European Online Journal of Natural and Social Sciences 2022;
Vol.11, No1 pp. 198-206
ISSN 1805-3602

The Effectiveness of Biopreparations on the Qualitative Properties of Feedstuff

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Received for publication: 14 January 2022.
Accepted for publication: 07 March 2022.

Abstract

It has been shown that in the process of enrichment of different green grass (silage), using a consortium endemic probiotic LAB strains (which sintesized bacteriocins) and yeast strain of Kluveromyces marxsianus 83(which synthesizing mycocin) led to increasing amounts of essential amino acids (in particular lysine and methionine) and protein. It has been shown that in the content of spore microflora, mold, bacteria contaminating silage was decreased with respect to the control. The technological parameters of silage have been determined.

Keywords: feedstuff, protein, amino acids, feedstuff

Introduction

Frequent uncontrolled use of feed antibiotics in intensive production of feedstuff has led to the formation of resistant strains of pathogenic bacteria. The use of feed antibiotics has resulted in the increased productivity of farm animals due to the suppression of the pathogenic microflora in the digestive system (Anadon A., Martinez-Larranga MR. et al., 2006).

The situation is exacerbated by animal stresses due to poor feed quality and poor conditions. Often there are cases of dysbacteriosis especially in young animals, decreased cow reproduction**,** excess live weight of young animals, increased infectious and alimentary (caused by defective unbalanced feeding) diseases.

The increase in the pace of production and output of livestock and poultry products is inextricably linked with the improvement and development of new resource-saving technologies, including through the involvement of innovative solutions in the field of biochemistry and microbiology. A special place among them is the use of probiotic preparations in animal husbandry and poultry farming (Hossain M. I., Sadekuzzaman M. et al, 2017, Israyelyan A., Tkhruni F., 2017).

These are live microbial feed additives that improve the microbial balance in the intestines of animals and birds and are biological regulators in the body. The effect of probiotics on the body is diverse. The principle of their action is that they inhibit the activity of pathogenic microbes, produce digestive enzymes, vitamins, amino acids, increase immunity, and inhibit toxins. An important result of the use of probiotics in animal husbandry is the environmental safety of food. The most modern is their separation into monocomponent (containing one bacterial strain), antagonist probiotics (mainly representatives of the genus Bacillus), combined preparations (containing several strains or additives that enhance their effect), and sorbed live bacteria. They produce different enzymes and biologically active substances that complement each other (Nekrasov, R. V., Chabaev, M. G., 2013). The products offered on the market differ not only in cost, but also in composition, quality, methods of use and dosage (Anadon A., Martinez-Larranga MR. et al., 2006, Gaggia F., Mattarelli P. et al., 2010).

The determining factor in the effectiveness of probiotics is largely the technology of their production. The modern approach to the development of probiotic drugs involves several elements, that is, first, the use of various types of microorganisms in certain combinations, then the release of them in a form that allows long-term storage at ordinary temperature, third, the preservation of properties when making them during the production of animal feed and feed additives. A new promising direction in the production of probiotics is their release in the form of a biologically active feed additive containing a biofilm of probiotic bacteria, which allows microorganisms to maintain their activity of animal feed during drying and granulation (http://subtilis.ru/products/laktofit).

We studied probiotic strains and yeasts which were isolated from dairy products of different regions of Artsakh Republic (cow matsoon, salt cheese, sheep milk, goat milk, buffalo milk). Our study showed the influence of the products of their metabolism on the ability to inhibit the growth of spores microflora, pathogenic microflora causing diseases for humans and animals and their antimicrobial activity against *Bacillus subtilis* and *Salmonella typhimurium* (Israyelyan A., 2017, Israyelyan A., Tkhruni F. et al., 2016).

Scientific experiments have been carried out to find out how endemic probiotic bacteria of Artsakh Republic appear in the process of silaging, taking into account their characteristics. Given the environmental and geological conditions of the Republic of Artsakh, as well as grain and agricultural crops used by the local population as feed**,** we used corn and esparcette for the study. Currently, there are no such ways to increase the nutritional value of feedstuff in Artsakh. (Mezhunts B., Sargsyan A. et al., 2016). Today, in terms of ensuring economic stability and food security for the population of Artsakh, it is important to address the issue of livestock development, for which there are sufficient domestic resources (large livestock and arable lands are a guarantee for a sustainable livestock base). Solving this problem also requires improving feedstocks, evaluating the nutritional integrity of feeds and identifying the risks of their use.

Materials and Methods

The growing of lactic acid bacteria

In the work we used *Ent. durans* P13, *L. acidophilus* 1991, *St. termophillus* 103, *Ent. faecium KE5, St. lactis* 62 probiotic LAB strains. For bacterial growth we used MRS (ISO) and MRS (Himedia) broth, MRS agar (Himedia), lactoagar, nutritional agar (Nutrient, Himedia, India), whey and pasteurized, sterilized fat milk (3.6%) produced by MYMY company, as well as dry skim milk (fat content: 1.5). Milk sterilization was performed under a sterilizing device for 15 minutes under a pressure of 0.8 mm/Pa.

For the preparation of antimicrobial preparation, we also used the nutritional medium prepared from cottage cheese whey with the following composition: yeast extract-0.30%, peptone-0.30%, trivalent sodium citric acid-0.60%, magnesium sulfate-0.2%, manganese sulfate-0.2%, dissociated ammonium sulfate-0.50-0.80%, potassium phosphate concentrated-0.10%. The bacteria were grown for 48 hours in 37°C in a prepared medium (Israyelyan A., Tkhruni F. et al, 2016, Karapetyan K., Tkhruni F. et al, 2017).

Yeast was grown on nutrition medium Saburo (Himedia), for 48 hours in 30^{o} C.

The strains were stored in the collection of the laboratory of Microbiology of Artsakh Scientific Center.

The process of making silage in the laboratory

For the study we used corn and esparcette which we gathered from two different communities: Avetaranotc and Herher

For the preparation of the silage, weather changes have been taken into account. Because the grass season is limited to late spring and summer, when the weather is favorable, the work has been done from late May to late July. The height of the grass should be 20-30 cm. After collecting the grass, the dried mass quickly was silaged within a day with two different preparations of lactic acid and yeast. We added 10 % preparations of consortium lactic acid (50%) and yeast (50%) to the silaging mass.

In laboratory conditions, food (silage) after the enrichment with probiotic bacteria was stored in appropriate containers for 45-60 days, after which we first checked their physicochemical parameters.

Table 1.Certain indicators of the received feedstuff

N	Feedstuff		Sample of feedstuff	Color	The smell	Form
1	Corn	control	I	dark green	sharp not pleasant	slightly moldy
2		*Ent. durans* P13 *L. acidophilus* 1991 *St.termophillus*103 *St. lactis*62	II	yellowish-green	like silage	like silage
3		*Ent. faecium* KE5 *L. acidophilus* 1991 *St. termophillus*103 *St. lactis*62	III	yellowish-green	not pleasant	like silage
4	esparcette	control	IV	green	like silage	slightly moldy
5		*Ent. durans* P13 *L. acidophilus* 1991 *St. termophillus*103 *St. lactis*62	V	yellowish-green	pleasant	like silage
6		*Ent. faecium* KE5 *L. acidophilus* 1991 *St. termophillus*103 *St. lactis*62	VI	dark green	pleasant	like silage

As can be seen from the data presented in Table 1 feedstuff with control and probiotic-producing bacteria are already different in appearance and odor. In addition, in the silos of the two different control groups, little mold was observed, which may already have a negative effect on animals.

Bacteriological analyses of feedstuff

Bacteriological analyses were carried out according to generally accepted methods (Биргер М., 2016).

The number of viable bacteria was determined by gradual dilution and characterized by the titration method. Microbiological parameters (fungi, mold, pathogens) were determined according to established methods and operating standards.

Protein determination method

Determination of protein in feedstuff is according to GOST PA13496.4-93

Amino acid determination

Determination of the amino acid content, thin-layer chromatography was used, followed by calculation of the content relative to the control data. For the quantitative determination of amino acids two methods of hydrolysylated samples of grass were used, esparcette and green mass of corn. The samples after silaging were hydrolyzed at 1 atm 120°C in the following ratio: 4 grams of the test 8 ml of 4 N HCl or 8 ml of 6 N HCl was added to the sample under a hydrolysis mode of 130°C for 20 min. Subsequently hydrolysis was selected hydrochloric acid embodiment hydrolysis at 130^0C for 20 minutes.

Based on the results on the amino acid content in all silage samples of three regions of Artsakh, thin-layer chromatography was used to transfer the selected samples to determine the amino acid content on an amino acid analyzer using High-performance liquid chromatography (HPLC) systems (Semi-preparative "Avex ODS" C18 column (8 by 250 mm, Waters and Shimadzu, Japan); Shimadzu LC-20 analytical C18 column (4.6 by 250 mm, Symmetry, USA, with a detector Diode array SPD-20 a, auto-sampler).

Results and discussion

The determination of the microflora contamination of the samples selected for silage is shown in Table 2.

The data show that after the use of probiotic bacteria, the quality of the feed increases. The activity of probiotic bacteria suppresses the growth of fungi and pathogenic microflora.

Table 2.Microbiological indicators of the received feedstuff

	Corn feedstuff			**Esparcette feedstuff**		
	Con-trol	*Ent. faecium* KE5 *L.acidophilus* 1991 *St.termophillus* 103 *St. lactis*62	*Ent. durans* P13 *L.acidophilus* 1991 *St.termophillus* 103 *St. lactis*62	Control	*Ent. faecium* KE5 *L.acidophilus* 1991 *St.termophillus* 103 *St. lactis*62	*Ent. durans* P13 *L.acidophilus* 1991 *St.termophillus* 103 *St. lactis*62
Contamination of feedstuff with fungi	Secondary growth	Absence of infection	Absence of infection	Secondary growth	Absence of infection	Absence of infection
Contamination of feedstuff with different pathogen bacteria	Secondary growth	Absence of infection	Absence of infection	Secondary growth	Absence of infection	Absence of infection
Content of probiotic LABs (CFU/ ml)	-	$2x10^7$	$8x10^7$	-	$1.16x10^9$	$3.2x10^6$

-No growth

The results of the content of amino acids and protein in the silage of grasses used in the farm conditions, are shown in Table 3.

Table 3.The content of amino acids and protein source-grass from the animal husbandry

Source-grass from the Animal husban-dary	Amino acids mg / ml											Pro-tein %
	Lys	Arg	Ala	Glut	Val	Isol	Tre	Met	Fal	Start	The amount of amino acids mg / ml	
Control	0,4	-	1,2	2,0	2,0	0,8	-	0,8	0,2	0,4	7,8	15,4
Consor-tium LAB+yeast	0,8	1,2	0,8	0,4	1,2	1,2	1,2	1,6	0,8	0,8	10,0	30,0
Consor-tiumLAB	0,8	1,2	1,2	0,8	1,6	1,6	1,6	1,6	0,8	0,8	12,0	27,2

However, the use of silos, the introduction of a certain consortium of lactic acid bacteria with yeast leads to an increase in protein content and the amount of amino acids. The lysine content in the study increased in the Avetaranots samples by an average of fourfold and in the Herher sample by an average of twice. As can be seen from the data presented in Table 3 after enriching with probiotic bacteria**,** the protein content increased in grass silage 76,6% and 94,8% after enriching with probiotic bacteria and yeast compare in the control version.

As can be seen from the data in Table 3, the addition of a mixture of LAB and yeast and a mixture of LAB when using green grass taken from the animal husbandary showed the best results for protein content during silaging.

The results of the content of amino acids and protein during silage of the green mass of corn are given in Table 4.

Table 4.The content of amino acids and protein source-grass from the Herher region

Source-corn from the Herher region	Amino acids, mg / ml											Pro-tein, %
	Lys	Arg	Ala	Glut	Val	Isol	Tre	Met	Fal	Start	The amount of amino ac-ids mg / ml	
Control	1,6	1,2	1,6	2,4	2,4	2,4	0,8	3,2	0,4	0,8	16,8	16,5
Consortium LAB	1,6	0,8	1,2	0,4	1,6	2,4	1,2	1,6	0,4	0,8	12,0	28,0
Consortium LAB+yeast	1,6	1,2	1,2	0,8	1,6	2,4	1,2	1,6	0,4	0,8	12,8	24,0

The data obtained show that during silage of the green mass of corn, the addition of a consortium consisting of yeast and LAB or only LAB can increase the protein content by an average of 50%, which is higher than when silaging a mixture of grass, regardless of the source of its use.

We studied the silage of esparcette, which is widely used in the regions of Artsakh as animal feed. The silaging was also carried out as described previously for silage of other grass.

Table 5.Content amino acid and protein from a mixture of grass sourced from Avetaranots and Herher region

Source-grass from the Avetaranots region	**amino acids,mg / ml**											**Protein %**
	Lys	**Arg**	**Ala**	**Glut**	**Val**	**Isole**	**Tre**	**Met**	**Fal**	**start**	**The amount of amino acids mg / ml**	
Control	0,8	-	1,6	2,4	3,2	0,8	1,6	3,2	0,4	0,4	14.4	17,0
Consortium LAB+yeast	3,2	-	3,2	3,2	3,2	2,4	2,4	3,2	0,4	0,4	21,6	27,0
Source-grass from the Herher region	amino acids,mg / ml											Protein %
	Lys	Arg	Ala	Glut	Val	Isole	Tre	Met	Fal	start	The amount of amino acids mg / ml	
Control	1,6	-	3,2	2,4	3,2	1,6	1,6	3,2	0,4	0,4	16,0	16,0
Consortium LAB+yeast	2,2	0,4	0,4	3,2	3,2	3,2	3,2	3,2	0,4	-	20,4	20,0

As can be seen from the data given in table 5, the used mixtures of grass from different sources differ in the content of some essential amino acids in the control and experimental samples. Thus, the content of the essential amino acid lysine in the control sample of grass silage from the Herher region is twice greater than in samples from the region of Avetaranots, but there is no difference in methionine content (The content of these 2 amino acids is the main requirement in feed).

However, the use of silage, the introduction of a certain consortium of lactic acid bacteria with yeast leads to an increase in protein content and the amount of amino acids. The lysine content in the studied increases in the Avetaranots samples by an average of fourfold and in the Herher sample by an average of twice. Thus, it is clear that the addition of LAB with yeast for silage increases the protein content by an average of 25% and essential amino acids by 30 % relative to silage sample of grasses without the use of biological additives.

Results of biochemical analysis of esparcette samples from different regions (thin layer chromatography method) are shown in Table 6.

As can be seen from the data, esparcette selected from the region of Avetaranots and Herher do not significantly differ in the quantitative content of protein, however, they differ in the total content of essential amino acids by 40%. The use of 2 methods of silage (addition of LAB and yeast and separately LAB) significantly increased the percentage of protein and the amount of essential amino acids.

Table 6.Comparative content amino acid and protein from the Esparcette source from Avetaranots and Herher region

Esparcette source from Avetaranots region			Esparcette source from Herher region	
Nomination	**protein %**	**The total number of amino acids, mg / ml**	**protein, %**	**The total number of amino acids, mg / ml**
Control	19.2	20.4	18.6	12.4
Consortium LAB+ yeast	35.0	20.0	26.25	15.6
Consortium LAB	33.6	19.2	19.25	14.4

The best result of silaging was noted in samples of esparcette from Averanots. As can be seen from the data, silage of esparcette and mixed grass selected from the Averanots region obtained by the proposed technology are more promising for use in animal feeding.

General view of the chromatogram of the ensiled sample with the addition of a consortium of probiotic bacteria and yeast in Figure 1.

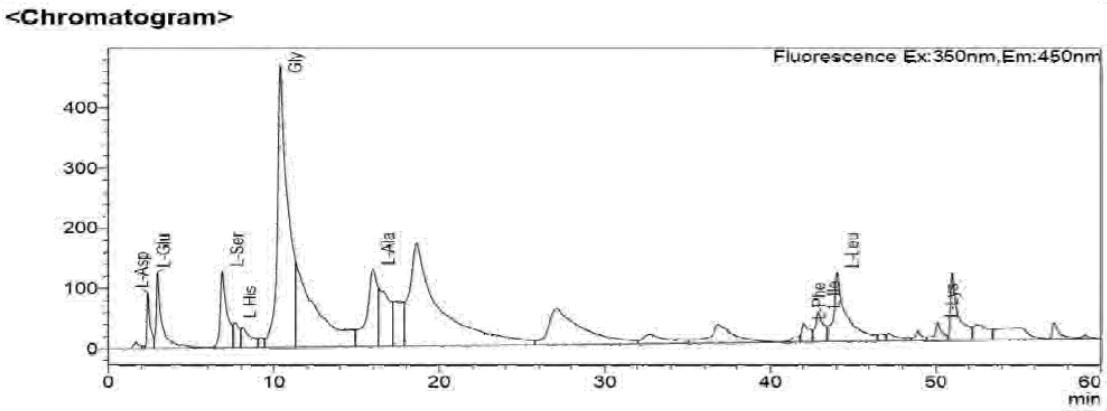

Figure 1. The chromatogram feedstuff with enrichment of the probiotics LABs and yeast

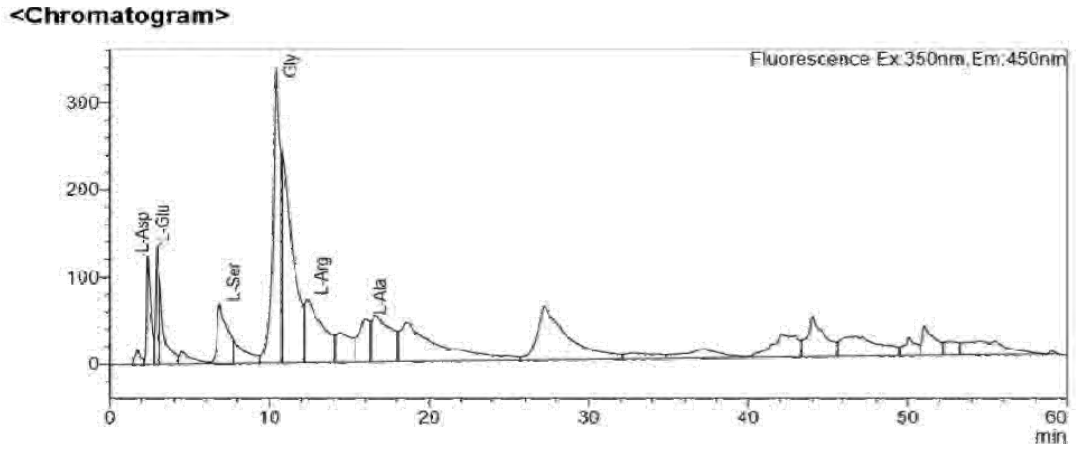

Figure 2. The chromatogram of feedstuff

General view of the chromatogram of the ensiled sample without the addition of a consortium of probiotic bacteria and yeast is shown in Figure 2.

The chromatograms show the differences in the results of the amino acid composition after ensiling the mixed green grass mass relative to the control.

Conclusions

The property of lactic acid bacteria to synthesize lactic acid is used for silage of green biomass. However, we have shown that the use of a consortium of LAB strains and yeast with probiotic properties leads to an increase in the content of protein and essential amino acids in silage, a decrease in its infectivity, and the effectiveness of the method used depends on the source and nature of the used method of green biomass and strains. Therefore, the use of starter culture from the consortium of investigated probiotics LAB and yeast in silage should be introduced as widely as possible, as they have a positive effect on the health of animals.

Acknowledgments

This work was supported in the frame of the research projects MESC AR scs 19AA-002, ANSEF biotech 52-52.

Conflict of interest

The authors declare that there is no conflict of interest

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