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The Effect of Delayed Ensiling and Application of Propionic Acid-Based Additives on the Nutritiive Value, Aerobic Stability and Degradability of Corn Silage

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Abstract: In order to study the effect of delayed ensiling and application of some organic acid-based additives on fermentation of corn silage, chopped whole plant corn mixed with 4 different additives that consists of propionic acid in addition to control treatment without any additive. The additives were: (1) propionic acid (2) propionic acid (85%) + formic acid (15%) (3) calcium propionate and (4) propionic acid (80%) + formic acid (15%) + ammonia (5%). Ten gram per kilogram dry matter of each additives were mixed with chopped corn forage in 2 different times (0, 24 h). Silages were assessed and evaluated for appearance; DM and pH. CP, NDF, ADF, TVFA, WSC, aerobic stability and DM degradation each of treatments were determined after 60 days. Silages that was exposed to air for 24 h, before ensiling had better appearance quality in Filg's method and whole additives had good effect on appearance quality compared with control group. In the method of DM and pH evaluation, all silages contained buffered propionic acid-based additive, were good and very good. These silages had lower pH than control ones ($p<0.05$). There was a significant interaction was between silages for concentration of dry matter ($p<0.05$). NDF in the silages (with propionic acid (85%) + Formic acid (15%)) was lower than the other silages while the contents of CP and TVFA increased ($p<0.05$) with addition of the additives especially additive containing propionic acid (85%) + formic acid (15%) ($p<0.05$). WSC in untreated silages was more than the other silage samples ($p<0.05$). Buffered propionic acid–based additives caused increase in the aerobic stability in treated silages compared with untreated silages. DM degradation (*in situ*) in untreated silages that delayed ensiled was lower other silages. Application of additives containing propionic acid (80%) + formic acid (15%) + ammonia (5%) resulted in highest DM degradation among experimental additives. Generally, treatment with propionic acid-based additives prevented the decrease of DM degradation (*in vitro*).

Key words: Corn silage, propionic acid, formic acid, fermentation, sealing time

INTRODUCTION

Ensiling is a preservation method for moist forage crops. Many chemical additives have been used to alter silage fermentation. For example, ammonia has been used to treat high moisture corn and corn silage. Moderate concentrations of ammonia have increased the concentrations of lactic and acetic acids, decreased proteolysis, improved DM recovery and improved the aerobic stability of corn silage (Kung *et al*., 2000). Unbuffered propionic acid-based preservatives have also been used to improve the aerobic stability of corn silages. In recent years, marked changes have been made to the formulations and recommended application rates of additives containing propionic acid. Current recommendations for use of buffered propionic acid additives are considerably lower (0.1 to 0.2% of fresh forage weight) than classical recommendations for use of the unbuffered acid (0.75 to 1.5%) (Kung *et al*., 2000). Data that directly compare the effects of buffered propionic acid based preservatives and ammonia on the aerobic stability of corn silage is lacking. A direct comparison is warranted to allow producers to make educated decisions on their relative usefulness (Kung *et al*., 2000). Moisture content at the time of harvest (too wet or too dry) may also be a problem if the proper maturity is not matched to the area. When the effects of grain content and stover digestibility are combined, very significant differences in corn silage quality can result due to hybrid differences. Maturity at harvest may affect corn silage quality because it influences grain and moisture content as well as stover digestibility. A good fermentation process is not only dependent on the type and quality of the forage crop, but

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also on the harvesting and ensiling technique. Attention to details such as speed of harvesting, moisture content, length of chop, silage distribution and compaction can greatly influence the fermentation process and storage losses. Efficient fermentation ensures a more palatable and digestible feed, which encourages optimal dry matter intake that translates into improved animal performance. Making consistent, high-quality silage requires sound management decisions and attention to details. Silage quality is achieved when lactic acid is the predominant acid produced, as it is the most efficient fermentation acid and will drop the pH of the silage the fastest. The faster fermentation is completed, the more nutrients will be retained in the silage (Johnson *et al*., 2002, 2003; Tapia *et al*., 2005). Chopping forages too long makes compaction difficult and air will remain trapped in the silage resulting in heating and spoilage (Pauly and Lingvall, 1999). The crop should be harvested and the silo filled as rapidly as possible. Filling delays will result in excessive respiration and increased silage losses. Packing should begin immediately when storing silage in bunker silos (Johnson *et al*., 2002, 2003; Tapia *et al*., 2005). Fermentation inhibitors could in theory be used for all types of forages. However, in practice, they are generally only used in wet crops with a low water-soluble carbohydrate content and/or high buffer capacity (McDonald *et al*., 1991). An advantage of the salts of propionic acid are that they are easier and safer to handle than their corresponding acids. Fermentation inhibitors can reduce clostridial spore counts. In wilted grass silages a decrease in spore counts by a factor 5 to 20 has been observed. A similar decrease in spore counts could be obtained by adding molasses, a fermentation stimulant (McDonald *et al*., 1991).

It is clear that to inhibit aerobic spoilage organisms, in particular the ones causing the onset of deterioration (i.e., yeasts and acetic acid bacteria) have to be inhibited in their activity and growth. Some additives which have proven to be effective in this respect include chemical additives based on volatile fatty acids such as propionic and acetic acid and biological additives based on bacteriocin producing micro-organisms such as lactobacilli and bacilli (McDonald *et al*., 1991; Phillip and Fellner, 1992; Weinberg and Much, 1996). The effect of formic acid as a silage additive for grass crops is well documented (McDonald *et al*., 1991). When added to crops that are difficult to ensile, a sufficient dose of formic acid will increase the lactic acid fermentation, reduce the fermentation to acetic, propionic and butyric acids, reduce proteolysis and reduce silage pH compared with untreated silage. In order to achieve the major goal in silage making that is to preserve silage material with minimum nutrient loss, formic acid is widely used. The addition of formic acid to silage material has been reported to have general positive effects on fermentation (Haigh, 1988; Snyman and Joubert, 1996). Formic acid as silage additive has anti-bacterial effect on many bacteria sp., including lactic acid bacteria.

Thus, the addition of formic acid into silage results in limited fermentation and reduction in organic acid content of silage. This type of silage contains a greater amount of water soluble carbohydrate, which is a better source of energy for rumen microbe than lactic acid. In some cases, a moderate dose of formic acid may inhibit lactic acid bacteria to a greater extent than yeasts and entrobacteria which are undesirable organisms in silage. Yeasts are highly tolerant to formic acid and when grown under anaerobic conditions, dependent on high sugar concentrations, which are usually present in formic acid treated silage (McDonald *et al*., 1991; Driehuis and Wikeselaar, 2000). Propionic acid-based additives have been used to inhibit yeasts that assimilate lactic acid when silages are exposed to air and thus, they improve aerobic stability (Kung *et al*., 2000, 2004). After chopping, the presence of air in forage mass delays the onset of fermentation and encourages the growth of undesirable microbes, which results in negative effects on the ensuing fermentation (McDonald *et al*., 1991). To eliminate air from the forage mass, forages should be packed rapidly and tightly into silos. However, poor management decisions, such as leaving chopped forage in wagons or piles, can end in substantial delays in filling silos; thus, forages may be exposed to air for prolonged periods of time. In addition, large bunk or trench silos sometimes require weeks to be filled, which expose forages to excessive amounts of air. Air is also detrimental during storage because it stimulates the growth of yeasts that metabolize lactic acid, which results in loss of nutrients (Woolford, 1990). High moisture grain silages are prone to spoil rapidly when exposed to air (McAllister *et al*., 1995). To improve the aerobic stability of these grains, methods to increase the concentration of propionic acid (because of its antifungal properties) in silages have been studied. The direct method of adding propionic acid to silages has resulted in more consistent improvements in aerobic stability (Kung *et al*., 1998, 2000), but the effects of adding this acid to forage that is or has been exposed to excessive amounts of air before ensiling has not been well studied. The objective of this study was to compare the effects of delayed ensiling on silage fermentation and also to investigate the application of buffered propionic acid-based additives on fermentation, aerobic stability and nutritive value of these silages.

MATERIALS AND METHODS

Experimental site: This research project was conducted from 2006 to 2007, in the Department of Animal Science Gorgan University of Agriculture Sciences and Natural Resources, Iran.

Silage preparation and the addition of additives: Whole-plant corn was harvested one half milk line and chopped to a theoretical length of 1-2 cm with a forage harvester. Forage without treatment (control), or treated with 0.1% of buffered propionic acid-based additives (propionic acid) (1), propionic acid (85%) + formic acid (15%) (2), calcium propionate (3), propionic acid (80%) + formic acid (15%) + ammonia (5%) (4) and then treated forages were ensiled immediately in quadruplicate 10 L micro silos. Portions of the chopped forage, untreated and treated, were left in loose piles on a clean concrete floor in a barn for 24 h before being packed into silos. Empty and full weights of silos were recorded and silos were stored in the dark at ambient temperatures ranging between 18 and 25°C. All of the silos for each treatment were opened after 60 days of ensiling. The DM content of fresh forage and silage samples was determined by drying (70 to 75 g) in a forced-air oven at 60°C for 48 h. After drying, samples were ground. Ten grams samples of silage from each treatment were diluted with 100 mL sterile deionized water and blended for 2 min. Silage pH was determined immediately. Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) were analyzed using sulfite and amylase (Van Soest *et al*., 1991). Total nitrogen was determined after total combustion (LECO Corporation, CNS 2000 Analyzer; St. Joseph, MI) and CP was calculated by multiplying total nitrogen by 6.25. Fresh forage (25 g) was added to a dilution bottle that contained 225 mL of sterile quarter-strength Ringer's solution (Oxoid BR54; Unipath, Basingstoke, UK) and homogenized in a blender for 1 min. After blending, water extracts were filtered through Whatman 54 filter paper (Whatman Inc., Clifton, NJ), acidified with 50% sulfuric acid and frozen before analysis of Water-Soluble Carbohydrates (WSC) (Nelson, 1944). The pH of silage extracts was determined within 20 min of homogenization. After 60 days of ensiling, silos were opened and silage was mixed well, then a 2 kg sample was returned to its respective silo. No physical packing of the silage took place. A thermometer was placed in the geometric center of each silage mass and temperatures were recorded every 3 h. A double layer of cheesecloth was placed on the top of each silo to prevent contamination but allowed for penetration of air because silos were incubated between 21 and 22°C. Aerobic stability was defined as the number of hours before a 2°C increase in the temperature of the silage mass relative to ambient temperature. The temperature and pH of silages were recorded for 144 h, each 24 h once. For the determination of *in situ* ruminal DM, dry corn silage samples were ground to pass through a 2 mm screen and 0.5 g of silage was weighed into Dacron bags (5×10 cm, 50 μm pore size; Ankom, Fairport, NY). Duplicate bags were placed in the rumen of a fistulated sheep fed a diet that was made up of 82.4% alfalfa hay/grass hay, 8.8% soybean meal and 8.8% dry rolled barely. Bags were incubated for 0, 4, 8, 16, 24, 48 and 72 h. After removal from the sheep, bags were washed with water until effluent was clear and then were dried at 55°C for 48 h. Dried bags were weighed and DM disappearance was determined. The chemical composition of forages and silages was analyzed as a factorial in a completely randomized design by the general linear models procedure of SAS (1988). Factors were ADF%, NDF%, CP%, %WSC, TVFA (Mmol kg^{-1}), DDM%, DE (Mcal kg^{-1}), ME (Mcal kg^{-1}) and % TDN. Means were separated using Duncan test at 0.05 probability level (Duncan, 1955). The kinetics of ruminal DM disappearance *in situ* was estimated by the Naway software.

RESULTS

Appearance evaluation: In this study, each of the factors including like, smell, color and structure (appearance characters) based on Filg's method evaluation were given a number (Horiguchi and Takahashi, 2007). Treated forage with the additive that was exposed to air for 24 h before ensiling, had better appearance than others. Control group had lower appearance quality than the other treatments in all of sealing times.

In this evaluation method, the maximum pH for stating in very good degree silage with 35% DM is 4.5. In the present study, average DM was 36.4% and pH ranged from 4.04 to 5.75, therefore, all of silages were stated in good and very good class (Table 1).

Table 1: Appearance evaluation based on dry matter and pH Filg's method

Delaying time[1] (h)	Additive type[2]	DM (g kg^{-1})	pH	Judgment
0	Control	339.8^{d}	5.75^{a}	Good
0	Additive 1	367.0^{bc}	4.36^{b}	Very good
0	Additive 2	360.6^{bcd}	4.04^{b}	Very good
0	Additive 3	356.2^{cd}	4.76^{b}	Good
0	Additive 4	365.6^{bcd}	4.35^{b}	Very good
24	Control	398.0^{ab}	4.58^{a}	Very good
24	Additive 1	341.6^{d}	4.17^{b}	Very good
24	Additive 2	360.4^{bcd}	4.14^{b}	Very good
24	Additive 3	416.4^{a}	4.10^{b}	Very good
24	Additive 4	337.1^{d}	4.33^{b}	Very good

[1]Delaying time of sealing = 0 and 24 h, [2]Control: Without any additive. Additive 1: Propionic acid. Additive 2: Propionic acid (85%)+formic acid (15%), Additive 3: Calcium propionate. Additive 4: Propionic acid (80%) +formic acid (15%) +ammonia (5%). Unlike superscript in a row differ significantly ($p<0.05$)

Table 2: Chemical composition (DM based) corn silage after 60 days of ensiling (sealing time is fixed)

	Treatments[1]				
Items	Control	Additive 1	Additive 2	Additive 3	Additive 4
Acid detergent fibre (g kg^{-1})	279.00[a]	256.10[a]	278.50[a]	269.50[a]	251.60[a]
Neutral detergent fibre (g kg^{-1})	595.60[a]	555.30[a]	456.50[b]	513.10[a]	540.80[a]
Crude protein (g kg^{-1})	99.40[ab]	95.60[ab]	104.60[a]	91.50[b]	96.90[ab]
Water soluble carbohydrates (g kg^{-1})	15.50[a]	11.60[b]	9.70[b]	11.70[b]	11.10[b]
Total volatile fatty acid (Mmol kg^{-1})	858.00[b]	863.70[b]	1552.30[a]	878.30[b]	847.70[b]
Digestible dry matter[2] (g kg^{-1})	671.60[a]	689.40[a]	672.00[a]	679.00[a]	692.90[a]
Digestible energy[3] (Mcal kg^{-1})	2.89[a]	2.97[a]	2.89[a]	2.92[a]	2.98[a]
Metabolizable energy[4] (Mcal kg^{-1})	2.37[a]	2.43[a]	2.37[a]	2.40[a]	2.45[a]
Total digestible nutrient[5] (g kg^{-1})	656.60[a]	673.80[a]	656.90[a]	663.70[a]	677.20[a]

[1]Control: Without any additive. Additive 1: Propionic acid. Additive 2: Propionic acid (85%)+formic acid(15%), Additive 3: Calcium propionate. Additive 4: Propionic acid (80%)+formic acid (15%)+ammonia (5%), [2]Digestable Dry Matter: 88.9-0.779 (ADF), [3]Digestable Energy: 0.027+0.0427 (%DDM). [4]Metabolizable Energy: DE *0.821. [5] Total digestible nutrition: DE / 0.04409 (Khalil *et al*., 1986). Unlike superscript in a row differ significantly (p<0.05)

Table 3: Apparent in situ DM digestion of corn silages (sealing time is fixed)

	Treatments[1]					Delaying time of sealing[2]	
Items	Control	Additive 1	Additive 2	Additive 3	Additive 4	0	24 h
DM digestion (g kg^{-1})	268.60[c]	403.40[ab]	340.90[bc]	331.60[bc]	426.90[a]	377.70[a]	359.00[a]
Kd[3]%/h	5.10	5.80	5.60	5.40	6.10	5.70	5.40
SE	1.22	1.61	1.68	1.68	1.78	1.37	1.84

[1]Control: Without any additive. Additive 1: Propionic acid. Additive 2: Propionic acid (85%) +formic acid (15%), Additive 3: Calcium propionate. Additive 4: Propionic acid (80%) +Formic acid (15%) +Ammonia (5%). [2]Delaying time of sealing : 0 h, 24 h. kd[3]: Rate of digestion. Unlike superscript in a row differ significantly (p<0.05)

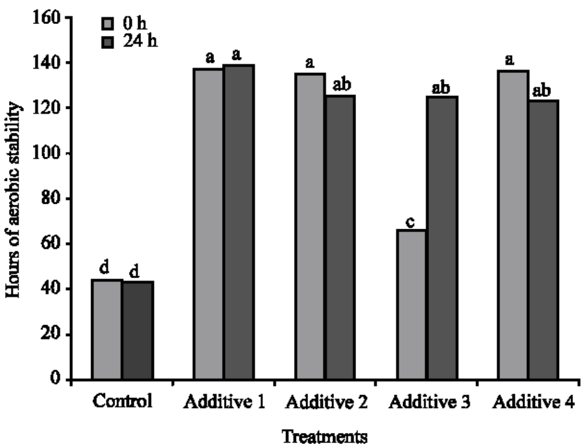

Fig. 1: The aerobic stability of corn silage. Control: Without any additive. Additive 1: Propionic acid. Additive 2: Propionic acid (85%) +formic acid (15%). Additive 3: Calcium propionate. Additive 4: Propionic acid (80%) +formic acid (15%) +Ammonia (5%). Delaying time of sealing: 0, 24 h. SE: 10. Bars with unlike letters differ (p<0.05)

Chemical composition: Untreated silage (control group) that was ensiled either immediately or after 24 h, had higher pH than treated ones (p<0.05). Significant interaction happened between silages for concentration of dry matter. The control group that was ensiled immediately had lower DM content compared with treated silage with additive 1. On the other hand in the control group* 24 h delayed ensiling, silage DM were higher than in additives 1, 4 (p<0.05). For this type of additive* 24 h delayed ensiling, silage with additive 3 had higher concentration of DM compared with the other treatments (p<0.05). It was observed that timing of treatment with buffered propionic acid either at chopping or after exposure to air made no difference in ADF content , but the amount of NDF in treated silage with additive 2 (propionic acid (85%)+formic acid (15%)) was lower than other treatments and control groups (p<0.05). The CP concentration of the additive 2 (104.6 g kg^{-1}) were significantly higher than the treatment 3 (p<0.05). The WSC concentration of silages without any additive control were higher than the other treatments (p<0.05). The TVFA concentration of treatment 2 (1552.3 Mmol kg^{-1}) were significantly higher than other treatments and control group (p<0.05). Concerning concentration of DDM, DE, ME and TDN in the silages had no significant effects on the type of additive and or the sealing time (Table 2).

***In situ* ruminal DM digestibility:** Addition of additives 1 and 4 to silages were positive affected on *in situ* ruminal DM digestibility of treated silages. The *in situ* ruminal DM digestion of control group (268.6 g kg^{-1}) was significantly lower than that of treatment 1(403.4 g kg^{-1}) and 4(426.9 g kg^{-1}) (p<0.05). Sealing time had no effect on *in situ* ruminal DM digestibility of silages (Table 3).

Aerobic stability: Significant interactions between additives and sealing time were found for pH and temperature (p<0.05). The temperature of the control silages in all of the sealing time after 48 h exposure to air 2°C increased and after 144 h to peak of temperature (immediately = 34.05°C, 24 h, delaying = 28.95°C). The aerobic stability of control groups were lower than the other ($p<0.05$). The acidity of the control silages in all of the sealing time after 24h exposure increased too (Fig. 1).

DISCUSSION

Appearance evaluation: In present research the applications of organic acid-based additives improved quality of fermentation corn silage and this supports earlier studies of Kung *et al*. (1998, 2000, 2004), Therefore, all of appearance quality of silages were in good and very good class.

Chemical composition: Untreated silage had higher pH than treated silages, similar to the results of Kung *et al*. (2000, 2004) and Randby (2000). Addition of the buffered propionic acid-based additive decreased pH, which suggests that these additives partially reduced the metabolism of some aerobic microorganisms (Kung *et al*., 1998).

Observations of DM were similar to those of Kung *et al*. (2004) and the reason related to the limiting fermentation power of formic acid and increasing DM content power of propionic acid unknown. However, Kung *et al*. (2000) reported that low levels of buffered propionic acid generally had no effect on silage fermentation. Addition and blending of some additives at time of ensiling improve fermentation and increase DM of silages (Harrison and Blauwiekel, 1994; Givens *et al*., 1993). Calcium propionate is the salt of propionic acid that increases DM with raising pH and limiting fermentation (Oude Elferink *et al*., 1999). We did not measure DM losses caused by delayed ensiling, but losses of DM during aerobic exposure before ensiling have been reported to be small relative to losses incurred in the subsequent ensiling and storage period (Henderson and McDonald, 1975; Mills and Kung, 2002).

Timing of treatment with buffered propionic acid either at chopping or after exposure to air made no difference in ADF content and the reason for decreased amount of NDF in treated silage with additive 2 (propionic acid (85%)+formic acid (15%)) compared with the other treatments and the control groups are unknown. Kung *et al*. (1998, 2000) reported that the application of the buffered propionic acid-based additives had no effect on fermentation end-products. In this study, the crud protein content of silages with additive 2 was higher than additive 3 (calcium propionate). Randby (2000) reported that application of formic acid in silages with 24 h delayed in ensiling, improved the quality of fermentation, increase amount of true protein and sugar but decreased the content of acetic acid, propionic acid, ammonia-N and pH. Additives that consisted of formic acid limit fermentation; decrease ammonia-N and increase quality of silage protein.

Mills and Kung (2002) reported that the content of WSC didn't have any difference between treatments that consisted of the buffered propionic acid-based additive and ensiling at 0 and 24 h after chopping forage. For type of additive* 24 h delayed ensiling, control silage had higher concentration of WSC compared with other treatments. In addition of preservative effect of buffered propionic acid-based additive, application of this additive resulted in little differences in end-productions of corn silage, but treating corn silages with 0.2% (w/w wet forage) of buffered propionic acid-based additive were increased amount of WSC (Kung *et al*., 2004). Of course, other factors are effective in WSC content of silages, including: variation of plant, stage of growth, effect of length of the day, effect of manure (McDonald *et al*., 1991), effect of witting and protein content of plant (Umana, 1991). This is a possible reason for the different results from earlier study related to the other effective factors in WSC.

TVFA concentrations of treatment 2 were significantly higher than others, which is similar to values reported by Kung *et al*. (2004). It has been mentioned that addition of buffered propionic acid-based additive to silage caused increase Volatile Fatty Acid (VFA) and the highest was for propionic acid content of silage. Timing of treatment with buffered propionic acid-based additive either at chopping or after exposure to air made difference in forage content of TVFA. Randby (2000) indicated that application of formic acid additive to silage improved quality of corn silage and when the sealing was delayed 24 h, additives could reduce the negative effects of delaying. Results from some studies detected that additive compound of formic acid influence amount of VFA and the content of propionic acid increase in corn silage treated with buffered propionic acid-based additive. Treating corn silage with 0.2% (w/w wet forage) with these additives caused an increasing of lactic acid, acetic acid and propionic acid contents compared with untreated silages (Kung *et al*., 2004).

The concentrate of DDM, DE, ME and TDN in the silages have influenced by content of ADF, which is similar to results reported by Kung *et al*. (1998, 2000) that mentioned the buffered propionic acid-based additive have little effect on some nutrients (e.g., ADF, NDF and starch).

Taking into consideration the effects of the buffered propionic acid-based additive on the chemical composition of silages in this study, it could be inferred that treating corn silage with this additives approximately improved quality of nutritive value.

***In situ* ruminal DM digestibility:** Sealing time had no effect on *in situ* ruminal DM digestibility of silages. Treating with the buffered propionic acid-based additive prevented the negative effects of exposing to air before ensiling on *in situ* ruminal DM digestibility (Mills and Kung, 2002). Collectively, these data showed that the buffered propionic acid-based products can improve the fermentation and increase nutritive value and final aerobic stability of silages.

Aerobic stability: Temperature and pH values of silages after opening demonstrated that wilting forage before ensiling, affected in inhibiting temperature raise of silage after exposing to air (Harrison and Blauwiekel, 1994). Among the experimental additives in all sealing time, additive 1 was more effective than others for inhibiting of raising temperature of silages, but generally all of the additives in all of the sealing time had significant effects on increasing aerobic stability, which is similar to results of Kung *et al.* (1998, 2004) and Mills and Kung (2002).

The objective of this study was to determine the effects of some different buffered propionic acid-based additive on the characters of storage corn silage including aerobic stability, that just as indicated these additives had benefit effect on this factor.

CONCLUSION

Present results shows that the use of a buffered propionic acid-based additive can partially, but not totally, compensate for poor silo management practices. However, rapid filling of silos and achieving adequate packing densities to exclude excessive air should still be high priorities for making excellent quality silage.

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REFERENCES

Driehuis, F. and P.G. Van Wikeselaar, 2000. The occurrence and prevention of ethanol fermentation in high dry matter grass silage. J. Sci. Food Agric., 80: 711-718.

Duncan, D.B., 1955. Multiple range and multiple F-tests. Biometrics, 11: 1-42.

Givens, D.I., A.R. Moss and A.H. Adamson, 1993. The digestion and energy value of whole crop wheat treated with urea. Anim. Feed Sci. Technol., 43: 51-64.

Haigh, P.M., 1988. The effect of wilting and silage additives on the fermentation of autumn made grass silage ensiled in bunkers on commercial farms in South wales. Grass Forage Sci., 43: 337-345.

Harrison, J.H. and R. Blauwiekel, 1994. Fermentation and utilization of grass silage. J. Dairy Sci., 77: 3209-3235.

Henderson, A.R. and P. McDonald, 1975. The effect of delayed sealing on fermentation and losses during ensilage. J. Sci. Food Agric., 26: 653-667.

Horiguchi, K.I. and T. Takahashi, 2007. Fermentation quality and nutritive value of green soybean stover silage. Grassland Sci., 53: 27-31.

Johnson, L.M., J.H. Harrison, D. Daridson, J.L. Robutti, M. Switt, W.C. Muhanna and K. Shinners, 2002. Corn silage management, I: Effect of hybrid, maturity and mechanical processing on chemical and physical characteristics. J. Dairy Sci., 85: 833-853.

Johnson, L.M., J.H. Harrison, D. Davidson, C. Hunt, W.C. Mahanna and K. Shinnerss, 2003. Corn silage management: Effects of hybrid, maturity, chop length and mechanical processing on rate and extent of digestion. J. Dairy Sci., 86: 3271-3299.

Khalil, J.K., W.N. Sawaya and S.Z. Hyder, 1986. Nutrient composition of Atriplex leaves grown in Saudi Arabiq. J. Ranye Manaye, 39: 104-107.

Kung, Jr. L., A.C. Sheperd, A.M. Smagala, K.M. Enders, C.A. Bessett, N.K. Ranjit and J.L. Glancey, 1998. The effect of preservatives based on propionic acid on the fermentation and aerobic stability of corn silage and a total mixed ration. J. Dairy Sci., 81: 1322-1330.

Kung, Jr. L., J.R. Robinson, N.K. Ranjit, J.H. Chen, C.M. Golt and J.D. Pesek, 2000. Microbial populations, fermentation end-products and aerobic stability of corn silage treated with ammunia or a propionic acid-based preservative. J. Dairy Sci., 83: 1479-1486.

Kung, L., C.L. Myers, J.M. Neylon, C.C. Taylor, J. Lazartic, J.A. Mills and A.G. Whiter, 2004. The effect of buffered propionic acid-based additive alone or combined with microbial inoculation on the fermentation of high moisture corn and whole-crop barley. J. Dairy Sci., 87: 1310-1316.

McAllister, T.A., L.B. Selinger, L.R. McMahon, H.D. Bae, T.J. Lysyk, S.J. Oosting and K.J. Cheng, 1995. Intake, digestibility and aerobic stability of barley silage inoculated with mixtures of *Lactobacillus plantarum* and *Enterococcus faecium*. Can. J. Anim. Sci., 75: 425-432.

McDonald, P., A.R. Henderson and S.J.E. Heron, 1991. The Biochemistry of Silage. 2nd Edn., Chalcombe Publications, Marlow, Bucks, UK., ISBN: 0-948617-22-5, pp: 340.

Mills, J.A. and L. Jr. Kung, 2002. The effect of delayed ensiling and application of a propionic acid-based additive on the fermentation of barly silage. J. Dairy Sci., 85: 1969-1975.

Nelson, N., 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem., 153: 375-380.

Oude Elferink, S.J.W.H., F. Driehuis, J.C. Gottschal and S.F. Spoelstra, 1999. Silage fermentation prosses and their manipulation. Proceedings of the FAO Electronic Conference on Typical Silage, 1999, AGPC., pp: 1-1.

Pauly, T. and P. Lingvall, 1999. Effects of mechanical forage treatments and surfactants on fermentation of grass silage. Acta Agric. Anim. Sci., 49: 197-205.

Phillip, L.E. and V. Fellner, 1992. Effect of bacterial inoculation of high moisture ear corn on its aerobic stability, digestion and utilization for growth by beef steers. J. Anim. Sci., 70: 3178-3187.

Randby, A.T., 2000. The effect of some acid-based additives applied to wet grass crops under various ensiling conditions. Grass Forage Sci., 55: 289-299.

SAS., 1988. SAS User's Guide: Statistics. Ver. 6.03, SAS Inst., Inc., Cary, NC.

Snyman, L.D. and H.W. Joubert, 1996. Effect of maturity stage and method of preservation on the yield and quality of forage sorghum. Anim. Feed Sci. Technol., 57: 63-73.

Tapia, M.O., M.D. Stern, A.L. Soraci, R. Meronuk, W. Olson, S. Gold, R.L. Koski-Hulbert and M.J. Murphy, 2005. Patulin-producing molds in corn silage and high moisture corn and effects of patulin on fermentation by ruminal microbes in continous culture. Anim. Feed Sci. Technol., 119: 247-258.

Umana, R.C., R. Staples, D.B. Bates, C.J. Wilcox and W.C. Mahanna, 1991. Effects of a microbial inoculant and (or) sugar cane molasses on the fermentation, aerobic stability and digestibility of bermudagrass ensiled at two moisture contents. J. Anim. Sci., 69: 4588-4601.

Van Soest, P.J., J.B. Robertson and B.A. Lewis, 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583-3597.

Weinberg, Z.G. and R.E. Muck, 1996. New trends and opportunities in the development and use of inoculants for silage. FEMS. Microbiol. Rev., 19: 53-68.

Woolford, M.K., 1990. The detrimental effects of air on silage. J. Applied Bacteriol., 68: 101-116.