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Effect of Biological and Chemical Additives on Fermentation Responses and Degradation Characteristics of Whole Crop Barely Silage

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Abstract: Whole crop barley was harvested (about 35% DM), chopped and then ensiled used laboratory scale silos (3.25±0.25 kg). The forage was ensiled as Untreated (UT) or treated using the following additives; formic acid (3.4 or 6.8 mL kg^{-1} DM; F3 or F6, respectively) acetic acid (3 or 4 mL kg^{-1} DM; A3 or A4, respectively) propionic acid (3 or 6 g kg^{-1} of DM; P3 or P6, respectively) ammonium propionate (0.75, 1 or 1.5 g kg^{-1} of DM; API 0.75 or AP1.5, respectively) *Lactobacillus plantarum* (8×1010 CFU (LP8) or 16×1010 CFU (LP16) per g of DM) or mixed with *Pediococcus pentosaceus* plus *Propionbacter freudenreichii* (5.5×1010 CFU (PP5.5) or 11×1010 CFU (PP11) per g of DM). Four replicates were performed for each treatment. Chemical composition, silage extracts pH and NH_3-N and *in situ* ruminal degradation parameters of DM, CP and NDF were determined. The additives caused a significant difference in the silage regarding chemical composition. Short chain organic acids did not have a significant effect on NH_3-N and CP but acetic acid decreased pH of the silages ($p<0.05$). Biological inoculants resulted to decrease pH and LP8 decreased significantly NH_3-N (LP8: 7.77 vs. untreated: 9.10 mg dL^{-1}). Adding the buffered propionic acid based additives decrease pH and increase concentration of ammonia-N in the silage. Data of dry matter degradable coefficients showed that the slowly degradable fraction of the silage was affected by the treatments. Degradable coefficients of NDF of the silages were affected by the additives used ($p<0.05$). The addition both quickly and slowly degradable coefficient of CP were influenced by the treatments used ($p<0.05$).

Key words: Whole crop barley, ammonium propionate, inoculants, organic acid additives, *in situ* degradability, Iran

INTRODUCTION

Ensiling is a preservation method for most forage crops and fermentation take place in every silo might be uncontrolled process. Many additives have been used to alter silage fermentation (Arbabi *et al*., 2008). Some additives which have proven to be effective in this respect include chemical additives based on volatile fatty acids such as propionic, formic and acetic acid and biological additives based on bacteriocin producing micro-organisms such as lactobacilli and bacilli (Arbabi *et al*., 2008; Phillip and Fellner, 1992).

In order to achieve a major goal in silage making that is to preserve silage material with minimum nutrient loss, formic acid is widely used (Arbabi *et al*., 2008). Addition of formic acid to silage material has been reported to have generally positive effects on fermentation (Arbabi *et al*., 2008; Haigh, 1988; Snyman and Joubert, 1996). Formic acid as silage additive has anti-bacterial effect on many bacteria species including lactic acid bacteria; thus, addition of formic acid into silage results in limited fermentation and reduction in organic acid content of silage. Whole crop cereal silage contain a greater amount of water soluble carbohydrate which is a better source of energy for rumen microbe than lactic acid (Arbabi *et al*., 2008). Formic acid treatment of silage induces antibacterial activity and reduces lactic acid production. Thus, a balance between sufficient lactic acid to preserve the silage effectively and maintaining as much carbohydrates as possible in the form of soluble sugars is required to obtain high quality silage (Aksu *et al*., 2006).

Of the short-chain fatty acid additives, propionic acid has the greatest antimycotic activity (Kung *et al*., 1998). In the past, aerobic stability was improved when large amounts of propionic acid (1-2% of the DM) were added to any silage (Huber and Soejono, 1976; Stallings *et al*., 1981) but the high percentage of acid often restricted fermentation in these cases. Many current products that are added to forage at ensiling for the purpose of improving aerobic stability contain several active

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ingredients; propionic acid usually constitutes the greatest percentage of these ingredients. The application rates of these products are relatively low (0.3-0.6% of the DM) (Kung *et al*., 1998).

On the other hand, un-buffered propionic acid-based preservatives have also been used to improve the aerobic stability of whole crop cereal silages (Kung *et al*., 2000; Britt *et al*., 1975). Therefore in recent years marked changes have been made to the formulations and recommended application rates of additives containing propionic acid (Kung *et al*., 2000). An advantage of salts from acids is that they are easier and safer to handle than their corresponding acids (Arbabi *et al*., 2008). For example, the corrosive nature of propionic acid has been reduced by buffering and many additives contain other antifungal compounds such as sorbic acid and benzoate (Kung *et al*., 2000). Current recommendation for using buffered propionic acid additives are considerably lower (0.1-0.2% of fresh forage weight) than classical recommendation for use of the un-buffered acid (0.75-1.5%; Arbabi *et al*., 2008; Kung *et al*., 2000).

Microbial inoculants are applied to forage at the time of ensiling to establish a desirable microbial flora in silage, accelerate the decline of pH during the initial stage of silage fermentation preserve plant carbohydrates through homo-fermentation and to preserve plant protein by decreasing proteolysis and deamination (Haigh, 1988; Hristov and McAllister, 2002). The inhibition of growth of undesirable bacteria is associated with the rate of lactic acid production following ensiling, which depends on the initial population of lactic acid bacteria and substrate availability at ensiling (Aksu *et al*., 2006). Bacterial inoculants generally increase lactic acid levels and reduce silage pH, acetic and butyric acid levels in the silages (Aksu *et al*., 2006; Kennedy, 1990).

Thus, inoculated silages are expected to improve animal performance (Hristov and McAllister, 2002). Barley forage has low buffering capacity and abundant fermentable carbohydrates and is considered relatively easy to ensile (Acosta *et al*., 1991). Despite its ease of ensiling, results of previous experiments have shown that lactic acid bacteria-based inoculants have the potential to improve barley silage fermentation (McAllister *et al*., 1995; Moshtaghi Nia and Wittenberg, 1999), digestibility of whole crop barley silage, nutrient intake and average daily gain by cattle (Aksu *et al*., 2006; Hristov and McAllister, 2002; McAllister *et al*., 1995).

Present study was conducted to evaluate the effect of various additives including short chain organic acids (formic, acetic and propionic acids), bacterial inoculants (*Lactobacillus plantarum* or mixed with *Pediococcus pentosaceus* plus *Propionbacter freudenreichii*) and ammonium propionate on fermentation responses and *in situ* ruminal degradation parameters of Whole Crop Barley Silage (WCBS).

MATERIALS AND METHODS

Ensiling procedures: Whole crop barley was harvested (about 35% DM) chopped, then ensiled. Approximately 3.25 kg (±0.25 kg) of the forage from each treatment was packed into a laboratory scale polyethylene tube silo. The forage was ensiled as Untreated (UT) or treated with the following additives; formic acid (3.4 or 6.8 mL Kg^{-1} DM; F3 or F6, respectively) acetic acid (3 or 4 mL Kg^{-1} DM; A3 or A4, respectively); propionic acid (3 or 6 g Kg^{-1} of DM; P3 or P6, respectively); ammonium propionate (0.75, 1 or 1.5 g Kg^{-1} of DM; API 0.75 or AP 1.5, respectively); *Lactobacillus plantarum* (8×10^{10} CFU (LP8) or 16×10^{10} CFU (LP16) per g of DM) or mixed with *Pediococcus pentosaceus* plus *Propionbacter freudenreichii* (5.5×10^{10} CFU (PP5.5) or 11×10^{10} CFU (PP11) per g of DM). Four replicates were performed for each treatment.

Chemical analysis: Representative samples of fresh chopped whole crop barley and the silages were collected, oven dried to a constant weight at 60°C and ground to pass through a 2 mm-screen for later analysis. Standard procedures were used to determine the chemical composition of the samples. Crude Protein (CP) was determined according to the Kjeldahl procedure (AOAC) on the Tecator Auto-analyzer (1030). Determination of Neutral Detergent Fiber (NDF) was made using the method of Van Soest. Samples of fresh silage (approximately 50 g) were mixed with 450 mL distilled water and the silage extraction was made. Then, silage pH was determined using a portable pH meter (Metrohm 691, Swiss). Five mL of the silage extract was mixed with 5 mL of 0.2 N HCL. Ammonia-N degradation of the acidified silage extract was determined using distillation method (Kjeltec, 2300 Autoanalyzer, FossTecator AB, Hoganas, Sweden).

***In situ* technique:** The ruminal degradable parameters of dry matter, NDF and CP of the silages were determined using *in situ* procedure. Four sheep (44±5) fitted with rumen fistulae were used in the present study. The bags (10×12 cm) were made of polyester nylon cloth with a pore size of 48 µm. About 5 g DM of each sample was placed in each bag and four bags for each treatment were incubated for each time (0.0, 2, 4, 8, 16, 24, 48, 72 ans 96 h). After removal the bags from the rumen, they were washed in cold running water and dried in a air-forced oven (60°C, 48 h), then weighted non ruminal incubated and incubated samples were analyzed to determine the CP and NDF concentration.

Calculation and statistical analysis: The equation of $P = a + b(1-e^{-ct})$ was applied to determine the coefficients of a = quickly degradable, b = slowly degradable, c = constant rate of degradation of the incubated samples (Orskov and McDonald, 1979). Effective Degradability (ED) of DM, CP and NDF was then calculated according to the equation of Orskov and McDonald (1979) where $ED = a + ((b \times c)/(k + c))$ where k is the rumen outflow rate assumed to be 2, 4 or 6% h^{-1} and a, b and c are as described before. Contrasts were used to determine the significance of the difference between control and the additive treated silages. Data of PH, NDF, NH_3-N and CP in each group of additives including acids, microbes and buffered ammonium were statistically analyzed using complete randomized design. The statistical model was:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where:

Y_{ij} = Dependent variable
μ = Dependent variable mean
T_i = Effect of treatment
E_{ij} = Residual error term

Means were compared using Tukey's Test (Version 9.1). An α level of $p<0.05$ was deemed significant.

RESULTS AND DISCUSSION

Chemical composition: Chemical compositions of the untreated and treated WCBS are shown in Table 1-3. Data shown in Table 1 indicated a significance difference between the acids used vs. untreated silage. Previous results indicated that short chain organic acids did not have a significant effect on NH_3-N and CP of the cereal silages (Jaakkola *et al*., 2006; Aksu *et al*., 2006; Baytok *et al*., 2005). Present study demonstrated that acetic acid had a significant effect on NDF content of WCBS compared with the untreated silage ($p<0.05$). The modifying effect of ensiling on carbohydrate concentrations of grass herbage is however, complicated because in addition to hydrolysis of NDF, the concentrations are affected by nutrient losses in respiration, effluent and fermentation (Jaakkola *et al*., 2006).

There are different reports about the effect of microbial inoculation on silage fermentation characteristics. It is generally reported that microbial inoculation to silage has a positive effect on the silage fermentation by decreasing pH (Kung *et al*., 1987; Kennedy, 1990; Rooke *et al*., 1998; Aksu *et al*., 2006). By use of inoculants in the present study, the pH ranged from 3.69-4.07 and was lowered by addition of LP8 and PP11 (Table 2). These data supported previous results (Kung and Ranjit, 2001) wich additive inoculants had a significant effect on WCBS.

Results of the present experiment showed that although treated silages had numerically lower ammonia-N content rather than untreated WCBS but only LP8 significantly decreased NH_3-N (LP8:7.77 vs. untreated: 9.10) and when ammonia-N content attended as percent of total-N (NH_3-N (mL dL^{-1})/CP (g kg^{-1} DM)), *Lactobacillus plantarum* additive was more effective in limiting the degradation of protein to ammonia rather than PP5.5 and PP11 as a homofermentative mixed bacteria or rather than untreated (Table 2). Kung and Ranjit (2001) reported that lower degradation of protein to ammonia in the silage may be resulted from higher rate of lactic and acetic fermentation via inoculants and greater amount of

Table 1: Chemical compositions of whole crop barley silage treated with different level of propionic, formic and acetic acid

Chemical composition of crop	Untreated	P3	P6	F3	F6	A3	A4	p-value	Contrast*
pH	4.07	4.06	4.03	4.00	3.95	3.96	3.83	0.0096	0.0348
NDF (g kg^{-1} DM)	0.55*	0.66*	0.66*	0.61*	0.64*	0.65*	0.61*	<0.0001	<0.0001
NH_3-N (mL dL^{-1})	9.10	11.01	8.56	8.46	8.28	9.61	9.62	0.0056	0.7546
CP (g kg^{-1} DM)	7.99	8.04	8.13	7.96	7.95	8.12	7.83	0.0009	0.7240

[a-c]Means in each row with unlike superscript differ ($p<0.05$); P3 = Propionic acid applied at 0.1% of fresh forage weight; P4 = Propionic acid applied at 0.2% of fresh forage weight; F3 = Formic acid applied at 3.4 mL kg^{-1} DM; F6 = Formic acid applied at 6.8 mL kg^{-1} DM; A3 = Acetic acid applied at 3 mL kg^{-1} DM; A4 = Acetic acid applied at 4 mL kg^{-1} DM; *contrast: (Pr>F) untreated vs. others

Table 2: Chemical compositions of whole crop barley silage treated with different level of *Lactobacillus plantarum* or mixed with *Pediococcus pentosaceus* plus *Propionbacter freudenreichii*

Chemical composition of crop	Untreated	LP8	LP16	PP5.5	PP11	p-value	Contrast*
pH	4.07	3.69*	3.98	3.97	3.88*	<0.0001	0.0003
NDF (g kg^{-1} DM)	0.55	0.58	0.63*	0.54	0.62*	<0.0001	<0.0001
NH_3-N (mL dL^{-1})	9.10	7.77*	8.62	8.80	8.48	0.0211	0.0264
CP (g kg^{-1} DM)	7.99	7.88	7.91	7.86	7.96	0.2731	0.1196
NH_3-N ((mL dL^{-1})/CP (g kg^{-1} DM))	1.14	0.99	1.09	1.12	1.07	-	-

[a-c]Means in each row with unlike superscript differ ($p<0.05$). LP8 = *Lactobacillus plantarum* (8×10^{10} CFU) per g of DM; or LP16 = *Lactobacillus plantarum* (16×10^{10} CFU) per g of DM; PP5.5 = *Lactobacillus plantarum* mixed with *Pediococcus pentosaceus* plus *Propionbacter freudenreichii* (5.5×10^{10} CFU) per g of DM; PP11 = *Lactobacillus plantarum* mixed with *Pediococcus pentosaceus* plus *Propionbacter freudenreichii* (11×10^{10} CFU) per g of DM; *contrast: (Pr>F) untreated vs. others

Table 3: Chemical compositions of whole crop barley silage treated with different level of ammonium propionate

Chemical composition of crop	Untreated	AP 0.75	AP1	AP 1.5	p-value	Contrast*
pH	4.07	3.94	3.83	3.93	0.0684	0.0232
NDF (g kg^{-1} DM)	0.55	0.58	0.58	0.59	0.0109	0.0031
NH_3-N (mL dL^{-1})	9.10	9.12	9.08	11.70*	0.0003	0.0520
CP (g kg^{-1} DM)	7.99	8.02	8.06	8.08	0.6269	0.3057

AP 0.75 = 0.75 g ammonium propionate per kg of DM; AP1 = 1 g ammonium propionate kg^{-1} of DM; or AP1.5 = 1.5 g ammonium propionate kg^{-1} of DM; *contrast: (Pr>F) untreated vs. others

Table 4: Dry matter degradable coefficients of whole crop barley silage treated with different chemical and biological additives

Items	a (%)	b (%)	c (%/h)	ED (0.02)	ED (0.04)	ED (0.06)
Untreated 2	36.3±1.70	44.7±2.31	4.52±0.69	67.2	60.1	55.5
P3	34.7±1.47	49.6±2.15	3.98±0.52	67.8	59.5	54.5
P6	34.6±1.36	53.6±3.46	2.46±0.42	64.3	55.1	50.3
F3	35.7±1.21	46.9±1.77	3.97±0.45	66.9	59.1	54.4
F6	35.3±1.68	48.5±2.94	3.25±0.59	65.3	57.0	52.4
A3	36.9±1.07	45.7±1.52	4.20±0.42	67.9	60.3	55.7
A4	34.5±1.18	48.3±1.79	3.78±0.42	66.1	57.9	53.2
LP8	36.1±1.62	48.3±2.13	4.88±0.63	70.4	62.7	57.8
LP16	32.9±1.22	48.8±1.53	5.63±0.51	68.9	61.4	56.5
PP5.5	34.2±1.52	47.4±1.91	5.72±0.66	69.3	62.1	57.3
PP11	32.8±1.64	49.4±2.10	5.29±0.65	68.7	60.9	55.9
AP0.75	35.2±1.30	46.3±1.71	4.85±0.53	68.0	60.6	55.9
AP1	34.4±1.74	47.7±2.18	5.71±0.74	68.9	62.4	56.5
AP1.5	33.6±1.40	49.3±1.83	5.00±0.54	68.8	61.0	56.0

a = rapidly degradable, b = slowly degradable, c = fractional degradation rate constant; P3 or P6: 3 or 6 g propionic acid kg^{-1} of DM, respectively; F3 or F6: 3.4 or 6.8 mL formic acid kg^{-1} DM, respectively; A3 or A4: 3 or 4 mL acetic acid kg^{-1} DM, respectively; LP8 or LP16: 8×10^{10} CFU *Lactobacillus plantarum* or 16×10^{10} CFU per g of DM, respectively; PP5.5 or PP11: 5.5×10^{10} CFU *Lactobacillus plantarum* mixed with *Pediococcus pentosaceus* plus *Propionbacter freudenreichii* or 11×10^{10} CFU per g of DM, respectively; AP 0.75, or AP1.5: 0.75 or 1 or 1.5 g ammonium propionate per kg of DM, respectively

Table 5: Crude protein degradable coefficients of whole crop barley silage treated with different chemical and biological additives

Items	a (%)	b (%)	c (%/h)	ED (0.02)	ED (0.04)	ED (0.06)
Untreated	40.9±1.79	39.0±3.05	3.33±0.78	65.2	58.6	54.8
P3	40.8±2.02	43.3±6.03	2.25±0.79	63.8	56.4	52.6
P6	38.2±2.10	43.9±5.57	2.41±0.81	62.1	54.6	50.7
F3	39.6±3.42	43.3±7.05	2.84±1.32	65.0	57.6	53.5
F6	38.1±2.90	43.9±5.47	3.04±1.11	64.6	57.1	52.9
A3	39.5±2.47	38.0±3.27	4.84±1.23	66.4	60.3	56.4
A4	39.8±2.43	36.1±3.11	5.31±1.32	66.0	60.3	67.0
LP8	42.2±2.03	36.5±2.76	4.55±1.02	67.6	61.6	58.0
LP16	41.3±1.74	41.2±2.41	4.38±0.77	69.6	62.9	58.7
PP5.5	40.6±2.39	38.4±3.46	4.05±1.10	66.3	59.9	56.1
PP11	39.2±3.04	41.8±6.07	2.91±1.22	63.9	56.8	52.8
AP0.75	39.8±3.14	43.9±7.18	2.64±1.20	64.8	57.2	53.2
AP1	42.1±2.84	42.8±10.2	2.03±1.15	63.7	56.5	52.9
AP1.5	42.3±2.19	40.4±6.65	2.23±0.92	63.6	56.8	53.3

a = rapidly degradable, b = slowly degradable, c = fractional degradation rate constant; P3 or P6: 3 or 6 g propionic acid kg^{-1} of DM, respectively; F3 or F6: 3.4 or 6.8 mL formic acid kg^{-1} DM, respectively A3 or A4: 3 or 4 mL acetic acid kg^{-1} DM, respectively LP8 or LP16: 8×10^{10} CFU *Lactobacillus plantarum* or 16×10^{10} CFU per g of DM, respectively; PP5.5 or PP11: 5.5×10^{10} CFU *Lactobacillus plantarum* mixed with *Pediococcus pentosaceus* plus *Propionbacter freudenreichii* or 11×10^{10} CFU per g of DM, respectively; API 0.75, or AP1.5: 0.75 or 1 or 1.5 g ammonium propionate per kg of DM, respectively

propionic acid which inhibits the growth of proteolytic bacteria. In the present study the inoculants did not have any significant effect on CP concentration of whole crop silage vs. untreated silage (Table 2).

Untreated silage had higher pH than ammonium propionate treated silages (Table 3). This finding supports previous results of Arbabi *et al.* (2008), Kung *et al.* (2000). Addition of the buffered propionic acid-based additive decrease pH which Kung *et al.* (1998) suggests that these additives partially reduced the metabolism of some aerobic microorganisms.

The concentration of ammonia-N of WCBS was increased when it was ensiled with ammonium propionate as 1.5 g kg^{-1} DM. It was predictable because the buffered propionic acid is in the form of ammonium propionate (Kung *et al.*, 1998). Silage CP ranged from 7.99 for untreated silage to 8.08% for AP 1.5 and were unaffected by the levels of ammonium propionate (Table 3). These data agreed with Mills and Kung (2002).

***In situ* degradation coefficients:** The data of *in situ* degradability of DM, CP and NDF are shown in Table 4-6, respectively. There is a lack of previous study regarding *in situ* degradation coefficients of the whole crop barley silage. Present data of dry matter degradable coefficients showed that fraction (b) was affected by the treatments. These results supported the finding of Zahiroddinia *et al.* (2004) who reported an increase in fraction of b by using of inoculants. The data of degradable coefficients of CP for various additives showed that there was not

Table 6: NDF degradable coefficients of whole crop barley silage treated with different chemical and biological additives

Items	a (%)	b (%)	c (%/h)	ED (0.02)	ED (0.04)	ED (0.06)
Untreated	12.1±2.60	68.9±4.90	3.04±0.63	0.54	0.42	0.35
P3	13.6±2.59	67.0±5.25	2.88±0.65	0.53	0.42	0.35
P6	13.9±2.31	67.4±4.93	2.77±0.57	0.53	0.41	0.35
F3	14.5±2.44	66.2±4.70	3.00±0.62	0.54	0.43	0.36
F6	14.2±2.21	64.3±3.91	3.22±0.58	0.54	0.43	0.37
A3	15.2±2.00	66.2±3.47	3.28±0.51	0.56	0.45	0.39
A4	14.8±2.18	65.2±3.61	3.42±0.57	0.56	0.45	0.38
LP8	14.9±1.94	66.6±4.23	2.73±0.49	0.53	0.42	0.36
LP16	15.1±1.81	66.1±3.65	2.89±0.46	0.54	0.43	0.37
PP5.5	15.4±2.05	66.4±3.91	3.02±0.52	0.55	0.44	0.38
PP11	15.5±2.00	67.4±4.47	2.69±0.50	0.54	0.43	0.36
AP0.75	13.3±2.47	68.0±4.53	3.11±0.61	0.55	0.43	0.37
AP1	13.7±2.69	66.9±5.31	2.94±0.67	0.53	0.42	0.36
AP1.5	16.1±1.90	63.9±3.10	3.47±0.51	0.57	0.46	0.40

a = rapidly degradable, b = slowly degradable, c = fractional degradation rate constant; P3 or P6: 3 or 6 g propionic acid kg^{-1} of DM, respectively; F3 or F6: 3.4 or 6.8 mL formic acid kg^{-1} DM, respectively; A3 or A4: 3 or 4 mL acetic acid kg^{-1} DM, respectively; LP8 or LP16: 8×10^{10} CFU *Lactobacillus plantarum* or 16×10^{10} CFU per g of DM, respectively; PP5.5 or PP11: 5.5×10^{10} CFU *Lactobacillus plantarum* mixed with *Pediococcus pentosaceus* plus *Propionbacter freudenreichii* or 11×10^{10} CFU per g of DM, respectively; AP 0.75, or AP1.5: 0.75 or 1 or 1.5 g ammonium propionate per Kg of DM, respectively

a significant difference between the treated and the untreated silage (Table 5). Data of NDF degradable coefficients of WCBS showed that the additives used to increase disappearance of fraction of (a) and decrease of fraction (b). In addition, data showed that effective degradability of NDF of treated silage increased about 0.06 in contrast with the untreated silage (Table 6). Additives including AP 1.5 and A4 had a most increased in ED of the treated silage. The data of effective degradability of NDF showed that the most difference between untreated and treated silage resulted by use of AP 1.5.

CONCLUSION

It was concluded that Acetic acid (A4) and Formic acid (F6) had the most effect on chemical composition of WCBS. Results of the present study indicated that the inoculants used in the present study had different effect on the fermentation responses of the WCBS. It was concluded that *Lactobacillus plantarum* was more effective in limiting the degradation of protein to ammonia rather than the untreated which caused to improve the silage quality. The data of *in situ* degradation coefficients showed that ammonium propionate had a most impact on effective degradability of NDF of WCBS between the used in the present study.

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