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Effect of Different Additives on the Nutrient Composition, *in vitro* Gas Production and Silage Quality of Alfalfa Silage

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ABSTRACT

This study was carried out to determine the effects of different additives on the nutrient composition, *in vitro* gas production and its parameters and the quality of alfalfa silage. Formic acid salts (F silofarm formiat dry; SD and F silofarm liquid; SL), biological inoculant (Sil All; SA) and molasses (M) were added to the raw material individually. Gas Production (GP) and its parameters were studied by using *in vitro* GP technique with ruminally fistulated Sakiz x Karayaka rams *in vitro* Krganic Matter Digestibility (IVOMD) and Total Energy (TE), Digestible Energy (DE), Metabolisable Energy (ME), Net Energy lactation (NE_L), Net Energy fat (NE_p), Net Energy maintenance (NE_M) were studied by using in pepsin-cellulase method. SL increased Dry Matter (DM) content of alfalfa silage compared the other group silages (p<0.05) but decreased Crude Fiber (CF) (p<0.01). Molasses numerically increased the CP but the difference is no statistically significant. Molasses increased the Ether Extracts (EE) compared with Control Silage (CS) (p<0.05). SA addition into silage increased the ash of alfalfa compared with CS (p<0.05). GP numerically is reduced by silages with additives. At 96 h incubation the total GP for SDS was significantly (p<0.05) higher than CS. The gas production from the insoluble fraction "b" value of silages was lower for MS compared with CS and SAS (p<0.05). MS and SAS improved the pH, lactic acid content and Flieg score of alfalfa silage but MS included butyric acid that it is undesirable in silages. The NH₃-N concentration of the silages were the highest for MS and CS. The different additives addition into alfalfa silage decreased the ME, NE_L and OMD values determined by using gas production technique. The energy values of alfalfa silages determined by using pepsin-cellulase method increased when they were ensiled with M and SL additives. Total energy values of silages determined by using pepsin-cellulase method different each other when they were ensiled with different additives.

Key words: Molasses, formic acid salts, inoculant, gas production, silage quality, energy value

INTRODUCTION

Alfalfa are widely used ensiling material as they are rich in protein. Alfalfa and their legumes are usually difficult to ensile because of low sugar content and high buffering capacity.

Silage additives can be used in order to enhance silage fermentation and their nutritional quality (Henderson, 1993). Chemical additives include various organic and mineral acids that lower

the pH artificially and inhibit specific microbial population (Ashbell *et al.*, 2002). Molasses, unlike grain, provides fermentable carbohydrate; therefore, molasses addition can improve the fermentation of some hay crop forages.

There are number of *in vitro* techniques available to evaluate the nutritive value of feeds at relatively low cost. The use of *in vitro* gas method to estimate the digestion of feed is based on measured relationships between the *in vivo* digestibility of feeds and *in vitro* gas production, in combination with the feed's chemical composition (Menke and Steingass, 1988).

The objective of the present study was to determine the effects of different additives on the nutrient composition, *in vitro* gas production and its parameters and the quality of alfalfa silage and to establish a suitable additive.

MATERIALS AND METHODS

In this study Alfalfa Silage (AS) were used. Alfalfa (Elci) harvested at dough stage were used for silage. Plants were ensilaged. The fresh material which will be ensilaged was chopped to approximately 1.5 cm particle length and were ensiled in experimental jar silos in quadruplicate repetitions. Alfalfa was harvested at dough stage. Five different silages were prepared from chopped forage. Silage treatments included control (no additives) (CS). The molasses was applied at a rate of 5% of the fresh weight of the lucerne (MS), 10 g t⁻¹ microbial inoculant (Sil-All 4×4 obtained from Alltech-Pioneer). As recommend by the manufacturer, inoculant was added at 1.0×10¹¹ cfu g⁻¹ of fresh forage (SAS). Sill-All from Alltech is formulated with four lactic acid-producing bacteria (*Lactobacillus plantarum*, *Enterococcus faecium*, *Pediococcus acidilactici* and *Lactobacillus salivarius*) and four enzyme (Cellulase, Hemicellulase, Pentosanase and Amylase). In the *in vitro* trial, enzyme cellulase obtained from the microorganism *Trichoderma viridae*, hemicellulase from the microorganism *Aspergillus niger*, α-amylase from the porcine pancrease and the enzyme pepsin were used 2.5-3.5 kg ton⁻¹ F silofarm formiat dry (sodium formiat) (SDS) and 5-7 kg ton⁻¹ F silofarm liquid (formic acid, sodium format and water) (SLS) as recommend by the manufacturer (from Farmavet), was added at 1.0×10¹¹ cfu g⁻¹ of fresh forage. After two months (60 d) storage, the silos were opened. Representative samples were dried at 48°C in a forced-air oven for 72 h.

After drying, silage samples were ground through a 1 mm screen for chemical analysis. Dry Matter (DM), Organic Matter (OM) Crude Protein (CP), Crude Fibre (CF) and Ether Extract (EE) were determined by the methods described by AOAC (1990). Volatile fatty acid and NH₃-N contents in rumen fluid were determined using Markham (1942) Steam Distillation procedure. Furthermore, quality analyses (Kilic, 1986), were made in silages and NFE was determined by calculation. All chemical analysis was carried out in triplicate. The pH of each sample was determined in triplicate using approximately 25 g wet ensilage added to 100 mL of distilled water. After hydration for 10 min using blender, pH was determined using digital pH meter (Hanna Instruments 1332 model). Flieg points were calculated in silages according to Kilic (1986):

$$\text{Flieg point} = 220 + (2 \times \% \text{ Dry Matter} - 15) - 40 \times \text{pH}$$

All silage organic acids analysis was accomplished by using gas chromatograph (Shimadzu, GC-14B) as described by Leventini *et al.* (1990).

***In vitro* gas production:** Three SakizxKarayaka rams aged 2 with ruminal cannulas were used in gas production technique. Rumen fluid was obtained from three fistulated 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 *et al.* (1979) Menke and Steingass (1988) and Blümmel and Orskov (1993). Approximately 200 mg dry weight of sample was weighed in triplicate into calibrated glass syringes of 100 mL. Rumen fluid was collected before the morning feeding. The syringes were prewarmed at 39°C before the injection of 30 mL rumen fluid-buffer mixture consisting of 10 mL rumen fluid and 20 mL digestion medium into each syringe followed by incubation in a water bath at 39°C. 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. Total gas values corrected for blank incubation. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979) by NEWAY computer package programme:

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

Where:

a = The gas production from the immediately soluble fraction (mL)

b = The gas production from the insoluble fraction (mL)

c = The gas production rate constant for the insoluble fraction (mL h⁻¹)

t = Incubation time (h)

y = gas produced at time "t".

Organic Matter Digestibility (OMD), Metabolisable Energy (ME) (Menke *et al.*, 1979) and Net Energy lactation (NE_L) (Menke and Steingass,1988) contents of forages were estimated using equations given below:

$$\text{OMD, \%} = 14.88 + 0.8893 \text{ GP} + 0.448 \text{ CP} + 0.651 \text{ A}$$

Where:

GP = 24 h net gas production (mL 200 mg⁻¹ DM)

CP = Crude protein (%)

A = Ash content (%)

$$\text{ME, (MJ/kg DM)} = 2.20 + 0.136 \text{ GP} + 0.057 \text{ CP} + 0.002859 \text{ EE}^2$$

$$\text{NE}_{L}, (\text{MJ/kg DM}) = 0.101 \text{ GP} + 0.051 \text{ CP} + 0.11 \text{ EE}$$

Where:

GP = 24 h net gas production (mL/200 mg 1 DM)

CP = Crude protein (%)

EE = ether extract (%)

A = Ash content (%)

Pepsin-cellulase method: *In vitro* digestibility of DM and OM were determining according to Robinson (1999). Total Energy (TE), Digestible Energy (DE), Metabolisable Energy (ME), Net Energy lactation (NE_L), Net Energy fat (NE_F) and Net Energy maintenance (NE_M) value were calculated according to the enzyme method (Jarrige, 1989; Malossini *et al.*, 1993). Calculated values were converted to MJ kg⁻¹ DM.

Statistical analysis: One-way analysis of variance (ANOVA) was carried out to compare gas production, gas production parameters, energy values, DMD and OMD values using General Linear Model (GLM) of SPSS 10.0 package programs. Significance between individual means were identified using the Duncan's multiple range test.

RESULTS AND DISCUSSION

Nutrient contents of silages: The nutrient composition of alfalfa silage was affected by additive type (Table 1). Dry Matter (DM) contents of alfalfa silage were significantly ($p < 0.01$) affected by additive. It was higher when alfalfa was ensiled with SL compared with other additive and control. The highest DM content of alfalfa ensiled with SL might be attributed to the fact that the use of SL as a silage additive prevented DM loss during ensiling because of the early decline in pH and silage stability (Khorsani *et al.*, 1993). In contrast to the present findings Lujia *et al.* (2004) reported reduced DM content when alfalfa was ensiled with molasses, Formic Acid (FA), inoculant and enzyme.

CP content of SDS was significantly ($p < 0.05$) lower than the other group silages. The reduction in CP content of silage was attributed to the extensive proteolysis during the ensiling process. The CP content of silage was not affected by the other additives. Florek *et al.* (2004) reported that DM and CP content of alfalfa silage decreased by FA additive but Weiss and Underwood (2006) reported that FA and mineral acids added will reduce pH quickly and greatly limit fermentation losses of protein and carbohydrates. Khorsani *et al.* (1993) reported that addition of fermentable carbohydrate should prevent the loss of nutrients in ensiling material due to early stabilisation of the material.

EE content of silage was significantly ($p < 0.05$) higher when it was ensiled with molasses compared with than the other group silages for except SAS. The ash content of SAS was significantly ($p < 0.05$) higher than CS.

The CF content of silage was significantly ($p < 0.05$) lower for alfalfa ensiled with molasses and SL compared with SDS and CS. These decreases in CF content may have resulted from increased cell wall degradation due to increased silage fermentation caused by addition of molasses and SL. This statement was in agreement with the reporting of Bolsen *et al.* (1996).

NFE content of SLS was significantly ($p < 0.05$) higher compared with the other groups except for CS. On the other hand Olt *et al.* (2005) who reported that chemical additive increased the NFE content of alfalfa silage.

In vitro gas production and its parameters: *In vitro* Gas Production (GP) of silage were significantly ($p < 0.05$) affected by additives type (Table 2). MS was observed GP lower than CS after

Table 1: Chemical composition of alfalfa silages (% DM)

Treatments	CS	MS	SAS	SDS	SLS	SEM	Significant
EE	3.04 ^b	4.20 ^a	3.61 ^{ab}	3.08 ^b	2.76 ^b	0.165	*
Ash	10.41 ^b	11.40 ^{ab}	14.25 ^a	11.91 ^{ab}	10.64 ^{ab}	0.539	NS
CP	18.59 ^a	19.06 ^a	18.70 ^a	16.62 ^b	18.62 ^a	0.313	*
CF	29.72 ^{ab}	27.53 ^c	28.93 ^{bc}	30.89 ^a	25.17 ^d	0.533	**
NFE	38.24 ^{ab}	37.82 ^b	34.50 ^b	37.50 ^b	42.81 ^a	0.885	*

EE: Ether extracts, CP: Crude protein, CF: Crude fiber, NFE: Nitrogen free extracts, a,b,...: Means with different superscript within same column significantly different at * $p < 0.05$ and ** $p < 0.01$. NS: Non-significant

Table 2: *In vitro* gas production, OMD and energy value of silage

Gas production	CS	MS	SAS	SDS	SLS	SEM	Sig
3	9.63	7.69	8.74	7.13	7.13	0.552	NS
6	15.91	12.06	14.95	12.47	12.47	0.732	NS
9	21.08	15.78	20.05	16.84	16.84	0.890	NS
12	25.34	18.93	24.24	20.42	20.42	1.016	NS
24	36.12 ^a	27.53 ^b	34.77 ^{ab}	29.37 ^{ab}	29.37 ^{ab}	1.279	*
48	43.46 ^a	34.47 ^{ab}	41.83 ^{ab}	35.29 ^{ab}	35.29 ^{ab}	1.378	*
72	45.06 ^a	36.44 ^{ab}	43.35 ^{ab}	36.53 ^{ab}	36.53 ^{ab}	1.381	*
96	45.42 ^a	37.01 ^{ab}	43.69 ^{ab}	36.80 ^b	36.80 ^b	1.380	*
pH	6.77	6.76	6.79	6.76	6.76	0.011	NS
Gas production parameters							
a (mL)	2.02 ^b	2.55	1.17	0.62	1.42	0.434	NS
b (mL)	43.50 ^a	34.70 ^c	42.62 ^{ab}	36.25 ^{bc}	36.74 ^{abc}	1.190	*
c (mL h ⁻¹)	0.06	0.06	0.06	0.07	0.06	0.001	NS
Energy contents and OMD							
ME (MJ kg ⁻¹ DM)	8.18 ^a	7.04 ^b	8.00 ^{ab}	7.15 ^{ab}	7.19 ^{ab}	0.175	*
NE _L (MJ kg ⁻¹ DM)	4.94	4.22	4.87	4.16	4.17	0.130	NS
OMD %	56.03	48.67	55.13	49.24	49.60	1.153	NS

Means with different superscript within same row significantly different at *p<0.05, NS: Not significant

24 h incubation (p<0.05a). The GP of CS after 96 h incubation was higher than SDS and SLS (p<0.05). Muck *et al.* (2007) reported reduced *in vitro* GP value when alfalfa was ensiled with inoculant but Bueno *et al.* (2005) reported that addition of inoculant into silage didn't affect. The GP of silage after 96 h incubation were numerically lower for alfalfa ensiled with molasses inoculant and FA salts compared with CS. The reduction GP value can be explained with limited degradability caused by acidity. The pH value of silage after 96 h incubation was not affected by additive type.

The gas production from the immediately soluble fraction "a" and the gas production rate constant for the insoluble fraction "c" value of silage were not affected by the additive type. While the gas production from the insoluble fraction "b" of silage was significantly (p<0.05) lower than when it was ensiled with molasses compared with CS and SAS. ME value of silage was significantly (p<0.05) lower when it was ensiled with molasses compared with control.

NE_L and OMD of silage were not affected by the additive type. The results obtained in this study are consistent with Bueno *et al.* (2005) who reported that OMD value of silage was not affected by the inoculant additive. In contrast to the present findings Florek *et al.* (2004) reported improved energy value of silage when alfalfa was ensiled with FA salts.

Silage fermentation kinetics and fleig score: The pH value of SAS and MS were lower than when alfalfa was ensiled with inoculant and molasses compared with the other groups. This could be due to the fact that the molasses is a relatively source of easily fermentable carbohydrate (Table 3). The results obtained in the present experiment are consistent with Man and Wiktorsson (2001) who reported significantly lower pH value of grass silage when molasses was added before ensiled. In this study pH value of silage was the highest for CS. Increased pH value may have caused by relatively higher nitrogen content of alfalfa. LuJia *et al.* (2004) reported that pH value was the highest for without additive but reduced pH value when alfalfa was ensiled with molasses. The addition of inoculant decreased pH value of silage. Similar findings have also been reported by others Kung *et al.* (1987), Ando *et al.* (2006), Charbel *et al.* (2005) and Bureenok *et al.* (2005).

Table 3: Fermentation kinetics and flieg scores of silages, %

	CS	MS	SAS	SDS	SLS	SEM	Sign.
DM	27.03 ^b	26.16 ^c	24.76 ^d	27.09 ^b	28.61 ^a	0.33	**
pH	5.45	4.35	4.85	5.80	5.37		
Lactic acid (%DM)	1.86 ^c	4.3 ^a	3.9 ^a	3.15 ^b	3.35 ^{ab}	0.46	*
Acetic acid (%DM)	1.10 ^{bc}	0.6 ^c	1.75 ^b	2.80 ^a	2.76 ^a	0.50	*
Butyric acid (%DM)	-	1.2	-	-	-	0.40	*
NH ₃ -N	6.40 ^a	4.05 ^{ab}	3.91 ^b	3.86 ^b	3.52 ^b	0.92	**
Flieg Score	40.97	83.29	60.39	27.14	46.64		

Means with different superscript within same row significantly different at *p<0.05 and **p<0.01

In this study, Flieg score of SAS and MS were numerically higher than the other groups. Similar findings have also been reported by others (Florek *et al.*, 2004; Olt *et al.*, 2005; Nadeau *et al.*, 2000).

Lactic Acid (LA) concentration of alfalfa silage were significantly (p<0.05) affected by additives. The lowest LA concentration was observed for CS (p<0.05). The addition of additive like a molasses improved the fermentable carbohydrate content of silages that provided a suitable environment for LA bacteria to lower the final pH of the silages (Bolsen, 1996; Man and Wiktorsson, 2001; Nisa *et al.*, 2008). Many researchers have reported that addition of inoculant into silage increased silage LA concentration (Baah *et al.*, 2005; Olt *et al.*, 2005; Charbel *et al.*, 2005; Ando *et al.*, 2006), however, there are some data indicating that inoculant decreased silage LA concentration (Lujia *et al.*, 2004). Low LA content without additive alfalfa silage may have resulted from the high N content of alfalfa, which is consistent with the results of Florek *et al.* (2004) and Olt *et al.* (2005). The LA content also increased when alfalfa was ensiled with FA salts. Charmley *et al.* (1990) and Nadeau *et al.* (2000) have reported that the addition of FA increased the silage LA concentration by limiting silage fermentation. However in contrast to the present finding, Florek *et al.* (2004) and Kennedy (1990) reported reduced LA concentration when alfalfa was ensiled with FA salts. In this study, the highest acetic acid concentration was observed when alfalfa was ensiled with FA salts, while the lowest LA concentration were observed for alfalfa ensiled with molasses (p<0.05), which is in agreement with the results reported in literature (Weiss and Underwood, 2006). Wolford (1984) reported that LA produced during ensiling is further fermented into acetic acid, resulting in a higher acetic acid concentration with addition of molasses to silage.

NH₃-N concentration of silages were significantly (p<0.05) affected by additive. The highest NH₃-N concentration was observed when alfalfa was ensiled without additive (p<0.05). The high NH₃-N of CS might be attributed to the fact that alfalfa is a very rich source of nitrogen. This statement is in agreement with the literature (Weiss and Underwood, 2006). The addition of additives like FA salts and inoculant decreased (p<0.05) the NH₃-N content compare with CS. It can be explained with limited fermentation caused by FA salts. This result is consistent with Florek *et al.* (2004), who reported that the NH₃-N concentration of silage reduced when alfalfa was ensiled with FA and inoculant.

Energy value (Pepsin-cellulase method): Energy content, *in vitro* dry matter (IVDM), *in vitro* organic matter (IVOM) of silages were given in Table 4. *in vitro* TE content of silages affected by all additives (p<0.05). In addition of SL into alfalfa increased TE, DE, ME, NE_L, NE_F, NE_M contents in their silages compared with CS, SAS and SDS (p<0.05).

Table 4: Energy value and digestibility, of alfalfa silages

Energy		CS	MS	SAS	SDS	SLS	SEM	Sig
TE	kcal kg ⁻¹ DM	4398.11 ^b	4390.10 ^c	4231.61 ^e	4297.88 ^d	4402.62 ^a	17.435	**
	(MJ kg ⁻¹ DM)	(18.41)	(18.38)	(17.71)	(17.99)	(18.43)		
DE	kcal kg ⁻¹ DM	1845.91 ^b	1918.34 ^{ab}	1818.63 ^b	1815.67 ^b	2009.88 ^a	27.585	**
	(MJ kg ⁻¹ DM)	(7.72)	(8.03)	(7.62)	(7.60)	(8.41)		
ME	kcal kg ⁻¹ DM	1481.07 ^b	1541.57 ^{ab}	1460.15 ^b	1461.70 ^b	1621.53 ^a	22.645	*
	(MJ kg ⁻¹ DM)	(6.20)	(6.45)	(6.11)	(6.12)	(6.79)		
NEL	kcal kg ⁻¹ DM	806.06 ^b	844.03 ^{ab}	797.56 ^b	796.45 ^b	894.61 ^a	14.032	*
	(MJ kg ⁻¹ DM)	(3.37)	(3.53)	(3.34)	(3.33)	(3.74)		
NEF	kcal kg ⁻¹ DM	398.98 ^b	431.66 ^{ab}	402.73 ^b	396.78 ^b	476.17 ^a	11.715	*
	(MJ kg ⁻¹ DM)	(1.67)	(1.80)	(1.68)	(1.66)	(1.99)		
NEM	kcal kg ⁻¹ DM	964.04 ^b	1009.46 ^{ab}	953.89 ^b	952.55 ^b	1069.95 ^a	16.782	*
	(MJ kg ⁻¹ DM)	(4.04)	(4.22)	(3.99)	(3.99)	(4.48)		
IVDMD		25.78	28.74	29.90	28.78	27.71	0.890	NS
IVOMD		41.97	43.70	42.98	42.25	45.65	0.563	NS

TE: Total energy, DE: Digestible energy, ME: Metabolisable energy, NEL: Net energy lactation, NEF: Net energy fat, NEM: Net energy maintenance, IVDMD: *in vitro* dry matter digestibility, IVOMD: *in vitro* organic matter digestibility. SEM: Standart error of means Means within the same row with differing superscript letters are different *: p<0.05; **: p<0.01. Means with different superscript within same row significantly differ (p<0.05). NS: Not significant

Addition of different additives to alfalfa no affected IVDMD and IVOMD contents of the silages (p>0.05). But the treatments showed numerically greater IVOMD and IVOMD value compared with CS. While some researchers reported that bacterial inoculant additive into silage had no effect neither on the IVOMD nor IVOMD (Hristov and McAllister, 2002), some other researchers reported that using inoculant in silage improved the DM digestibility of silages (Mandevbu *et al.*, 1999).

In conclusion, The nutrient composition were affected by all additives. *in vitro* gas production decreased for SDS and SLS at 96 h incubation (p<0.05). The additives did not affect the ME, NE_L and OMD. The addition of additives like innoculant and molasses improved the silage quality. The effects of additives on alfalfa silage fermentation, gas production and its parameters, energy, OMD and silage quality showed further be studied to determined proper silage additive.

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REFERENCES

- AOAC, 1990. Official Method of Analysis of Association of Official Analytical Chemist. 15th Edn, Association of Official Analytical Chemists, Arlington, Virginia, USA.
- Ando, S., M. Ishida, S. Oshio and O. Tanaka, 2006. Effects of isolated and commercial lactic acid bacteria on the silage quality digestibility, voluntary intake and ruminal fluid characteristics. Asian-Aust. J. Anim. Sci., 19: 386-389.
- Ashbell, G., Z.G. Weinberg, Y. Hen and I. Filya, 2002. The effects of temperature on the aerobic stability of wheat and corn silages. J. Ind. Microbiol. Biotechnol., 28: 261-263.
- Baah, J., T.A. McAllister, L. Bos, F. van Herk and R.C. Charley, 2005. Effect of Lactobacillus buchneri 40788 and buffered propionic acid on preservation and nutritive value of alfalfa and timothy high-moisture hay. Asian-Aust. J. Anim. Sci., 18: 649-660.

- Blummel, M. and E.R. Orskov, 1993. Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting feed intake in cattle. *Anim. Feed Sci. Technol.*, 40: 109-119.
- Bolsen, K.K., G. Ashbell and Z.G. Weinberg, 1996. Silage fermentation and silage additives. *Asian-Aust. J. Anim. Sci.*, 5: 483-493.
- Bueno, I.C.S., S.L.S. Cabral Filo, S.P. Gobbo, D.M.S.S. Vitti and A.L. Abdalla, 2005. Influence of inoculum source in a gas production method. *Anim. Feed Sci. Technol.*, 123-124: 95-105.
- Bureenok, S., T. Namihira, M. Tamaki, S. Mizumachi, Y. Kawamoto and T. Nakada, 2005. Fermentative quality of guineagrass silage by using fermented juice of the epiphytic lactic acid bacteria (FJLB) as a silage additive. *Asian-Aust. J. Anim. Sci.*, 18: 807-811.
- Charbel, R., A.F. Mustafa and L.E. Phillip, 2005. Effects of inoculation of high dry matter alfalfa silage on ensiling characteristics, ruminal nutrient degradability and dairy cow performance. *J. Sci. Food Agric.*, 85: 743-750.
- Charmley, E., M. Gill and C. Thomas, 1990. The effect of formic acid treatment and the duration of the wilting period on the digestion of silage by young steers. *Anim. Prod.*, 51: 497-504.
- Florek, S., P. Purwin, D. Minakowski, M. Stanek and F. Tredowicz, 2004. The influence of formic acid additives on the quality of silage from different plant material. *Veterinářja Ir Zootechnika. T.*, 26: 23-28.
- Hristov, A.N. and T.A. McAllister, 2002. Effect of inoculants on whole-crop barley silage fermentation and dry matter disappearance *in situ*. *J. Anim. Sci.*, 80: 510-516.
- Jarrige, R., 1989. Ruminant Nutrition. Recommend Allowances and Feed Tables. Libbey Eurotext, Paris, London, Rome.
- Kennedy, S.J., 1990. Comparison of the fermentation quality and nutritive value of sulphuric and formic acid treated silage feed to beef cattle. *Grass Forage Sci.*, 45: 17-28.
- Khorsani, G.R., E.K. Okine, J.J. Kennelly and J.H. Helm, 1993. Effect of whole crop cereal grain silage substituted for alfalfa silage on performance of lactating dairy cows. *J. Dairy Sci.*, 76: 3536-3546.
- Kilic, A., 1986. Silo Feed (Instruction, Education and Application Proposals). Bilgehan Press, Izmir, pp: 327.
- Kung, L., L.D. Satter, B.A. Jones, K.W. Genin, A.L. Sudoma, G.L. Enders and H.S. Kim, 1987. Microbial inoculation of low moisture alfalfa silages. *J. Dairy Sci.*, 70: 2069-2077.
- Leventini, M.W., C.V. Hunt, R.E. Roffler and D.G. Casebolt, 1990. Effects of dietary levels of barley-based supplements and ruminal buffer on digestion and growth by beef cattle. *J. Anim. Sci.*, 68: 4334-4344.
- Lujia, H., L. Xian, H. Shinichiro and N. Kazuhisa, 2004. Effect of different additives on the quality of alfalfa silage. *J. China Agric. Univ.*, 9: 25-30.
- Malossini, F., S. Bartocci, G.M. Terzano, E. Tibaldi and S. Bovolenta, 1993. Estimation of gross energy in forages from chemical composition. *NAR Ser. B*, 63: 61-61.
- Man, N.V. and H. Wiktorsson, 2001. Cassava tops ensiled with or without molasses as additive effects on quality, feed intake and digestibility by Heifer. *Asian-Aust. J. Anim. Sci.*, 4: 624-630.
- Mandebvu, P., J.W. West, M.A. Froetschel, R.D. Hatfield, R.N. Gates and G.M. Hill, 1999. Effect of enzyme or microbial treatment of bermudagrass forages before ensiling on cell wall composition end products of silage fermentation and *in situ* digestion kinetics. *Anim. Feed Sci. Technol.*, 77: 317-329.

- Markham, R., 1942. A steam distillation apparatus suitable for micro-kjeldahl analysis. *Biochem. J.*, 36: 790-791.
- Menke, K.H. and H. Steingass, 1988. Estimation of the energetic feed value obtained from chemical analysis and gas production using rumen fluid. *Anim. Res. Dev.*, 28: 7-55.
- Menke, K.H., L. Raab, A. Salewski, H. Steingass, D. Fritz and W. Schneider, 1979. The estimation of the digestibility and metabolisable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. *J. Agric. Sci.*, 93: 217-222.
- Muck, R.E., I. Filya and F.E. Contreras-Govea, 2007. Inoculant effects on alfalfa silage: *in vitro* gas and volatile fatty acid production. *J. Dairy Sci.*, 90: 5115-5125.
- Nadeau, E.M.G., D.R. Buxton, J.R. Russell, M.J. Allison and J.W. Young, 2000. Enzyme, bacterial inoculant and formic acid effects on silage composition of orchardgrass and alfalfa. *J. Dairy Sci.*, 83: 1487-1502.
- Nisa, M., M.A. Shahzad, M. Sarwar and N.A. Tauqir, 2008. Influence of additives and fermentation periods on silage characteristics, chemical composition and *in situ* digestion kinetics of Jambo silage and Its fodder in Nili buffalo bulls. *Turk. J. Vet Anim. Sci.*, 32: 67-72.
- Olt, A., O. Kart, H. Kaldmae, M. Ots, E. Songisepp and I. Smidt, 2005. The effect of additive and dry matter content on silage protein degradability and biogenic amine content. http://www.eau.ee/~aps/pdf/20052/Olt_i.pdf.
- Orskov, E.R. and I. McDonald, 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. Agric. Sci.*, 92: 499-503.
- Robinson, P.H. and G. Getachew, 2002. A Practical gas production technique to determine the nutritive value of forages: The UC Davis approach. Cooperative Extension University of California, Davis.
- Robinson, P.H., M.C. Mathews and J.G. Fadel, 1999. Influence of storage time and temperature on *in vitro* digestion of neutral detergent fiber at 48 h and comparison to 48 h in sacco neutral detergent fiber digestion. *Anim. Feed Sci. Technol.*, 80: 257-266.
- Weiss, B. and J. Underwood, 2006. Silage additives. Ohio State University Extension department of Horticulture and Crop Science 2021 Coffey Road, Columbus, Ohio 43210-1044, AGF-018-92. <http://ohioline.osu.edu/agf-fact/0018.html>.
- Woolford, M.K., 1984. *The Silage Fermentation*. Marcel Dekker, Inc., New York.