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THE EFFECT OF HARVEST DATE AND BACTERIAL-ENZYMATIC ADDITIVES ON CHEMICAL COMPOSITION AND AEROBIC STABILITY OF SORGHUM SILAGE

J. B. Pyś[1], F. Borowiec[1], A. Karpowicz[1], V. Vlizlo[2]

[1]Department of Animal Nutrition, Agricultural University of Kracow
[2]Institute of Animal Biology, Ukrainian Academy of Agrarian Science

The experiment was carried out to determine the effect of harvest date and addition of bacterial-enzymatic inoculants on chemical composition and aerobic stability of sweet sorghum silages. Harvest date did not effect (P < 0,05) on dry matter content, fermentation quality and aerobic stability of the silages obtained. The addition of preparations used, regardless of harvest date of sorghum forage, increased fermentation quality and resistance to aerobic deterioration of sorghum silages. The highest (P < 0,05) level of crude protein was found in SI-D and SI-P silages, while the highest starch and water soluble carbohydrates content was observed in SII, SII-D and SII-P silages. The greatest resistance to aerobic deterioration was characteristic of silages made with a mixture of Lactobacillus buchneri bacteria, Pediococcus acidilactici bacteria with cellulose and hemicellulase.

Key words: SORGHUM SILAGES, HARVEST DATE, ADDITIVES, CHEMICAL COMPOSITION, AEROBIC STABILITY

In Poland in recent years, the cultivation of maize for both silage and grain has encountered several problems. The main of these include extremely adverse weather conditions such as drought and lack of precipitation, which are increasingly common all over Poland. The water shortage in Poland prevents the artificial irrigation of maize crops. Another problem are maize diseases, which are gradually increasing all over Poland. The most common diseases are fusariosis (Fusarium sp.), eyespot (Aureobasidium zeae), left spot (Trichometasphaeria tarcica Luttr.), common smut (Ustilago maydis), smut head (Sphacelotheca reiliana) and crazy top downy mildew (Sclerophthora makrospora) [Kita and Puszcz, 2007; Korbas, 2007]. Maize crops are also threatened by the European corn borer (Ostrinia nubilalis Hbn.). A new problem for maize growers is the corn root worm (Diabrotica virgifera LeConte), which has been subject to compulsory eradication in Poland since 2005. The increasing problems in maize cultivation prompted a search for alternative plants, suitable for cultivation in Poland's climate and soil conditions.

Sweet sorghum (*Sorghum saccharatum* L.) is an alternative plant that has been grown in Poland for several years. In the Polish climatic conditions, sweet sorghum blooms in early autumn and cannot produce a panicle with ripe grains. For this reason, it is grown for fodder, which can be an alternative or supplementary ensilage material to maize. Sorghum cut for silage at the heading stage and at the early blooming stage (20 September–20 October) is characterized by a higher content of water soluble carbohydrates (WSC), crude fiber, neutral detergent fiber (NDF) and acid detergent fiber (ADF) compared to maize forage. Sorghum is a plant that is very easy to ensile thanks to the high WSC content in relation to the crude protein content and the low buffering capacity. A disadvantage of sorghum is the low dry matter content of less than 250 $g \cdot kg^{-1}$ during harvest [Pyś and Borowiec, 2007]. When ensiling sorghum forage that is so low in dry matter and high in WSC, the fermentation process is very intensive and the silages contain high amounts of lactic acid. Silages high in lactic acid are particularly susceptible to aerobic deterioration and secondary fermentation after exposure of the silage heap [Ohmomo et al., 2002]. The resistance of sorghum silage to aerobic deterioration is an important issue, especially when it is the only bulky feed in the ration for heifers or dry cows.

Fermentation quality and aerobic stability of sorghum silages can be improved by ensiling forage with addition of lactic acid bacteria and cellulolytic enzymes [Rodriguez et al., 1997; Schmidt et al., 1997].

The aim of the present study was to determine the effects of the harvest date of sweet sorghum forage and the addition of bacterial-enzymatic inoculants containing lactic bacteria and cell wall polysaccharide-degrading enzymes on the fermentation quality, chemical composition and aerobic stability of the silages obtained.

Materials and methods

Sorghum forage [*Sorghum saccharatum* cv. Sucrosorgo 506] cut on three dates (12 Sep, 28 Sep and 13 Oct 2006) was used as ensiling material. Sorghum forage was mowed using self-propelled forage harvester and cut into 15–20 mm particles. The chemical composition of sorghum forage is given in Table 1.

Sorghum forage from every harvest date was ensiled without additives — SI, SII and SIII and with the addition of bacterial-enzymatic inoculants: Lalsil Dry (*Lactobacillus buchneri* — $1{,}5\times10^5$ cfu·g^{-1}, *Pediococcus acidilactici* — $1{,}5\times10^5$ cfu·g^{-1} of forage; cellulose and hemicellulase — 0,1 IU·g^{-1} of forage) — SI-D, SII-D and SIII-D or Lalsil PS (*Lactobacillus plantarum* — $1{,}75\times10^6$ cfu·g^{-1}, *Pediococcus acidilactici* — $7{,}5\times10^5$ cfu·g^{-1} of forage; cellulose and hemicellulase — 0,1 IU·g^{-1} of forage) — SI-P, SII-P and SIII-P. Sorghum was ensiled in 15-l polyethylene microsilos. A total of 14,0–14,4 kg sorghum forage was compacted in microsilos. Ensiled biomass were stored for 60 days in an enclosed room at 15 ± 2° C.

Sorghum forage and silage samples were analysed for dry matter content using the drying method at 105° C for 12 h. Buffering capacity of sorghum forage was determined according to Playne and McDonald [1966]. Silage samples were analysed for dry matter content using the drying method. In silages determined pH level using a pH/Ion Analyser MA 235 [Mettler Toledo, Switzerland]. The level of lactic, acetic, and butyric acids in the silages was determined using liquid chromatography. The analysis was performed using an LC 5000 liquid chromatograph with a UV/VIS detector [INGOS, Czech Republic] and an Ostion LG KS 0800 H^+ column [Tessek, Czech Republic]. Column operating temperature was 50° C, with mobile phase of 0.005 M H_2SO_4. Ethanol content of the silages was determined using gas chromatography. The analysis was performed using a Varian Star 3400 CX gas chromatograph [Varian, USA] with an FID detector and a DB-FFAP capillary column (30 m long, 0,53 mm in diameter), using argon as the carrier gas. Operating temperature was 90–205° C for the column, 200° C for the sample injector, and 240° C for the detector. The NH_3-N content of the silages was determined using the Conway method described by Skulmowski [1974].

Sorghum forage and silages samples intended for further chemical analyses were dried at 50° C for 48 h and ground [Fritsch Pulversette 15 mill, Germany] into 1,0 mm sized particles. These samples were analysed for the level of crude ash [AOAC, 2007], crude fat [AOAC, 2007] using an Ankom XT15 extractor [Ankom, USA], and crude fibre [AOAC, 2007] using an Ankom^{220} Fibre Analyser [Ankom, USA]. The crude protein (% total-N × 6,25) content was determined according to Kjeldahl [AOAC, 2007] using a Kjeltec 2200 unit [Foss, Denmark], NDF, ADF and ADL [Goering and Van Soest, 1970] using an Ankom^{220} Fibre Analyser [Ankom, USA], starch [Faisant et al., 1995] and WSC using the colourimetric method [Dubois et al., 1956].

The aerobic stability of sorghum silages was tested for 7 days in an air-conditioned room in the ambient temperature of 20 ± 1° C, according to the method of Honig [1985]. Aerobic stability was measured by the number of hours during which the temperature of the silages subjected to aerobic exposure did not exceed the ambient temperature in the air-conditioned room by 2° C [Honig, 1985].

The results of experiment were analysed statistically using two-way analysis of variance and Tukey's test [SAS, ver. 912, 2001–2003].

Results and discussion

The nutrient content and buffering capacity of sorghum forage are shown in Table 1. The NDF, WSC and starch content of sorghum forage increased with progressing vegetation. The crude protein and crude fat content and the buffering capacity of sorghum forage decreased as the date of harvest was delayed. The date of sorghum forage harvest had no effect on the ADF content.

Table 1

Chemical composition and buffering capacity of sorghum forage

Item	Harvest date		
	12 Sep 2006	28 Sep 2006	13 Oct 2006
Dry matter ($g \cdot kg^{-1}$)	205,2	231,5	234,5
	($g \cdot kg^{-1}$ DM)		
Crude ash	74,6	61,4	61,8
Crude protein	97,5	81,2	77,2
Crude fat	24,4	25,1	20,9
Crude fiber	283,6	298,5	285,9
NDF [1]	510,2	598,0	619,2
ADF [2]	351,9	351,4	359,0
ADL [3]	32,9	38,8	44,6
Starch	45,7	48,7	80,8
WSC [4]	142,8	159,9	198,3
Buffering capacity (meq·$100g^{-1}$ DM)	31,7	24,7	22,2

[1] NDF — neutral detergent fiber, [2] ADF — acid detergent fiber,
[3] ADL — acid detergent lignin, [4] WSC — water soluble carbohydrates

Table 2 presents the chemical composition of sorghum silages. Harvest date of sorghum forage did not effect ($P < 0,05$) on dry matter content.

Table 2

The nutrients content in sorghum silages

Type of silage	Dry matter	Crude protein	Crude fiber	NDF [1]	ADF [2]	Starch	WSC [3]
	($g \cdot kg^{-1}$)	*($g \cdot kg^{-1}$ DM)*					
SI	199,0 ab	87,3 ab	302,3 a	510,6 b	354,1	43,3 b	40,8 d
SII	201,3 ab	73,6 bc	306,9 a	599,7 ab	361,8	42,7 b	55,1 c
SIII	210,8 ab	67,4 c	300,5 a	620,1 a	365.7	76,3 a	83,4 ab
SI-D	203,2 ab	93,8 a	278,0 b	505,2 b	349,4	44,0 b	58,5 c
SII-D	212,2 a	80,5 ab	270,1 b	576,3 ab	341.9	43,3 b	65,8 bc
SIII-D	211,9 a	75,6 bc	271,0 b	588,4 a	353,9	76,3 a	91,9 a
SI-P	188,6 b	90,5 a	279,1 b	500,3 b	357,5	43,3 b	42,9 cd
SII-P	192,1 ab	77,7 bc	273,0 b	573,6 ab	350,0	41,4 b	57,9 c
SIII-P	193,6 ab	71,3 c	275,1 b	593,3 a	358,2	76,3 a	88,9 a
Mean (n=36)	210,3	79,7	284,0	564,5	354,7	54,1	65,0
SD	25,61	8,52	13,96	43,2	6,72	15,72	17,95
Effect:							
date of harvest	NI	*	NI	*	NI	*	*
inoculant	NI	*	*	NI	NI	NI	*
date × inoculant	NI	*	NI	NI	NI	NI	*

[1, 2, 3] — as in Table 1; SD — standard deviation; a, b, c, d — (in columns) — $P < 0,05$; * — $P < 0,05$;
NI $P > 0,05$

The crude protein content of the sorghum silages was dependent on the date of harvest and the inoculant used. All the silages, with or without the inoculants, made from sorghum cut on the first date had a higher ($P < 0,05$) crude protein content compared to the crude protein content of silages made from sorghum cut on the third date. In addition, the highest amount of crude protein

was found in silages supplemented with L. buchneri and P. acidilactici bacteria as well as cellulose and hemicellulase.

The WSC content of silages increased ($P < 0,05$) with each successive harvest and was the highest ($P < 0,05$) for the variants made using the inoculant containing L. buchneri and P. acidilactici bacteria as well as cellulase and hemicellulase. The bacterial-enzymatic inoculants used significantly ($P < 0,05$) reduced the crude fiber content of the sorghum silages. NDF was the highest in silages from sorghum cut on the third date and differed significantly ($P < 0,05$) only in relation to silages made from sorghum cut on the first date. Compared to untreated silages, lower NDF content was characteristic of silages with bacterial-enzymatic inoculants, but the differences were not significant ($P > 0,05$). In the present study, no significant ($P > 0,05$) effect of sorghum harvest date or the inoculants used on the ADF content of the silages was found.

The highest amount of starch was found in silages from sorghum cut on the third date and differed significantly ($P < 0.05$) from the starch content of silages made from sorghum cut on the first and second date.

The fermentation parameters and the aerobic stability of sorghum silages are shown in Table 3. Harvest date had no effect on pH level or NH_3-N and ethanol content of the silages. Ensiling sorghum with the bacterial-enzymatic inoculants significantly ($P < 0,05$) reduced the above parameters of fermentation quality.

Table 3

Fermentation parameters and aerobic stability of sorghum silages

Type of silage	pH	NH_3-N	Ethanol	Lactic acid	Acetic acid	Butyric acid	Aerobic stability
		(% of total-N)	($g \cdot kg^{-1}$DM)				(h)
SI	3,89 b	52,4 a	17,0 a	91,6 b	35,1 c	0,0	49 c
SII	3,92 b	58,9 a	16,4 a	85,7 b	36,0 c	0,0	45 c
SIII	4,08 a	50,9 a	20,9 a	84,7 b	37,6 c	0,0	50 c
SI-D	3,73 c	34,3 b	9,4 b	80,4 b	75,4 a	0,0	96 a
SII-D	3,74 c	37,1 b	11,1 b	86,3 b	71,5 ab	0,0	103 a
SIII-D	3,72 c	35,6 b	10,5 b	88,9 b	69,8 ab	0,0	91 a
SI-P	3,69 c	37,3 b	18,9 a	116,9 a	57,4 b	0,0	68 b
SII-P	3,70 c	38,8 b	20,4 a	113,3 a	58,9 b	0,0	69 b
SIII-P	3,72 c	40,0 b	21,2 a	118,0 a	60,1 a	0,0	72 b
Mean (n=36)	3,80	42,8	16,2	96,2	55,8	0,0	71,4
SD	0,13	8,35	4,44	14,37	14,94	--	20,17
Effect:							
harvest date	NI	*	NI	NI	NI	--	NI
inoculant	*	*	*	*	*	--	*
date × inoculant	NI	*	NI	NI	NI	--	NI

SD — standard deviation; a, b, c — in columns — $P < 0,05$; * — $P < 0,05$; NI $P > 0,05$

Sorghum forage harvest date ($P > 0,05$) had no significant effect on the lactic acid content of the silages. Ensiling sorghum forage with L. buchneri and P. acidilactici bacteria as well as cellulase and hemicellulase had no significant ($P > 0,05$) effect on the lactic acid content of the silages. Lactic acid was found to increase in the sorghum silages supplemented with L. plantarum, P. acidilactici bacteria, cellulase and hemicellulase.

The level of acetic acid was the highest in silages with L. buchneri, P. acidilactici bacteria cellulase and hemicellulase. Lower ($P > 0,05$) rate of acetic fermentation was characteristic of silages with L. plantarum, P. acidilactici bacteria and cellulolytic enzymes.

Silages with L. buchneri, P. acidilactici bacteria, cellulase and hemicellulase were the most resistant to aerobic deterioration during aerobic exposure. In these silages, the period of aerobic

stability was on average twice or three times that of silages with L. plantarum, P. acidilactici bacteria, cellulase and hemicellulase, as well as untreated silages.

In the present experiment, the dry matter content of sweet sorghum silages was not determined by harvest date. A different tendency was reported by Karsli et al. [2002]. In the present study, the lowest dry matter content was characteristic of sorghum silages with L. plantarum, P. acidilactici bacteria, cellulase and hemicellulase. It was due to considerable nutrient degradation, which is necessary for producing the maximum amount of organic acids in these silages.

The crude protein content of the sorghum silages decreased with each harvest date. A consistent reduction in the crude protein content of sorghum with each maturity stage of plants was also reported by Karsli et al. [2002].

In the present study, the crude protein content of the sorghum silages was also dependent on bacterial-enzymatic inoculants used. The lowest protein degradation during the fermentation process was characteristic of silages with L. buchneri bacteria. The metabolic products of these bacteria limit or eliminate the activity of undesirable microorganisms that degrade protein during the fermentation process [Holzer et al., 2003].

The WSC content of sorghum forage increased at each harvest date. The same relation was true for the silages made. A higher WSC content was characteristic of the silages made with the inoculants, which was due to the activity of cellulolytic enzymes that degrade structural cell wall polysaccharides of plants into carbohydrates that are easily soluble in water. Schmidt et al. [1997] reported that ensiling sorghum forage with the addition of cellulolytic enzymes alone increased the amount of WSC in the silages obtained.

In the present experiment, WSC were the highest in sorghum silages with L. plantarum, P. acidilactici bacteria, cellulase and hemicellulase. These silages had the lowest concentration of lactic acid. Therefore, it is suggested that the lactic bacteria population found in the ensiled biomass did not have to degrade large amounts of nutrient substrate in the form of easily soluble carbohydrates to produce enough lactic acid for optimum acidification of the environment. The increased amount of WSC in the sorghum silages made with L. plantarum, Streptococcus faecium bacteria and cellulolytic enzymes was also reported by Schmidt et al. [1997].

The crude fiber, NDF and ADF content of the sorghum silages resulted from the amount of these components in sorghum forage prior to ensiling and from the additives used. The content of these components in sorghum forage increased at each harvest date, as reflected in the silages made. The same relationship was reported by Karsli et al. [2002].

The sweet sorghum silages with bacterial-enzymatic inoculants were characterized by a considerably lower concentration of crude fiber and NDF compared to untreated silages. The lower amount of these components was due to the activity of cellulolytic enzymes found in the preparations and degraded cellulase and hemicellulase. The results obtained were consistent with the findings of Schmidt et al. [1997], who reported NDF and ADF fractions to decrease in sorghum silages made with lactic bacteria and cellulolytic enzymes.

In the present study, ensiling sorghum forage with bacterial-enzymatic inoculants caused a significant decrease ($P < 0,05$) of the pH in the silages obtained. This was due to the increased amount of acetic acid in silages with L. buchneri and P. acidilactici bacteria and to the increased amount of lactic acid in silages with L. plantarum and P. acidilactici bacteria. Different results were obtained in the studies of Rodriguez et al. [1997] and Schmidt et al. [1997], in which the addition of enzymes alone or together with homofermentative lactic bacteria to the ensiled sorghum did not significantly affect the pH of the silages obtained.

Ensiling sorghum forage with the bacterial-enzymatic inoculants significantly limited the degradation ($P < 0,05$) of protein to ammonia. Intensive lactic and acetic fermentation during the ensilage of sorghum with these additives caused a rapid and strong acidification of the environment, which restricted the activity of bacteria degrading protein to ammonia. The lowest amount of ammonia was found in silages with L. buchneri and P. acidilactici bacteria. During fermentation,

L. buchneri produce large amounts of acetic acid, which inhibits the growth of bacteria that break down protein to ammonia [Oude Elferink et al., 2001; Holzer et al., 2003]. This action of the lactic bacteria strain was confirmed by the present study. The favourable effect of the inoculant containing L. plantarum, Streptococcus faecium and cellulolytic enzymes on limiting the amount of ammonia in sorghum silages was not found by Schmidt et al. [1997].

Silage materials with a low dry matter and high WSC content are particularly susceptible to alcohol fermentation caused by yeast activity [McDonald et al., 1991]. In the present study, ensiling sweet sorghum forage with L. buchneri, P. acidilactici bacteria, cellulase and hemicellulase significantly reduced this undesirable type of fermentation. The high content of organic acids [especially acetic acid produced by the L. buchneri strain] in these silages has limited yeast activity through strong acidification of the environment. No inhibition of alcohol fermentation was obtained by Rodriguez et al. [1997] who ensiled sorghum with cellulolytic enzymes alone or by Schmidt et al. [1997] who ensiled sorghum with L. plantarum and Streptococcus faecium bacteria and cellulolytic enzymes. However, the silages made in these two studies contained much lower amounts of organic acids.

In the present study, ensiling sorghum forage with L. buchneri, P. acidilactici bacteria, cellulase and hemicellulase did not increase the rate of lactic fermentation, but increased acetic fermentation, resulting in the highest concentration of acetic acid in the silages. With easy access to nutrient substrate, L. buchneri bacteria produce large amounts of acetic acid [Oude Elferink et al., 2001; Holzer et al., 2003], as confirmed by the present findings.

The inoculants containing homofermentative lactic bacteria are used to increase the rate of lactic fermentation in the ensiled material [Filya, 2003]. This action of these bacteria was confirmed in the present experiment. Sweet sorghum silages with L. plantarum and P. acidilactici bacteria and cellulolytic enzymes contained the highest amounts of lactic acid.

Rodriguez et al. [1997], who ensiled sorghum with cellulolytic enzymes alone did not find their significant effect on the concentration of lactic and acetic acids in the silages. In a study by Schmidt et al. [1997], the addition of the same enzymes alone or together with homofermentative lactic bacteria to the ensiled sorghum caused a slight [statistically non-significant] increase in the concentration of lactic acid in the silages. In the same study [Schmidt et al., 1997], sorghum ensiled with lactic bacteria and enzymes reduced the amount of acetic acid in the silages.

In the present experiment, the concentration of organic acids in the sorghum silages had an effect on the resistance of the silages to aerobic deterioration after exposure of the silage heap. Silages with L. buchneri and P. acidilactici bacteria and cellulolytic enzymes were the most resistant to secondary fermentation as a result of air activity. During the fermentation process, L. buchneri produce several metabolites, which reduce the population of yeasts and moulds responsible for aerobic deterioration, thus increasing the aerobic stability of the silages [Oude Elfering et al., 2001; Holzer et al., 2003]. This activity of the bacterial strain was confirmed by the present findings.

In the present experiment, sweet sorghum silages without additives were the least resistant to aerobic deterioration. These silages were characterized by an unfavorable profile of organic acids, resulting from the lowest amount of acetic acid in relation to lactic acid, which did have an effect on their aerobic stability.

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THE EFFECT OF HARVEST DATE AND BACTERIAL-ENZYMATIC ADDITIVES ON CHEMICAL COMPOSITION AND AEROBIC STABILITY OF SORGHUM SILAGE

S u m m a r y

Sorghum forage harvest date determined the nutrient content of the silages made, but had no effect on the quality of the fermentation process occurring in the ensiled biomass. The addition of *L. buchneri* and *P. acidilactici* bacteria as well as the celullase and hemicellulase to the ensiled sorghum forage made it possible to obtain silages with the best parameters of fermentation and the greatest resistance to aerobic deterioration.

Й. Б. Пис, Ф. Боровєц, В. Влізло

ВПЛИВ ДАТИ ЗБОРУ УРОЖАЮ І БАКТЕРІАЛЬНО-ФЕРМЕНТНИХ ДОБАВОК НА ХІМІЧНІЙ СКЛАД ТА АЕРОБНУ СТАБІЛЬНІСТЬ СИЛОСУ СОРГО

Р е з ю м е

Метою проведення дослідів було визначення результату впливу дати збору урожаю і бактеріально-ферментних добавок на хімічній склад та аеробну стабільність силосу сорго. Дата збору урожаю не впливала ($P < 0.05$) на хімічний склад та аеробну стабільність свіжого силосу сорго. Додавання препаратів, що використовуються, незалежно від дати збору урожаю, підвищило якість ферментації та аеробну та опірність аеробному псуванню силосів сорго. Найвищий ($P < 0.05$) рівень загального білка виявлено в силосах SI-D і SI-P, в той час як найвищий вміст вуглецїв крохмалю і розчинних у воді карбонатгідратів спостерігався в силосах SII, SII-D і SII-P. Найвища опірність до аеробного псування була характерна для силосів, виготовлених з додаванням суміші бактерій Lactobacillus buchneri, Pediococcus acidilactici з целюлозою і геміцелюлози.

И. Б. Пыс, Ф. Боровец, В. Влизло

ВЛИЯНИЕ ДАТЫ СБОРА УРОЖАЯ И БАКТЕРИАЛЬНО-ФЕРМЕНТНЫХ ДОБАВОК НА ХИМИЧЕСКОЙ СОСТАВ ТА АЭРОБНУЮ СТАБИЛЬНОСТЬ СИЛОСА СОРГО

А н н о т а ц и я

Целью проведения опытов было определение результата влияния даты сбора урожая и бактериально-ферментных добавок на химический состав та аэробную стабильность силоса сорго. Дата сбора урожая не влияла ($P < 0.05$) на химический состав та аэробную стабильность свежего силоса сорго. Добавление препаратов, что используются, независимо от даты сбора урожая, повысило качество ферментации и аэробную та сопротивляемость аеробному порче силосов сорго. Наивысший ($P < 0.05$) уровень общего белка выявлен в силосах SI-D и SI-P, в то время как наивысшее содержание углеродов крахмала и растворимых в воде карбонатгидратов наблюдался в силосах SII, SII-D и SII-P. Наивысшая сопротивляемость к аэробногй порчи был характерный для силосов, изготовленных с добавлением смеси бактерий Lactobacillus buchneri, Pediococcus acidilactici с целлюлозой и гемицелюлозой.

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Рецензент: завідувач лабораторії фізіології та патології відтворення тварин, кандидат біологічних наук Шаран М. М.