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Preserving Herbs with *Lactobacillus plantarum-52* Lactic-acid Bacterium and Their use in Forage For Cows

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In order to preserve the nutritional value of herbs, 4 options of combined silos have been designed: option I (reference) - maize, option II (experimental) - maize (70%), Sudan grass (30%), option III (experimental) - maize (70%), alfalfa (30%) and option IV (experimental) - maize (60%), Sudan grass (20%), and alfalfa (20%). Options II - IV of combined silage contained lactic -acid bacterium *Lactobacillus plantarum-52* at the rate of 1 billion cells per kg of green mass. Study of the combined silos chemical composition 120 days later showed that in the experimental options, as compared to the reference one, protein content was higher (II-11.4%. III - 12.2%. IV -12.91%) and, conversely, fibre content decreased. Feeding cows with combined silage that contains probiotics has a positive effect on their milk production. Within 100 days of the experiment, cows in experimental groups gave milk more by 60-230 kg.

Key words: combined silage, cow, *Lactobacillus plantarum-52* culture, Milkiness, maize, Sudan grass, alfalfa, milk, fat.

According to the data from Donaldson E. [1], Luck E.[2], Wiegmann C. [3], Spoelstra S.F.et. Al.[4], increasing attention in foreign countries is paid to combined silos obtained with the use of various bacteria, which improve the quality of ensilaged cultures to a certain extent.

At the same time, many of the bacteria used proved to be unsuitable for hardly ensilaged grass, particularly legumes. Inefficiency of preparations based on osmotolerant lactic-acid bacteria in ensilaging high-protein legumes can be explained by the lack of sugar required for formation of a sufficient amount of organic acids.

Various preparations and biological preservatives have been used for ensilaging hardly ensilaged herbs[5,6]. In recent years, works appeared that evidence a positive solution to the problem of ensilaging high protein legumes with the use of the *Lactobacillus plantarum-52* lactic acid bacterium[7-9]. According to G.Y.Laptev[10] and V.A.Ramensky[11], *Lactobacillus plantarum-52* is suitable for preserving nutrients and for improving quality of grass silage of virtually any plant material. Besides, *Lactobacillus plantarum-52* possesses antibacterial properties together with well expressed fungicidal abilities, as well.

The purpose of the research was to develop combined silage in the Southern region of Kazakhstan, using the *Lactobacillus plantarum-*

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52 culture, from a variety of forage crops, including legumes, that stably maintains nutritional assets of freshly cut grass and, when subsequent used for feeding lactating cows, contribute to increased milk production.

MATERIALS AND METHODS

The research was carried out in the laboratories of the South-Kazakhstan State University named after M. Auezov and in production conditions in accordance with "Guidelines for studying preservative effects of chemicals used in silages in the laboratory"[12]. The study of combined silage influence on dairy cows was made using Auliatinsk breed. The digital material obtained from the studies was mathematically processed by N.A. Plokhinsky[13].

Main part

In laying silage, the following crops were used: maize, Sudan grass and alfalfa. These crops were used for laying 4 kinds of silage. Silage of option I (reference) consists of maize, option II (experimental) - of maize (70%) and Sudan grass (30%), option III (experimental) - of maize (70%) and alfalfa (30%), and option IV (experimental) - of maize (60%), Sudan grass (20%), and alfalfa (20%). Experimental options of combined silage contain lactic-acid bacterium *Lactobacillus plantarum-52*.

Green plants were ensilaged using containers (1 dm^3) with 4 repetitions of each option. Freshly mowed green mass was milled and chemically analyzed in a laboratory for 24 hours. 10 ml of preservatives diluted in water at the ratio of 1:1 were added to 1 kg of freshly mowed mass. After introducing bacterial cultures, raw materials were mixed and loaded into a laboratory container rammed to profuse juice discharge from the silage mass.

The containers were weighed, capped, sealed with paraffin and stacked for storage in an unlit, dry and cool room. The silages were allowed to ripen for 120 days, then they were evaluated by organoleptic characteristics, their acidity was determined, and the content of solids was analyzed.

The results of silos chemical analysis showed (table.1) that complex silage with probiotics (groups 2 through 4) features a higher content of nutrients, as compared to maize silage. Thus, the amount of dry matter in silage of maize,

Table 1. Characteristics of the compared combined silages by chemical composition

Indicators	Maize silage I (reference)	Complex silage (70% maize +30% Sudan grass+ Lactobacillus plantarum-52) II (experimental)	Complex silage (70% maize +30% alfalfa+ Lactobacillus plantarum-52) III (experimental)	Complex silage (60% maize +20% Sudan grass+20% alfalfa+Lactobacillus plantarum-52) IV (experimental)
Dry matter,% including:	27.76±1.2	26.34±0.97	26.54±0.93	29.36±0.68
Protein, %	9.52 ±0.01	11.61±0.08	12.18±0.3 7*	12.91±0.78*
Fat, %	3.49 ± 0.02	4.14 ±0.03*	4.83 ±0.05**	4.86 ±0.06**
Fibre,%	37.03 ±1.04	35.93± 0.92*	32.34 ± 1.31**	30.82 ± 1.74**
Ash, %	1.97±0.15	1.89±0.13	1.92±0.13	1.96±0.17
Nitrogen-free extractive substances %	47.99± 1.74	46.43 ±2.14	48.73 ±2.76	49.45 ±1.43
pH	3.8 ±0.03	3.9 ±0.04	3.9 ±0.08	3.9 ±0.07
Acids ratio, %				
lactic	71.7 ±2.08	79.6±2.47	80.5±2.11	81.1±2.43
acetic	28.3±0.7	20.4±2.36	19.5±3.21	18.9±1.62
oleic	0.0	0.0	0.0	0.0
Carotene, mg/kg	19.40±1.84	22.42	26.7 ±2.42	29.8 ±2.19
Forage units in 1kg of natural forage	0.19±0.007**	0.22±0.005	0.23±0.006	0.24±0.008

*p<0.05; **-p<0.01

Sudan grass and alfalfa was 29.36%, or by 1.60% higher, as compared to option I (P <0.01). Content of dry matter in option IV is also higher than in option II by 2.02% (P<0.05), and in option III by 2.82%. Content of crude protein was also significantly higher in silage of option IV. At the same time, crude fat content in the compared silages containing *Lactobacillus plantarum-52* (Groups 2-4) is virtually the same (4.7-4.8%). By this indicator, the experimental groups outperform the reference group by 1.4 to 1.5%. The highest content of crude fibre was seen in the reference option of silage - 7.67%, in the option with Sudan grass and alfalfa, fibre content is less by 0.46%, and in the maize and alfalfa option- by 0.89%. Acidity of the reference and experimental options of complex silages was 3.8 to 3.9.

Table 2. Preservation of nutrients in silages (% of initial weight)

Indicators	Maize silage	Complex silage (70% maize + 30% Sudan grass+ Lactobacillus plantarum-52)	Complex silage (70% maize +30% alfalfa+ Lactobacillus plantarum-52)	Complex silage (60% maize +20% Sudan grass+20% alfalfa + Lactobacillus plantarum-52)
Dry matter	81.32±2.53	82.87±2.69	82.72±2.59	87.89±2.49*
Organic matter	81.21±2.67	85.62±3.83	85.92±3.27	86.78±2.12
Protein	85.23±1.98	86.63±3.02	87.54±3.05	92.51±2.32*
Fibre	93.26±2.64	83.28±3.34	83.84±3.57	83.72±2.91
Ash	81.92±3.62	83.87±3.51	85.92±2.78	85.96±2.89
Nitrogen-free extractive substances	74.99±2.39	75.73±3.12	76.43±3.41	78.49±2.83
Carotene, mg/kg	52.29±5.29	61.44±2.02	61.72±1.56	65.71±1.89
Forage units	82.61±1.45	84.64±3.51	85.81±3.38	94.37±2.29*

Table 3. Influence of combined silages on cows' milk production

Indicators	I reference	Combined silage with *Lactobacillus plantarum-52* lactic-acid bacterium		
	(no preservative)	II experimental	III experimental	IV experimental
Daily milk yield, kg	13.9±0.84	15.1±0.71	15.7±0.76	16.4±0.83
Milk fat,%	3.83±0.06	3.93±09	3.91±0.07	3.90±0.08
Obtained in 100 days of experiment: milk	1390.0±62.2	1510.0±49.2	1570.0±53.6	1640.0±67.4
milk fat	53.2±1.51	55.4±1.59	61.3±2.04	63.9±1.84

An equally important quality characteristic of the harvested silages is preservation of nutrients (Table 2). Results in Table 2 show that the maize & alfalfa & Sudan grass option (IV experimental) is the best in preserving dry matter (87.89%), organic matter (86.78%), protein (92.51%), ash (85.96 %), nitrogen-free extractives (78.49%) and carotene (65.71%). Fibre is best preserved in maize silage (83,72%).

Based on the above data about qualitative and quantitative composition of ready silages, it can be stated that *Lactobacillus plantarum-52* is suitable for the preservation of green mass of cereals and legumes. In all options, studies with the use of probiotics gave results superior to mono-component silage. It was established that combined silages containing maize, Sudan grass and alfalfa with probiotic added feature the least loss of nutrients. Especially, silaged grass proteins were preserved in sufficient quantities (86.63 to 92.51%). Cereals mixed with legumes and lactic acid bacterium *Lactobacillus plantarum-52* make a silage with good organoleptic characteristics, high degree of

preservation of dry and organic substances, and less loss of carotene of observed during storage.

Feeding complex silage made of maize, Sudan grass and alfalfa to lactating cows results in increased milk production by animals (Table 3). Introduction of silages of maize and Sudan grass with probiotic (II experimental) increases milk production by 8.6% in comparison with maize silage. A similar 11.3% increase in cows' milk yield was found in case of feeding them with maize and alfalfa silage with probiotic. Milk productivity in cows that received combined silage (group IV) consisting of: maize (60%), Sudan grass (20%) and alfalfa (20%) with *Lactobacillus plantarum-52* was higher than that in other groups. Milk production in cows of the fourth group was higher by 17.9% than that in the reference group, and by 8.6% higher than that in the second group, and by 4.4% higher than the one in the third group. Eatability by cows of the silage from group I was 90.2%, from groups II and III - 92.6%. The best combined silage eatability was found in cows in group IV- 94.3%.

The study of the qualitative composition of milk from experimental cows showed that they were practically identical in chemical composition and technological properties at the beginning of the experiment. However, after 100 days of receiving combined silage with lactic-acid ferment (Lactobacillus plantarum-52), improvement in milk quality indicators in all experimental groups was found.

A great increase in milk quality indicators was found in test group IV. Cows from this group gave milk with an increased fat content to 0.18 or 9.3%, protein -0.18 or 10.5% (P> 0.999), casein - 0.16 or 10.6% (P>0.99), sugar - 0.17 or 10.4% (P> 0.99), dry matter - 0.64, or 10.5%, and SNF - 0.28 or 10.3 % (P> 0.95) (Table 4).

Table 4. Qualitative characteristics of milk from experimental cows, % (it the end of the experiment)

Indicator	Group			
	Ireference	IIexperimental	IIIexperimental	IVexperimental
Fat	3,65±0,02	3.75±0.02	3.78±0.02	3.83±0.04
Protein	3,24±0,03	3.33±0.02	3.35±0.02	3.42±0.04
including casein	2,73±0,02	2.84±0.01	2.86±0.03	2.89±0.03
Sugar	4,71±0,04	4.81±0.04	4.84±0.05	4.88±0.03
Dry matter	12,54±0,16	12.86±0.07	12.91±0.06	13.18±1.19
SNF	8,73±0,04	8.93±0.04	8.94±0.06	9.01±0.26
Ash	0,74±0,004	0.75±0.003	0.75±0.003	0.76±0.004
Density, g/cm3	1,032±0,007	1.033±0.005	1.033±0.005	1.034±0.008
Titratable acidity, 0T	17,30±1,04	17.26±0.10	17.26±0.11	17.24±0.14
Rennet coagulation, min.	39,78±1,69	41.32±2.63	41.54±2.65	42.53±2.97

DISCUSSION

The obtained experimental results show the possibility of ensilaging hardly ensilaged alfalfa with the *Lactobacillus plantarum-52* preservative that has antibacterial properties and fungicidal action and is present in maize and Sudan grass. Bactericidal properties of *Lactobacillus plantarum-52* were first reported by A. Polnomochnov *et al.*,[14-15] who suggested using this culture in the production of antibiotics. More detailed studies of morphological, cultural, and biological properties of *Lactobacillus plantarum-52* was performed by N.P. Tarabukina[16], which became the basis for developing the possibility of using herbs for preserving animal forage[17-18]. V. Duborezov, V. Vinogradov[19] studied cellular features of *Lactobacillus plantarum-52* in detail and characterized their zymoplastic features. Our research has shown that silage combined with Lactobacillus plantarum-52 by its protein and fat content exceeds the reference group and has a positive effect on cows' milkiness, which also confirms previous findings of some authors[20].

CONCLUSIONS

1. The studies have shown high preservative activity of the *Lactobacillus plantarum-52* strain in ensilaging legumes and cereals. In

combined silages containing this probiotic, protein and fat content increases, and fibre content decreases.

2. Feeding combined silage with the *Lactobacillus plantarum-52* strain to lactating cows results in an increase in milkiness by 11.3% -17.9%, and an increase in protein content by 3.32%.

REFERENCES

1. Donaldson, E., Getting the right silage. Machinery for silage. Hurley, 1985; 12-17.
2. Boyarsky LG Production of high-quality feed. MM: Rosagropromizdat, 2002; 317s.
3. Wiegmann, S., Konservierungvon Herbstgrasernmiteinem Silierzusatzaus Lactobacterien. Diplomarbeitan der TechnichenUniversität. Berlin, 1986; 95.
4. Spoelstra, SF, MGCourtin andJ.ACBeers, Acetic acid bacteria can initiateaerobic deterioration of whole crop maize silage. *Journal of Agricultural Science*., 1988; **1**: 127-132.
5. Levakhin, VI, Use of conservatives in forage silaging. Kazan: «Adelaide», 2001; 291.
6. Pobednov, Y.A. and V.V. Khudokormov, New preparation for ensilaging air-dried herbs. *Forage production*, 2000; **6**: 30-31.
7. Victorious, JA, Khudokormov VV On the theory of drug use of lactic acid bacteria in silage grass, Grassland: Research and Production magazine. 1999; **12**: C. 29-32.
8. Mamaev, AA, Efficacy preserving herbs culture Bacillus subtilis and use of the resulting feed ration cattle, thesis of Candidate of Agricultural Sciences, National Research Institute im.V.R.Vilyamsa, Moscow, 2005.
9. Kostin, DN, Meat efficiency of calves when used in canned rations of alfalfa silage Moscow: Kolos, 2008; 113.
10. Laptev, GY, Biotroph microbiology for livestock production. Agricultural News, 2003; **1**: 10.
11. Ramensky, VA, Comparative characteristics of bacterial ferments and chemical conservatives in herbs ensilaging thesis of Candidate of Agricultural Sciences, National Research Institute of beef cattle Rosselkhozakademia, Moscow, 1991; 205.
12. Guidelines for the study in the laboratory preservative effects of chemicals used in the silage (AUIAB), Dubrovicy, 1983; 9.
13. Plohinsky, HA, Biometrics Guide for Animal Husbandry. Moscow: Kolos, 1969; 25.
14. Polnomochnov, A., Storing silage with biological preservative. Livestock Production in Russia, 2001; **6**: 36-37.
15. Panov, AA, Development and improvement of technologies for ensilaging forage crops green mass with the use of chemical and biological agents, abstract from thesis of Doctor of Agricultural Sciences, National Research Institute of beef cattle Rosselkhozakademia, Moscow, 1998; 38.
16. Tarabukina, NP, Morphological, cultural, and biological properties of Bacillus subtilis "TNT-3" .Scientific support veterinary problems in livestock: Sat scientific. tr. / RASKHN.Sib.otd-nie.IEVSiDV.- Novosibirsk, 2000; 264-266.
17. Allaberdin, IL, Scientific and practical basis for the use of chemical, biological and vegetable conservatives in silaging and using it in feeding cattle, abstract from PhD thesis, Bashkir Research Institute of Agriculture, Ufa, 1999; 46.
18. Hudokormov, VV, The effectiveness of air-dried herbs conservation with Biotroph preparation and use of resulting forage in rations for cattle, abstract of thesis of Candidate of Agricultural Sciences, National Research Institute im.V.R.Vilyamsa, Moscow, 2002; 16.
19. Duborezov, V. and V. Vinogradov, Biological conservatives increase nutritional value of forage. *Livestock Production in Russia*, 2004; **5**.1: 9.
20 Bezborodov, IN, Full-value feeding of cattle. Belgorod: Publishing House of BSAA, 2001; 35.