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Combined Silage for Pigs with the Use of *Bacillus subtilis* Probiotic

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In order to increase productivity of pigs, combined silage including *Bacillus subtilis* probiotic has been developed. The studies showed that when 20 % of the combined silage (75 g of the *Bacillus subtilis* culture per 1 ton of alfalfa, 50 kg of pumpkin and 60 kg of grape pomace) is introduced into the nutrient rations of boars, their ejaculate volume increases by 20.9 %, the number of sperms - by 22.1 %, preservation of SH groups - by 13.6 %, operation of power supply system in the gametes - 2.6 times, and fertility of sows from the first insemination - by 25.0 %. Introduction of combined silage with *Bacillus subtilis* into the diet of sows (1 ton of alfalfa + 90 kg of grape pomace + 50 kg of pumpkin + 100 g of the probiotic) increases the number of weaned piglets by 0.6 animals, weight of weaned piglets by 2.3 kg, and increases piglets viability by 5.0 %.

Key words: Silage, Pigs, *Bacillus subtilis*, alfalfa, Pomace, Pumpkin, Sperm.

Kazakhstan has adopted feeding pigs with concentrates, and probiotics that are widely used abroad have not been tested on cultivated plants and plant waste, and their preserving action has not been studied. In this regard, studying their preserving action on plants that occupy a vast territory of the region becomes especially urgent, as well as studying the possibility of increasing energy conservation and protein nutritional value of green mass with the use of probiotics.

From many probiotics, for ensilaging of green plants the *Bacillus subtilis* culture was selected, which combines equally high fermenting activity and antibiotic properties. Studying the fermenting activity, Woolford MK[1], H.K. Tewari, D.S. Chahal[2] noted that *Bacillus subtilis* is one of the few microorganisms capable of decomposing hemicellulose. S.F. Jansen 8 D.J. Hirschmann[3] separated the subtilin antibiotic from Bacillus subtilis, which deteriorates development of phytopathogenic microorganisms, staphylococci, micrococci and protozoa. Later V.A. Johnson *et al*.[4] described the bacitracin antibiotic.

According to A.A.Mamaev[5], A.Senthil *et al*[6], E. Baca *et al*[7], S. Bunting *et al*[8], introduction of sugar-containing waste (1.3 %) into ensilaged alfalfa and other hardly ensilaged plants stimulates proliferation of lactic acid bacteria, especially when these ferments are able to suppress extraneous micro-flora.

The aim of this study is to develop the combined silage with the use of *Bacillus subtilis* culture, on the basis of green plants and their waste, in order to obtain bulky food that would be equal or slightly inferior to the initial green mass in terms of energy value, and would contribute to increasing pigs' productivity.

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MATERIALS AND METHODS

The work was performed in laboratories of the South Kazakhstan and the West Kazakhstan State Universities. Silage quality was assessed in accordance with GOST 23638-90 "Silage made of green plants". 4 groups were formed, with 5 boars and 10 sows of large white breed in each. We used *Bacillus subtilis* provided by the "Biotroph" company in St. Petersburg. Forages were analyzed according to Lukashik and Toschilin[9] and guidelines [10-11]. Quality of sperm was assesses by the usual method of freezing and thawing proposed by N.V. Korban[12], content of SH-groups - according to Torchinskiy [13] and preservation of acrosomes - according to V.G. Pursel[14]. The results of the experimental research were processed according to the methods proposed by N.A. Plohinskyi[15].

RESULTS

Chemical composition of plants was studied, and it was found that alfalfa contains 18.2 % of dry matter and 81.8 % of water. Dry matter contains, in %: crude protein - 18.3, crude fat - 2.9, crude fibre - 25.2, ash - 10.3 and nitrogen-free extractive substances (NFES) - 43.3. Fresh grape pomace contains 69.6 % of water and 30.4 % of dry matter. Dry matter contains, in %: crude protein - 3.4, crude fat - 2.5, crude fibre - 9.8, NFES - 11.8 and ash - 2.9. Calculations showed that, on the average, the pomace contains: exchange energy - 1.67, forage units - 0.31, crude protein - 3.4 g. 1 kg of pumpkin contains: 0.16 forage units and 153 g of dry matter. Dry matter contains, in %: 11 g of crude protein, 27 g of crude fat, 4g of sugar, 104 g of NFES, 4 grams of starch and 3 % of ash. The schedule of the experiments is shown in Table 1.

The silages were opened 90 days after ensilaging. According to organoleptic assessment, the silages were of good quality: they had averagely acid taste, smell of fermented vegetables, and color of the original stock with well-preserved particles structure. These assessments are confirmed by biochemical parameters and chemical composition of silage prototypes (table 2).

The highest quality indicators in boar ejaculates were observed in the second experimental group (table 3). The average volume of boar ejaculate is 319.3 ml, which is higher than in the reference group by 20.9 %, the number of spermatozoa in the ejaculate is 76.8 billion or higher by 22.1 %, respectively, preservation of SH groups is 13.6 %, functioning of the power system, i.e., respiration and phosphorylation, in gametes is higher 2.6 times, and fertility of sows from the first insemination is higher by 25.0 %.

Introduction of combined silage with higher nutrition value by 15-25 % into the diet of bars, with introduction of various doses of the *Bacillus subtilis* culture, grape pomace and pumpkin makes up for missing nutrients, improves metabolism and physiological condition of the organism, thereby increasing their reproductive qualities. The total volume of ejaculate increases from 12.6 to 20.9 %, and, respectively, the lifetime of spermatozoa increases from 19.6 to 35.3 %,

Table 1. Schedule of feeding pigs with combined silage produced with the use of the *Bacillus subtilis* culture

Groups	Structure of rations	Combined silage composition
I - reference	mixture of concentrates - 88 %, safflower press-cake - 4 %, cotton cake - 3 %, forage of animal origin (meat and bone scraps, natural return) -5 %.	-
II -experimental	mixture of concentrates - 75 %, safflower press-cake - 4 %, cotton cake - 3 %, forage of animal origin - 3 %, combined silage - 15 %.	1 ton of alfalfa + 30 kg of grape pomace + 50 kg of pumpkin + 50 g of Bacillus subtilis
III - experimental	mixture of concentrates - 70 %, safflower press-cake - 4 %, cotton cake - 3 %, forage of animal origin - 3 %, combined silage - 20 %.	1 ton of alfalfa + 60 kg of grape pomace + 50 kg of pumpkin + 75 g of Bacillus subtilis
IV - experimental	mixture of concentrates - 65 %, safflower press-cake - 4 %, cotton cake - 3 %, forage of animal origin - 3 %, combined silage - 25 %.	1 ton of alfalfa + 90 kg of grape pomace + 50 kg of pumpkin + 100 g of Bacillus subtilis

fertility of sows from the first insemination with cryopreserved sperm was between 8.3 - 33.4 %.

An important qualitative indicator of high quality of spermatozoa is their motility after thawing. The highest sperm motility after freezing and thawing was in boars from the second experimental group. This indicator is better than in the reference group by 26.8 %, and is better by 10.2 % and 4.8 % than in the first and the second groups, respectively.

Analysis of information about reproductive qualities of sows and preservation of their piglets during the experiment made it possible to establish the positive effect of combined silage prepared with the use of the *Bacillus subtilis* culture (table 4).

Using the combined silage obtained using the *Bacillus subtilis* culture did not lead to an increase in the number of piglets, from these

Table 2. Correlation of acids, composition and nutritive value of combined silages with the use of the *Bacillus subtilis* culture

Indicators	Experimental groups		
	II	III	IV
@”	4.2	4.18	4.27
Acids ratio, %			
lactic	73.9±0.04	75.3±0.05	74.1±0.05
acetic	26.1±0.01	24.7±0.01	25.9±0.01
oleic	0.00	0.00	0.00
Dry matter, %	17.57±0.52**	19.23±0.78**	19.87±0.86**
Protein, %	2.94*	3.21*	3.41*
Fat, %	0.89	0.98	1.01
Fibre, %	5.29*	5.80*	5.88*
Ash, %	1.74	1.91	2.01
NFES %	6.71	7.33	7.56
Carotene, mg/kg	25.20±2.03	26.14±2.12	27.04±2.31
Fodder units in 1 kg of natural forage	0.21±0.005	0.22±0.004	0.23±0.006

* <0.05; ** <0.01

Table 3 . The effect of combined silage on quality indicators of undiluted boar sperm after 12 hours of incubation

Indicators	I – reference group (without silage)	Experimental groups		
		II	III	IV
Activity of spermatozoa, points	5.4	5.9	6.4	6.1
Average volume of ejaculate, ml	283.6	319.3	342.9	321.7
Concentration of spermatozoa, million/ml	232.7	231.8	224.2	229.1
Number of spermatozoa in the ejaculate, billions	62.9	71.2	76.8	73.7
Specific electrical conductivity at 30 °C, 10^{-4} Ohm	127.9	127.4	127.4	127.4
Speed of spermatozoa movement, µm/s	26.2	28.6	29.1	29.0
Preservation of SH groups in the gametes, %	50.7	62.8	64.3	62.6
Number of damaged acrosomes, %	42.1	33.9	29.8	37.6
Time of methylene blue reduction, minutes	14.5	11.7	7.9	8.8
Activity of common dehydrogenases, minutes	79.2	76.7	63.3	67.2
Activity of cytochrome oxidase, minutes	92.1	87.7	78.4	79.1
Amount of absorbed O^2 and phosphorus in µg-atoms per 109 cells	0.46	1.08	1.21	1.12
Fertilizing capacity of spermatozoa, %	33.3	41.6	58.3	50.0

Table 4. The influence of the combined silage obtained with the use of the *Bacillus subtilis* culture on reproductive qualities of sows

Indicators	I – reference group (without silage)	Experimental groups		
		II	III	IV
Number of sows, animals	22	21	21	21
Number of piglets in kidling, total animals	10.2±0.18	10.2±0.12	10.3±0.11	10.3±0.15
incl.: alive	10.1±0.14	10.1±0.13	10.2±0.14	10.2±0.17
nonviable	0.1±0.001	0.1±0.002	0.1±0.02	0.1±0.001
Fetus weight, kg	1.22±0.01	1.22±0.01*	1.23±0.02**	1.23±0.01
Nest weight at birth, kg	12.3±0.25	12.3±0.32	12.5±0.16	12.5±0.43
average weight of one piglet, kg	4.9±0.09	5.2±0.07	5.3±0.09	5.5±0.08
Milkiness, (weight of piglets on the 21st day), kg	50.3±1.8	53.3±2.02	54.4±1.04**	55.9±1.33
At the age of 30 days:				
Number of piglets in kidling, total animals	9.3±0.31	9.4±0.19	9.6±0.38	9.8±0.37
weight of the whole kindle, kg	56.7±1.6	62.0±1.1**	65.3±1.4**	69.6±1.7
average weight of one piglet, kg	6.1±0.5	6.6±0.7	6.8±0.6	7.1±0.5
At the age of 60 days:				
number of piglets in the nest, animals	9.1±0.4	9.3±0.4	9.6±0.3	9.7±0.3
average weight of one piglet, kg	17.1±1.1	18.3±1.3**	18.6±1.5**	19.4±1.3**
Nest weight at the age of 2 months, kg	155.6±4.2	170.2±5.9*	178.6±4.8**	188.2±6.1
Preservation, %	89.2±1.6	94.8±2.2	93.2±4.8	94.2±5.3

* <0.05; ** <0.01

sows, which in group II was 10.2; in group III – 10.3 and in group IV – 10.3 animals. The number of non-viable piglets in experimental groups III and IV is caused by improved protective properties of the organism, followed by improved metabolic processes that resulted in higher live weight at birth. Analysis of piglets' weight in the age of 2 months showed a trend to steady progress, when the sows received silage combined with the *Bacillus subtilis* culture. So, in experimental groups II, III and IV, piglet weight was higher than that of counterparts in the reference group by 10.7; 10.8 and 11.3 %, respectively. Higher live weight of the nest in 60 days was found in experimental groups III and IV - 178.6 and 188.2 kg, which exceeded the reference group by 11.5 and 12.1 %, respectively.

High piglets' preservation rate was noted in nests of the sows that received combined silage with the *Bacillus subtilis* culture. Therefore it can be stated that this is the effect of the combined silage on the organism of sows; it improved the milk formation process and ensured higher viability of the offspring.

CONCLUSION

The findings testify about the possibility of ensilaging hardly ensilaged alfalfa with introduction of grape pomace, pumpkins and the *Bacillus subtilis* culture in order to increase the productivity of pigs. High enzymatic and antibiotic activity of the *Bacillus subtilis* culture, and its ability to function equally well in both aerobic and anaerobic conditions is a prerequisite for using it in ensilaging herbs in order to improve preservation and quality of thus obtained forage. Our information about the possibility of improving silage quality is consistent with works of authors[16-20]. In particular, in works of Miiller Th. and Fehrmann E. [16]] improvement of silage quality is treated in relation to epiphytic bacteria of lactic acid; Ohyama Y., Masaki S.[17] noted an increase in water-soluble carbohydrates, Gallo M., et. al[18] studied the effect of adding various biological additives to clover silage, Khorvash M. *et al*.[19] used adsorbents and modifiers, and Polat E. *et al*.[20] used natural enrichers. Works of V. N. Sidorov[21], A. A. Nekrasov[22], and M.I. Slozhenkina[23] also

witness the possibility of using *Bacillus subtilis* in ensilaging. According to N.P. Tarabukina [24], over 70 various antibiotics have been discovered so far that are produced by Bacillus subtilis. According to W. Loeffer *et al*[25], the most studied of these are bacilisyn and chlortetain that inhibit growth of bacteria and fungi, as well as rysocticin, inturin, miArobaAillin and fungimiAin that predominantly inhibit growth of fungi. G.Yu. Laptev[26] also consider important the fact that the *Bacillus subtilis* bacteria produce the amylase exoenzyme, which degrades starch to glucose, maltose, xylose and oligoglycosides with subsequent fermentation of the latter to organic acids, mainly lactic acid, without any noticeable increase of gas production.

Deductions

Summarizing the results of researching the effect of the combined silage obtained with the use of the *Bacillus subtilis* culture on reproductive qualities of pigs, the following conclusions were made:

1. The developed combined silage by its organoleptic and chemical qualities meets the requirements.

2. When 20 % of the combined silage (75 g of the *Bacillus subtilis* culture, 50 kg of pumpkin and 60 kg of grape pomace per 1 ton of alfalfa) is introduced into the nutrient rations of boars, their ejaculate volume increases by 20.9 %, the number of spermatozoa - by 22.1 %, preservation of SH groups - by 13.6 %, functioning of energy system in the gametes - 2.6 times, and fertility of sows from the first insemination - by 25.0 %.

3. After the combined silage was introduced to the diet of sows (1 ton of alfalfa + 90 kg of grape pomace + 50 kg of pumpkin + 100 g of Bacillus subtilis), the number of weaned piglets increased by 0.6 piglets per sow, weaned piglets were heavier by 2.3 kg, and viability of piglets increased by 5.0 %.

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