.80	0.75±1.01

In the studied samples. an increase in dry matter occurred in relation to the initial indicators. so the level of dry matter was 22.18-41.26% Nitrogen in biohumus is reduced and is in the range of 0.4-0.7%. A similar picture is noted for the content of macronutrients in zoohumus and is 0.060-0.069 mg/kg for calcium. 0.33-0.48 mg/kg for phosphorus. The level of iron and manganese in biohumus increases and amounts to 143.8-117.0 mg/kg. and the content of copper. cobalt and zinc decrease.

When Bacillus subtilis is added to the substrate. the period of droppings and manure processing by larvae is reduced by 2 days and is 6 days. while the mass of processed manure decreases to 1.5 kg. and the larvae increase by 200 g. compared with the control group I. The conversion of a substrate with the addition of Bacillus subtilis reaches 68.5%.

4 Conclusion

In pig manure and chicken droppings. there was a gradual decrease in the amount of opportunistic microflora, more than half from the first day of research. Thus, the positive vector of correction of microbiocenosis in manure and droppings is directly related to a single application of Bacillus subtilis. due to the high antagonistic activity of microorganisms.

Processing of droppings with the help of the Black soldier fly (Hermetia Illucens) larvae is environmentally friendly. since instead of methane. only a certain amount of carbon dioxide is released into the atmosphere. Methane is an air pollutant that causes a global greenhouse effect [8].

We propose to use the results of the conducted research in agriculture when processing the poultry and pig waste products. as well as to use larval biomass in feeding animals and birds. A huge amount of nitrogen and phosphorus enter the environment and pose a great threat, as a rule, this is due to an imperfect system of disinfection. storage and disposal of manure and droppings, having a negative impact on the environment.

Larvae efficiently process organic waste. such as pig manure and chicken droppings. accumulating various substances in their bodies. The percentage of which depends on what they have to process.

Considering that currently, the ecological problem is in the first place. and agricultural enterprises, especially industrial farms and complexes. pollute the ecosystem as much as possible. It is expected that the results of our work will find their application in the agriculture of the Chelyabinsk region.

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