Published December 12, 2014

Effects of feeding corn silage inoculated with microbial additives on the ruminal fermentation, microbial protein yield, and growth performance of lambs[1]

F. C. Basso,*[2] 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

 J. Anim. Sci. 2014.92:5640–5650
doi:10.2527/jas2014-8258

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 obligatory 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

[1]The authors wish to express their appreciation to Sao Paulo Research Foundation – FAPESP for their financial support.

[2]Corresponding author: fcarvalhobasso@yahoo.com.br

Received July 12, 2014.
Accepted October 8, 2014.

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 1×10^5 cfu/g of fresh forage of *L. buchneri* NCIMB 40788 (Lallemand Animal Nutrition, Milwaukee, WI; **LB**) or LB combined with 1×10^5 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

Table 1. Chemical composition (g/kg of DM) and fermentation profile of inoculated and uninoculated corn plants at silo filling and corn silages[1]

	Corn plants				Corn silages				
Item[2]	Control	LB	LBLP	SEM[3]	Control	LB	LBLP	SEM[3]	*P*-value
Chemical composition									
DM	320	355	314	0.7	317[b]	334[a]	299[c]	6.3	0.002
OM	952	949	947	2.3	965[a]	958[b]	956[b]	1.3	0.001
CP	87	80	89	2.0	82	88	82	2.3	0.083
EE	27	26	27	1.9	27	28	28	1.0	0.758
NDF	580	569	587	12.6	507	492	498	8.4	0.467
iNDF					143[b]	143[b]	157[a]	3.0	0.020
ADF	272	261	284	8.8	292[b]	280[b]	300[a]	5.3	0.040
Cellulose					242	230	250	5.8	0.070
Hemicellulose	309	308	303	11.0	215	212	198	6.6	0.191
Lignin	54	42	59	2.7	43	41	44	2.0	0.472
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	4.1	4.0	4.1	0.1	0.197
Ammonia N, % TN	2.3	2.4	2.3	0.1	2.6	2.4	2.9	0.2	0.076

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Corn 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).

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

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

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 26 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 *N* H_2SO_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

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 1×10^5 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).

[2]EE = 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 1d 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 H_2SO_4 (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 cm^2) were made of nonwoven textile (100 g/m^2) material (Valente et al., 2011) and a sample weight to bag surface area ratio of 20 mg DM/cm^2 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% H_2SO_4 (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, St. Joseph, MI). The CP concentration was calculated as N × 6.25. Gross energy was determined with a bomb calorimeter (PARR 6200; Parr Instrument Company, 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]:

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

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

$$\text{apparent digestibility} = [(\text{nutrient intake} - \text{fecal nutrient output})/\text{nutrient intake}] \times 100, \quad [2]$$

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]):

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

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)] × BW0.75.

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 × 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 Toeplitz and autoregressive average were the best covariance structures for the data as these had the lowest Aikaike information criterion scores. Differences between means were determined using the PDIFF, 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 $0.05 \leq P < 0.1$.

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 MEwere 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

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[1]

Item[2]	Control	LB	LBLP	SEM[3]	*P*-value
Intake, g/d					
DM	1,001[b]	1,056[a]	1,058[a]	53.9	0.001
DM (Exp. 2)	1,139 [b]	1,349[a]	1,372[a]	64.2	0.036
OM	958[b]	1,015[a]	1,023[a]	46.8	0.022
CP	134[c]	141[b]	148[a]	7.5	0.001
EE	30	31	31	1.6	0.206
NDF	411[b]	442[a]	446[a]	21.8	0.002
CHO	783[b]	822[a]	840[a]	41.5	0.026
NSC	405	421	423	21.7	0.212
GE, MJ/d	16.5[b]	17.2[a]	17.3[a]	0.9	0.001
Apparent digestibility, %					
DM	71.9[a]	68.3[b]	67.2[b]	0.6	0.001
OM	72.5[a]	70.0[b]	68.1[c]	0.6	0.001
CP	72.2[a]	66.5[b]	66.8[b]	1.3	0.006
EE	88.9	87.5	87.0	0.7	0.052
NDF	54.2[a]	54.9[a]	50.3[b]	0.6	0.001
CHO	72.1[a]	69.8[b]	67.5[c]	0.5	0.001
NSC	87.7[a]	86.2[b]	85.2[b]	0.4	0.001
GE	71.2[a]	67.9[b]	66.5[b]	0.6	0.001
Energy value, MJ/kg DM					
DE	11.7[a]	11.0[b]	11.0[b]	0.1	0.001
ME	9.6[a]	9.1[b]	8.9[b]	0.1	0.001

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Corn 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).

[2]EE = ether extract; CHO = total carbohydrates; NSC = nonstructural carbohydrates.

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

Table 4. Effect of bacterial inoculation of corn silage on ruminal microbial N synthesis in lambs. All data are from Exp. 1[1]

Item	Control	LB	LBLP	SEM[2]	*P*-value
Microbial N synthesis					
Allantoin, mmol/d	8.5[b]	8.9[a]	8.6[b]	0.4	0.001
Uric acid,[3] mmol/d	1.9	1.9	1.9	0.1	0.855
PD,[4] mmol/d	10.4[b]	10.7[a]	10.5[ab]	0.5	0.018
Microbial N supply, g/d	8.9[b]	9.2[a]	9.0[ab]	0.4	0.030
DOMR,[5] g/d	437[b]	457[a]	452[a]	20.8	0.022
Microbial N/kg of DOMR	20.6	20.4	20.1	1.0	0.525

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

[1]Corn 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).

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

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

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

[5]DOMR = 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,

Table 5. Effect of bacterial inoculation of corn silage on growth performance and carcass yield of lambs[1]

Item[2]	Control	LB	LBLP	SEM[3]	*P*-value
IBW, kg	20.2	20.6	20.4	1.3	0.350
ADG, g/d	198[b]	199[b]	206[a]	6.2	0.030
G:F	0.199[b]	0.190[a]	0.195[ab]	0.0	0.017
SBW, kg	32.0	32.5	32.7	0.4	0.373
HCW, 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	68[b]	75[b]	83[a]	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

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

[1]Corn 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).

[2]IBW = 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.

[3]Standard 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 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

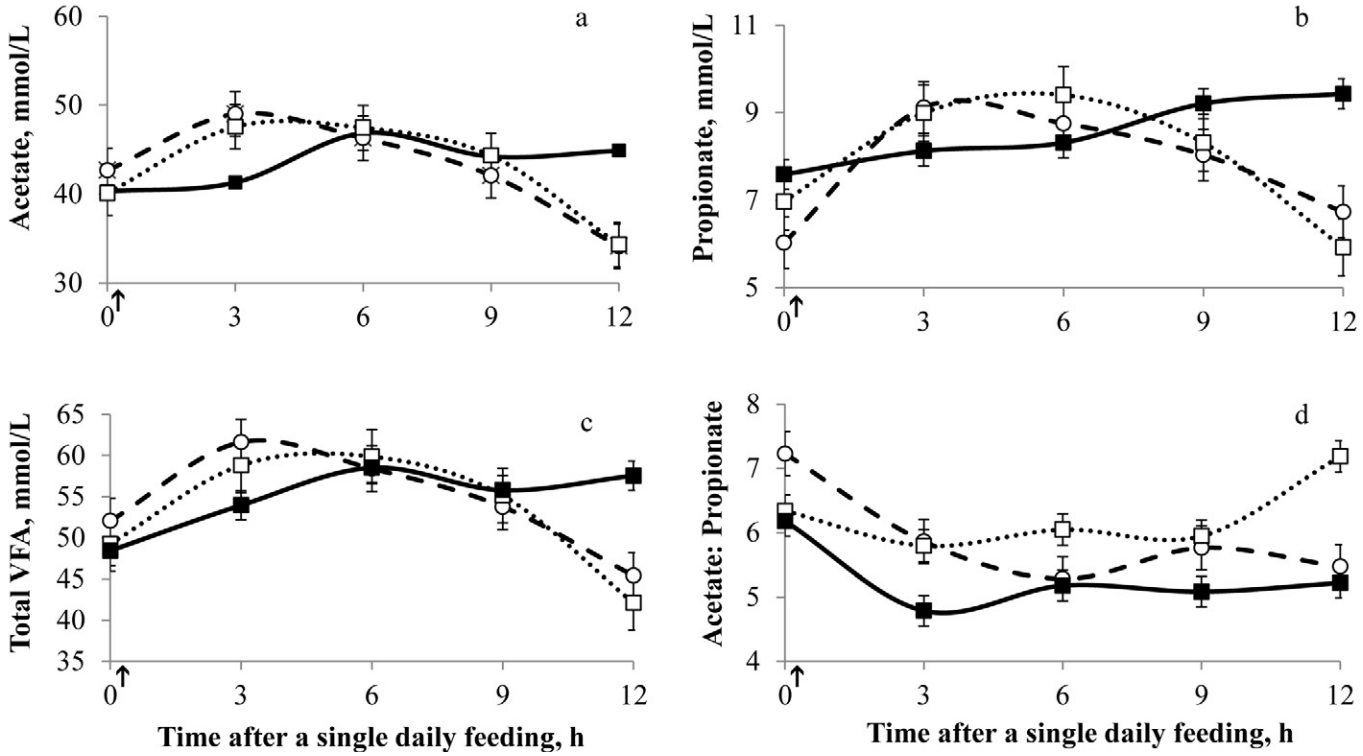

Figure 1. Effect of microbial inoculation of corn silage on acetate (a), propionate (b), total VFA concentration (c), and acetate:propionate ratio (d) in the ruminal fluid of wethers (Control silage, ○; *Lactobacillus buchneri* silage, □; *Lactobacillus buchneri* and *L. plantarum* silage, ■). ↑ = feeding. Vertical bars are SEM.

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.

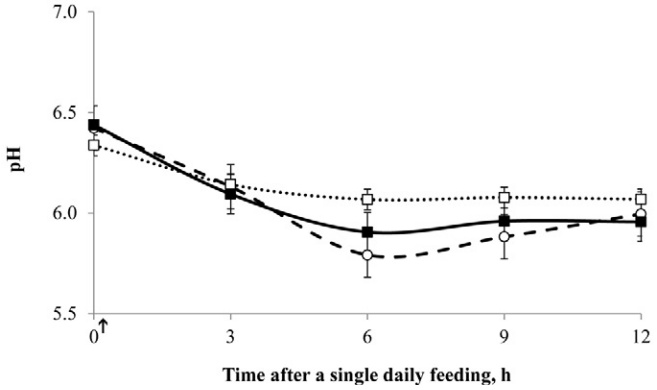

Figure 2. Effect of microbial inoculation of corn silage on ruminal pH of wethers (Control silage, ○; *Lactobacillus buchneri* silage, □; *Lactobacillus buchneri* and *L. plantarum* silage, ■). ↑ = feeding. Vertical bars are SEM.

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 only in lambs fed 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.

LITERATURE CITED

Aksu, T., E., Baytok, and D. Bolat. 2004. Effects of a bacterial silage inoculant on corn silage fermentation and nutrient digestibility. Small Ruminant Res. 55:249-252.

Agricultural Research Council. 1984. The nutrient requirements of ruminant livestock. Commonwealth Agricultural Bureau, Farnham Royal, UK.

AOAC. 1996. Official methods of analysis. 16th ed. AOAC Int., Arlington, VA.

Arriola, K. G., S. C. Kim, C. R. Staples, and A. T. Adesogan. 2011. Effect of applying bacterial inoculants containing different types of bacteria to corn silage on the performance of dairy cattle. J. Dairy Sci. 94:3973–3979.

Barker, S. B., and W. H. Summerson. 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138:535–554.

Basso, F. C., T. F. Bernardes, A. P. T. P. Roth, B. N. Lodo, T. T. Berchielli, and R. A. Reis. 2012. Fermentation and aerobic stability of corn silage inoculated with *Lactobacillus buchneri*. R. Bras. Zootec. 41:1789–1794.

Bayatkouhsar, J., A. M. Tahmasebi, and A. A. Naserian. 2011. The effects of microbial inoculation of corn silage on performance of lactating dairy cows. Livest. Sci. 142:170–174.

Casali, A. O., E. Detmann, S. C. Valadares Filho, J. C. Pereira, L. T. Henriques, S. G. Freitas, and M. F. Paulino. 2008. Influence of incubation time and particles size on indigestible compounds contents in cattle feeds and feces obtained by *in situ* procedures. R. Bras. Zootec. 37:335–342.

Charmley, E. 2001. Towards improved silage quality – A review. Can. J. Anim. Sci. 81:157–168.

Chen, X. B., Y. K. Chen, M. F. Franklin, E. R. Ørskov, and W. J. Shand. 1992. The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep. J. Anim. Sci. 70:1534–1542.

Chen, X. B., and M. J. Gomes. 1995. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives – An overview of the technical details. Occasional Publication. Rowett Research Institute, Bucksburn, Aberdeen, UK.

Chen, X. B., A. T. Mejia, D. J. Kyle, and E. R. Ørskov. 1995. Evaluation of the use of the purine derivative: Creatinine ratio in spot urine and plasma samples as an index of microbial protein supply in ruminants: Studies in sheep. J. Agric. Sci. 125:137–143.

Contreras-Govea, F. E., R. E. Muck, G. A. Broderick, and P. J. Weimer. 2013. *Lactobacillus plantarum* effects on silage fermentation and in vitro microbial yield. Anim. Feed Sci. Technol. 179:61–68.

Contreras-Govea, F. E., R. E. Muck, D. R. Mertens, and P. J. Weimer. 2011. Microbial inoculant effects on silage and in vitro ruminal fermentation, and microbial biomass estimation for alfalfa, bmr corn, and corn silages. Anim. Feed Sci. Technol. 163:2–10.

Detmann, E., and S. C. Valadares Filho. 2010. On the estimation of non-fibrous carbohydrates in feeds and diets. Arq. Bras. Med. Vet. Zootec. 62:980–984.

Fellner, V., L. E. Phillip, S. Sebastian, and E. S. Idziak. 2001. Effects of a bacterial inoculant and propionic acid on preservation of high-moisture ear corn, and on rumen fermentation, digestion and growth performance of beef cattle. Can. J. Anim. Sci. 81:273–280.

Fenner, H. 1965. Method for determining total volatile bases in rumen fluid by stem distillation. J. Dairy Sci. 48:249–251.

Filya, I. 2003. The effect of *Lactobacillus buchneri* and *Lactobacillus plantarum* on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. J. Dairy Sci. 86:3575–3581.

Gollop, N., V. Zakin, and Z. G. Weinberg. 2005. Antibacterial activity of lactic acid bacteria included in inoculants for silage and in silages treated with these inoculants. J. Appl. Microbiol. 98:662–666.

Hu, W., R. J. Schmidt, E. E. McDonell, C. M. Klingerman, and L. Kung Jr. 2009. The effect of *Lactobacillus buchneri* 40788 or *Lactobacillus plantarum* MTD-1 on the fermentation and aerobic stability of corn silages ensiled at two dry matter concentrations. J. Dairy Sci. 92:3907–3914.

Keady, T. W. J., and W. J. Steen. 1994. The effects of treating low dry-matter, low digestibility grass with bacterial inoculant on the intake and performance of beef cattle, and studies of its mode of action. Grass Forage Sci. 50:217–226.

Keles, G., and U. Demirci. 2011. The effect of homofermentative and heterofermentative lactic acid bacteria on conservation characteristics of baled triticale–Hungarian vetch silage and lamb performance. Anim. Feed Sci. Technol. 164:21–28.

Kristensen, N. B., K. H. Sloth, O. Højberg, N. H. Spliid, C. Jensen, and R. Thøgersen. 2010. Effects of microbial inoculants on corn silage fermentation, microbial concentrations, aerobic stability, and milk production under field conditions. J. Dairy Sci. 93:3764–3774.

Kung, L., Jr., D. B. Grieve, J. W. Thomas, and J. T. Huber. 1984. Added ammonia or microbial inoculant for fermentation and nitrogenous compounds of alfalfa ensiled at various percents of dry matter. J. Dairy Sci. 67:299–306.

Kung, L., Jr., and R. E. Muck. 1997. Animal response to silage additives. In: Silage: Field to feedbunk. Northeast Regional Agricultural Engineering Service, Ithaca, NY. p. 200–210.

Kung, L., Jr., and R. Shaver. 2001. Interpretation and use of silage fermentation analysis reports. Focus Forage 3:1–5.

Kung, L., Jr., M. R. Stokes, and C. J. Lin. 2003. Silage additives. In: D. R. Buxton, R. E. Muck, and J. H. Harrison, editors, Silage science and technology. Agronomy Monograph. ASA, CSSA, and SSSA, Madison, WI. p. 305–360.

McDonald, P., A. R. Henderson, and S. J. E. Heron. 1991. The biochemistry of silage. 2nd ed. Chalcombe Publications, Marlow, Bucks, UK.

Mertens, D. R., and L. O. Ely. 1982. Relationship of rate and extent of digestion to forage utilization – A dynamic model evaluation. J. Anim. Sci. 54:895–905.

Moon, N. J. 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. J. Appl. Bacteriol. 55:453–460.

Muck, R. E. 2010. Silage microbiology and its control through additives. R. Bras. Zootec. 39:183–191.

Nkosi, B. D., R. Meeske, T. Langa, and R. S. Thomas. 2011. Effects of bacterial silage inoculants on whole-crop maize silage fermentation and silage digestibility in rams. S. Afr. J. Anim. Sci. 41:350–359.

Nkosi, B. D., R. Meeske, D. Palic, T. Langa, K. J. Leeuwa, and I. B. Groenewald. 2009. Effects of ensiling whole crop maize with bacterial inoculants on the fermentation, aerobic stability, and growth performance of lambs. Anim. Feed Sci. Technol. 154:193–203.

Nkosi, B. D., R. Meeske, H. J. Van Der Merwe, and I. B. Groenewald. 2010. Effects of homofermentative and heterofermentative bacterial silage inoculants on potato hash silage fermentation and digestibility in rams. Anim. Feed Sci. Technol. 157:195–200.

Nocek, J. E. 1988. In situ and other methods to estimate ruminal protein and energy digestibility: A review. J. Dairy Sci. 71:2051–2069.

NRC. 1985. Nutrient requirements of sheep. 6th ed. Natl. Acad. Press, Washington, DC.

NRC. 2007. Nutrient requirements of small ruminants. Sheep, goats, cervids and New World camelids. Natl. Acad. Press, Washington, DC.

Oude Elferink, S. J. W. H., J. Krooneman, J. C. Gottschal, S. F. Spoelstra, F. Folkert, and F. Driehuis. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by *Lactobacillus buchneri*. Appl. Environ. Microbiol. 67:125–132.

Pina, D. S., S. C. Valadares Filho, E. Detmann, R. F. D. Valadares, J. M. S. Campos, K. A. K. Moraes, A. S. Oliveira, and M. L. Paixão. 2006. Comparison of internal markers and extent of total fecal collection to estimate nutrient digestibility and energy value of feeds on dairy cows fed different protein sources. R. Bras. Zootec. 35:2461–2468.

Queiroz, O. C. M., A. T. Adesogan, K. G. Arriola, and M. F. S. Queiroz. 2012. Effect of a dual-purpose inoculant on the quality and nutrient losses from corn silage produced in farm–scale silos. J. Dairy Sci. 95:3354–3362.

Ranjit, N. K., C. C. Taylor, and L. Kung Jr. 2002. Effect of *Lactobacillus buchneri* 40788 on the fermentation, aerobic stability and nutritive value of maize silage. Grass Forage Sci. 57:73–81.

Rooke, J. A., and R. D. Hatfield. 2003. Biochemistry of ensiling. In: D. R., Buxton, R. E. Muck, and H. J. Harrison, editors, Silage science and technology. American Society of Agronomy, Madison, WI. p. 95–140.

Rooke, J. A., F. M. Maya, J. A. Arnold, and D. G. Armstrong. 1988. The chemical composition and nutritive value of grass silages prepared with no additive or with the application of additives containing either *Lactobacillus plantarum* or formic acid. Grass Forage Sci. 43:87–95.

Rowghani, E., M.J. Zamiri, M. Khorvash, A. Abdollahipanah. 2008. The effects of *Lactobacillus plantarum* and *Propionibacterium acidipropionici* on corn silage fermentation, ruminal degradability and nutrient digestibility in sheep. Iran. J. Vet. Res. 9:308-315.

Russell, J. B., and D. B. Wilson. 1996. Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? J. Dairy Sci. 79:1503–1509.

Senger, C. C. D., G. V. Kozloski, L. M. B. Sanchez, F. R. Mesquita, T. P. Alves, and D. S. Castagnino. 2008. Evaluation of autoclave procedures for fibre analysis in forage and concentrate feedstuffs. Anim. Feed Sci. Technol. 146:169–174.

Silva Sobrinho, A. G., and J. C. S. Osório. 2008. Aspectos quantitativos da produção de carne ovina. In: A. G. Silva Sobrinho, C. Sañudo, J. C. S. Osório, M. M. C. Arribas, and M. T. M. Osório, editors, Produção de carne ovina. (In Portuguese.) Funep, Jaboticabal, SP, Brazil. p. 1–68.

Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. J. Anim. Sci. 70:3562–3577.

Valente, T. N. P., E. Detmann, S. C. Valadares Filho, M. Cunha, A. C. Queiroz, and C. B. Sampaio. 2011. In situ estimation of indigestible compounds concentrations in cattle feed and feces using bags made from different textiles. Rev. Bras. Zootec. 40:666–675.

Van Soest, P. J., and J. B. Robertson. 1985. Analysis of forages and fibrous foods. Cornell University, Ithaca, NY.

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

Verbič, J., D. Babnik, V. Žnidaršič-Pongrac, M. Resnik, A. Gregorčič, and V. Kmecl. 2005. The effect of dent versus flint maize genotype on site and the extent of starch and protein digestion, ruminal fermentation and microbial protein synthesis in the rumen of sheep. Anim. Res. 54:443–458.

Weinberg, Z. G., G. Ashbell, Y. Hen, A. Azrieli, G. Szakacs, and I. Filya. 2002. Ensiling whole-crop wheat and corn in large containers with *Lactobacillus plantarum* and *Lactobacillus buchneri*. J. Ind. Microbiol. Biotechnol. 28:7–11.

Weinberg, Z. G., Y. Chen, and M. Gamburg. 2004a. The passage of lactic acid bacteria from silage into rumen fluid, in vitro studies. J. Dairy Sci. 87:3386–3397.

Weinberg, Z. G., R. E. Muck, and P. J. Weimer. 2003. The survival of silage inoculant lactic acid bacteria in rumen fluid. J. Appl. Microbiol. 94:1066–1071.

Weinberg, Z. G., R. E. Muck, P. J. Weimer, Y. Chen, and M. Gamburg. 2004b. Lactic acid bacteria used in inoculants for silage as probiotics for ruminants. Appl. Biochem. Biotechnol. 118:1–9.

Weinberg, Z. G., O. Shatz, Y. Chen, E. Yosef, M. Nikbahat, D. Ben-Ghedalia, and J. Miron. 2007. Effect of lactic acid bacteria inoculants on in vitro digestibility of wheat and corn silages. J. Dairy Sci. 90:4754–4762.