Indian J. Anim. Nutr. 2021. 38 (2): 167-172
doi: 10.5958/2231-6744.2021.00024.4

Indian Journal of Animal Nutrition

Effect of Different Additives on Bale Silage Quality of Maize Fodder

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ABSTRACT

The present study was conductedto evaluate the effectof different additives on the bale silage quality of maize fodder. An experiment was conducted with four different treatments of maize fodder before bailing them. These treatments consisted of; control without additive (MF_0), *Lactobacillus casei* at 10^6 CFU/g (NCDC-17) of fresh fodder (MF_{LAB}), Silotan, a commercial product at 15ml/100 kg of fresh maize fodder (MF_{ST}) and formaldehyde at 0.5% of fresh maize fodder (MF_{FA}) as silage additives. The bale silage of four such treatments MF_0, MF_{LAB}, MF_{ST} and MF_{FA},in replications (3*9 treatments) was prepared by using a high power 100 kg bailing machine. Each treatment was evaluated after 60 days of ensiling in terms of quality parameters like chemical composition, fermentation characteristics, microbial (LAB) counts and *in vitro* DM degradability. The evaluation of bale silage revealed a significant increase (P<0.001) in pH and decrease (P<0.001) in ammonia nitrogen in MF_{FA}. There was no significant effect on DM, CP, EE and ADF contents of any treatment, however a significant increase (P<0.001) in WSC was observed in MF_{FA}, with a decrease (P<0.001) in WSC of MF_{LAB}. The TVFAs were significantly increased (P<0.001) in MF_{LAB} and MF_{ST}, with a decrease (P<0.001) in MF_{FA}. There was a significant decrease (P<0.001) in acetate content of MF_{FA} while a significant reduction (P<0.001) in butyrate was observed in MF_{ST} and MF_{FA}. There was a significant decrease (P<0.001) in Lactic acid content of MF_{FA}. The *in vitro* DM degradability was significantly increased (P<0.05) in MF_{LAB}. There was no significant effect of treatments on methane (ml/g DM) production in any of the treatments. The microbial counts were found significantly increased (P<0.05) in MF_{LAB}, whilst a decrease (P<0.05) of microbial count was noted in MF_{FA}, Yeast counts were significantly decreased (P<0.05) in MF_{ST} with increase in MF_{FA}. It was concluded that Silotan or MF_{LAB} can be used to improve the quality of bale silage.

Key words: Bale Silage, Formaldehyde, *Lactobacillus casei,* Silotan

INTRODUCTION

The principle of silage fermentation is to achieve a sufficient quantity of lactic acid to inhibit the growth of undesirable epiphytic microorganisms and the activity of endogenous plant catabolic enzymes, thus maximizing nutrient preservation. In previous times, pit/silo methods of making silage were preferred as the basic conditions required for the effective preservation of silage (*i.e.* anaerobic storage, high density and low pH) were difficult to achieve in big bale silage, because the herbage was not properly chopped due to which it was difficult to achieve proper density in the bales. However with the advancement in bailing machines, high power bailing machines are available now to chaff the fodder and consequently densify/ensile it compactly to make good quality silages. Silage additives may be chemical or biological, and can be categorized as stimulants, inhibitors, nutrients or absorbents (McDonald *et al.,* 1991). Information regarding the effects of the additives on the quality of these bales is limited. However, it is known that silage quality can be improved by adding LAB, molasses, formic acid and other organic acids, or mixtures of organic acids and salt to the bales. Limited information is available about use of "Silotan" as a silage additive. Our objective was to study the effect of three different additives viz *Lactobacillus casei*, formaldehyde and Silotan (a commercial product containing Chest nut extract) on the quality of the bale silage.

MATERIALS AND METHODS

Treatments consisted of different additives as MF_0 as control group (without the use of any additive), MF_{LAB} with addition of *Lactobacillus casei* at 10^6 CFU/g of fresh fodder, MF_{ST} with the use of Silotan, a commercial product made from chest nut extract at 15 ml/100 kg of fresh maize fodder and MF_{FA} with the

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addition of formaldehyde at 0.5% of fresh maize fodder. The bale silage was prepared by using a 100 kg bailing machine. Duration of ensiling was 60 d. The silage was then evaluated for silage quality, namely chemical composition, and microbial quality and *in vitro* digestibility and methane production.

The silage sample was analyzed for colour, texture, and smell. The texture was observed by pressing the silage between two fingers. Colour was observed visually. Mildly ammonical, pleasantly acidic and natural yogurt smell was preferred (Breirem and Ulvesli, 1960). Silage samples were oven dried at 60°C for 72 h. The dried samples were ground to pass through a 1 mm screen for subsequent analyses of neutral detergent fibre (NDF), acid detergent fibre (ADF), water soluble carbohydrates (WSC), crude protein (CP), and *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility IVOMD. NDF and ADF were determined according to the method of Van Soest *et al.* (1991). WSC was determined by the method of Yemm and Willis (1954). CP was determined according to the method of Association of Official Analytical Chemists (AOAC, 2005). Ash was estimated by combustion of the samples in a muffle furnace at 550°C for 5 h. The IVDMD, IVOMD and *in vitro* gas production was measured following methods of Menke and Steingass (1988). Approximately 200 mg of sample was weighed, placed into a graduated glass syringe provided with a piston, and ûlled with 30 ml of buffered rumen ûuid. Each of the incubation was run in triplicate. The glass syringes containing samples, rumen ûuid and buffer mixtures were incubated in a water bath at 39°C and the gas production was subsequently measured before incubation (0 h) and at 8, 24, and 48 h after incubation. Total gas values were corrected for blank. After the incubation of feeds, suitable aliquot of gas was withdrawn from the tip of the syringe using Hamilton gas tight syringe and was analyzed methane, using Gas chromatograph (Nucon 5700, India). For the measurements of pH, ammonia nitrogen, total volatile fatty acid (TVFA), water extract of silage was prepared by adding 90 ml of distilled water to 10 g of fresh samples in a beaker and homogenized by mechanical homogenizer. A few drops of 0.1% mercuric chloride were added in the sample, stirred thoroughly and kept in refrigerator at 4ºC. After 48 hours, the material was filtered through four layers of cheese cloth and stored in refrigerator at 4ºC. The pH was measured by using a Eutech pH meter (Oakton Instruments, IL USA) and the buffering capacity of the sample was measured as per the method of Playne and McDonald (1996). For determining individual VFA, 5 ml of water extract was taken in a beaker and 1 ml of 25 % metaphosphoric acid (prepared in 5N H_2SO_4) was added. Samples were kept overnight and centrifuged at 3500-4000 rpm for 15-20 minutes. The supernatant was injected in gas chromatograph (Nucon 5700, Nucon Engineers, New Delhi) equipped with flame ionization detector and stainless steel column packed with Chromosorb-101 to serve as a stationary phase. Analytical conditions for fractionation of VFA were as follows: Injection port temperature, 250ºC; column temperature, 190ºC and detector temperature, 260ºC. The flow rate of carrier gas (nitrogen) was 40 ml/min; hydrogen 30 ml/min; air 300 ml/min. Injection volume was 3 µl. The injection was performed by means of 10 µl Hamilton syringe (Hamilton, Nevada, USA). Different VFA's of the samples were identified on the basis of their retention time and their concentration (mM) was calculated by comparing the retention time as well as the peak area of standards after deducting the corresponding blank values. The nitrogen (N) content was measured using the Kjeldahl method (AOAC, 1990). The CP was calculated as N*6.25. Lactic acid (LA) concentration was determined following Barker and Summerson (1941) method. Estimation of lactic acid bacteria and yeast and moulds was done by plate count method after using Rogosa agar medium (selective medium for *Lactobacillus*) and potato dextrose agar (selective medium for yeast and molds).

The effects of the treatment on the quality characteristics of silage were evaluated by analyses of

variance (ANOVA). If the ANOVA was significant, the means of variables were further compared using the procedure of Tukey-Kramer comparison. All these analyses were performed by SAS program (SAS Inst. Inc. Cary, NC, 2001).

RESULTS AND DISCUSSION

The chemical composition and pH, buffering capacity, TSC, and microbial counts of maize fodder have been presented in Table 1. After opening of the silage a preliminary sensory evaluation was performed by an expert. The silage was evaluated for colour, smell, visible fungal growth and acidic tastes. The results are presented in Table 2. The fermented aroma and acidic taste of the treatments were related to the amount of fermented acids generated. None of the treatments had visible fungal growth and/or sliminess. Absence of fungal growth and sliminess in all the treatment were because of preservative effect of fermented acids produced during ensiling or very low pH of the ensiled products, which prevented the growth of fungi and moulds.

Table 3 is showing the DM content, moisture, weight loss and ammonia nitrogen of bale silage. There was no difference among different group in comparison to control group for all these parameters *i.e.* DM, moisture and weight loss. The DM (%) content ranged from 28.16 to 28.89 % (MF_{FA} Vs MF_0). A significant weight loss per 100 kg bale was observed ($P<0.05$) with the treatment of *Lactobacillus* culture. It seems that silage made by bales wrapping in plastic densely compact the fodder leaving no air pocket behind for any DM losses. Nishino *et al.* (2007) observed that the DM of the maize silage was 28.4 in comparison to the control group *i.e.* 28.1, when treated with *Lactobacillus casei*. The pH in treatment groups was quite good at 3.85 in MF_{LAB}, 3.87 in MF_{ST} and as compared to 3.91 in control MF_0. It indicates that supplementation of both *Lactobacillus casei* and silotan are quite effective to bring down the pH of bale silage. Herremans *et al.* (2019) observed the addition of oak and chestnut tannin extract at different levels did not influence pH values of silages but reduced the ammonia nitrogen levels. However, a significant increase ($P<0.001$) in pH was observed with in MF_{FA}. The ammonia- N was within the range of permissible limits

Table 1. Chemical composition of maize fodder before ensiling

Parameter	Values
DM (%)	30.82±1.07
Ash (%DM)	7.33±0.10
CP (%DM)	9.09±0.002
EE (%DM)	3.62±0.001
ADF (%DM)	33.95±0.28
NDF (%DM)	56.99±0.006
TSC g/100g DM	13.9±0.6
BC meq. /100g FM	27.00±0.01
LAB (log_{10}cfu/g)	5.66±0.04
Yeast and mold (log_{10}cfu/g)	5.02±0.08
pH	6.65±0.029

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; TSC, total soluble carbohydrates; BC, buffering capacity; FM, fresh matter

Table 2. Sensory evaluation of the silage

Treatment	Treatments[#]			
	MF_0	MF_{LAB}	MF_{ST}	MF_{FA}
Colour	Dark Brown	Dark Brown	Dark Brown	Dark Brown
Smell	Ammonical	Ammonical	Ammonical	Mild Ammonical
Acidic	Highly Acidic	Highly Acidic	Mild Acidic	Mild Acidic
Visible	No moulds	No moulds	No moulds	No moulds
Sliminess	Little slimy	Little slimy	Slimy	Little slimy

[#]Bale silage containing either no additive, or *Lactobacillus casei*, Silotan, Formaldehyde as additive were denoted as MF_0, MF_{LAB}, MF_{ST}, MF_{FA}, respectively.

Table 3. Effect of Different Additives on DM, weight Loss, pH, ammonia nitrogen and buffering capacity of bale silage

Parameters	Treatments≠				P value
	MF_0	MF_{LAB}	MF_{ST}	MF_{FA}	
Dry matter (%)	28.89^{a}±1.46	28.36^{a}±0.79	28.73^{a}±1.11	28.16^{a}±1.04	0.965
Weight loss (kg/bale)	3.24^{ab}±0.69	4.87^{b}±0.66	2.33^{a}±0.08	1.70^{a}±0.53	< 0.05
pH	3.91^{a}±0.05	3.85^{a}±0.01	3.87^{a}±0.03	4.44^{b}±0.05	<0.001
NH_3-N (g/100 g DM)	0.22^{b}±0.02	0.26^{b}±0.02	0.24^{b}±0.02	0.17^{a}±0.01	<0.05
Total (g/100 g DM)	1.42^{a}±0.01	1.43^{a}±0.02	1.39^{a}±0.01	1.40^{a}±0.01	0.221
BC (mEq NaOH/100 g)	81.26^{b}±3.88	75.77^{b}±2.62	72.22^{b}±3.73	40.89^{a}±2.50	<0.001

BC, buffering capacity; ≠Bale silage containing either no additive, or *Lactobacillus casei*, Silotan, Formaldehyde as additive were denoted as MF_0, MF_{LAB}, MF_{ST}, MF_{FA}, respectively; $^{a, b}$ Values bearing different alphabet in a row differ significantly (P<0.05)

at 0.26 in MF_0, 0.27 in MF_{LAB} and 0.24 in MF_{ST}, however a significant decrease (P<0.05) in ammonia nitrogen levels was observed in MF_{FA} (0.17). Kaiser *et al.*(1981) observed that the addition of formaldehyde increased the pH and decreased the ammonia nitrogen levels of the maize silage. Supplementation of lactobacilli culture and Silotan were not able to alter buffering capacity of the bale silage. However, supplementation of formaldehyde significantly reduced (P<0.001) the buffering capacity of bale silage.

Data pertaining to chemical composition of bale silage is presented in Table 4.The crude protein content of MF_0 (8.08), MF_{LAB} (8.94), MF_{ST} (8.80) and MF_{FA} (8.94) were not statistically significantly different. The treatment of *Lactobacillus casei* significantly (P<0.001) decreased the EE and WSC, with no effect on CP, NDF and ADF content of silage. The treatment of Silotan significantly (P<0.05) reduced NDF content, with no significant effect on the protein, organic matter, EE, ADF and TSC content of the silage. The treatment of formaldehyde significantly (P<0.001) increased the ash, NDF, WSC, hemicellulose, with no prominent effect on CP, EE, and ADF content of silage.

Data pertaining to VFA content of bale silage are presented on Table 5. Treatment of *Lactobacillus casei* significantly (P<0.001) increased TVFAs, with no significant effect on acetic acid, lactic and butyric acid content of silage. Nishino *et al.* (2005) found similar

Table 4. Effect of different additives on chemical composition of bale silage

Parameters	Treatments≠				P value
	MF_0	MF_{LAB}	MF_{ST}	MF_{FA}	
Dry matter	28.89^{a}±1.46	28.36^{a}±0.79	28.73^{a}±1.11	28.16^{a}±1.04	0.965
On % dry matter basis					
Crude protein	8.08^{a}±0.08	8.94^{a}±0.05	8.80^{a}±0.73	8.94^{a}±0.96	0.217
Ether extract	3.19^{a}±0.22	2.52^{a}±0.22	2.51^{a}±0.42	3.04^{a}±0.29	0.261
Ash	5.92^{a}±0.07	5.92^{a}±0.15	6.02^{a}±0.21	6.66^{a}±0.09	0.012
Organic matter	93.34^{a}±0.21	94.08^{a}±0.15	93.68^{a}±0.07	93.94^{a}±0.09	0.033
NDF %	24.97^{a}±0.96	25.15^{a}±0.63	22.33^{a}±0.62	26.43^{a}±0.36	0.433
ADF %	52.67^{b}±0.30	53.14^{b}±0.74	47.06^{a}±1.30	53.76^{b}±1.66	<0.05
TSC	4.75^{b}±0.17	3.30^{a}±0.11	4.64^{b}±0.11	9.46^{c}±0.40	<0.001

≠Bale silage containing either no additive, or *Lactobacillus casei*, Silotan, Formaldehyde as additive were denoted as MF_0, MF_{LAB}, MF_{ST}, MF_{FA}, respectively; NDF, neutral detergent fibre; ADF, acid detergent fibre; TSC, total soluble carbohydrates; $^{a, b}$ Values bearing different alphabet in a row differ significantly (P<0.05)

Table 5. Effect of different additives on volatile fatty and lactate content of bale silage

Parameters	Treatments≠				P value
	MF_0	MF_{LAB}	MF_{ST}	MF_{FA}	
Total volatile fatty acids	30.98^{b}±3.09	38.07^{c}±2.20	34.51^{c}±1.83	14.90^{a}±0.89	<0.001
Acetate	23.83^{b}±2.38	28.31^{b}±1.57	24.86^{b}±1.46	12.07^{a}±0.67	<0.001
Propionate	8.68^{c}±0.78	6.17^{b}±0.65	6.02^{b}±0.61	1.28^{a}±0.17	<0.05
Butyric acid	0.49^{a}±0.06	0.56^{a}±0.04	0.26^{b}±0.20	0.49^{a}±0.60	<0.001
Lactate	6.48^{b}±0.02	6.59^{b}±0.06	6.76^{b}±0.02	3.69^{a}±0.15	<0.001

≠Bale silage containing either no additive, or *Lactobacillus casei*, Silotan, Formaldehyde as additive were denoted as MF_0, MF_{LAB}, MF_{ST}, MF_{FA}, respectively; ^{a,b,c} Values bearing different alphabet in a row differ significantly (P<0.05)

results with *Lactobacillus casei* on both direct cut and wilted grass silages. Treatment of Silotan significantly (P<0.001) increased TVFAs, with no significant effect on acetic acid and lactic acid content of silage. Treatment of formaldehyde significantly (P<0.001) decreased TVFAs, particularly acetic acid and propionic acid, with a significant (P<0.001) reduction in lactic acid content of silage.

Table 6 is showing the data on *in vitro* digestibility of bale silage. The data revealed that the *in vitro* DMD ranged from 67.45 to 69.68 with no significant difference in the treatment of Silotan and formaldehyde, while a significant (P<0.05) increase was observed in *in vitro* DMD with the treatment of *Lactobacillus casei* (MF_{LAB}). There was no significant difference in their methane production per

Table 6. Effect of different additives on *in-vitro* digestibility and gas production of bale silage

Parameters	Treatments≠				P value
	MF_0	MF_{LAB}	MF_{ST}	MF_{FA}	
IVDMD (%)	68.96^{a}±0.87	69.85^{b}±0.95	67.89^{a}±0.80	67.45^{a}±1.20	<0.05
IVOMD (%)	72.39^{b}±0.57	71.23^{ab}±1.96	68.70^{ab}±1.52	67.50^{a}±0.52	<0.05
ME (MJ/kg DM)	6.46^{b}±0.09	5.89^{ab}±0.05	5.67^{a}±0.24	6.38^{b}±0.16	<0.05
CH_4(% total gas)	36.11^{b}±1.80	30.36^{a}±0.58	27.92^{a}±0.12	27.30^{a}±1.01	<0.05

IVDMD, *in vitro* dry matter digestibility; IVOMD, *in vitro* organic matter digestibility; ME, metabolizable energy; ≠Bale silage containing either no additive, or *Lactobacillus casei*, Silotan, Formaldehyde as additive were denoted as MF_0, MF_{LAB}, MF_{ST}, MF_{FA}, respectively; $^{a, b}$ Values bearing different alphabet in a row differ significantly (P<0.05)

Table 7. Effect of different additiveson microbial counts of bale silage

Parameters	Treatments≠				P value
	MF_0	MF_{LAB}	MF_{ST}	MF_{FA}	
On the day of opening					
Microbial Counts	8.41^{b}±0.01	8.48^{c}±0.02	8.45^{bc}±0.01	7.91^{a}±0.02	<0.05
Yeast and Moulds	4.04^{b}±0.09	4.07^{bc}±0.02	4.02^{a}±0.04	4.15^{c}±0.03	<0.05
pH	3.91^{a}±0.05	3.85^{a}±0.01	3.87^{a}±0.03	4.44^{b}±0.05	<0.001
After three days of exposure					
Yeast moulds	5.52^{a}±0.05	5.31^{a}±0.14	6.32^{b}±0.01	6.40^{b}±0.06	<0.001
pH	4.66^{a}±0.17	5.53^{b}±0.15	4.55^{a}±0.06	5.71^{b}±0.18	<0.001

≠Bale silage containing either no additive, or *Lactobacillus casei*, Silotan, Formaldehyde as additive were denoted as MF_0, MF_{LAB}, MF_{ST}, MF_{FA}, respectively; $^{a, b, c}$ Values bearing different alphabet in a row differ significantly (P<0.05)

gram DM and ME values. The percent methane gas of total gas production was reduced significantly (P<0.05) in MF_{LAB}, MF_{ST} and MF_{FA}.

Table 7 is showing the data on microbial quality of the silage at the time of opening silage and three days after exposure to the air. There was a significant (P<0.001) reduction in the microbial counts in MF_{ST} (7.91) in comparison to control group MF_0 (8.41). Supplementation of *Lactobacillus casei* and Silotan significantly (P<0.001) increased the microbial counts to 8.48 in MF_{LAB} and 8.45 in MF_{ST}. Further, a significant (P<0.001) increase in the yeast and mould counts in MF_{FA} (4.15) was observed,while no effect was observed in MF_{LAB} and a significant (P<0.001) decrease in the yeast and mould counts was observed in MF_{ST} (4.02). There was a significant (P<0.001) increase in yeast and moulds counts after 3 days exposure to the air in MF_{ST} (6.32) and MF_{FA} (6.40) treatments in comparison to MF_0 (5.52) group, however no significant difference was observed in MF_{LAB} (5.31) treatment. A significant (P<0.001) increase in pH after 3 days exposure to the air was observed in MF_{FA} (5.71) and MF_{LAB} (5.53) with no significant difference in MF_{ST} (4.55) treatment.

CONCLUSION

Silotan or MF_{LAB} supplementation improved the bale silage quality of maize fodder in terms of acetic acid, lactic acid and microbial quality, and further reduced the production of butyric acid. Further it also reduced the growth of yeast in the silage.

ACKNOWLEDGEMENT

The authors are thankful to the Director, ICAR-NDRI, Karnal for providing required fund, infrastructure and facilities to carry out the work. Authors are also thankful to Dr. Praminder, Nurture organics Pvt. Ltd. for providing Silotan and facilities for production of bale silage at Khanna, Punjab, India.

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Received on 14.09.2021 and accepted on 08.11.2021