Development of Mixed Silo for Boars and Investigation of Its Effect on Quality Indicators of Ejaculates

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Abstract: Combined silage consisting of alfalfa, pumpkin and husks of grapes with addition of lactic acid bacteria Bacillus subtilis was developed. According to sensory evaluation the stacked silos with added lactic acid bacteria Bacillus subtilis, pumpkin and husks of grapes were of good quality, had medium sour taste, the smell of pickled vegetables, the color of raw materials, and intact structure of the particles. The best option to make silage is to add 75 g of probiotic, 50 kg of pumpkin and 60 kg of husks of grapes per 1 ton of green mass of alfalfa. 1 kg of such a silo contained 0.33 fodder units, 385 g of dry matter, 32 g of digestible protein, 18 g of fat, 50 mg of carotene and 51 g of crude fiber. The inclusion of 20% of the total nutritional value of such mixed silage in the diets of boars increases the palatability and improves the physiological condition of the body and reproductive capacity of boars. In this case, the volume of ejaculate increased by 12.6 % and total sperm - by 13.2 %, pathological forms decreased by 19.5 %, the safety of the sulfhydryl groups (SH) improved by 12.1%, and functioning of energy system in 2.3 times. It was found that feeding boars with mixed silage increases sperm cryostability. Compared with the control group damaging of sperm with acrosomes is 8.6% lower, the survival time is longer by 19.6-35.3 %, the sperm motility is higher by 14.7-26.5 %, as well as the safety of SH-groups – by 8.1%, functioning of the energy system - by 46.9-77.5 %, fertilizing capacity of gametes – 8.3-33.4%. The highest results for cryostability of sperm were found in boars of second experimental group.
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1. Introduction

In Kazakhstan, the livestock industry is one of the priorities; therefore, the problem of production of high-quality forage becomes the most acute and needs new and evidence-based decision-making. Forage production is a priority issue in pork production. In many farms pigs are fed mainly with concentrated fodder, and tuberous roots, silage, green and crude fodder are used very little and irrationally. Monotonous feeding, as we know, is one of the major causes of the deterioration of animal health, reduction of their productivity and consumptive spending of feeds. The experience of many countries with the developed pork production shows that the use of various full and balanced diets allows identifying possible genetic potential of animals' productivity.

Further enhancement of the reproductive qualities of pigs requires the creation of a strong and well-organized forage base, intensification of fodder production and usage of the world's scientific achievements. One of the most important ways to increase the content of nutrients in feed is a progressive technology for preservation of feed, especially stacking of mixed silage, which provides the most complete preservation of their physiologically beneficial properties at a minimum input of labor and material resources.

Scientific bases of feed silage were adequately developed and examined in the works of A.I. Virtanen [1], O. Richard [2], S.Ya. Zafren [3]. Siloing is a complex microbiological process. A wide variety of microorganisms get in silo with the green mass. According to O. Knabe et al. [4] putrescent, butyric micro flora and yeast develop on the raw materials. In this case, the total loss of nutrients can reach 20%.

The results of scientific research of P. Mc Donald [5], D.C. Patterson et al. [6], and the practical use of chemical and bacterial mixtures at ensilage of green plants as preservatives-additives to improve the quality of silage, in general have given positive results.

Development of scientific and practical aspects of the adaptation of modern techniques of preparation and use of animal feed is an urgent problem. Currently, one of the best recognized preservatives is inorganic and organic acids, but they are quite expensive, deficient and non-technological

due to their aggressiveness, which limits their use in fodder production.

The results of the works of M.K. Woolford [7], A.P. Mansurov [8] and others indicate that these drawbacks are not specific for probiotics - preparations produced, as a rule, on the basis of combinations of different strains of lactic acid bacteria. However, the vast majority of them are recommended for conserving easily ensilaged crops, which do not include legumes. Legumes are characterized by low sugar content and high buffering capacity, which can affect the efficiency of probiotics at their silage.

In preparation and feeding with mixed silage on the basis of alfalfa there is still very much unclear: the technology of preparation of various sugar-containing food waste has not been developed yet, the effectiveness of their use for pigs has not been developed, and the effect of silage in the ration on the physiological state and sperm productivity of breeding boars has not been examined.

2. Materials and methods of investigation

The work was performed at the Department of "Biotechnology" of M. Auezov South Kazakhstan State University, in the laboratory of reproduction of the All-Russian Scientific Research Institute of Animals Genetics and Breeding in South Regional laboratory and breeding piggery farm "Shubar." In the experiments we used Semirechinskaya grade of alfalfa, pomace of Aliquot and Terbash grape grades and Mozoleevskaya pumpkins. New-mown alfalfa was grinded to 1.5 cm, moisture of silage mass was within 65-75%.

The object of research was adult breeding boars of large white breed with a live weight of 286-312 kg. Animals were selected on the basis of analogs and divided into four groups, 8 animals in each. The first group was the control group. Animals of this group had basic diet used in many pig farms in the region. Feeding of experimental animals was conducted according to the study scheme (Table 1). In the daily diets of boars there was 1.5 kg of silage. All feed rations were completely eaten. Duration of experimental work was 6 months.

Alfalfa was mowed in the budding stage with humidity - 60-65 %. At silo stacking pure culture of lactic acid bacteria Bacillus subtilis (strain 111) was used on the basis of 105 cells of viable bacteria per 1 g of silage mass. Assessment of the quality of silage was performed according to the requirements of OST 10202-97 [9].

Assessment of the quality of sperm was performed by the standard technique: freeze-thaw by Corban [10], the content of SH-groups - according to Torchinsky [11], dehydrogenase and cytochrome oxidase - by Semakova [12], and acrosome intactness - by Pyrsel and Johnson [13]. Determination of phosphorus-oxygen ratio and measurement of oxygen and inorganic phosphorus uptake were performed according to Moroz et al. [14]. The sperm for preserving was diluted in glucose-chelate-citrate-viteline-potassium-unitiol-urea (GCCVPUU) medium. Freezing of sperm was performed on fluoroplastic plate in vapors of liquid nitrogen at -85C° temperature on the plate. To assess the fertilizing capacity of boar semen in each group 12 adult sows were inseminated.

Table 1. Scheme of investigation

No. of group	Structure of diets	The dose of complexes "Biotorf-111" + husks of grapes + pumpkin per 1 ton of alfalfa
Control	mix of concentrates 88%, safflower oil cake - 4%, cotton seed meal - 3%, feed of animal origin (meat and bone meal, natural skim) - 5%.	-
First experimental	concentrates mix 75%, safflower oil cake - 4%, cotton seed meal - 3%, animal feed -3%, the combined silage 15%	50 g of probiotics + 30 kg of husks of grapes + 50 kg of pumpkin
Second experimental	mix of concentrates 70%, safflower oil cake - 4%, cotton seed meal - 3%, animal feed - 3%, the combined silage 20%	75 g probiotic + 60 kg husks of grapes + 50 kg pumpkin
Third experimetnal	mix of concentrates 65%, safflower oil cake - 4%, cotton seed meal - 3%, animal feed -3%, the combined silage 25%	100 g probiotic + 90 kg of husks of grapes + 50 kg pumpkin

The experimental results were processed by the methods of variation statistics according to Merkurieva [15].

3. Key part

Alfalfa has a very high buffering capacity of sap and low content of sugars, which are necessary for the development of lactic acid bacteria. Besides

its main spare polysaccharide is starch, which is insoluble in cold water and, therefore, not available for the majority of lactic acid bacteria as a substrate for fermentation in silage. This may reduce the effectiveness of the preparation in the preservation of alfalfa. However, adding sugar in its silaged green mass we may create necessary conditions for the development of lactic acid bacteria during ensilage. To determine the suitability of husks of grapes for mixed silage we have conducted some analysis. Chemical analysis of the composition of fresh husks of grapes showed the presence of dry matter - 28.4%, crude fat – 2.7%, crude protein – 3.2%, crude fiber - 10.8, carbohydrates - 3,2 , nitrogen-free extractives (NFE) - 11.3 and 2.8% of ash. Husks of grapes made up 28.2 % of the initial amount of the processed grapes, and slurry amounted to 16.1%, seeds - 10.4% and bunches - 1.7% of pomace mass. Calculations have shown that on average pomace contains: exchange energy - 1.67, feeding units - 0.06, digestible protein – 3.3 g.

Analyses of pumpkin showed that 1 kg contains: 0.16 feed. units, 153 g of dry substance, 0.11 g of digestible protein, 21 g of crude fiber and 4 g of sugar, 102 g of biological extractives, and 4.4 g of starch.

According to the analysis mixed silage includes freshly cut alfalfa, husks of grapes, pumpkin and pure culture of lactic acid bacteria Bacillus subtilis (strain 111). 1 kg of such silo contained 0.33 feeding units, 385 g of dry matter, 32 g of digestible protein, 18 g of fat, 50 mg of carotene and 51 g of crude fiber.

According to the sensory and biochemical evaluation the stacked silos were of good quality: had medium sour taste, smell of pickled vegetables, color of raw materials, and intact structure of the particles. The content of organic acids in mixed silos at storage for 6 months ranged within 2.43-2.57 %, of which the proportion of lactic acid was 77.3-79.1 %, the proportion of acetic acid was 22.7-20.9%, and the butyric acid was absent.

Inclusion of mixed silage with 15% nutrient value in the diets of breeding boars, with the introduction of 50 g of probiotic + 30 kg of husks of grapes + 50 kg of pumpkin (first test), contributed to the increase in the ejaculate volume by 12.6%, the total number of sperm in the ejaculate - by 13.2%, decrease in pathological forms - by 19.5%, increase in the safety of sulfhydryl groups (SH) by 12.1% and functioning of the energy system - respiration and phosphorylation in gametes - more than 2.3 times (Table 2). Such intactness of quality indicators has increased the fertilizing capacity of sperm by 8.3 %, compared with control animals.

Table 2. Influence of fed mixed silage in different options on the qualitative indicators of undiluted sperm of boars after 12 hours storage.

Indicators	Control	Alfalfa with different doses of the complex "Biotrof-111" + husks of grapes + pumpkin		
	(without silage)	1 (first)	2 (second)	3 (third)
Activity of sperm, points	5.4	5.9	6.4	6.1
Average volume of ejaculate, ml	283.6	319.3	342.9	321.7
Concentration of sperm, mln/ml	232.7	231.8	224.2	229.1
Number of sperm in ejaculate, bln	62.9	71.2	76.8	73.7
Specific electro-conductivity at 30^0C, 10^{-4} Ohm	127.9	127.4	127.4	127.4
Motion velocity, μm/s	26.2	28.6	29.1	29.0
Safety of SH-groups in gametes, %	50.7	62.8	64.3	62.6
Number of damaged chromosomes, %	42.1	33.9	29.8	37.6
Time of reductions of methylene-blue, min	14.5	11.7	7.9	8.8
The activity of common dehydrogenase, min	79.2	76.7	63.3	67.2
The activity of cytochrome oxidase, min	92.1	87.7	78.4	79.1
The amount of absorbed O_2 and phosphorus in mg-atoms per 10^9 cells	0.46	1.08	1.21	1.12
Fertilizing capacity, %	33.3	41.6	58.3	50.0

The highest quality indicators of boar ejaculates are observed in the second test group, where feeding includes 20% of mixed silage, with added 75 g of the probiotic, 50 kg of pumpkin and 60 kg of husks of grapes. The average volume of boars ejaculate was 319.3 ml: higher than in the control group by 20.9%; the number of sperm in the ejaculate was 76.8 billion or 22.1% respectively, the safety of SH groups - 13.6%, functioning of the energy system - respiration and phosphorylation in gametes - more than 2.6 times, the fertility of sows from the first insemination - by 25.0%. In boars of the third experimental group the quality indicators of ejaculates occupy an intermediate position.

An important qualitative indicator characterizing high quality evaluations of sperm is sperm mobility after thaw. As it is apparent from Table 3, the largest mobility of sperm after freezing-melting (4.3 points) is in the boars of the second group. This indicator is higher than in the control group by 26.8%, and by 10.2% and 4.8% compared with the first and second groups respectively.

Table 3. Influence of the fed mixed silage of different options on the quality of frozen-thawed sperm of boars.

Indicators	Control	First experimental	Second experimental	Third experimental
Mobility, points	3.4	3.9	4.3	4.1
Survival time, h	5.1	6.1	6.9	6.3
Motion velocity, μ/s	26.8	29.1	34.4	32.9
Safety of SH-groups in gametes, %	66.5	68.3	74.6	71.8
Number of damaged acrosomes, %	30.3	30.2	21.7	23.6
Time of methylene-blue reduction, min	14.9	11.9	8.6	9.9
Most common dehydrogenase, min	94.6	81.7	67.6	70.9
The activity of cytochrome oxidase, min	106.3	103.1	79.8	94.4
The amount of absorbed O_2 and phosphorus in μg-atoms per 10^9 cells	0.49	0.72	0.87	0.73
Fertilizing capacity, %	8.3	16.6	41.7	33.3

Inclusion of 20% mixed silage in the diets of boars, where 75 grams of probiotic + 50 kg of pumpkin and 60 kg of husks of grapes, contributes to the fact that sperm is less exposed to damage after freezing and thawing (21.7 %), and time of experiences of frozen-thawed sperm was the highest (6.9 h), and the safety of SH groups in the gametes as higher - 74.6 %. To determine the connection between the better safeties of the structure, the effect of the increase in the time of experience and oxidative phosphorylation, the study of fertilizing capacity of gametes was conducted. It was found that the fertility of sows from the first insemination was at the level of 41.7 % or higher than in control at 33.4%, and compared with the first and second experimental groups, by 25.1% and 8.4 %, respectively.

Thus, inclusion of 15-25% mixed silage in the diets of breeding boars with various added doses of probiotics, the amount of husks of grapes and pumpkin makes up the missing nutrients, improves metabolism and physiological condition of the body, thereby increasing their reproductive qualities. All this contributes to the improvement of sexual activity, improvement of sperm quality parameters of boars, stabilizes cell membrane structure (acrosome, protein SH groups), due to this the sperm is less exposed to damage by freezing and thawing, retain enzymatic activity, and as a consequence, the time of their experiences after thawing and affects relatively high fertilizing capacity.

4. Conclusion

The problem of legumes harvesting for their use in the autumn-winter period for feeding animals is studied in a number of papers. The results of experiments of P. Mc Donald [5], who proved that from the vegetative mass of chopped green alfalfa at appropriate conditions it is possible to produce high-quality silage with minimal loss of nutrients in relation to raw material. H. Honig, G. Pahlow [16], describing production of high quality silage from grass, believe that a good-quality silage is directly related to green mass preservation. H.M. Dodd and M.J. Gasson [17] M. Vescovo et al. [18] describe a number of strains of lactic acid bacteria with

antagonistic properties against in relation to various microorganisms, including phyto-pathogenic ones.

As preservatives of legumes many preparations were tested. According to the data of M.K. Woolford [19] and other researchers the common feature was that they represented either organic or mineral acids (or their derivatives) that rather rapidly acidified initial plant mass, preventing the creation of conditions for the development of undesirable microorganisms.

According to the works of R. Jones, M.K. Woolford [20], F. Driehuis et al. [21], food prepared with the use of biological preservatives is not affected by fungi, does not fester, is juicy and well consumed by animals; the composition of organic acids is improved, and dry matter losses are reduced. Among the biological preservatives the particularly special are lactic acid bacteria (LAB). Criteria for inclusion of lactic acid bacteria in silage additives were formulated by R. Whittenbury [22].

In the laboratory experiments, A.R. Henderson [23] showed that bacterial silage additives comprising such types of the mixture of lactic acid bacteria are capable of causing a rapid drop in pH – for 24 hours. To suppress the dominant cocci at early stage of silage C.M. Carpintero et al. [24] added Lactobacillus planterum. In the works of F. Gross [25] it was noted that any bacterial silage additive must contain a sufficient number of viable lactic acid bacteria to dominate over the local micro flora at adding at least 10^{-5} -10^{-6} bacteria per gram of cut grass.

According to Anon [26, 27] Cermak [28] in Western Europe, there have been successful tests of biological additives for silage - Silo-Bak, Biomax, Lacktosil and others. As a result, the western market has sufficient range of biological products to improve the silage conditions.

In the research, for the preservation of green mass of alfalfa we used probiotic" Biotrof-111", which, according to the recommendations of the manufacturer must be used for the mass of wilted green beans. In order to avoid wilting (drying) phase, extra husks of grapes was used to create a nutrient medium for the development of lactic acid bacteria in the preparation and to strengthen the preservative effect of probiotic.

These silos are characterized by the fact that during their maturation pure culture of probiotic due to the shortage of sugars in the initial mass of alfalfa can not suppress the development of clostridia, which at high pH medium can use alfalfa proteins. The proof was that in the control sample there was a high content of ammonia nitrogen, which level exceeded the samples preserved with formic acid and complex "Biotrof-111" + molasses 2.9 and 2.2 times respectively.

The intensity of the metabolic processes in the body of animals can be seen in the reproductive ability of boars characterizing the metabolism of energy and nutrients coming from the feed.

Inclusion in diets of boars of 15-25% mixed silo on nutritional value of, with the introduction of various doses of probiotics, husks of grapes and pumpkin makes up the missing nutrients, improves metabolism and physiological condition of the body, thereby increasing their reproductive qualities. Total volume of ejaculate increased from 12.6 to 20.9 %, while the convolution time of sperm - from 19.6 to 35.3%, respectively, fertility of sows from the first insemination of cryo-preserved sperm varied from 8.3 to 33.4 %.

In summary, we can conclude that the inclusion of 20% mixed silage of total nutrition value with introduced 75 g of probiotic, 60 kg of husks of grapes and 50 kg pumpkin at silageing of 1 ton of green mass of alfalfa can increase the reproductive capacity of boars, semen quality parameters and fertilizing capacity of sperm.

1. Conservation of grinded green mass of alfalfa, husks of grapes, pumpkin with lactic acid bacteria Bacillus subtilis (strain 111) allows obtaining mixed silage of good quality. The content of organic acids in mixed silo at 6 months storage ranged within 2.43-2.57 %, of which the share of lactic acid was 77.3-79.1%, the share of acetic acid was 22.7-20.9%, and butyric acid was virtually non-existent.

2. Inclusion of 15-25% mixed silage on nutrient value in the diets of boars, with the introduction of various doses of probiotics, husks of grapes and pumpkins, improves overall physiological condition of the body and increases the reproductive qualities.

3. The most positive effect of mixed silage is observed at inclusion in the diet of 20% mixed silage on the nutritional value with addition of 75 g of probiotic, 50 kg of pumpkin and 60 kg of husks of grapes. The volume of ejaculate increased by 20.9 % and the number of sperm cells - by 22.1 %.

4. Inclusion of mixed silage in boars' diets contributes to the sperm cryostability, improves cell viability after freezing and thawing, and preserves rather high fertilizing capacity of gametes.

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