RESEARCH ARTICLE

Hamid Paya [1]
Akbar Taghizadeh [1]
Saman Lashkari [2]

Effects of *Lactobacillus plantarum* and hydrolytic enzymes on fermentation and ruminal degradability of orange pulp silage

Authors' addresses:
[1] Department of Animal Science,
Faculty of Agriculture,
University of Tabriz, Tabriz, Iran.
[2] Department of Animal Science,
Faculty of Agriculture,
University of Kurdistan, Sanandaj, Iran.

Correspondence:
Saman Lashkari
Department of Animal Science, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran.
e-mail: S.Lashkari@hotmail.com

Article info:
Received: 19 April 2015
Accepted: 5 October 2015

ABSTRACT

The current study was carried out to examine the effect of inoculants, enzymes and mixtures of them on the fermentation, degradability and nutrient value of orange pulp silage. Orange pulp was treated with water (control), inoculant (*Lactobacillus plantarum*), enzymes (multiple enzyme) or inoculants + enzymes prior to ensiling (denoted C, I, E and I+E). For ensiled orange pulp, 84 kg of orange pulp were mixed with 16 kg of wheat straw as an absorbent. Three mini-silos were prepared for each treatment and ensiled for 90 days. Data of each silo within each silage treatment was averaged and used as an experimental unit in a completely random design. Silage pH, total fatty acid and ammonia nitrogen were determined. Silage pH and lactic acid concentration were lowest and highest respectively for I and I+E ($p<0.01$), while the lowest ($p<0.01$) NH_3N concentration (49.8 g/kg total N) was observed in I compared to the control. The lowest acetic and butyric acid concentrations were observed in I and I+E compared with the control ($p<0.01$). The highest metabolizable energy (ME), net energy lactation (NEl), digestible organic matter in dry matter (DOMD), short chain fatty acid (SCFA) and microbial protein (MP) values were observed for I+E ($p<0.01$). The *in vitro* degradability of dry matter (IVDMD) was highest ($P<0.01$) in I+E, while the highest ($P<0.01$) effective degradability of DM (EDDM) was observed for E and I+E treatments. These results indicated that the bacterial inoculants and combination of enzyme and bacterial inoculants clearly improved silage fermentation characteristic. In addition, the ME, DOM, MP and IVDMD of I+E were significantly improved.

Key words: orange pulp silage, degradability, enzymes, *Lactobacillus plantarum*

Introduction

Orange pulp is the by-product obtained when orange fruits are pressed to make juice. Orange pulp is a proper energy source, and also has high potential rumen degradability, high apparent digestibility and it is considered as pectin-rich feeds (Lashkari & Taghizadeh, 2015). Orange pulp can be fed fresh, ensiled or dried. Storage of fresh orange pulp is difficult due to its high moisture content. The process of drying is costly and often inconvenient, but using the ensiling orange pulp is cheaper than dry processing and can be easily done by the farmer. The ensiled orange pulp has been fed successfully to dairy cattle (Volanis et al., 2006, Volanis et al., 2004) and fattening lamb (Gado et al., 2011).

Using of bacterial inoculants as starters for silages have been recommended to ensure rapid fermentation during the early stages of ensiling, to minimize the loss of nutrients, dry matter and to accelerate the decline of pH by promoting homo-fermentation of major water soluble carbohydrates (WSC) to lactate. Rapidly decreasing pH conserves WSC and declining proteolysis and deamination by inhibiting prolonged fermentation (Muck, 1993). Positive outcomes such as higher lactate: acetate ratios, lower ammonia N, decreased DM losses (Henderson, 1993), increased

DOI: 10.69085/jbb20153349

digestibility and degradability, improved aerobic stability and enhanced growth performance (McAllister et al., 1998; Muck, 1993) have been reported by adding microbial inoculation. Most biological additives contain homo-fermentative lactic acid bacteria to obtain the highest possible amount of lactic acid in silage.

Exogenous fibrolytic enzymes can improve the rate of ruminal degradability of dry matter and neutral detergent fiber and also increase the availability of WSC to use as a substrate for lactic acid producing bacteria (LAB) (McDonald et al., 1991; Weinberg et al., 1995). Gado et al. (2011) investigated the effects of addition of exogenous enzymes during ensilage of orange pulp on performance of lamb and reported that growth of fattening lamb was improved by enzyme addition. Nadeau et al. (2000) evaluated effects of inoculants and enzymes and reported that quality of legume and grass silage was improved. However, information is rare on effects of addition of exogenous enzymes, inoculants, and inoculants plus enzyme mixtures during ensilage of orange pulp. The objective of this experiment was to evaluate the effect of inoculants, enzymes and enzyme plus inoculants mixture ensiled with orange pulp on silage fermentation characteristics, in vitro digestibility and DM disappearance.

Materials and Methods

Silage preparation and treatment

Orange pulp (OP) was collected from a food industrial company in Northwestern Iran. The average dry matter content of OP was 170 g/kg. Due to the high moisture content and the physical property (after chopping) of OP (approximately 900 mm^3), wheat straw was added as an absorbent. Eighty-four kilograms of chopped OP were mixed with 16 kg of wheat straw (fresh weight basis). The mixture was divided into equal portions for application of four treatments: (1) distilled water (control), denoted as treatment C; (2) inoculant (Lalsil containing *L. plantarum* MA-18/5U, 3×10^6 and *Propionibacterium acidipropionici* MA-26, 3×10^6 CFU/g of fresh silage. The inoculants were dissolved in distilled water before application to the silage), treatment I; (3) enzymes (Natuzyme Plus; a mixture of enzymes: cellulase, xylanase, β-qlucanase, α-amylase, pectinase, phytase, proteases and lipase activities of 6000000, 10000000, 700000, 700000, 70000, 500000, 3000000 and 30000 U/kg, respectively), treatment E. Enzymes were applied at a rate of 1 g/kg of fresh forage; (4) inoculants plus enzymes, treatment I+E. Treatment I+E was applied in a manner that achieved the same concentrations of inoculants and enzymes as in treatments I and E. An appropriate amount of each product was dissolved in 50 ml of distilled water and applied to 25 kg of freshly mixed OP and wheat straw using a hand sprayer. Three mini-silos were prepared for each treatment and silages were ensiled for 90 days.

Chemical composition

Silage samples were taken from each silo after opening and mixing of contents. For measurement of pH, silage (15 g) was blended with 135 ml of deionized water for 30 s. The homogenate was filtered through two layers of cheesecloth and pH was immediately measured (Zahiroddini et al., 2004). The pH value was measured using a digital pH meter and ammonia-N concentration was determined by direct distillation in Kjeldahl equipment according to Cajarville et al. (2006). The filtrate was used for the determination volatile fatty acids (VFA) and NH_3-N. Subsamples of filtrate were prepared for analysis of VFA by adding 1 ml of 25% (wt/vol) meta-phosphoric acid to 5 ml of filtrate. Lactic acid in water extracts of the silages was determined spectrophotometrically according to Barker & Summerson (1941). In order to analyse VFA and NH_3-N, samples were stored on ice and then stored at –40°C. Before analysis, the samples were thawed overnight at 4°C. Silage VFAs were quantified using gas chromatography (WCOT Fused Silica Capillary, chorompack CP 9002) flame ionization detection and crotonic acid was the internal standard.

Silage samples were dried in a forced air oven at 55°C until constant weight and for chemical analyses they were milled through a 1.0 mm sieve. The chemical compositions of silages were determined using the methods recommended by AOAC (2005). Determinations of N were conducted using the Kjeldahl method in an automated Kjelfoss apparatus (Foss Electric, Copenhagen, Denmark). Neutral-detergent fiber (NDF) and Acid-detergent fiber (ADF) were determined by the detergent procedures of Van Soest et al. (1991).

In situ ruminal procedure

Three wethers fitted with rumen cannula were used to measure rumen degradability of silages. The wethers were fed a diet composed of (on DM basis), 385 g/day alfalfa hay, 280 g/day barley grain, 35 g/day wheat bran and 1.5 g/day lime stone at maintenance (NRC, 1985). The wethers were kept in individual tie-stalls with individual feed bins in an animal house and had continuous access to water. Diets were given as total mixed ration with fresh feed offered twice each day (08:30 and 15:30 h). The nylon bag technique (Ørskov &

DOI: 10.69085/jbb20153349

McDonald, 1979) was used to measure the DM degradation of feeds in the rumen. Nylon bags (4×8 cm polyester bag; poor size 45-50 µm) containing 3 g of feed ground through a 2 mm screen were incubated in the rumen for 4, 8, 16, 24, 48, 72 and 96 h for feeds, immediately after the morning feeding. Six replicates were incubated for each feed sample. Immediately after removal from the rumen, the bags were washed in cold water and frozen at −18°C. The residues were weighed and submitted for analysis. The kinetics of *in situ* DM and crude protein (CP) disappearance were estimated using a non-linear procedure of SAS (1999). The model of Ørskov & McDonald was fitted to the percentage of DM and CP disappearance as:

$$Y= a+ b\,(1 - e^{-ct})$$

where '*a*' is the soluble fraction,'*b*' the slowly disappearing fraction,'*c*' the fractional rate of disappearance (per h) and t is the incubation time (h). Effective ruminal disappearance was estimated using the model:

$$Y= a + bc/(c + k),$$

where *k* is the fractional rate of particulate passage, assumed to be 0.03 (Ørskov and McDonald, 1979)

In vitro gas production

Silage samples (300 mg) were weighed into 100 ml serum vial. McDougall (1948) buffer solution was prepared and placed in a water bath at 39°C. Rumen liquor samples were obtained from the three wethers that were used for *in situ* technique. Rumen fluid was collected after the morning feeding. Rumen fluid was pumped with a manually operated vacuum pump and transferred into pre-warmed thermos flask, combined, filtered through four layers of cheesecloth and flushed with CO_2. Each feed sample was incubated in six replicates with 20 ml of rumen liquor and buffer solution (1:2). Five vials containing only the rumen fluid/buffer solution and no feed sample was included with each test and the mean gas production value of these vials was termed the blank value. The vials were sealed immediately after loading and were affixed to a rotary shaker platform (lab-line instruments Inc Melors dark, USA) set at 120 rpm housed in an incubator. Gas production was measured in each vial after 2, 4, 8, 12, 16, 24, 36, 48, 72 and 96 h of incubation using a water displacement apparatus (Fedorak & Hrudey, 1983).

In vitro dry matter digestibility

The *in vitro* dry matter digestibility (IVDMD) procedure was carried out according to Holden (1999). Rumen liquor samples were obtained from the three wethers that used for *in situ* and gas production technique. Rumen fluid was collected after the morning feeding, pumped with a manually operated vacuum pump and transferred into pre-warmed thermos flask, combined, filtered through four layers of cheesecloth and flushed with CO_2. Samples weighted into ash-free and N-free filter bags. The following reagents were used: solution A and B that prepared according to recommendation of Holden (1999). Each of the four silage samples was digested in eight replicates. Sixteen bags were placed in each digestion vessel, 1600 ml of the buffer solution and 400 ml of the rumen inoculums were added to each vessel. Vessels were then flushed with CO_2 and were placed in the DAISY for incubation at 39°C for 48h. Then, the bags were incubated for 24 in pepsin/HCl solution. Bags were rinsed thoroughly and dried at 100°C for 24h and dry weight was recorded. Also, blank bags were used to correct for bacterial contamination.

Calculations and statistical analysis

Rate and extent of gas production was determined for each feed by fitting gas production data to the one component France et al. (2000). Parameters A and c were estimated by an iterative least square method using a non-linear regression procedure of the statistical analysis systems (SAS, 1999).

The metabolizable energy, net energy for lactation and digestible organic matter in dry matter content of feeds was calculated using equation of Menke & Steingass (1987) as:

$$\text{ME (MJ/kg DM)} = 2.2+0.136\text{GP}+0.0057\text{CP} +0.000286\text{CF}^2$$

$$\text{NE}_L \text{ (MJ/kg DM)} = 0.54+0.096\text{GP} +0.0038\text{CP} +0.000173\text{CF}^2$$

$$\text{DOMD (\%)} = 16.49 + 0.9042\text{GP} +0.0492\text{CP} +0.0387\text{CA}$$

The short chain fatty acid (SCFA) was calculated using equations of Menke et al. (1979):

$$\text{SCFA (mmol/200 mg DM)} = 0.0222\text{ GP} - 0.00425$$

where, GP is 24 h net gas production (ml/200 mg DM); CP, CF and CA are crude protein, crude fat and crude ash (% DM), respectively.

Microbial protein was calculated as 19.3 g microbial nitrogen per kg OMD (Czerkawski, 1986).

Data on *in situ* DM degradability, gas production parameters and *in vitro* dry matter digestibility were subjected to one-way analysis of variance using the analysis of variation model (ANOVA) of SAS (1999). Multiple comparison tests used Duncan's multiple-range test (Snedecor & Cochran, 1989).

DOI: 10.69085/jbb20153349

Results

Chemical composition before ensiling and silage characteristics in mini silos

The chemical composition of ensiled orange pulp prior to ensiling, and after 90 days of ensiling in laboratory silos are presented in Table 1. Concentrations of NDF and ADF were highest ($p<0.01$) in C silage. The pH of the inoculated silages (I and I+E) were lower ($p<0.01$) than the pH of the uninoculated silages (C and E).

The lactic acid concentration of the inoculated silages (I and I+E) were higher ($p<0.01$) than that of the uninoculated silages (C and E). Also, lowest acetic and butyric acid concentration were observed ($p<0.01$) in the inoculated silages (I and I+E). The concentration of propionic acid was unaffected by enzyme and inoculations (P= 0.06). The concentration of NH_3-N (as g/kg of total N; $p<0.01$) in I and I+E silages were lower than in silages C and E. Total fatty acid concentrations were higher in I+E and I than in E or C ($p<0.01$).

In situ dry matter degradability and estimated parameter

The mean values for DM disappearance of silages at different rumen incubation times, estimated parameter and effective degradability of DM (EDDM) are presented in Table 2. At 0 h incubation time DM disappearance of C silage was significantly ($p<0.01$) lower than the other silages. At 48, 72 and 96 h incubation time DM disappearance of E silage was significantly ($p<0.01$) higher than the other silages.

There were no significant difference between degradation rate (c) of DM ($p=0.24$) and rapidly soluble DM fraction (a) ($p=0.059$). Highest and lowest potentially degradable DM fraction (b) values were observed for E and I silages, respectively ($p<0.01$). The EDDM of silages with enzyme (E and E + I) were significantly higher than the other silage ($p<0.01$).

Table 1. *Chemical composition of orange pulp at time of ensiling and after 90 days of ensiling in mini silos.*

Items[†]	At time of ensiling	Treatment				SEM	*p*-value
		C	E	I	I + E		
pH	4.1 ± 0.03	3.8[a]	3.7[a]	3.5[b]	3.4[b]	0.03	0.001
DM	296.6 ± 1.7	276.2[b]	303.3[a]	302.4[a]	245.3[c]	0.22	0.001
CP	64.4 ± 1.0	69.6	70.6	68.3	68.9	0.15	0.76
Ash	89.8 ± 0.9	89.5	90.7	89.7	90.1	0.18	0.96
EE	13.5 ± 0.5	13.0	12.9	13.9	13.0	0.04	0.99
NDF	401.3 ± 2.8	351.3[a]	312.6[d]	334.7[b]	327.4[c]	0.22	0.001
ADF	307.3 ± 1.2	316.4[a]	267.5[d]	304.3[b]	283.1[c]	0.18	0.001
NH_3-N	2.7 ± 1.1	86.6a	84.7[a]	57.2[b]	49.8[b]	2.92	0.001
Lactic acid	1.2 ± 0.03	36.42[c]	41.25[b]	47.26[a]	51.05[a]	1.44	0.001
Acetic acid	3.44 ± 0.09	9.50[b]	14.47[a]	6.85[c]	7.13[c]	0.12	0.01
Propionic acid	1.23 ± 0.06	0.57	0.64	0.68	0.63	0.02	0.065
Butyric acid	0.05 ± 0.008	1.39[a]	1.21[a]	0.75[b]	0.73[b]	0.08	0.001
Total Fatty acid	5.95 ± 0.09	47.00[c]	56.36[a]	54.79[a]	58.82[a]	1.49	0.04
Lactic acid:Acetic acid ratio	0.35 ± 0.17	3.88[c]	3.26[d]	6.02[b]	7.51[a]	0.17	0.01

[†] DM is expressed as g/kg of as-fed diet, and all other items are expressed as g/kg DM. DM, Dry matter; CP, Crude protein; EE, Ether extract; NDF, Neutral detergent fiber; ADF, Acid detergent fiber; NH_3-N, Ammonia nitrogen.
C, control silage; E, enzymes silage; I, inoculant silage; I + E, inoculant + enzymes silage.
Means within a row with different subscripts differ. SEM, standard error of means (n=6).

DOI: 10.69085/jbb20153349

RESEARCH ARTICLE

In vitro gas production and in vitro dry matter digestibility

There was a difference ($p<0.05$) in gas production among silages (Table 3). Potential gas production (A) and rates of gas production (c) differed ($p<0.001$) among silages and I+E has highest amount. Also, ME, NE_L, SCFA, DOMD and MP values were high for I+E treatment. The IVDMD of silages were 505.3, 542.3, 565.5 and 674.3 for C, E, I and I+E treatment respectively (Table 4). Highest and lowest potentially IVDMD value was observed for I + E and C silages, respectively ($p<0.001$).

Discussion

Chemical composition before ensiling and silage characteristics in mini silos

Compared with the controls, enzymatic treatment decreased NDF concentration by 11% in E, by 6.80% in E + I. The lower level of ADF in E silage shows that the enzymes contributed to the hydrolysis of the plant cell walls in the orange pulp silage. The lower NDF concentration in I silage than in C can be explained by acidic hydrolysis of microbial inoculants. However, Moshtaghi Nia and Wittenberg (1999) reported no effects of inoculant on cell-wall concentrations of cellulase-treated orchardgrass and alfalfa silages. Averaged across plant species, cellulase degraded 25% of the cellulose and 13% of the hemicellulose. Less cell-wall degradation in the current study is likely due to the greater lignin concentration in wheat straw.

All four silages appeared to be of good quality, as supported by pH values below 4, high concentration of lactic acid and low concentration of acetic and butyric acid. Lashkari et al. (2014) reported that orange pulp contains high amount of pectin and soluble carbohydrates; resulting the fermentation of high soluble carbohydrates in this by-product lead to low final pH in the all four silages (McDonald et al., 1991). Our result is in agreement with Gado et al. (2011) who found that final pH of orange pulp silage was 3.85. Lowest pH values were observed for inoculation silages (I and I+E). Addition of LAB inoculants at ensiling ensured rapid and vigorous fermentation which resulted in faster accumulation of lactic acid, lower pH at earlier stages of ensiling and improved forage conservation. Many studies have shown the advantage of such inoculants (Schmidt et al., 2009).

Table 2. *Ruminal DM disappearance (g/kg DM) at different times of ruminal incubation*

	Treatment					
Time of incubation (h)	**C**	**E**	**I**	**I + E**	**SEM**	***p*-value**
0	294.5^{b}	310.2^{a}	310.2^{a}	308.0^{a}	1.85	0.001
4	314.5	317.2	324.7	319.2	2.85	0.14
8	342.7	346.2	351.7	345.0	3.35	0.32
16	373.0	377.5	382.0	379.7	2.85	0.20
24	429.5	440.2	437.7	438.2	3.30	0.16
48	528.0^{b}	551.0^{a}	529.9^{b}	538.7^{b}	3.35	0.001
72	561.2^{bc}	587.5^{a}	557.5^{c}	569.2^{b}	3.03	0.001
96	585.7^{b}	613.2^{a}	582.5^{b}	592.5^{b}	4.25	0.001
DM degradation characteristics †						
a (g/kg)	291.6	300.2	291.2	294.1	2.25	0.05
b (g/kg)	338.0^{b}	383.9^{a}	324.3^{c}	347.3^{b}	4.00	0.01
c (/h)	0.020	0.024	0.022	0.021	0.75	0.24
EDDM (3%)	434.0^{b}	446.6^{a}	439.0^{b}	440.9^{ab}	2.20	0.01

C, control silage; E, enzymes silage; I, inoculant silage; I + E, inoculant + enzymes silage.
†a, rapidly degraded fraction; b, slowly degraded fraction; c, rate of degradation; EDDM (3%), effective degradability of DM with passage rate of 0.03 h^{-1}.
Means within a row with different subscripts differ. SEM, standard error of means (n=6).

DOI: 10.69085/jbb20153349

RESEARCH ARTICLE

Table 3. *In vitro gas production characteristics.*

Time of incubation (h)	Treatment				SEM	*p*-value
	C	E	I	I + E		
2	47.2[c]	60.6[bc]	65.3[b]	101.8[a]	5.261	0.001
4	96.3[b]	101.0[b]	104.3[b]	140.8[a]	6.999	0.008
6	126.1[b]	127.0[b]	129.9[b]	174.9[a]	7.646	0.004
8	153.4[b]	1151.8[b]	151.8[b]	204.4[a]	8.522	0.005
12	187.7[b]	183.3[b]	187.1[b]	239.5[a]	9.078	0.007
16	215.4[b]	210.7[b]	213.3[b]	264.8[a]	8.741	0.006
24	248.1[b]	243.8[b]	240.0[b]	294.0[a]	8.676	0.008
36	269.3[b]	265.2[b]	261.2[b]	312.8[a]	8.613	0.012
48	279.5[b]	275.2[b]	269.7[b]	321.2[a]	8.748	0.018
72	284.4[b]	279.3[b]	273.5[b]	325.2[a]	8.845	0.020
96	288.5[b]	284.7[b]	278.5[b]	329.5[a]	9.127	0.031
Gas production constants						
A (ml/g DM)	283.9[b]	281.1[b]	274.6[b]	324.7[a]	8.530	0.019
C (h^{-1})	0.089	0.082	0.085	0.095	0.004	0.253
Lag time (h)	0.68[a]	0.097[b]	0.027[c]	0.015[c]	0.06	0.001
Estimated parameters						
ME (MJ/kg DM)	9.3[b]	9.2[b]	9.1[b]	10.5[a]	0.23	0.008
SCFA (mmol/200mg DM)	1.0[b]	1.0[b]	1.0[b]	1.3[a]	0.03	0.008
DOMD (gr/kg DOM)	606.2[b]	597.8[b]	590.0[b]	697.9[a]	15.6	0.008
NE_L (MJ/kg DM)	5.3[b]	5.2[b]	5.1[b]	6.2[a]	0.16	0.008
MP (gr/kg DOM)	11.9[b]	11.8[b]	11.7[b]	13.5[a]	0.30	0.008

C: control silage; E, enzymes silage; I: inoculant silage; I + E, inoculant + enzymes silage. *A*, Potential gas production; c, fractional rate of gas production (/h); ME, metabolizable energy; SCFA, short chain fatty acid; DOMD, digestible organic matter in dry matter; NE_L, net energy lactation; MP, microbial protein. Means within a row with different subscripts differ. SEM, standard error of means (n=6).

Table 4. *In vitro dry matter digestibility*

	Treatment					
IVDMD (g/kg)	**C**	**E**	**I**	**I + E**	**SEM**	***P*-value**
	505.3[c]	542.3[b]	565.5[b]	674.3[a]	10.68	<0.001

C: control silage; E, enzymes silage; I: inoculant silage; I + E, inoculant + enzymes silage. IVDMD, *In vitro* dry matter digestibility.Means within a row with different subscripts differ. SEM, standard error of means (n=6).

Forages inoculated with homolactic bacteria before ensiling usually had a lower pH than uninoculated silage (Muck, 1993). The result of this study is in agreement with results of Zhang et al. (2000) who reported that inoculating alfalfa silages with *Lactobacillus plantarum* decreased the pH compared with alfalfa silages without *L. plantarum*. In contrast to these findings, Hristov et al. (2000a) observed similar terminal pH in inoculated and uninoculated barley silages.

Lactic acid concentrations were higher ($p<0.01$) in I and I+E than in E or C. These findings are in agreement with those of Zahiroddini et al. (2004) who reported that the addition of bacterial inoculants and enzyme to forage at ensiling resulted in increase in lactic acid content compared with untreated silages. Acetic and butyric acid concentrations were lower in I+E and I than in E or C ($p<0.01$). In agreement with these findings, Xu et al. (2011) reported combination of *L. plantarum* and enzyme decreased acetic acid and butyric acid the silage. In the present study, propionic acid concentration was unaffected by bacterial inoculants and enzyme (p =0.06). In contrast to our finding, Xu et al. (2011) showed that *L. plantarum*, enzyme and combination of *L. plantarum* and enzyme decreased propionic acid compared with control silage.

Addition of enzyme to silage significantly increased lactic acid concentration compared with C ($p<0.01$). The greater NDF degradation by adding of enzyme during ensiling and sufficient sugars available to stimulate fermentation cause to increase lactic acid concentration (Moshtaghi Nia & Wittenberg, 1999).

It has been suggested that inoculation of silages with homofermentative LAB case to decline concentrations of an NH_3-N (Muck & Kung, 1997). After 90 days of ensiling, silages inoculated with *Lactobacillus plantarum* (I and I+E) had lower concentrations of NH_3-N than C and E. When

DOI: 10.69085/jbb20153349

RESEARCH ARTICLE

homofermentative LAB dominate the ensiling process NH3-N levels are reduced as a result of suppressed deamination and proteolysis (Heron et al., 1989). Our findings support the hypothesis of Muck & Kung (1997) that the silages supplemented with homofermentative LAB have lower concentrations of an NH_3-N. As well, Moshtaghi Nia & Wittenberg (1999) studied microbial inoculation on silage quality and suggested the rapid decline in pH partially inhibited plant proteolysis and probable proteolytic clostridia deamination of amino acids. The result of this study shown NH_3-N level for E was higher than the I and E + I. The higher level of NH_3-N for E silage was expected because the enzyme used in this study contains proteases that degrade protein to amino acids with further breakdown to NH_3-N by microbial activity.

In situ dry matter degradability and estimated parameter

The inoculation with *Lactobacillus plantarum* did not alter the silage EDDM. These findings agree with previous experiments (Zhang et al., 2000) showed that EDDM was not affected by inoculation with *L. plantarum*. However, Mandebvu et al. (1999) found that application of microbial inoculant treatment to bermudagrass forages silage increased the *in situ* rumen DM degradability. As well, Harrison et al. (1994) proposed that improvements in extent of DM from the addition of microbial inoculant to forage at ensiling have been small. It can be concluded that the result of microbial inoculation could be affected by factors such as different plant species treated as well as differences in microbial inoculant products.

There were no significant differences between degradation rate (c) of DM (p=0.24). This result is in agreement with Hristov et al. (2000b) who found that the degradation rate was not influenced by enzyme addition. The result of this study showed that EDDM of E and E + I were higher than the C. This is consistent with research by Van Vuuren et al. (1989) who reported increased *in situ* DM degradability in grass silage. It has been reported that supplementation of exogenous enzymes to feed enhances ruminal DM and fiber digestion by modifying the structural fractions of the feed thereby making it more available to microbial degradation (Beauchemin et al., 2001). Exogenous enzymes treatment increase bacterial colonization and improves DM disappearance of forage (Yang et al., 1999). Also, Wang et al., (2001) proposed that exogenous enzymes increased microbial attachment of ruminal microbes to feed particulate and enhance activity of enzymes associated feed particles. The higher level of EDDM in I + E silages may reflect both the effectiveness of the enzyme preparation and the more efficient fermentation (Zahiroddini et al, 2004).

In vitro gas production and in vitro dry matter digestibility

Adding enzyme and inoculants increased *in vitro* gas production and this positive effect agrees with previous reports (Wallace et al., 2001). Wallace et al. (2001) used six enzyme products to examine the relationship between enzyme activities and *in vitro* gas production using grass and corn silage. A significant positive correlation was reported between cellulase activity and gas production from grass silage. There is increasing evidence that plant cell wall degrading enzymes stimulate fiber digestion in the rumen (Feng et al., 1996; Yang et al., 1999). An increased gas production might be related to improve the silage quality (Hetta et al., 2007), which would also determine the microbial access to fermentable carbohydrates in the rumen. Lower gas production in silages treated with *Lactobacillus plantarum* agrees with results of Muck et al. (2007) and Hashemzadeh-Cigari et al. (2011) who found that silages treated with inoculants generally produced less gas per unit of incubated DM than the control silages.

These findings showed that inoculants enhanced silages *in situ* degradability, but did not affect its gas production. These results may suggest a superior action of inoculants in the real ruminal environment. This finding agrees with a previous report (Hashemzadeh-Cigari et al., 2011). Zhang et al. (2000) reported that *Lactobacillus plantarum* increased the D-isomer of lactate which is metabolized more slowly than its L-isomer (Giesecke & Stangassinger, 1980). Thus, it can be inferred that lactate and other by- or end-products of ensilage are metabolized more effectively under the dynamic *in situ* rumen conditions than in batch cultures of the *in vitro* laboratory bottles where some digestion products can easily accumulate. For E+I silage high content of ME, SCFA, DOMD, NEl and MP can result from its high rate of gas production, extent of gas production at 24 h and its nutrient composition.

Conclusion

Despite the proper fermentation characteristics of orange pulp silage (control), results of this study shows that treatment with microbial inoculants had beneficial effects on preserving orange pulp ensiled. Treatments of I + E significantly improved silage *in vitro* dry matter digestibility and *in vitro* gas production. Including enzymes improved *in*

DOI: 10.69085/jbb20153349

situ rumen degradability and estimated parameters. However, addition of microbial inoculant to the enzymes did not improve *in situ* rumen degradability.

References

AOAC. 2005. Association of official chemists. 18th Edition. Washington, DC.

Barker SB, Summerson WH. 1941. The colorimetric determination of lactic acid in biological material. J. Biological Chem., 138: 535–554.

Beauchemin KA, Morgavi DP, McAllister TA., Yang WZ, Rode LM. 2001. The use of enzymes in ruminant diets. In: Recent Advances in Animal Nutrition (Ed. P. C. Garnsworthy and J. Wiseman). Nottingham University Press. Nottingham, 298-322.

Cajarville C, Aguerre M, Repetto JL. 2006. Rumen pH, NH_3-N concentration and forage degradation kinetics of cows grazing temperate pastures and supplemented with different sources of grain. Anim. Res., 55: 511-520.

Czerkawski JW. 1986. An introduction to rumen studies. Pergamon Press. Oxford. New York.

Fedorak PM, Hrudey SE. 1983. A simple apparatus for measuring gas production by methanogenic cultuvesin serum bottles. Environmental Technol. Let., 4: 425-435.

Feng P, Hunt CW, Pritchard GT, Julien WE. 1996. Effect of enzyme preparations on in situ and in vitro degradation and in vivo digestive characteristics of mature cool-season grass forage in beef steers. J. Anim. Sci., 74: 1349–1357.

France J, Dijkstra J, Dhanoa MS, Lopez S, Bannink A. 2000. Estimating the extent of degradation of ruminant feeds in vivo from a description of their gas production profiles observed in vitro. 1. Derivation of models and other mathematical considerations. Brit. J. Nut., 83: 143–150.

Gado HM, Salem AZM, Odongo NE, Borhami BE. 2011. Influence of exogenous enzymes ensiled with orange pulp on digestion and growth performance in lambs. Anim. Feed Sci. Technol., 165: 131–136.

Giesecke D, Stangassinger M. 1980. Lactic acid metabolism. In: Digestive Physiology and Metabolism in Ruminants. Eds Ruckebush, Y. & Thivend, P., AVI Publishing, Westport, CT. 213-239.

Harrison JH, Blauwiekel R, Stokes MR, 1994. Fermentation and utilization of grass silage. J. Dairy Sci., 77(10): 3209-3235.

Hashemzadeh-Cigari F, Khorvash M, Ghorbani GR, Taghizadeh A. 2011. The effects of wilting, molasses and inoculants on the fermentation quality and nutritive value of lucerne silage. S. Afri. J. Anim. Sci., 41: 377-388.

Henderson N, 1993. Silage additives. Anim.Feed Sci. Technol., 45: 35–56.

Heron SJE, Edwards RA, Phillips P. 1989. The effect of pH on the activity of ryegrass proteases. Sci. Food Agric., 46: 267-277.

Hetta M, Cone JW, Bernes G, Gustavsson AM, Martinsson K. 2007. Voluntary intake of silages in dairy cows depending on chemical composition and in vitro gas production characteristics. Livestock Prod. Sci., 106: 47-56.

Holden A. 1999. Comparison of method of in vitro dry matter digestibility for ten feeds. J. Dairy Sci., 82: 1791-1794.

Hristov AN, McAllister TA, Graham S. 2000a. Effect of inoculants on whole-crop barley silage fermentation and dry matter degradability in the rumen. J. Anim. Sci 78: (Suppl. 2), pp. 107 (abstract). In: Recent Advances in Animal Nutrition (Ed. P. C. Garnsworthy and J. Wiseman). Nottingham University Press. Nottingham. pp. 298-322.

Hristov AN, McAllister TA, Cheng KJ. 2000b. Intraruminal supplementation with increasing levels of exogenous polysaccharide-degrading enzymes: Effects on nutrient digestion in cattle fed a barley grain diet. J. Anim. Sci., 78: 477-487.

Lashkari S, Taghizadeh A, Seifdavati J, Salem AZM. 2014. Qualitative characteristics, microbial populations and nutritive values of orange pulp ensiled with nitrogen supplementation. Slovak J. Anim. Sci., 47(2): 90-99.

Lashkari S, Taghizadeh A. 2015. Digestion kinetics of carbohydrate fractions of citrus by-products. Vet. Res. Forum, 6: 41 – 48.

Mandebvu P, West JW, Froetschel MA, Hatfield RD, Gates RN, Hill GM. 1999. Effect of enzyme or microbial treatment of bermudagrass forages before ensiling on cell wall composition, end products of silage fermentation and in situ digestion kinetics. Anim. Feed Sci. Technol., 77: 317-329.

McAllister TA, Feniuk R, Mir Z, Mir P, Selinger LB, Cheng KJ. 1998. Inoculants for alfalfa silage: effects on aerobic stability, digestibility, and the growth performance of feedlot steers. Livestock Prod. Sci., 53: 171–181.

McDonald P, Henderson AR, Heron SJE. 1991. The Biochemistry of Silage, 2nd ed. Chalcombe Publications, Marlow, Bucks, UK.

McDougall EI. 1948. The composition and output of sheep in saliva. Biochem. J., 43: 99-109.

Menke KH, Rabb L, Saleweski A, Steingass H, Fritz D, Schnider W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feed stuffs from the gas production when they are incubated with rumen liquor In vitro. J. Agric. Sci., 93: 217-222.

Menke KH, Steingass H. 1987. Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. Anim. Res., 28: 7-12.

Moshtaghi-Nia SA, Wittenberg KM. 1999. Use of forage inoculants with or without enzymes to improve preservation and quality of whole crop barley forage ensiled as large bales. Can. J. Anim. Sci., 79: 525–532.

Muck RE. 1993. The role of silage additives in making high quality silage. In: Proceedings of the National Silage Production Conference on Silage Production from Seed to Animal, Syracuse, NY, USA, 106–116.

Muck RE, Kung JRL. 1997. Effects of silage additives on ensiling. In: Proceedings of Silage: Field to Feedbunk. North. Amer. Con. NRAES, 99: 187-199.

Muck RE, Filya I, Contreras-Govea FE. 2007. Inoculant effect on lucerne silage: In vitro gas and volatile fatty acid production. J. Anim. Sci., 90: 5115-5125.

Nadeau EMG, Russel JR, Buxton DR. 2000. Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs. J. Anim. Sci., 78: 2980–2989.

NRC. 1985. Nutrient Requirements of sheep. The National Academies Press, Washington DC, USA

Ørskov ER, McDonald Y. 1979. The estimation of protein degradability in the rumen from determining the digestibility of feeds in the rumen. J. Agric. Sci., 92: 499-503.

DOI: 10.69085/jbb20153349

SAS. 1999. Static Analysis Software, Version release 8/0, SAS Institute Inc., Cary, NC, USA.

Schmidt RJ, Hu W, Mills JA, Kung JRL. 2009. The development of lactic acid bacteria and *Lactobacillus buchneri* and their effects on the fermentation of alfalfa silage. J. Dairy Sci., 92: 5005-5010.

Snedecor GW, Cochran WG. 1989. Statistical Methods. Iowa State University Press, USA.

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci., 74: 3583–3597.

Van Vuuren AM, Bergsma K, Frol-Kramer F, Van Beers JAC. 1989. Effects of addition of cell wall degrading enzymes on the chemical composition and the in sacco degradation of grass silage. Grass and Forage Science, 44(2): 223–230.

Volanis M, Zoiopoulos P, Tzerakis K. 2004. Effects of feeding ensiled sliced oranges to lactating dairy sheep. Small Rumin. Res., 5: 15–21.

Volanis M, Zoiopoulos P, Panagou E, Tzerakis K. 2006. Utilization of an ensiled orange pulp mixture in the feeding of lactating dairy ewes. Small Rumin. Res., 64: 190–195.

Wallace RJ, Wallace SJA, McKain N, Nsereko VL, Hartnell GF. 2001. Influence of supplementary fibrolytic enzymes on the fermentation of corn and grass silages by mixed ruminal microorganisms in vitro. J. Anim. Sci., 79: 1905–1916.

Wang Y, McAllister TA, Rode LM, Beauchemin KA, Morgavi DP, Nsereko VL, Iwaasa AD, Yang W. 2001. Effects of an exogenous enzyme preparation on microbial protein synthesis, enzyme activity and attachment to feed in the Rumen Simulation Technique (Rusitec). Brit. J. Nut., 85: 325-332.

Weinberg ZG, Ashbell G, Hen Y, Azrieli A. 1995. The effect of propionic acid bacterial inoculant applied at ensiling on the aerobic stability of wheat and sorghum silages. J. Industr. Microbiol. Biotech., 15: 493–497.

Xu Ch, Wang H, Yang F, Yu Zh. 2011. Effect of an inoculant and enzymes on fermentation quality and nutritive value of erect milkvetch (*Astragalus adsurgens* Pall.) silages. Journal of Animal and Feed Sciences, 20: 449–460.

Yang WZ, Beauchemin KA, Rode LM. 1999. Effects of enzyme feed additives on extent of digestion and milk production of lactating dairy cows. J. Dairy Sci., 82: 391–403.

Zahiroddini H, Baah J, Absalomb W, McAllister TA. 2004. Effect of an inoculant and hydrolytic enzymes on fermentation and nutritive value of whole crop barley silage. A. Feed Sci. Technol., 117: 317–330.

Zhang JG, Cai Y, Kobayashi R, Kumai S. 2000. Characteristics of lactic acid bacteria isolated from forage crops and their effects on silage fermentation. J. Sci. Food Agric., 80: 1455-1460.

DOI: 10.69085/jbb20153349