Effects of feeding corn silage inoculated with microbial additives on the ruminal fermentation, microbial protein yield, and growth performance of lambs

F. C. Basso,* A. T. Adesogan,† E. C. Lara,* C. H. S. Rabelo,* T. T. Berchielli,* I. A. M. A. Teixeira,* G. R. Siqueira,‡ and R. A. Reis*

*Department of Animal Science, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil 14883-900; †Department of Animal Science, Institute of Food and Agricultural Sciences, University of Florida, Gainesville 32611; and ‡Sao Paulo Agency for Agribusiness Technology – Alta Mogiana, Colina, Sao Paulo, Brazil 14770-000

ABSTRACT: This study aimed to examine the effects of feeding corn silage inoculated without or with either Lactobacillus buchneri (LB) alone or a combination of LB and Lactobacillus plantarum (LBLP) on the apparent digestibility, ruminal fermentation, microbial protein synthesis, and growth performance of lambs. Thirty Santa Inês × Dorper crossbred intact males lambs weighing 20.4 ± 3.8 kg were blocked by weight into 10 groups. Lambs in each group were randomly assigned to 1 of the following 3 dietary treatments: untreated (Control), LB, and LBLP silage. Lambs were fed experimental diets for 61 d. The apparent digestibility was indirectly estimated from indigestible NDF measured on d 57 to 59. Spot urine samples were collected from all animals on d 59 to estimate microbial protein synthesis. Lambs were slaughtered for carcass evaluation on d 61 when they weighed 32.4 ± 5.2 kg. Six additional ruminally cannulated Santa Inês × Dorper crossbred wethers weighing 40.5 ± 1.8 kg were used to examine dietary effects on ruminal fermentation. Average daily gain was increased when lambs were fed LBLP silage (P < 0.05) but not LB silage. The LBLP silage had the highest (P < 0.05) lactic acid concentration and both inoculated silages had greater acetic acid concentrations than the Control silage (P < 0.05). Inoculation of corn silage increased intakes of DM, OM, CP, NDF, total carbohydrate (CHO), and GE by the lambs but decreased digestibility of DM, OM, CP, total and nonstructural carbohydrates, and concentration of GE and ME (P < 0.05). Nevertheless, lambs fed inoculated silages had greater microbial N supply than those on the Control treatment (P < 0.05). The acetate to propionate ratio was lower in ruminal fluid of wethers in LBLP treatment than LB and Control treatment (P < 0.05) and ruminal pH tended to be greater in LB lambs than in LBLP and Control wethers (P < 0.10). Finally, the inoculation with both bacteria combined enhanced the silage fermentation. The intakes of DM, OM, CP, NDF, and GE were improved in the lambs fed corn silage inoculated with L. buchneri alone or combined with L. plantarum. The microbial N supply was enhanced in the lambs fed corn silage inoculated with L. buchneri. The inoculation of L. buchneri combined with L. plantarum reduced the acetate to propionate ratio in ruminal fluid and improved the ADG of lambs.

Key words: corn silage, digestibility, growth performance, heterofermentative inoculants, sheep

INTRODUCTION

Aerobic spoilage by yeasts and molds is a major cause of reduced nutritional value and wastage of silage. This problem can be severe in corn silage that is not preserved with additives or fed out at an appropriate rate, especially in warm weather. Yeasts utilize lactic acid produced by lactic acid bacteria (LAB) during aerobic exposure for growth. Depletion of lactic acid by yeasts increases the pH, which makes the silage conducive for the growth of spoilage-enhancing molds. Both yeasts and molds can reduce the nutritional quality of silage (Kung et al., 2003). An obligate heterofermentative LAB, Lactobacillus buchneri, has been successfully used as an additive to improve the aerobic stability of corn silages (Queiroz et al., 2012). This bacteria converts glucose and fructose into lactic acid, acetic acid, and other...
products (McDonald et al., 1991) and also converts lactic acid into acetic acid and 1,2-propanediol (Oude Elferink et al., 2001). The presence of acetic and or propionic acid protects the silage against spoilage by aerobic microorganisms (Moon, 1983) because of their antifungal nature. However, heterolactic fermentations are less desirable than homolactic fermentations because of the greater DM loss (McDonald et al., 1991). So the Lactobacillus plantarum, a facultative heterofermentative LAB, has been combined with L. buchneri in silage inoculants to reduce DM losses and protein degradation during the fermentation by faster and greater lactic acid production.

Furthermore, Weinberg et al. (2004a,b, 2007) suggested that LAB used in inoculant for silage could survive in ruminal fluid, to interact with ruminal microorganisms, to change ruminal fermentation, and to enhance rumen functionality as well as provide a probiotic effect in the small intestine. However, only few studies have examined effects of feeding silage treated with the L. buchneri and L. plantarum on the ruminal fermentation and animal performance (Nkosi et al., 2009; Ranjit et al., 2002). Therefore, this study was aimed at evaluating the apparent digestibility, ruminal fermentation, microbial protein synthesis, and growth performance of lambs fed corn silage inoculated with microbial additives.

**MATERIAL AND METHODS**

Animal feeding and ruminal fermentation trials were conducted at Sao Paulo State University (Jaboticabal, Sao Paulo, Brazil), located at 21°14′14.04″ S and 48°17′27.92″ W. According to the Köppen classification, the climate is of the “Aw” type characterized as tropical wet and dry, rainy in summer and dry in winter season. The animal care and handling procedures were followed according to the Sao Paulo State University’s Animal Care Committee (project number 022704/09).

Two experiments were conducted, one to measure apparent digestibility and growth performance and the other to measure ruminal fermentation products.

**Silage Production**

A corn hybrid (cv. Maximus; Syngenta, Matao, Sao Paulo, Brazil) was harvested at approximately 32% DM using a Premium Flex forage harvester (MentaMit; Cajuru, Sao Paulo, Brazil) and chopped without wilting to achieve a theoretical length of cut of 10 mm. The corn plants were treated with water (2 L/t; Control) or with either $1 \times 10^5$ cfu/g of fresh forage of L. buchneri NCIMB 40788 (Lallemand Animal Nutrition, Milwaukee, WI; LB) or LB combined with $1 \times 10^5$ cfu/g of fresh forage of L. plantarum MA18/5U (Lallemand Animal Nutrition, Milwaukee, WI; LBLP). The inoculants were dissolved in water (2 L/t) and then sprayed on piles of fresh forage under constant mixing. The application rate of the inoculant was determined in accordance to Basso et al. (2012) in tropical conditions. Ten metric tons of forage from each treatment was conserved in 22.6 ± 0.8 m$^3$ stack silos at a packing density of 442 ± 15.3 kg/m$^3$ (width: 4.1 ± 0.3 m; height: 0.60 ± 0.1 m; and length: 10.2 ± 0.8 m). Each silo was sealed with black-on-white plastic film (200 μm thick), which were weighed down with sand bags. Samples were taken to characterize the chemical composition of the corn plant at silo filling (Table 1).

After 165 d of ensiling at ambient temperature (21°C), silos were opened and fed out at a rate of approximately 10.5 cm/d. The silage was removed from vertical face of silo by hand using a fork. Samples of all silages were collected weekly and stored at –20°C for later analysis or stored after air drying (55°C) for 72h.

**Experiment 1: Animal Feeding Experiment**

Average ambient temperature during the trial ranged from 23.5 to 24°C. Thirty Dorper × Santa Ines crossbred intact male, weaned lambs with average initial BW of 20.4 ± 3.8 kg were blocked by weight into 10 groups. Lambs in each group were randomly assigned to 1 of the following 3 dietary treatments: Control, LB, and LBLP silage.

All diets were formulated to meet nutrient requirements of lambs gaining 200 g/d (NRC, 2007). Diets consisted of 80% of the respective corn silage, 11.5% of soybean meal, 1.0% of cottonseed meal (380 g/kg of CP), 1.4% of wheat meal, 3.2% of corn meal, 2.5% of citrus pulp, and 0.4% of mineral supplement (80 g/kg of P, 130 g/kg of Ca, 1,500 mg/kg of Na, 1,000 mg/kg of Mg, 60 mg/kg of Zn, 5 mg/kg of Cu, 40 mg/kg of Mn, 42 mg/kg of Fe, and 1.50 mg/kg of I) on a DM basis. The chemical composition estimated of the diets is shown in Table 2.

**Determination of Growth Performance**

Lambs were housed in individual wooden pens (0.5 m$^2$) with slated floored, each fitted with a feed and water container in a well-ventilated covered barn. Lambs were adapted to diets for 14 d and fed for 47 d of measurement. The diet during adaptation and measurement periods was the same. Diets were fed ad libitum twice daily (0700 and 1700 h). Water was available in ad libitum amounts. Refusals were weighed daily before the morning feeding and DM intake was calculated. Samples of offered feed and refusals were collected twice a week and stored at –20°C for later analysis.

Initial and final BW was measured after a 16-h fast and ADG was calculated by subtracting the initial BW from final BW and dividing the difference by the trial duration of 47 d. The G:F was calculated by dividing
daily ADG by DM intake. Average daily gain and G:F also were expressed relative to hot carcass yield.

**Determination of Apparent Digestibility and Microbial Protein Synthesis**

Apparent digestibility was determined indirectly using indigestible NDF (iNDF) as an indigestible marker (Valente et al., 2011). Fecal grab samples were collected from each lamb every 24 h from d 57 to 59 (Pina et al., 2006). Samples of silage, concentrate, and refusals were also collected daily. The samples were composited and analyzed.

On d 59, spot urine samples were collected for 2 h with bowls attached with a harness to each animal 4 h after the morning feeding. Subsamples (10 mL) of urine were collected and stored at −20°C for later analysis of purine derivatives (PD) to estimate microbial protein synthesis. The 10-mL urine sample was diluted with 40 mL of a 0.036 $\text{NH}_2\text{SO}_4$ solution before storage. Creatinine concentration in the urine was determined using a commercial kit (Labtest, Lagoa Santa, MG, Brazil).

**Determination Carcass Traits**

After the 61-d trial, the animals were anesthetized by electronarcosis (220 V and 0.5 A) and jugular veins and carotid arteries were severed. After evisceration, carcasses were weighed, hung by the gastrocnemius tendons at −4°C for 24 h, and weighed again to calculate carcass quantitative parameters according to Silva Sobrinho and Osório (2008). The subcutaneous fat thickness on the

| Item $^2$ | Control | LB | LBLP | SEM $^3$ | P-value |
|-----------|---------|----|------|---------|---------|
| **Chemical composition** | | | | | |
| DM | 320 | 355 | 314 | 0.7 | |
| OM | 952 | 949 | 947 | 2.3 | |
| CP | 87 | 80 | 89 | 2.0 | |
| EE | 27 | 26 | 27 | 1.9 | |
| NDF | 580 | 569 | 587 | 12.6 | |
| iNDF | 143 $^b$ | 143 $^b$ | 157 $^a$ | 3.0 | 0.020 |
| ADF | 272 | 261 | 284 | 8.8 | |
| **Cellulose** | | | | | |
| Hemicellulose | 309 | 308 | 303 | 11.0 | |
| Lignin | 54 | 42 | 59 | 2.7 | |
| CHO | 856 $^a$ | 841 $^b$ | 846 $^b$ | 3.2 | 0.008 |
| NSC | 394 | 386 | 385 | 5.9 | 0.555 |
| **Fermentation profile** | | | | | |
| Lactic acid | 67 $^b$ | 62 $^b$ | 93 $^a$ | 7.5 | 0.018 |
| Acetic acid | 27 $^b$ | 42 $^a$ | 55 $^a$ | 5.9 | 0.011 |
| Lactate:acetate ratio | 2.6 | 1.5 | 2.9 | 0.4 | 0.169 |
| pH | 5.1 | 5.2 | 5.3 | 0.1 | |
| Ammonia N, % TN | 2.3 | 2.4 | 2.3 | 0.1 | 0.076 |

$^a,b$ Means within a row with different superscripts differ ($P < 0.05$).

1Corn silage inoculated or uninoculated at ensiling with either $1 \times 10^5$ cfu/g of fresh forage of *Lactobacillus buchneri* NCIMB 40788 (Lallemand Animal Nutrition, Milwaukee, WI; LB) or LB combined with $1 \times 10^5$ cfu/g of fresh forage of *Lactobacillus plantarum* MA18/5U (LBLP).

2EE = ether extract; iNDF = indigestible NDF; CHO = total carbohydrates; NSC = nonstructural carbohydrates; TN = total nitrogen.

3Standard errors of least squares means and $P$-values represent statistical comparison between LB, LBLP, and Control silages.

**Table 2. Estimation of chemical composition (g/kg of DM) and energy values (MJ/kg of DM) of the experimental diets$^1$**

| Item $^2$ | Control | LB | LBLP |
|-----------|---------|----|------|
| DM | 440 | 454 | 426 |
| OM | 949 | 943 | 943 |
| CP | 130 | 135 | 130 |
| EE | 26 | 27 | 27 |
| NDF | 436 | 436 | 441 |
| ADF | 260 | 253 | 264 |
| CHO | 792 | 780 | 785 |
| NSC | 382 | 375 | 374 |
| GE | 16.5 | 16.3 | 16.5 |
| DE | 11.8 | 11.0 | 11.0 |
| ME | 9.7 | 9.0 | 9.0 |

$^1$Corn silage inoculated or uninoculated at ensiling with either $1 \times 10^5$ cfu/g of fresh forage of *Lactobacillus buchneri* NCIMB 40788 (Lallemand Animal Nutrition, Milwaukee, WI; LB) or LB combined with $1 \times 10^5$ cfu/g of fresh forage of *Lactobacillus plantarum* MA18/5U (LBLP).

2EE = ether extract; CHO = total carbohydrates; NSC = nonstructural carbohydrates.
left side carcasses was measured with a caliper over the loin-eye muscle between the 12th and 13th ribs.

**Experiment 2: Determination of Ruminal Fermentation Products**

Six Dorper × Santa Ines crossbred ruminally cannulated wethers, each fitted with a silicone, 6.4-cm ruminal cannula, were used in an experiment arranged as a replicated 3 × 3 Latin square. The initial BW of the wethers was 40.5 ± 1.8 kg and they were housed individually in 0.9-by-2.0-m pens, each fitted with individual feed and water containers. The sheep were fed ad libitum once a day (0700 h) with same diets as described above. Water was available in ad libitum amounts.

Ruminal measurements were taken over 3 experimental 10-d periods, each consisting of 9 d for diet adaptation and 1 d for ruminal fluid collection. A 50-mL sample of ruminal fluid was collected from each sheep before feeding and 3, 6, 9, and 12 h afterward and squeezed through 2 layers of cheesecloth and the pH was immediately measured. Subsequently, 1 mL of H₂SO₄ (1:1) was added to the ruminal fluid and the solution was stored at −20°C for analysis of concentrations of ammonia N and VFA.

**Chemical Analysis**

A water extract was made from fresh silage samples according to Kung et al. (1984) and an electrode (MA522 model; Marconi Laboratory Equipments, Piracicaba, SP, Brazil) was used to measure the pH. Volatile fatty acids were measured in a gas chromatograph (GC2014; Shimadzu Corporation, Kyoto, Japan) using a HP-INNO wax capillary column (30 m by 0.32 mm; Agilent Technologies, Loveland, CO) at an initial temperature of 80°C and a final temperature of 240°C. Lactic acid was determined by a colorimetric method (Barker and Summerson, 1941). Ammonia N was measured by distillation (AOAC, 1996; method number 941.04).

Samples of silage, concentrate, refusals, and feces were oven dried (55°C for 72 h), ground in a knife mill (Wiley; A. H. Thomas, Philadelphia, PA) to pass through a 1-mm screen, and analyzed for DM (105°C for 12 h) and ash (500°C for 5 h). The NDF and ADF concentrations were determined using the method of Van Soest et al. (1991) in an ANKOM 2000 Fiber Analyzer (ANKOM Technologies, Macedon, NY) without sodium sulfite. Heat-stable α-amylase was used in the NDF assay. The NDF also was corrected for ash and protein.

Indigestible NDF was measured by incubating bags containing the feed, refusals, and feces samples in the rumens of 2 bulls for 264 h and determining the iNDF content in the residual feed and feces after the incubation (Casali et al., 2008). The bags (25 cm²) were made of nonwoven textile (100 g/m²) material (Valente et al., 2011) and a sample weight to bag surface area ratio of 20 mg DM/cm² was used (Nocek, 1988). The bulls were fed diets consisting of 60% of corn silage and 40% of concentrate on a DM basis. The iNDF concentration was determined using an autoclave at the temperature of 110°C for 40 min (Senger et al., 2008).

Lignin concentration was measured after hydrolysis of the cellulose in ADF residues in a 72% H₂SO₄ (Van Soest and Robertson, 1985). Ether extract was measured according to the AOAC (1996; method number 920.39). The N concentration was determined by rapid combustion using a Leco F528 N analyzer (LECO Corporation, Milone, IL) and DE was calculated by subtracting the GE excreted in feces from the GE consumed. The ME was estimated as 0.82 from DE (NRC, 1985).

Total carbohydrate (CHO) and nonstructural carbohydrate (NSC) concentrations were estimated according to Sniffen et al. (1992) and Detmann and Valadares Filho (2010), respectively.

Purine derivatives were measured as the sum of allantoin, uric acid, xanthine, and hypoxanthine according to Chen and Gomes (1995). Daily PD excretion and microbial N supply was calculated according to Chen et al. (1995; Eq. [3] to [5]). The efficiency of microbial N synthesis (EMNS) was expressed as grams of microbial N per kilogram of digestible OM fermented in the rumen (DOMR; calculated as digestible OM intake × 0.65, according to the Agricultural Research Council (1984)). The pH of ruminal fluid was measured using a pH meter (MA522 model; Marconi Laboratory Equipments). Ammonia N was measured by distillation with 2 N KOH according to Fenner (1965). Aliquots of strained ruminal content collected were thawed in the refrigerator overnight and centrifuged at 4°C and 20,000 × g for 30 min, and the supernatant was analyzed for VFA by gas chromatography as described earlier.

**Calculation and Statistical Analysis**

The fecal output and apparent digestibility were calculated using Eq. [1] and [2]:

\[
\text{fecal DM output} = \frac{\text{iNDF intake}}{\text{fecal iNDF}} \times 100, [1]
\]

in which iNDF intake is expressed in grams and fecal iNDF is expressed as a percent, and

\[
\text{apparent digestibility} = \left[\frac{\text{nutrient intake} - \text{fecal nutrient output}}{\text{nutrient intake}}\right] \times 100, [2]
\]
in which nutrient intake and fecal nutrient output is expressed in grams.

To estimate microbial protein synthesis, the daily PD excretion and microbial N supply were calculated according to Chen et al. (1995; Eq. [3] to [5]):

$$\text{PDC index} = \left( \frac{\text{daily PD}}{\text{daily creatinine}} \right) \times \text{BW}^{0.75} \times \text{BW}^{0.75} \times \text{BW}^{0.75} \times \text{BW}^{0.75} \times \text{BW}^{0.75}, [3]$$

in which daily PD and daily creatinine is expressed in millimoles per day and Purines derivatives Creatinine (PDC) index = [PD (in spot urine samples; mmol)/creatinine (in spot urine samples; mmol)] \times \text{BW}^{0.75}.

Microbial N supply (Eq. [4]) and intestinal flow of microbial N (Eq. [5]) were estimated according to Chen and Gomes (1995):

$$\text{daily PD} = 0.84 \times \text{AP} + [0.150 \times \text{BW}^{0.75} \times \exp(-0.25 \text{AP})], \quad [4]$$

in which daily PD is expressed in millimoles per day, AP is the absorption of microbial purines (mmol/d), 0.84 represents the recovery of absorbed purines as PD in urine, and the component within brackets represents the endogenous contribution, which decreases as exogenous purines become available for utilization by the animal, and

$$\text{microbial N} = (\text{AP} \times 70)/(0.116 \times 0.83 \times 1,000) = 0.727 \times \text{AP}, \quad [5]$$

in which microbial N is expressed in grams N per day, the N concentration of purines is 70 mg N/mmol, the ratio of purine-N:total N in mixed ruminal microbes is taken as 11.6:100, and the digestibility of microbial purines is assumed to be 0.83.

Efficiency of microbial N synthesis was calculated by dividing microbial N supply (g/d) by the amount of OM apparently digested (DOMR; kg).

The corn silage chemical composition data were as analyzed as a completely randomized design with 9 replicates (a compound sample per week of study). Data on the effects of treatments on digestibility, microbial protein synthesis, and performance were analyzed as a randomized block design (with 10 replicates). Ruminal parameter data were analyzed as a replicated 3 \times 3 Latin square design. All data were analyzed using the MIXED procedure of SAS (version 9.0; SAS Inst. Inc., Cary, NC). The treatments were considered as fixed effect and the animals were considered as random effect. The initial BW was used as a covariate for analyzing performance and carcass data. The model for analyzing ruminal fermentation data included a repeated measures statement; the treatments were the fixed effect and the animals and periods were considered random effects. Banded Toepplitz and autoregressive average were the best covariance structures for the data as these had the lowest Akaike information criterion scores. Differences between means were determined using the PDdiff, which differentiates means based on Fisher’s F-protected least significant difference test. Significance was declared at $P < 0.05$ and tendencies to significance at $0.05 \leq P < 1$. 

**RESULTS**

The DM concentration of the LB silage was greater than those of the other silages ($P < 0.05$; Table 1). Total carbohydrate concentration was lower in inoculated silages ($P < 0.05$). Inoculated silages had lower OM concentrations than the Control silage ($P < 0.05$). The ADF and iNDF concentrations were greater in the LBLP silage than the others ($P < 0.05$), but cellulose and lignin concentrations were unaffected by treatment.

Inoculation with LBLP increased the lactic and acetic acid concentrations for the silage, whereas inoculation with LB increased only the acetic acid concentration compared to the Control silage ($P < 0.05$). Inoculation had no effect on other silage fermentation characteristics.

Lambs fed diets containing the LB and LBLP silages had greater intake of DM, OM, CP, NDF, CHO, and GE than those fed the Control silage ($P < 0.05$; Table 3). These measures of intake did not differ among diets containing inoculated silages except that lambs fed LBLP silage had greater CP intake than those fed LB silage ($P < 0.05$). However, apparent digestibilities of DM, OM, CP, CHO, NSC and GE, and concentrations of DE and ME were less for the diets containing LB and LBLP silages compared to that of the Control diet ($P < 0.05$).

Lambs fed the diet containing LB silage had similar NDF digestibility to those fed the Control diet and 4.6% higher values than those fed the LBLP silage diet ($P < 0.05$). Hence, lambs that consumed the LB silage diet had greater digestible NDF intake (242 g/d) than fed other diets (LBLP: 225 g/d, and Control: 222 g/d; $P < 0.05$). Intake of digestible OM and CHO were greater in lambs fed inoculated silage diets instead of the Control diet, yet DE intake was lower in lambs fed inoculated silage diets instead of the Control diet.

Lambs fed the diet containing LB silage had greater concentrations of urinary allantoin and PD, higher DOMR, and greater microbial N supply than those fed the Control silage diet ($P < 0.05$; Table 4). Lambs fed LBLP silage also had greater DOMR than those fed Control silage diet but their PD and microbial N supply values did not differ from those of the other treatments.

Average daily gain was increased by 4% in lambs fed the diet containing LBLP silage instead of the Control silage ($P < 0.05$; Table 5). This increase was also evident when ADG was expressed in relation to hot carcass yield. Lambs fed the Control silage diet had greater G:F than those fed the diet containing the LB silage and similar
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Table 3. Effect of bacterial inoculation of corn silage on intake (g/d), apparent digestibility of nutrients (%), and energy concentration (MJ/kg) in lambs. All data are from Exp. 1 unless otherwise stated.

| Item | Control | LB | LBLP | SEM | P-value |
|------|---------|----|------|-----|---------|
| Intake, g/d | | | | | |
| DM (Exp. 2) | 1,001b | 1,050a | 1,058a | 53.9 | 0.001 |
| OM | 958b | 1,015a | 1,023a | 46.8 | 0.022 |
| CP | 134c | 141b | 148a | 7.5 | 0.001 |
| EE | 30 | 31 | 31 | 1.6 | 0.206 |
| NDF | 411b | 442a | 446a | 21.8 | 0.002 |
| CHO | 783b | 822a | 840a | 41.5 | 0.026 |
| NSC | 405 | 421 | 423 | 21.7 | 0.212 |
| GE, MJ/d | 16.5b | 17.2a | 17.3a | 0.9 | 0.001 |
| Apparent digestibility, % | | | | | |
| DM | 71.9a | 68.3b | 67.2b | 0.6 | 0.001 |
| OM | 72.5a | 70.0b | 68.1c | 0.6 | 0.001 |
| CP | 72.2a | 66.5b | 66.8b | 1.3 | 0.006 |
| EE | 88.9 | 87.5 | 87.0 | 0.7 | 0.052 |
| NDF | 54.2a | 54.9a | 50.3b | 0.6 | 0.001 |
| CHO | 72.1a | 69.8b | 67.5b | 0.5 | 0.001 |
| NSC | 87.7a | 86.2b | 85.2b | 0.4 | 0.001 |
| GE | 71.2a | 67.9b | 66.5b | 0.6 | 0.001 |
| Energy value, MJ/kg DM | | | | | |
| DE | 11.7a | 11.0b | 11.0b | 0.1 | 0.001 |
| ME | 9.6a | 9.1b | 8.9b | 0.1 | 0.001 |

a,bMeans within a row with different superscripts differ (P < 0.05).

1Corn silage inoculated or uninoculated at ensiling with either 1 × 105 cfu/g of fresh forage of Lactobacillus buchneri NCIMB 40788 (Lallemand Animal Nutrition, Milwaukee, WI; LB) or LB combined with 1 × 105 cfu/g of fresh forage of Lactobacillus plantarum MA18/5U (LBLP).

2Standard errors of least squares means and P-values represent statistical comparison between LB, LBLP, and Control silages.

3Xanthine and hypoxanthine were converted into uric acid (xanthine oxidase enzyme).

4PD = purine derivatives (sum of allantoin and uric acid).

5DOMR = digestible OM fermented in the rumen.

Values to lambs fed the LBLP silage diet. However, when G:F was expressed relative to hot carcass yield, no differences among treatments were evident. Other carcass yield measures were unaffected by treatment.

Most ruminal fermentation indices (Fig. 1 and 2) were not affected by dietary treatment. However, acetate to propionate ratio was lower in the ruminal fluid of lambs fed the LBLP silage diet versus those fed other diets (P < 0.05). Unlike those of others, ruminal fluid pH values of wethers fed the LB silage diet remained above 6 throughout the monitoring period. Ammonia N concentration was not affected by dietary treatment (P > 0.10).

DISCUSSION

The DM concentrations of silages differed among treatments reflecting similar differences in the DM concentrations of the forages at ensiling (Table 1). However, the reduction in DM concentration between that in the plant and the silage was greater in inoculated silages (15 to 20 g/kg of DM), presumably reflecting the increased water production due to greater fermentative activity (McDonald et al., 1991).

Inoculation with LB or LBLP resulted in the lower OM and NSC concentrations in the silages. These responses likely reflect greater fermentation of carbohydrates in inoculated silages. Lactic acid bacteria need carbohydrates as energy and carbon sources and these microorganisms metabolize NSC into organic acids (Rooke and Hatfield, 2003).

As expected, when L. buchneri was combined with facultative heterofermentative L. plantarum, lactic acid concentration increased. The latter increased the supply of substrates for conversion into acetic acid by L. buchneri; hence, the LBLP silage had the highest acetic acid concentration. In agreement, Weinberg et al. (2002), Filya (2003), and Hu et al. (2009) also found increases in the lactate concentration of corn silage inoculated with L. buchneri and L. plantarum and increases in the acetic acid concentration of corn silage inoculated with L. buchneri alone or combined with L. plantarum.

All pH values were low as they fell within the range of 3.7 to 4.2 (Kung and Shaver, 2001). Low pH values inhibit protein degradation in silage (McDonald et al., 1991) and ammonia N concentrations were below the upper limit of the range (5 to 7% Total Nitrogen) beyond which unsuitable preservation of silage occurs (Kung and Shaver, 2001).

Although pH values during feed-out were similar between silages and no difference was observed among concentrations of NDF, the NDF concentration of LBLP silage decreased by 89 g/kg of DM during ensiling, which unsuitable preservation of silage occurs (Kung and Shaver, 2001).
Table 5. Effect of bacterial inoculation of corn silage on growth performance and carcass yield of lambs

| Item* | Control | LB | LBLP | SEM* | P-value |
|-------|---------|----|------|------|---------|
| IBW, kg | 20.2 | 20.6 | 20.4 | 1.3 | 0.350 |
| ADG, g/d | 198bc | 199bc | 206a | 6.2 | 0.030 |
| G:F | 0.190b | 0.190a | 0.195ab | 0.0 | 0.017 |
| SBW, kg | 32.0 | 32.5 | 32.7 | 0.4 | 0.373 |
| HCV, kg | 13.9 | 14.2 | 14.6 | 0.2 | 0.139 |
| CCW, kg | 13.5 | 13.7 | 14.2 | 0.2 | 0.126 |
| HCY, % | 41.4 | 42.4 | 43.2 | 0.7 | 0.064 |
| CCY, % | 42.9 | 43.8 | 44.6 | 0.7 | 0.092 |
| LC, % | 3.2 | 3.0 | 3.1 | 0.1 | 0.198 |
| ADGc, g/d | 68ab | 75b | 83a | 3.5 | 0.027 |
| G:Fc | 68 | 73 | 78 | 3.4 | 0.134 |
| FT, mm | 1.9 | 1.8 | 1.8 | 0.2 | 0.905 |

*Means within a row with different superscripts differ (P < 0.05).

1Corn silage inoculated or uninoculated at ensiling with either 1 × 10^5 cfu/g of fresh forage of Lactobacillus buchneri NCIMB 40788 (Lallemand Animal Nutrition, Milwaukee, WI; LB) or LB combined with 1 × 10^5 cfu/g of fresh forage of Lactobacillus plantarum MA18/5U (LBLP).

2BW = initial body weight; SBW = shrunk body weight; CCW = cold carcass weight; HCY = hot carcass yield; CCY = cold carcass yield; LC = losses due to cooling; ADGc = ADG expressed in relation to HCY; G:Fc = feed efficiency expressed in relation to HCY; FT = fat thickness.

3Standard errors of least squares means and P-values represent statistical comparison between LB, LBLP, and Control silages.

whereas those for LB and Control silages decreased by 77 and 73 g/kg of DM, respectively. This may be related to partial acid hydrolysis of hemicellulose (McDonald et al., 1991), which might have resulted in greater iNDF concentration in this silage since hemicellulose is the potentially digestible fraction of forage NDF.

Despite the fact that inoculated silages had higher levels of acetic acid, intake of diet containing such silages was greater than that of Control silages (Table 1). According to Muck (2010), levels of acetic acid above 50 g/kg of DM might negatively affect silage intake. However, Charmley (2001) reported that silage intake is not affected by high organic acid concentrations.

The greater nutrient intake of diet containing inoculated silages by lambs may be due to various factors. In vitro studies show that LAB originating from silage inoculant can survive in ruminal fluid, interact positively with ruminal microorganisms, alter ruminal fermentation, and increase ruminal microbial biomass yield (MBY), enhancing the functionality of the rumen (Weinberg et al., 2004a,b; Contreras-Govea et al., 2011, 2013).

Our findings in this study are in agreement with those of Nkosi et al. (2009, 2011), who found higher DM intake in lambs fed corn silage inoculated with L. buchneri than that in animals fed an untreated silage diet. These authors attributed the greater DM intake to greater levels of water-soluble carbohydrates in the inoculated corn silage.

Apparent digestibilities of DM, OM, CP, CHO, NSC, and GE were decreased in lambs fed inoculated silages (Table 3). As reported by Chen et al. (1992), this is likely a response to the increase in intake, because there are linear negative relationships between intake and digestibility in lambs. The high rate of passage of lambs (Mertens and Ely, 1982) could also be implicated. Chen et al. (1992) noted that the apparent digestibility of DM and OM decreases as DM intake increases or as retention time decreases and rate of passage increases (Mertens and Ely, 1982). In the current study, the greater intake of DM, OM, CP, CHO, NSC, and GE by lambs fed inoculated silages explains the lower digestibility of the respective components by such lambs at least partly.

Rowghani et al. (2008) observed no effects of inoculation of corn silage on nutrient digestibility in sheep. However, Aksu et al. (2004) and Nkosi et al. (2010, 2011) reported increased silage digestibility in sheep due to inoculation of the silage. These contrasting results could be due to differences among bacterial strains and application rates and differences in the magnitude of the intake responses as well as differences among animals and their inherent passage rates (Mertens and Ely, 1982) among the studies.

That NDF digestibility in lambs fed the diet containing LB silage was greater than that of lambs fed LBLP silage is probably related to the fact that ruminal pH did not drop below 6 in lambs fed LB silage but did in lambs fed LBLP silage (Fig. 2). Ruminal pH below 6 is detrimental to the growth cellulolytic bacteria (Russell and Wilson, 1996). In addition, greater concentrations of iNDF in LBLP may have limited the NDF digestibility of this silage.

In the rumen, silage lactate can be used as energy, because it is metabolized primarily to propionate by some bacteria (Charmley, 2001). Therefore, the greater lactic acid concentration of the LBLP silage resulted in numerically greater ruminal propionate proportion and lower acetate to propionate ratio in ruminal fluid of lambs fed this silage relative to the others (Table 4). Keady and Steen (1994) also found lower acetate to propionate ratio in the ruminal fluid of steers fed grass silage inoculated with L. plantarum than in the animals that consumed control silage.

Acetate, butyrate, and total VFA concentrations of ruminal fluid were not affected by silage inoculation (Table 4). Keles and Demirci (2011) also did not find differences in the ruminal parameters of lambs fed a triticale-Hungarian vetch herbage inoculated with L. plantarum or L. buchneri. On the other hand, Fellner et al. (2001) observed greater concentration of acetate in the ruminal fluid of steers fed high-moisture corn inoculated with L. plantarum and Enterococcus faecium than in those fed control silage. Some of these results are not consistent with those in the preceding paragraph because of differences in the forages, inoculum strains, and study conditions.

Although the average ruminal pH values were similar among treatments, the pH of ruminal fluid of lambs in LB treatment was at least numerically greater than those of
other treatments from 6 to 9 h after feeding. These findings agree with results obtained in vitro by Weinberg et al. (2004a,b, 2007), who showed greater pH values occurred in ruminal fluid of animals fed diets inoculated with LAB. Silage LAB might survive in ruminal fluid and they are likely to be more tolerant to ruminal acidity than ruminal lactate-producing microorganisms. Therefore, they may compete more efficiently for readily fermentable substrates resulting in lower ruminal lactic acid concentrations and a greater ruminal pH, which would favor ruminal fibrolytic bacteria (Weinberg et al., 2007).

The ruminal ammonia N concentration was similar between treatments. Bayatkouhsar et al. (2011) also found no differences in the ruminal ammonia N (average of 15.85 mg/dL) of cows fed corn silage inoculated with or without LAB.

Inoculations of the corn silage with LB resulted in greater microbial N supply and inoculation with LBLP resulted in a similar numerical response. Therefore, inoculation with LB and LBLP may have increased N retention by the lambs. This is supported by the fact that lambs fed the respective silages had greater or numerically greater ADG than those fed the Control silage. Rooke et al. (1988) also found improvements in N retention due to improved microbial N synthesis within the rumen of sheep fed grass silage inoculated with LAB. Efficiency of microbial N synthesis did not differ among treatments because both microbial N supply and DOMR values were greater in lambs fed inoculated versus Control silages (Table 5). The values of EMNS observed in the present study are within the range of values (14 to 49 g/kg of DOMR) reported by the Agricultural Research Council (1984). Verbič et al. (2005) reported an EMNS of 24.5 g/kg of DOMR in sheep fed corn silage alone.

Contreras-Govea et al. (2011, 2013) found greater MBY in silage inoculated with LAB in in vitro studies. Contreras-Govea et al. (2013) suggested that the improvements in MBY are due to enhanced protein preservation during ensiling. Nkosi et al. (2010, 2011) found greater N retention in lambs fed silage inoculated with LAB and attributed this to better digestibility of CP, which resulted in greater N absorption and N use efficiency in lambs. Contreras-Govea et al. (2011) noted
that LAB can survive in the rumen and could affect rumen MBY either through a direct effect on ruminal fermentation (Weinberg et al., 2003, 2004a,b) or through inhibition of undesirable bacteria by bacteriocins from the inoculant (Gollop et al., 2005), whereas Chen et al. (1992) reported that increasing DM intake may increase microbial N supply. In this study, the increased microbial N supply in lambs fed inoculated silage diets may have been due to survival of inoculant bacteria in the rumen or to increased DMI.

Although inoculation of corn silage with the heterofermentative LB alone or with LB and facultative heterofermentative LP resulted in increases in nutrient intake, digestibility, and microbial N supply, ADG and ADG expressed relative to hot carcass yield were greater which indicates relatively efficient feed utilization by forage to concentrate ratios of 50:50. NDF concentrations of the inoculated silages, which reduced ruminal acetate proportion. Collectively, both of these factors likely resulted in the lower acetate to propionate ratio in the LBLP silage. This may be due at least partly to the greater efficiency of ruminal energy utilization by lambs fed the LBLP silage due to metabolism of the greater lactate concentrations in the LBLP silage to propionate in the rumen as well as the lower NDF concentrations of the inoculated silages, which reduce ruminal acetate proportion. Collectively, both of these factors likely resulted in the lower acetate to propionate ratio of lambs fed the LBLP silage diet and to their greater ADG. Keady and Steen (1994) reported that animal performance increases due to feeding inoculated silage might be caused by improved efficiency of energy utilization due to higher levels of propionate in the rumen caused by the inoculant. Kung and Muck (1997) reviewed the effects of silage inoculants on animal performance and reported that in 53 and 47% of the 15 and 36 experiments they examined, ADG and milk production in beef and dairy cattle were increased by silage inoculation, with average increases of 5 and 3%, respectively. Recent studies showed no effects of inoculation on animal performance. For instance, Kristensen et al. (2010) and Arriola et al. (2011) found no effect of microbial inoculation of corn silage on DMI and milk production of dairy cows. In contrast, Bayatkouhsar et al. (2011) found greater intake of corn silage inoculated with microbial additive by lactating dairy cows, but milk production was not affected. As in this study, Nkosi et al. (2009) reported greater ADG in lambs fed corn silage inoculated with LAB instead of Control silage.

The average G:F in the present study was 0.192, which indicates relatively efficient feed utilization by the lambs fed diets containing 0.80 of forage. Nkosi et al. (2009) reported G:F values near 0.200 in lambs fed corn silage inoculated with or without LAB in diets with forage to concentrate ratios of 50:50.

### Conclusions

The inoculation with both bacteria combined enhanced the silage fermentation. The intakes of DM, OM, CP, NDF, and GE were improved in the lambs fed corn silage inoculated with *L. buchneri* alone or combined with *L. plantarum*. The microbial N supply was enhanced in the lambs fed corn silage inoculated with *L. buchneri* alone. The inoculation with both bacteria combined reduced the acetate to propionate ratio in ruminal fluid and improved the ADG of lambs.

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