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The Study of Possibility Corn Silage Quality Improvement and Ensiling Period Reduction by Using Microbial Additives

H. Mansoori Yarahmadi and J. Fakhraei
Department of Animal Science, Faculty of Agriculture, Islamic Azad University of Arak, Iran

Abstract: This experiment was done for quality improvement and decrease ensiling period from 21 to 14 day. Experimental design was completely randomized design with 5 treatments by adding *Lactobacillus Plantarum* (LP) and *Saccharomyses Cervicea* (SC) and 3 repeat. Treatments included: 1-without any additive as control (LP0-SC0), 2-0.5 g *Lactobacillus Plantarum* in 100 kg corn forage (LP1-SC0), 3-0.75 g *Lactobacillus Plantarum* in 100 kg corn forage (LP2-SC0), 4-0.5 g *Saccharomyses Cervicea* in 100 kg corn forage (LP0-SC1) and 5-0.5 g *Lactobacillus Plantarum* and 0.5 g Saccharomyses *Cervicea* in 100 kg corn forage (LP1-SC1). Silo construction simulated by dark barrel bung. Sampling and evaluating was done for pH, DM, lactic acid, acetic acid and apparent specifications at the 7, 14, 21 days. Treatment LP2-SC0 had lowest pH and highest lactic acid. Results showed that corn silage inoculants by *L. plantarum* had prepared completely and had high score at 14 and 21 day. pH and lactic acid at 21 day in 3rd treatment was significant with other treatment ($p<0.05$). Therefore by using of suitable microbial additives, can decrease ensiling period from 21-14 day and prevent from harmful facts. But corn silage consumption is not recommended less than 21 day.

Key words: Silage, *lactobacillus plantarum*, ensiling period, quality improvement

INTRODUCTION

Silages are provided with fermentation of green forages especially corn forage. For production of H^+ ion and pH reduction, it is necessary to establish an anaerobic conditions and prevention or reduction from inappropriate process fermentation and to stimulate growth of lactic acid bacteria (Liminkung, 2003).

For prevention of plant respiration and heat increase, it is necessary to create an anaerobic condition quickly. Protein degradation will reduce associated with pH depression of corn silage. Lactic acid bacteria produce lactic acid from water-soluble carbohydrates (Sufisiavash, 2001).

Decrease of pH will prevent *Clostridia* and *Entobacteria* activity, which result in Butyric acid production and increasing of pH. *Entrobacteria* can produce ammonia and increase pH. Also some of the Fungi such as *Pyromonia Communis* spoilage corn silage. Supplemented forage silage with additives such as stimulators of lactic acid bacteria has fine population, which is sufficient for satisfactory fermentation. Overcoming lactic acid bacteria rapidly will cause water-soluble carbohydrates to be utilizable efficiently. Such silages have lowest pH, more water-soluble carbohydrate and more lactic acid concentration and lowest acetic acid and ethanol concentration.

These factors can improve animal feeding and their efficiency (McDonald *et al*., 1991) and (Muck, 2000). Today some commercial additives exist that consist of species of lactic acid bacteria. On the other hand, evidence showed using additives cause an increasing in feed value, favorability and preservation of the corn silage (Hashemi, 1997). Microbial additives are included *Lactobacillus Acidophilus*, *Lactobacillus Buchneri* and *Lactobacillus Plantarum* (Kung and Ranjit, 2001).

Several studies have suggested improvement of fermentation case by microbial additives. Kung and Ranjit (2000) reported silage inoculation by bacteria caused pH reduction, increase of ratio lactic acid to acetic acid and decrease of ammonia nitrogen. Silage enduring improved more than 30% and also dry matter digestibility. Ranjit and Kung (2000) confirmed positive animal interaction to bacteria adding in corn silage. On the base results of Shaver (2004), *Lactobacillus* bacteria are caused rapidly decrease of pH and protein degradation. Increasing of lactic acid production, silage will be acidity and is prevent from dry matter loss due the decrease in fermentation. Prevention of harmful protozoa activity reported by Muck (2000) due the pH reduction has been confirmed previously.

Corresponding Author: H. Mansoori Yarahmadi, Department of Animal Science, Faculty of Agriculture, Islamic Azad University of Arak, Iran

The objective of this study, was to preventing of harmful factors activity and to accelerate the fermentation process using *Lactobacillus plantarum* as lactic acid bacteria and pH reducer. Meanwhile by adding a yeast as *Saccharomyses cervicea* to many experimental material for ethanol and CO2 production, was reviewed their effect and mechanism on the unsuitable fermentation and also useful role of Lactobacillus bacteria to ability of overcoming on yeasts and neutralization their effect harmful on silage. Despite of company producer recommendations to using of *Lactobacillus plantarum*, but no advice is at a condition which is found yeast and fungi activity in the silage (Hashemi, 1997) and (Sufisiavash, 2001).

MATERIALS AND METHODS

Forage and treatments: This experiment was done in Azad University of Arak-Iran dairy farm. Corn plant was harvested at one-half milk line and chopped to a length of almost 5 cm for corn ensiling, it is used from dark barrel bangs with 50 kg capacity (Taghizadeh *et al*., 2007). Chemical composition of corn forage was determined by A.O.A.C (1995) and is presented in Table 1.

Treatments included: 1-Forage without any additive such as control (LP0-SC0). 2-0.5 g *L. plantarum* in 100 kg forage (LP1-SC0). 3-0.75 g *L. plantarum* in 100 kg forage (LP2-SC0). 4-0.5 g S.*cervicea* and without *L. plantarum* in 100 kg forage (LP0-SC1). 5-0.5 g *S. cervicea* and 0.5 g *L. plantarum* in 100 kg forage (LP1-SC1). *Lactobacillus Plantarum* with its commercial named (ICOSYL) made in England and *Saccharomyses cervicea* with its commercial named (BIOSAFF) made in France was provided from Makiandaroo Co. of Iran.

Amounts of adding were calculated according to company producer recommendations, but with nir changes. ICOSYL and BIOSAFF were weighted by digital scale (100±0.01) and dissolved in water and spread on the each treatment layer by layer then forage was added in bungs. Corn forage was ensiled in 15 dark barrel bungs (in each bung, 50 kg). Forages in bungs were compacted for air exit, then their doors were closed and kept in a room 25°C (Kung *et al*., 2000).

Table 1: Chemical composition of chopped corn silage before ensiling

Chemical composition	% of DM
Dry matter	30.08
Crude protein	5.99
Crude fat	1.52
Ash	6.08
Crude fiber	22.36
ADF	24.10
NDF	45.29

Sampling and analyses: The sampling was done for evaluation and determination of silage prepare at 7, 14 and 21 days. Variables like DM, pH, lactic acid and acetic acid concentration and visible assessments by Bates method (2004) evaluated at each those time. Fifty gram of fresh forage (after treated) from each treatment were collected for chemical analyses and diluted in 450 mL sterile de-ionized water and blended for 1 min. silage pH was determined immediately (digital pH-meter HTW-320 model) (Taghizadeh *et al*., 2007) and (Taghizadeh *et al*., 2006). Collected samples were filtered (Whatman no.45 filter paper) and centrifugation (5700×g for 10 min). Lactic and acetic acid concentration were measured by NaOH titration according of A.O.A.C (1995). For this purpose 50 g fresh forage mixed with 900 mL distilled water. After filtration, 200 mL from those solutions have been reached 250 mL by distilled water. Then 10 mL $Ca(Co3)_2$ and 10 mL $Cu(So4)_2$ and 30 mL distilled water were added to those solutions. Finally solutions have been distilled in 300°C. 100 mL of first distilled and 50 mL of second distilled solution were titrated by NaOH for determination of lactic and acetic acid. Samples were then dried in oven at 55°C until a constant weight (Okuda *et al*., 1965; Weinberg *et al*., 1999).

Statistical model was completely randomized design with 3 repeat. Data were analyzed using the General Linear Model (GLM). Procedure of (SAS institute Inc) with Duncan's multiple range test used for the comparison of means. The test treatments were the only sources of the variation considers analytical variability was included in the error variance (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

The chemical composition of the treatments in this experiment is presented in Table 2. The DM content of the LP0-SC1 was lower and LP1-SC1 was higher than other treatments and the CP content was contrary. pH, DM, lactic acid and acetic acid as four important performance have been determined in this study. The results of mean pH, % DM, lactic acid and acetic acid (mL/kg/DM) are presented in Fig. 1-4.

Table 2: Chemical composition of the experimental treatments (%) in 21 day

Item	LP0-SC0	LP1-SC0	LP2-SC0	LP0-SC1	LP1-SC1
DM	31.25	31.37	30.85	30.73	32.42
CP	6.20	6.22	6.37	6.78	6.15
N	0.99	0.99	1.02	1.04	0.98
NDF	43.22	44.03	42.85	42.29	43.96
ADF	24.00	23.72	23.53	23.30	23.65
Hemicelluloses*	19.22	20.31	19.32	18.99	20.31

*Hemicelluses = NDF-ADF

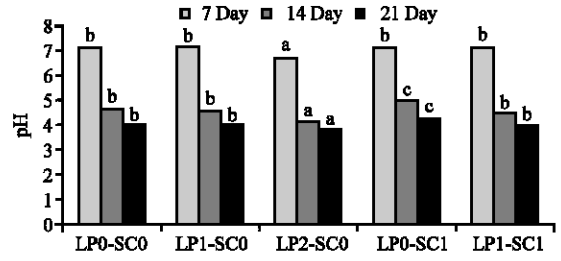

Fig. 1: pH of corn silage at 7-14-21 days

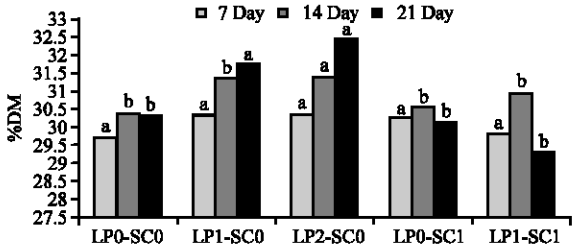

Fig. 2: DM of corn silage at 7-14-21 days

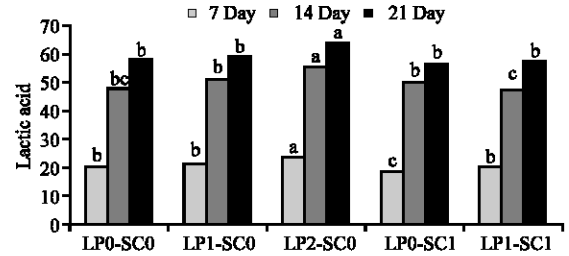

Fig. 3: Lactic acid of corn silage (mL kg^{-1}) at 7-14-21 days

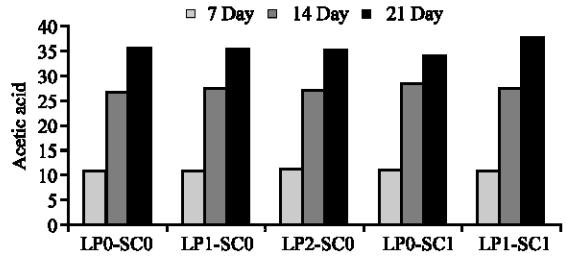

Fig. 4: Acetic acid of corn silage (mL kg^{-1}) at 7-14-21 days (non significant)

Results demonstrated that the best performance for pH obtained 6.72-4.15 and 3.81 with LP2-SC0 and lactic acid was 23.38-55.42 and 63.28 (mL/kg/DM) in 7-14 and 21 days, respectively.

The changes of pH were significant between treatments. This variation was lower in LP2-SC0 in 21 day. Also increase of lactic acid concentration was significant in LP2-SC0 in comparison to other treatments ($p<0.05$). Control treatment (LP0-SC0) did not show any significant different compared to other treatments except for pH in LP2-SC0 in total period and in LP0-SC1 in 14 and 21 days and also for lactic acid concentration in LP2-SC0 at total period ($p>0.05$). Supplemented silage with yeast (LP0-SC1) had undesirable silage specific in the manner pH and lactic acid concentration in that treatment (LP0-SC1) was 4.24 and 56.49 in 21 day. This silage shown more pH and less lactic acid concentration compared to other silages. Although it has exist any reason why this silage doesn't be consumption. In LP1-SC1 treatment that contained bacteria and yeast together, pH in 14 and 21 days were 4.52 and 3.99 and lactic acid were 47.25 and 57.31 (mL/kg/DM), respectively. In this treatment is observed acceptable symptoms for feeding to animal because did not show significant different to control. Amounts of acetic acid hadn't significant different (Table 3 and Fig. 1-4).

Silage visible assessments that considered as Bates method (2004) is presented in Table 4. Results showed that LP2-SC0 with 0.75 g *L.plantarum* in fresh forage has obtained 78 and 83 score and LP1-SC0 with 0.50 g *L.plantarum* 73 and 81 score in 14 and 21 days. Those treatments had fine quality (at 21 day) and moderate quality (at 14 day). Corn silage with LP0-SC0, LP1-SC1 and LP0-SC1 had 77, 73 and 67 score at the end of 21 day, respectively (Fig. 5).

Obtained results showed pH in 14 day is closest to pH in 21 day in all treatments except for LP0-SC1. These results have obtained by Adesogan *et al.* (2003), Charalampopoulos *et al.* (2002) and Dellagio (2002 and 1995). The pH reduction have caused with increasing of acid concentration. Lactic acid increasing is considerable in the groups that contained additives too. Base upon this result, lactic acid are produced 54.42 and 63.78 (mL/kg/DM) in LP2-SC0 at the 14 and 21 days. Therefore,

Table 3: pH, DM, lactic acid and acetic acid of corn silage at 7-14-21 days

	pH			DM			Lactic acid			Acetic acid (ns*)		
	7	14	21	7	14	21	7	14	21	7	14	21
LP0-SC0	7.12^{b}	4.63^{b}	4.01^{b}	29.64^{a}	30.05^{a}	30.31^{b}	20.44^{b}	48.28^{bc}	58.31^{b}	10.72	26.75	35.62
LP1-SC0	7.16^{b}	4.57^{b}	3.98^{b}	30.35^{a}	31.34^{a}	31.72^{b}	21.25^{b}	50.77^{b}	59.18^{b}	11.15	27.43	35.13
LP2-SC0	6.72^{b}	4.15^{a}	3.81^{a}	30.35^{a}	31.34^{a}	32.40^{a}	23.38^{a}	54.42^{a}	63.78^{a}	11.17	27.31	34.86
LP0-SC1	7.15^{b}	4.98^{c}	4.24^{c}	30.22^{a}	30.52^{a}	30.12^{b}	18.75^{c}	50.42^{b}	56.49^{b}	11.94	28.33	35.21
LP1-SC1	7.15^{b}	4.52^{b}	3.99^{b}	29.76^{a}	30.90^{a}	29.29^{b}	20.34^{b}	47.25^{c}	57.31^{b}	10.68	27.67	35.55
SEM			0.64			2.43			3.78			0.82

*Non significant between treatment

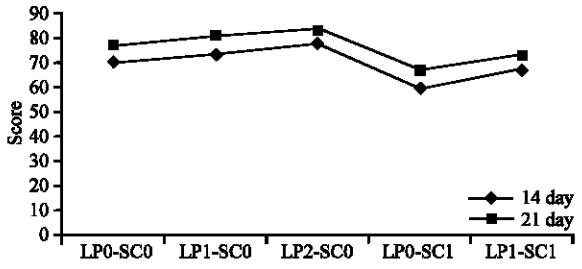

Fig. 5: Apparent value of corn silage with Bates method at 14 and 21 days

Table 4: Apparent value and quality characteristics of corn silage by Bates method at 14 and 21 days*

	LP0-SC0	LP1-SC0	LP2-SC0	LP0-SC1	LP1-SC1
Grain amount					
14 day	30	30	30	30	30
21 day	30	30	30	30	30
Color					
14 day	7	7	8	5	6
21 day	8	9	10	5	7
Odor					
14 day	20	23	25	12	17
21 day	24	26	27	15	20
Moisture					
14 day	6	6	8	6	7
21 day	8	9	9	10	9
Particle size					
14 day	7	7	7	7	7
21 day	7	7	7	7	7
Sum of scores					
14 day	70	73	78	60	67
21 day	77	81	83	67	73

* 90 and more than: Excellent 80-90: Well 65-79: Middle under 65: Poor

pH reduction can relate to increasing of lactic acid. Heristov *et al.* (2001) and Heron *et al.* (1999) have been reported that pH reduction could be achieved due to increase in acetic acid and butyric acid concentration. In those treatments that used *L.plantarum*, apparent appearance showed that lactic acid had the highest amount and acetic acid and butyric acid had the lowest values (Charalampopoulos *et al.*, 2002) and (Hristov *et al.*, 2001). Also Shaver (2004) reported that adding lactic acid bacteria such as *L. plantarum* to corn forage could improve fermentation and produce more lactic acid in comparison to other bacterial additives that produce butyric acid and CO^2.

Moreover results showed that animals can consume treated corn silage with lactic acid produced bacteria after 14 day, because pH and lactic acid were equal 4.15 and 54.42 (mL/kg/DM) in LP2-SC0. Adding *Saccharomyces Cervicea* (LP0-SC1) produced poor quality corn silage in 14 and middle quality in 21 days. Better results obtained in LP1-SC1 to LP0-SC1. In LP1-SC1 treatment acceptable signs are considered for feeding to animals at 21 days but it must be of concern.

These results are in agreement with Kung and Ranjit (2001), Ranjit and Kung (2000) and Weinberg *et al.* (1999) reports. By reviewing these experimental results and compare to other researchers experiments, we can say that adding of suitable amounts of bacterial additives especially lactic acid bacteria such as *L. plantarum,* the ensiling period could decrease from 21-14 days and corn silage quality will protect and prevent from damaging agents such as yeasts that produce butyric acid and CO2.

CONCLUSION

This is important that must be caution to feeding of silage that produced by 14 days (Liminkung, 2003). Usually consumption of silage at less than 21 days isn't recommended even with all prepare and provide condition of corn silage. On the other hand, must be notice to buy cost of commercial bacteria. Besides it is request to analyses of Non-Ammonia Nitrogen (NAN), Acid Detergent Insoluble Nitrogen (ADIN), butyric acid and Ethanol in later researches.

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