The Effects of Different Silage Additives on *in vitro* Gas Production, Digestibility and Energy Values of Sugar Beet Pulp Silage

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INTRODUCTION

Sugar beet pulp is a valuable by-product obtained from the sugar industry. The fiber in sugar beet pulp is highly digestible and has an energy content close to that of starch. For a longer storage it is recommended to preserve this feed in the form of silage.

Both fresh and ensiled sugar beet pulp has a high feeding value and shows a positive dietetic effect on ruminal fermentation.

The aim of this study

The aim of this study was to determine the effects of different silage additives on the silage quality, *in vitro* gas production, gas production kinetics, energy values, organic matter digestibility and dry matter digestibility of sugar beet pulp silage.

MATERIALS & METHODS

In this study, sugar beet pulp samples were obtained from Samsun sugar factory in Turkiye (dry matter contents 13.98% - 14.12%).

Fresh pulp samples were wilted for 24 h in the laboratory then the samples were ensiled in experimental jar silos in quadriplicate repetitions.

Eight different silages were prepared from the sugar beet pulp samples.

Silage Additives

1) Silage treatments included control (no additives) (**CONT**).

2) The AIV solution (Artturi Imarın Virtanen) concludes **1 part H_2SO_4, 1 part HCl and 6 parts water** which it was applied at a 80g, per kg of the fresh weight of the sugar beet pulp (**AIV**)

3) Urea was applied 1% of the fresh weight of the sugar beet pulp (**UREA**).

4) Formic acid was applied at 2.2–2.5 lt/ton of the fresh weight of the sugar beet pulp (**FAS**).

5) 10 g/t **microbial inoculant** (**Maize All** was applied obtained from Alltech-Pioneer). As recommend by the manufacturer, inoculant was added at $1.0x10^{11}$ cfu/g of fresh forage **(MAL).**

6) 10 g/t **microbial inoculant (Sil All** was applied obtained from Alltech-Pioneer). As recommend by the manufacturer, inoculant was added at 1.0x1011 cfu/g of fresh forage **(SAL).**

7) 0.5 kg/ton **F silofarm formiat dry** was applied (**sodium formiat**) (**SFD**)

8) 5–7 kg/ton **F silofarm liquid** was applied (**formic asid, sodium format and water**) (**SLI**) as recommend by the manufacturer (from Farmavet), was added at 1.0x1011 cfu/g of fresh forage.

Chemical and Quality Analyses

After two months storage, the silos were opened. Representative samples were dried at 48 ˚C in a forced-air oven for 72 hours. After drying, silage samples were ground through a 1-mm screen for chemical analyses.

Crude Protein (CP) was calculated as N x 6.25
Crude Fibre (CF) and
Ether Extract (EE) were determined by the methods described by AOAC (1998).

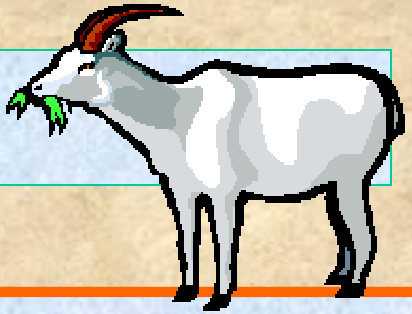
Quality analyses (Kilic, 1986), were made in silages, and NFE was determined by calculation. All chemical analyses were carried out in triplicate.

Total volatile fatty acids and NH_3-N contents in rumen fluid were determined using Markham Steam Distillation procedure (1942).

Total points (DLG) were determined and **Fleig points** were calculated in silages according to Kilic (1986) (Fleig Point = 220+(2 x % Dry Matter - 15) – 40 x pH).

The pH of each sample was determined in triplicate using approximately 25 g wet ensilage added to 100 ml of distiled water. After hydration for 10 min using blender, **pH was determined using digital pH meter** (HANNA INSTRUMENTS 1332 model).

Cellulase Method



In the **Cellulase method**, **cellulase**, **hemicellulase**, **α- amylase** and **pepsin enzyme** were used.

In vitro digestibility of DM and OM were determined according to Alcicek and Wagener (1995) as follows;

DMD, %=(S1 (T1-T0)/ S1) x 100
OMD, % = 1-((T1-T2)/ (S1-A1)) x100
Where; S1: sample amount (as DM), T0: weight of crucible (105°C, 48 hours), T1: dry sample (105°C, 24 hours) + T0, T2: ashed sample (550°C, 4 hours) + T0, A1: crude ash amount of sample, g

Total energy (TE), digestible energy (DE), metabolisable energy (ME), net energy lactation (NE_L), net energy fat (NE_F) and net energy maintanence (NE_M) value were calculated according to the enzyme method (Jarrige, 1989 and Malossini et.al. 1993). Calculated values were converted to MJ/kg DM.

TE(kcal/kgDM)= 5.99CP+6.71 EE+4.28CF+4.73NFE
DE(kcal/kgDM)= (TEXOMD) /100
ME(kcal/kgDM)=[(86.82-0.0099CF-0.0196CP)DE]/ 100
q= ME/TE
NE_L (kcal/kgDM) =kxME, (k=0.60+0.24 (q-0.57))
NE_F (kcal/kgDM)=kxME, (k=0.78q+0.006)
NE_M (kcal/kgDM)= kxME, (k=0.287q+0.554)

in vitro gas production technique

Three SakızxKarayaka rams aged 2 with ruminal cannulas were used in this technique. The sheep fed twice daily (08.30-16.30) with a diet containing **grass hay (60%)** and **concentrate (40%).**

The samples (milled through a 1-mm sieve) were incubated in vitro rumen fluid in calibrated glass syringes following the procedures of Menke and Steingass, (1988).

Rumen fluid was collected before the morning feeding. Triplicates of each sample were used in two separate runs. Readings of gas production recorded before incubation and 3, 6, 9, 12, 24, 48, 72 and 96 h after incubation.

Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) by NEWAY computer package programme.

$$y = a+b(1-e^{-ct})$$

Estimated Organic Matter Digestibility (OMD) and Energy Values

Organic matter digestibility (Menke et al., 1979), ME (Menke et al., 1979) and NE_L (Menke and Steingass,1988) contents of silages were estimated by using these equations:

OMD, % = 14.88+ 0.8893 GP + 0.448 CP + 0.651 A

Where; GP: 24 h net gas production (ml/200mg DM), CP: Crude protein (%), A: Ash content (%)

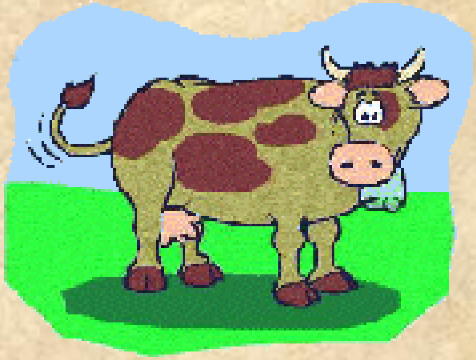
ME, (MJ/kg DM) = 2.20+0.136 GP + 0.057 CP+0.002859 EE^2

NE_L, (MJ/kg DM) = 0.101 GP + 0.051 CP + 0.11 EE

Where; GP: 24 h net gas production (ml/200mg DM), CP: Crude protein (%), EE: ether extract (%), A: Ash content (%)

Statistical Analysis

In this study, one-way analysis of variance (ANOVA) was carried out to compare in vitro gas production, gas production kinetics, energy values, DMD and OMD values using General Linear Model (GLM) of SPSS 10.0 package programs. Significances among individual means were identified using the Duncan's multiple range test.



RESULTS & DISCUSSION

Chemical composition content and silage qualities of SBPS were given in Table 1.

Table 1: The effects of different silage additives on chemical composition of SBPS (g/kg DM).

Treatments	DM	EE	Ash	CP	CF	NFE
CONT	129,1	10,2b	67,1c	115,8bcd	253,4bc	553,5b
AIV	125,4	30,6a	167,8a	99,4de	259,8bc	442,5d
FAS	140,1	12,5b	62,1c	88,8e	223,9d	612,7a
MAL	115,8	10,3b	57,8c	121,7bc	263,9b	546,3b
SAL	119,2	12,7b	54,8c	118,5bc	249,9c	564,0b
SFD	109,5	11,2b	71,6c	127,4b	279,6a	510,2c
SLI	139,0	12,7b	77,5c	105,5cde	229,4d	574,7b
UREA	129,2	11,7b	107,0b	212,3a	249,4c	419,7d
SEM		0,116	0,565	0,578	0,283	0,969
Significant		**	**	**	**	**

The silage additives significantly affected the nutrient contents (P<0.01). The addition of AIV resulted in the highest EE and ash values in silages (P<0.01). The highest content of CP was found with UREA, whereas that of NFE was determined with FAS (P<0.01).

In spite of the reduction in NFE content, UREA treated SBPS as roughage source would like to be prefered in ruminant nutrition as compared to other additive treated silages.

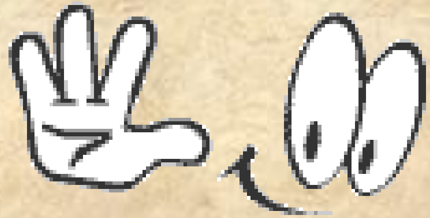
It is known that the quality of silage can change depending upon the type of additive (Kilic, 1986; Filya, 2001).The differences in nutrient composition of SBPS between the results of the present study and other studies can be attributed to the nature of the additives used.

Table 2. The effects of different silage additives on silage pH and silage qualities

Treatments	PH	smell	structure	colour		DLG point quality		Flieg point quality
CONT	3,44	9,00	3,00	1,50	13,50	moderate	93,36	excellent
AIV	**1,45**	8,00	3,00	1,50	**12,50**	moderate	172,09	excellent
FAS	3,08	9,00	3,00	1,50	13,50	moderate	109,66	excellent
MAL	3,35	10,00	3,00	1,50	**14,50**	**good**	94,29	excellent
SAL	3,38	11,00	3,00	1,50	**15,50**	**good**	93,51	excellent
SFD	3,55	9,00	3,00	1,50	13,50	moderate	84,90	excellent
SLI	3,32	10,00	3,00	1,50	**14,50**	**good**	100,15	excellent
UREA	3,17	9,00	3,00	1,50	13,50	moderate	103,84	excellent

It can be seen that the lowest pH value was found for AIV treatment. But there are not differences among other treatments. Based on Flieg point evaluation, all the silages were classified as excellent quality silages. The quality of MAL, SAL and SLI silages was better than the others when total point (DLG) was taken into account.

Silage fermentation is thought to be benefited from biological inoculants containing lactic acid bacteria and SLI.



The lowest DLG value (total point) was observed in AIV treated silage with **its smell worse than other silages**. It is probable that this silage would be less preferred by the animals due to **its smell, low NFE and high ash contents**.

The increase in ash content of AIV treated silages is thought to due to HCl and H_2SO_4. In addition, the intake of AIV treated silages cause physiological problems due to their very low pH (1.45). Therefore these silages should be offerred together with other roughages, which would increase the pH.

Table 3. The effects of different silage additives on energy values (kcal/kg DM and MJ/kg DM), DMD (%) and OMD (%) of sugar beet pulp silage by using cellulase method

	TE	DE	ME	NE_L.	NE_F	NE_M	DMD	OMD
CONT	4464,98[d]	1670,96[bc]	1370,88[bc]	736,34[bc]	337,61[c]	880,66[bc]	44,02[a]	37,42[ab]
	(18,69)	(7,00)	(5,74)	(3,08)	(1,41)	(3,69)		
AIV	3996,34[h]	1123,32[d]	924,55[d]	481,52[d]	178,68[d]	575,90[d]	36,63[ab]	28,11[c]
	(16,73)	(4,70)	(3,87)	(2,02)	(0,75)	(2,41)		
FAS	4471,90[c]	1696,11[bc]	1405,46[abc]	757,14[bc]	353,35[bc]	905,54[bc]	36,61[ab]	37,93[ab]
	(18,72)	(7,10)	(5,88)	(3,17)	(1,48)	(3,79)		
MAL	4511,77[b]	1964,45[a]	1607,37[a]	882,05[a]	456,56[a]	1054,93[a]	32,14[b]	43,54[a]
	(18,89)	(8,22)	(6,73)	(3,69)	(1,91)	(4,42)		
SAL	4532,70[a]	1910,59[ab]	1567,11[ab]	855,96[ab]	432,16[ab]	1023,73[ab]	38,62[ab]	42,15[ab]
	(18,98)	(8,00)	(6,56)	(3,58)	(1,81)	(4,29)		
SFD	4447,93[e]	1699,48[bc]	1386,01[bc]	746,38[bc]	347,56[bc]	892,67[bc]	33,58[ab]	38,21[ab]
	(18,62)	(7,12)	(5,80)	(3,12)	(1,46)	(3,74)		
SLI	4418,55[f]	1736,69[abc]	1432,44[abc]	775,33[abc]	372,02[abc]	927,29[abc]	41,71[ab]	39,30[ab]
	(18,50)	(7,27)	(6,00)	(3,25)	(1,56)	(3,88)		
UREA	4402,64[g]	1600,86[c]	1283,73[c]	684,56[c]	300,01[c]	818,74[c]	40,42[ab]	36,36[b]
	(18,43)	(6,70)	(5,37)	(2,87)	(1,26)	(3,43)		
SEM	33,36	54,72	44,95	26,39	18,48	31,56	1,21	1,06
Sig.	**	**	**	**	**	**	*	**

The highest total energy (TE) occurred with SAL addittion, whereas the lowest was found with AIV (P<0.01). TE of all the silages differred significantly (P<0.01). The highest DE, ME and NE_L values were determined with SAL, MAL and SLI (P<0.01).

Only DMD of MAL was different from that of CONT (P<0.05). The OMD of AIV was found the lowest (P<0.01) and all the treatments significantly differred. In addition, a significant difference was found between the OMD of UREA and MAL (P<0.01).

The lowest energy value estimated with <u>cellulase method compared to in vitro gas production technique</u> and OMD value were found in AIV treated silages ($P<0.01$). This can be attributed to the highest ash and the lowest NFE contents. The differences in total energy of all silage treatments are thought to depend on the nutrient contents of silages.

Dry matter digestibility (Table 3) of **MAL added silages was lower** than that of the CONT silages ($P<0.05$), whereas no differences were found between the other silages. ($P>0.05$). Of the silages with the highest DLG points, MAL, SAL and SLI groups had generally higher energy values.

<u>The lowest values were determined with AIV silages, which also had the lowest DLG point</u>

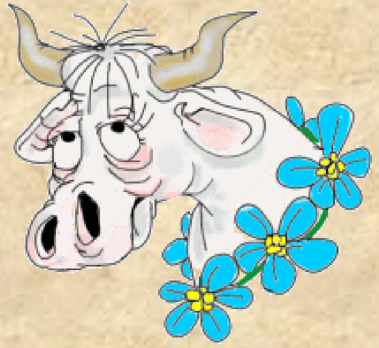
Table 4. The effects of different silage additives on in vitro gas production and estimated parameters of sugar beet pulp silage

İncubation time, h	CONT	AIV	FAS	MAL	SAL	SFD	SLI	UREA	SEM	Sig
				Gas production, ml/200 mg DM						
3	8,91[b]	12,50[ab]	11,27[ab]	11,79[ab]	10,63[ab]	9,13[b]	15,34[a]	8,71[b]	0,62	*
6	16,82[ab]	14,86[b]	15,58[b]	19,68[ab]	18,73[ab]	16,20[ab]	21,88[a]	15,68[b]	0,72	*
9	23,71[abc]	16,89[c]	19,54[bc]	26,46[ab]	25,72[ab]	22,43[abc]	27,63[a]	21,86[abc]	0,92	*
12	29,73[ab]	18,65[c]	23,18[bc]	32,27[a]	31,76[a]	27,90[ab]	32,70[a]	27,35[ab]	1,13	**
24	47,05[a]	23,62[c]	35,04[b]	48,54[a]	48,80[a]	44,00[ab]	47,57[a]	43,77[ab]	1,76	**
48	63,15[a]	28,06[c]	49,62[b]	62,80[a]	63,89[a]	59,50[a]	61,98[a]	60,33[a]	2,30	**
72	68,88[a]	29,54[c]	57,11[b]	67,54[ab]	68,91[a]	65,12[ab]	67,33[ab]	66,77[ab]	2,48	**
96	70,99[a]	30,06[b]	60,99[a]	69,19[a]	70,67[a]	67,16[a]	69,35[a]	69,28[a]	2,55	**
				Gas production kinetics						
a, ml	-0,18[c]	9,78[a]	6,58[ab]	2,58[bc]	1,24[c]	1,10[c]	7,90[a]	0,85[c]	0,80	**
b, ml	72,55[a]	20,57[d]	58,62[c]	67,54[abc]	70,50[ab]	67,24[abc]	62,75[bc]	70,08[ab]	3,01	**
c, ml/h	0,04[ab]	0,05[a]	0,03[b]	0,05[a]	0,05[a]	0,04[ab]	0,04[ab]	0,04[ab]	0,001	*
				Estimated parameters						
ME, MJ/kg DM	9,26[a]	5,98[c]	7,48[b]	9,50[a]	9,52[a]	8,92[a]	9,28[a]	9,37[a]	0,25	**
NE_L, MJ/kg DM	5,46[a]	3,23[b]	4,13[b]	5,64[a]	5,67[a]	5,22[a]	5,49[a]	5,63[a]	0,18	**
OMD, %	62,35[a]	41,44[c]	50,43[b]	63,88[a]	63,95[a]	60,19[a]	62,42[a]	64,04[a]	1,63	**

Gas production values of 24, 48, 72 and 96 h incubations were lowest in AIV treated silages and differred significantly from other silages ($P<0.01$). The decrease in the pH of incubations after 96 h could have caused such a difference.

Low silage pH probably prevented acetic acid formation and reduced *in vitro* gas production (Wolin, 1960). However, the variations in the nutrient compositons of feedstuffs are also known to influence in vitro gas production and related parameters (Kilic and Saricicek, 2006). The increase in ash and NFE content reduces gas production (Menke and Steingass, 1988). Lower gas production in AIV and FAS groups can be associated with high ash content of AIV silage and high NFE content of FAS.

AIV resulted in the lowest in vitro gas production values at 24, 48, 72 and 96 h and differred significantly from other treatments (P<0.01).



However, AIV addition, which had high “a value”, had the lowest “b value” (P<0.01). The rates of gas production (c value) of MAL, SAL and AIV were significantly higher than that of FAS (P<0.05) and no significant differences were found between other treatments.

AIV resulted in the lowest values of ME and OMD estimated from in vitro gas production values, which was followed by FAS (P<0.01). No differences were observed between other feedstuffs (P>0.05). NE_L of AIV did not differ from that of FAS, however these treatments caused lower values than did the other additives (P<0.01).

The lowest ME and OMD values determined using in vitro gas production technique of AIV treatment and NE_L of AIV and FAS additions can be associated with high ash content.

Estimated ME and NE_L values by using in vitro gas production technique were higher and more reliable than the values estimated with cellulase method.

The energy values estimated using in vitro gas production technique are similar to those calculated by using nutrient contents (Karabulut and Canbolat, 2005).

Conclusions

Addition of AIV increased ash content of the SBP silage but decreased sensorial observation based quality, energy, OMD and *in vitro* gas production values.

In spite of high Flieg point, addition of AIV to SBPS is not recommended.

However, addition of UREA increased the CP content of silages but did not affect silage quality. The in vitro gas production and energy values and OMD contents of the silages were high.

UREA can be used as an additive for SBPS due to the increment in the CP content in spite of a reduction in NFE content.

Conclusions

- **The addition of FAS decreased CP content but increased NFE content of the silage. In addition, FAS added silages had high quality points with a low rate of gas production.**
  Therefore this silage would not cause a rapid gas production and could be fed with feedstuffs with high gas production.

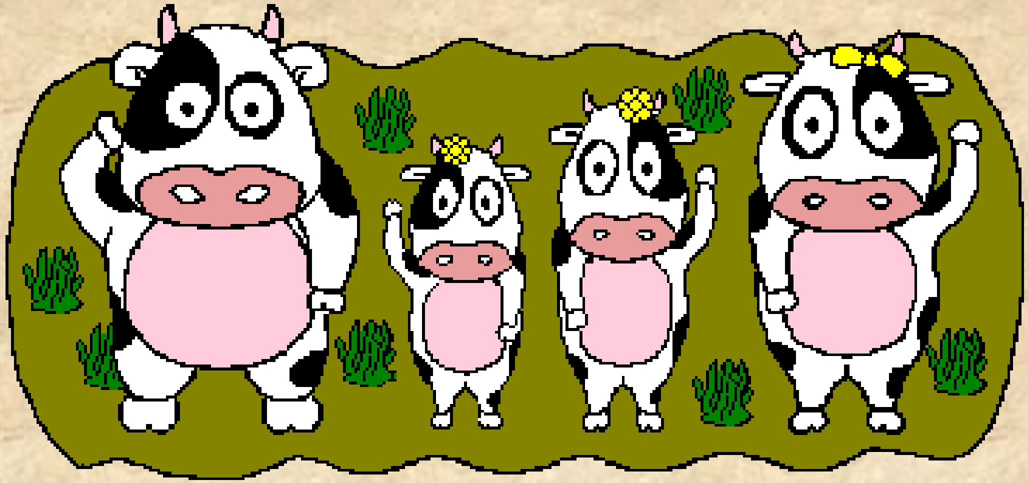
- **MAL and SAL biological inoculants showed the highest energy values and gas productions.**

- **SLI and SFD had high gas production, energy and OMD values.**

- **The use of in vitro gas production technique can be recommended in the estimation of ME and NE_L values of SBPS since this technique provides more reliable estimates as compared to cellulase method.**

Conclusions

In conclusion, suitable additives should be selected following the consideration of economical prenciples. Besides, *in vivo* trials are also required in the future.



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