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RUMINANT NUTRITION

Effects of inoculation of corn silage with *Lactobacillus hilgardii* and *Lactobacillus buchneri* on silage quality, aerobic stability, nutrient digestibility, and growth performance of growing beef cattle

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Abstract

This study evaluated the effects of inoculation of whole crop corn silage with a mixture of heterofermentative lactic acid bacteria (**LAB**) composed of *Lactobacillus hilgardii* and *Lactobacillus buchneri* on ensiling, aerobic stability, ruminal fermentation, total tract nutrient digestibility, and growth performance of beef cattle. Uninoculated control corn silage (**CON**) and silage inoculated with 3.0×10^5 cfu g^{-1} of LAB containing 1.5×10^5 cfu g^{-1} of *L. hilgardii* CNCM I-4785 and 1.5×10^5 cfu g^{-1} of *L. buchneri* NCIMB 40788 (**INOC**) were ensiled in silo bags. The pH did not differ ($P > 0.05$) between the two silages during ensiling but was greater ($P < 0.001$) for CON than INOC after 14 d of aerobic exposure (**AE**). Neutral detergent insoluble crude protein (**NDICP**) content (% of DM and % of CP basis) of terminal INOC silage was greater ($P \leq 0.05$) than that of CON. In terminal silage, concentrations of total VFA and acetate were greater ($P < 0.001$), while water-soluble carbohydrates were lower ($P < 0.001$) for INOC than CON. Yeast and mold counts were lower for INOC than CON ($P \leq 0.001$) in both terminal and aerobically exposed silages. The stability of INOC was greater ($P < 0.001$) than that of CON after 14 d of AE. Ruminal fermentation parameters and DMI did not differ ($P > 0.05$) between heifers fed the two silages, while there was a tendency ($P \leq 0.07$) for lower CP and starch digestibility for heifers fed INOC than CON. Total nitrogen (N) intake and N retention were lower ($P \leq 0.04$) for heifers fed INOC than CON. Dry matter intake as a percentage of BW was lower ($P < 0.04$) and there was a tendency for improved feed efficieny (**G:F**; $P = 0.07$) in steers fed INOC vs. CON silage. The NE_m and NE_g contents were greater for INOC than CON diets. Results indicate that inoculation with a mixture of *L. hilgardii* and *L. buchneri* improved the aerobic stability of corn silage. Improvements in G:F of growing steers fed INOC silage even though the total tract digestibility of CP and starch tended to be lower for heifers fed INOC are likely because the difference in BW and growth requirements of these animals impacted the growth performance and nutrient utilization and a greater proportion of NDICP in INOC than CON.

Key words: corn silage, growth performance, *Lactobacillus hilgardii*, nutrient digestibility

Abbreviations

ADICP	acid detergent insoluble crude protein
G:F	feed efficieny
MRS	de Man, Rogosa, and Sharpe agar
NDICP	neutral detergent insoluble crude protein
SDA	Sabouraud's dextrose agar
TMR	total mixed ration
UIP	undegradable intake protein
WSC	water-soluble carbohydrate

Introduction

Corn silage production has steadily been increasing in feedlot and dairy operations in western Canada, thanks to the availability of short-season corn hybrids (Guyader et al., 2018). However, corn silage is prone to aerobic deterioration during feed out because of the activity of spoilage microorganisms, including yeast and mold (McDonald et al., 1991). Aerobic deterioration of corn silage results in increased DM and energy losses and lowered nutritive value, while the production of mycotoxins can potentially impact animal health (Woolford, 1990; Ferrero et al., 2019). Under some conditions, DM losses can be severe with upto 70% of the DM lost in the peripheral areas and near the sidewalls of the bunkers (Bernardes et al., 2012; Borreani et al., 2018). It has been reported that silage inoculants containing heterofermentative lactic acid bacteria **(LAB)** such as *Lactobacillus buchneri* improved the aerobic stability of silages due to the conversion of lactic acid **(LA)** to acetic acid (**AC**; Driehuis et al., 1999). However, these inoculants have not been consistently effective in improving aerobic stability (Blajman et al., 2018), possibly due to variation in application rate, type of forage ensiled, the epiphytic LAB population, and the nature of the bacterial strains in the inoculant (Filya and Sucu, 2010). A combination of obligate heterofermentative strains of *Lactobacillus hilgardii* and *L. buchneri* has been shown to improve the aerobic stability of corn silage ensiled within laboratory-scale silos (Reis et al., 2018; Ferrero et al., 2019). However, the impact of these inoculants on corn silage ensiled in commercial-scale silo bags and the effects on nutrient digestion and growth performance of growing beef cattle have not been well evaluated. It was hypothesized that the combination inoculant containing *L. hilgardii* and *L. buchneri* would improve the aerobic stability of corn silage and the backgrounding performance of growing beef cattle. The objectives of this study were to assess the effects of a novel silage inoculant containing *L. hilgardii* and *L. buchneri* on ensiling and aerobic stability of corn silage and to elucidate its impact on nutrient digestion and growth performance of beef cattle.

Materials and Methods

Animal care and management

Experimental protocols involving animals were reviewed and approved by the Lethbridge Research and Development Centre (**LeRDC**) animal care committee (protocol # 1901), and animals were cared for as per the guidelines of the Canadian Council on Animal Care (CCAC, 2009).

Forage production and harvesting

Corn (*Zea mays*; AS1047RR; Pride Seeds, Pain Court, ON) was planted on May 16, 2018, under irrigation near Lethbridge, AB, and harvested on October 16, 2018, at two-thirds milk line (34.9% DM). Corn was planted at a depth of 5 to 6 cm, with 38-cm row spacing at a density of 89,500 seeds ha^{-1}. The forage was harvested and chopped to 9.5 mm with a forage harvester (Claas Jaguar 950 Forage Harvester, Harsewinkel, Germany) equipped with a kernel processor with the rollers adjusted to 1.0 mm clearance.

Preparation of whole-crop corn silage in silo bags

Chopped and kernel processed corn forage was ensiled in Silobolsa Plastar premium silo bags (2.7 × 60.0 m; Plaster, San Luis, SA) using Ag-Bag bagger (Ag-Bag, St. Nazianz, WI). The inoculant (Lallemand Specialties Inc., Milwaukee, WI) was applied with ATV sprayers (AG Spray Equipment, Hopkinsville, KY) at a rate of 40 mL t^{-1}, providing 3.0×10^5 cfu g^{-1} fresh forage strains of LAB consisting of a mixture of 1.5×10^5 cfu g^{-1} of *L. hilgardii* CNCM I-4785 and 1.5×10^5 cfu g^{-1} of *L. buchneri* NCIMB 40788 (**INOC**). Corn sprayed with deionized water at a rate of 40 mL t^{-1} served as uninoculated control (**CON**) silage (Table 1). To reduce cross-contamination between bags, spray systems were flushed with deionized water each time the treatments were changed. Both CON and INOC forages were ensiled in two silo bags, with each bag containing both the treatments. Untreated corn forage was introduced into the bags (~3 m) to separate CON and INOC silages with this region marked with spray paint on the outside of the bags. To minimize differences in forage composition due to harvest location and time of harvest, forage was chopped at random locations within the field. Upon delivery to the bagger, forage was sampled from each truckload with ~200 t of each treatment compressed into the two silo bags. The forage was ensiled for 120 d prior to opening for the evaluation of silage quality and aerobic stability as described below.

Aerobic stability of corn silage

Aerobic stability was assessed in four runs with a 3-wk interval between runs. Silage for runs 1 and 2 was sampled from silo bag 1 and for runs 3 and 4 was sampled from silo bag 2 (2 runs per silo bag), with runs evenly timed over the feed-out period. After opening of the bags, hand grab subsamples (~1.2 kg each) from about 0.5 m depth from the silo face and at least 0.5 m away from the edges were collected from five locations across the silo face. Subsamples were combined and placed into four 4-liters insulated containers (13.5 cm in diameter × 30.9 cm in height) per treatment. The silage was covered with two layers of cheesecloth and placed in a room at 20 °C for 14 d. Two Dallas Thermochron iButton temperature sensors (Embedded Data Systems, Lawrenceburg, KY) were embedded in the silage at ~9.0 and ~18.0 cm within each container and set to record the temperature every 15 min. Two sensors were also placed in the room to measure ambient temperature. The contents of each container were subsampled on day 0 (terminal silage) and after 3, 7, and 14 d of aerobic exposure (**AE**) for determinations of pH, chemical, and microbial compositions. Aerobic stability was defined as the number of hours before the temperature of aerobically exposed silage exceeded ambient temperature by 2 °C (Teller et al., 2012).

Ruminal fermentation and total tract nutrient digestibility

Animals, treatments, experimental design, and feeding management

Eight ruminally cannulated Angus × Hereford crossbred beef heifers (751.0 ± 53.7 kg; mean ± SD) were individually housed at the metabolism barn of LeRDC. Heifers were housed in tie stalls and were provided with daily exercise in an open dry lot, except during the sample collection period. During total collection in each period, heifers were tethered in stalls with rubber mats.

Table 1. Chemical composition, fermentation products, and microbial populations of fresh corn forage and CON or INOC corn sillage ensiled in silo bags

		Silage after 120 d of ensiling[1]			
Item[2]	Corn forage[3]	CON	INOC	SEM[4]	*P*-value
pH	5.84 ± 0.07	3.77	3.78	0.023	1.00
DM	34.9 ± 1.0	34.2	33.5	0.235	0.08
OM, % DM	95.6 ± 0.6	95.7	95.3	0.044	<0.001
CP, % DM	8.19 ± 0.22	8.61	8.71	0.116	0.55
ADICP, % DM	ND	0.67	0.65	0.025	0.45
ADICP, % CP	ND	7.82	7.41	0.247	0.26
NDICP, % DM	ND	1.45	1.57	0.038	0.05
NDICP, % CP	ND	16.8	18.0	0.31	0.02
ADF, % DM	25.6 ± 2.0	28.0	28.9	0.38	0.09
NDF, % DM	44.2 ± 1.6	49.4	52.0	0.72	0.02
Starch, % DM	23.7 ± 2.8	23.1	24.3	0.82	0.34
WSC, mg g^{-1} DM	34.9 ± 14.1	37.2	6.8	2.885	<0.001
Fermentation products mg g^{-1} DM					
Acetate (AC)	0.70 ± 0.07	13.9	26.2	0.88	<0.001
Propionate	0.37 ± 0.03	ND	ND	NA	NA
Butyrate	0.38 ± 0.07	0.01	ND	NA	NA
Total VFA	1.76 ± 0.14	14.0	26.2	0.88	<0.001
Lactate (LA)	0.23 ± 0.03	49.3	48.0	1.49	1.00
LA:AC ratio	0.33 ± 0.05	3.64	1.85	0.130	<0.001
Ethanol	0.08 ± 0.03	0.43	0.15	0.067	<0.01
NH_3-N	0.33 ± 0.06	1.07	1.07	0.022	1.00
Microbiology, $\log_{10}$ cfu g^{-1} DM					
TB	8.68 ± 1.05	8.55	8.59	0.087	1.00
LAB	5.61 ± 0.17	8.50	8.65	0.090	0.94
Yeast	7.46 ± 0.12	4.32	0.91	0.479	<0.001
Mold	6.58 ± 0.18	ND	ND	NA	NA

[1]CON, control; INOC, inoculated.
[2]AC, acetate; LA, lactate; TB, total bacteria; LAB, lactic acid bacteria; NA, not applicable; ND, not detected.
[3]Values for fresh forage were not included in statistical analysis (n = 3).
[4]SEM, pooled standard error of mean (n = 4).

Heifers were fed total mixed rations (**TMR**) consisting of 65.0% of either CON or INOC corn silage, plus 20.0% barley grain, 10.0% canola meal, and 5.0% of a vitamin–mineral supplement (DM basis; Table 2). Both diets (CON and INOC) were formulated to meet or exceed the National Academies of Sciences, Engineering, and Medicine (NASEM), (2016) nutrient requirements for CP, energy, minerals, and vitamins for beef heifers. Diets were also formulated for calcium to phosphorus ratio of 2:1 across treatments. Monensin sodium was provided at 33 mg kg^{-1} (DM basis) in the vitamin–mineral supplement. The experiment was a crossover design with four heifers per treatment receiving either CON or INOC diet.

The study was conducted for 42 d with two 21-d periods in a crossover design. Each period consisted of 8 d of dietary adaptation, 6 d of voluntary intake, 5 d of total collection of feces and urine, and 2 d of ruminal sampling. Silage was fed from one silo bag and then switched to the other bag at the halfway (after day 21) of the digestibility study. Diets were mixed using a Calan Data Ranger (American Calan, Northwood, NH) and delivered daily at 0900 hours targeting 5% feed refusal during the adaptation period. Bunks were cleaned before the morning feeding, and orts weighed and recorded and subsampled for

Table 2. Formulation and nutrient composition of diets used in digestibility and performance studies containing CON or INOC corn sillage ensiled in silo bags

	Treatments[1]			
Item	CON	INOC	SEM[2]	*P*-value
Diet formulation for the digestibility and performance study, % DM basis				
Corn silage	65.0	65.0	—	—
Barley grain	20.0	20.0	—	—
Canola meal	10.0	10.0	—	—
Supplement[3]	5.0	5.0	—	—
Nutrient composition of the diets for the digestibility study, % DM basis				
OM	93.4	93.6	0.19	0.47
CP	16.0	15.2	0.36	0.13
ADF	24.3	24.2	0.90	0.91
NDF	42.4	43.3	0.78	0.43
Starch	25.0	23.1	0.83	0.13
Nutrient composition of the diets for the performance study, % DM basis				
OM	93.3	93.3	0.13	0.94
CP	17.0	16.8	0.41	0.67
ADF	22.3	23.1	0.43	0.14
NDF	42.3	43.7	0.60	0.12
Starch	21.0	20.2	0.43	0.21

[1]CON, control; INOC, inoculated.
[1]SEM, pooled standard error of mean (n = 3).
[2]Supplement (as fed basis) contained 30.0% ground barley, 29.1% canola meal,26.0% limestone, 6.5% urea, 5.0% salt, 1.5% canola oil, 1.5% vitamin–mineral premix, 0.3% Rumensin 200, and 0.1% Vitamin E 500.

the determination of DM content during the 14-d adaptation period. From day 15 of each period, heifers were fed at 90% of voluntary intake to ensure consumption of all feed. All heifers were weighed at the beginning and end of the adaptation period to calculate DMI as a percentage of BW. Silage samples were collected weekly and DM was determined and adjustments for DM content were undertaken as necessary to ensure similar dietary composition between diets throughout the study. Samples of TMR were collected daily from each heifer during the collection period. All samples of TMR and orts were composited on a period basis for chemical analysis.

Determination of total tract digestibility and nitrogen (N) retention

The total collection of urine and feces was carried out from day 15 to 19 of each period as described by Nair et al. (2019b). Briefly, heifers were fitted with bladder catheters (Bardex 75 cc Lubricath 2-way Foley catheter, C. R. Bard Inc., Covington, GA) 2 h before the start of the total collection. Urinary catheters were attached to 20-liters plastic cans containing 500 mL of 4.0 N sulfuric acid to prevent the volatilization of ammonia. Urine output was recorded daily, mixed thoroughly, and 1% was subsampled throughout the total collection period and stored at –20 °C. At the end of each period, composite urine samples were thawed, mixed thoroughly, and subsampled for the analysis of urinary N. Total fecal collection was carried out by scraping feces off the floor every 2 h from 0600 to 2200 hours and every 4 h thereafter. Daily fecal output was recorded, mixed thoroughly, and 2.5% was subsampled throughout the collection period into plastic bags. At the end of each period, the composite fecal samples were thawed, mixed thoroughly, and subsampled for each animal within each period for chemical analysis.

Measurement of rumen metabolites

Ruminal fluid samples were collected at 1, 3, 8, and 24 h after feeding on days 20 and 21 of each period as described by Nair et al. (2019b). Briefly, ruminal fluid (~250 mL) was collected from the ventral, anterior, and posterior sacs of the rumen and strained through four layers of cheesecloth. The pH of the ruminal fluid was measured and recorded immediately using a portable pH meter (Model 265A, Orion Research Inc., Beverly, MA). Two 5-mL samples of ruminal fluid were collected with one sample being mixed with 1 mL of 25% (wt/vol) metaphosphoric acid for VFA analysis and another with 1% (vol/vol) aqueous solution of 18.4 M sulfuric acid for the measurement of ammonia.

Growth performance of feedlot cattle

Animal, treatments, and experimental design

A total of 40 Angus × Hereford crossbred steers (381.6 ± 21.5 kg; mean ± SD) were purchased from commercial sources and housed in individual feeding pens at the LeRDC individual feeding barn. Upon arrival, steers were ear-tagged and processed as described by Wang et al. (2017). Steers were stratified by weight and assigned to two blocks of 20 steers each. Steers in each block were randomly assigned to either the CON or INOC diets described in the digestibility study, resulting in 20 steers per treatment in a completely randomized block design.

Feeding management and measurements

Feed was delivered to each pen using a Calan Data Ranger (American Calan, Northwood, NH) once daily at 0900 hours. The steers were fed to a target of 5% feed refusal. Individual feeders were examined each morning, and the daily feed allotted was based on the residual feed in the feeder and the amount fed the previous day. Orts were collected weekly, weighed, and subsampled for DM to adjust for DMI. Steers were weighed before the morning feeding on two consecutive days at the beginning and end of the study and every 28 d throughout the 84-d growth experiment. As for the digestibility experiment, silage was fed from one silo bag and then switched to the other bag at the halfway (after day 42) of the growth performance study. Samples of corn silage were collected weekly to measure DM and adjustments for DM content were undertaken as necessary as in the digestibility study. Diets were sampled weekly and composited monthly for chemical analysis.

Laboratory analyses

Microbial analyses of corn silage

Microbial analyses were conducted as described by Addah et al. (2011). Briefly, samples (10 g) of fresh corn forage collected on the day of ensiling, terminal silage, and silage after 3, 7, and 14 d of AE were added to 90 mL of sterile 70 mM potassium phosphate buffer (pH = 7.0) and agitated for 30 s at 260 × rpm in a Stomacher 400 Laboratory Blender (Seward Medical Limited, London, UK). The suspension was serially diluted (10^{-2} to 10^{-7}), and 100 µL aliquots of each dilution were spread in triplicate onto de Man, Rogosa, and Sharpe agar (**MRS**; Dalynn Biologicals, Calgary, AB) for enumeration of LAB, onto nutrient agar (**NA**; Dalynn Biologicals, Calgary, AB) for enumeration of total bacteria (**TB**), and onto Sabouraud's dextrose agar (**SDA**; Dalynn Biologicals, Calgary, AB) for enumeration of yeast and mold. Both MRS and NA plates contained 200 µg mL^{-1} of cycloheximide (Dalynn Biologicals, Calgary, AB), while SDA plates contained 100 µg mL^{-1} each of tetracycline and chloramphenicol. Both MRS and NA plates were incubated at 37 °C for 24 to 48 h, while SDA plates were incubated at ambient temperature for 72 h. Colonies were counted from plates containing a minimum of 30 and a maximum of 300 colonies.

Chemical analyses

For the determination of fermentation products during ensiling and AE, the procedure described by Addah et al. (2011) was adopted. Briefly, samples (15 g fresh wt) were mixed with 135 mL of deionized water and blended at full speed for 30 s in a Waring blender (Waring Commercial, Torrington, CT). The suspension was filtered through two layers of cheesecloth, and the pH of the collected fluid was measured twice using a Symphony pH meter (SB70P, VWR, Mississauga, ON). Subsamples of the filtrate were immediately boiled for 10 min to stop fermentation and stored at –20 °C for subsequent analysis of water-soluble carbohydrates **(WSC)** by the Nelson–Somogyi method (Nelson, 1944) using a Dynatech MRX microplate reader (Dynatech Laboratories Inc., Chantilly, VA). Another sample of the filtrate was centrifuged at 10,000 × *g* for 15 min at 4 °C (Thermo electron Corporation, Gormley, ON). Two 1.5-mL samples each of the supernatant were collected with one sample being mixed with 0.3 mL of 25% (wt/vol) metaphosphoric acid solution for VFA analysis and the second with 0.3 mL of 1% (vol/vol) aqueous solution of 18.4 M sulfuric acid for the measurement of ammonia.

Samples of silage extract and ruminal fluid for VFA concentrations were analyzed as described by Addah et al. (2016). The concentration of each VFA (mM) was measured by comparing their peak areas with that of malonic acid as an internal standard. LA was methylated and quantified as described by Kudo et al. (1987), using the same column and chromatograph used for VFA analysis. Samples for ammonia concentrations were measured by the phenol-hypochlorite procedure of Broderick and Kang (1980).

Samples of silage, TMR, orts, and feces were ground to pass through a 1.0-mm screen using a Wiley Mill (model 4, Arthur H. Thomas Co., Philadelphia, PA) and analyzed for DM, OM, total N, ADF, NDF, and starch as described by Addah et al. (2016) and Wang et al. (2017). Urinary N was analyzed using the NA1500 Nitrogen/Carbon analyzer (Carlo Erba Instruments, Milan, Italy). Crude protein was calculated as total N × 6.25. Protein associated with ADF (acid detergent insoluble crude protein [**ADICP**]) and NDF (neutral detergent insoluble crude protein [**NDICP**]) residues was calculated as described above for CP.

Data calculations and statistical analyses

Microbial populations were estimated as cfu g^{-1} silage DM and were log-transformed before statistical analyses. Dietary NE_m content was calculated based on animal performance using the retained energy formula for medium frame yearling steers [RE = $(0.0493 \times BW^{0.75}) \times ADG^{1.097}$; NRC, 1996] as per Zinn et al. (2002). The net energy of gain was calculated from NE_m assuming $NE_g = NE_m \times 0.877 - 0.41$ as per Zinn and Shen (1998). Apparent total tract nutrient digestibility **(ATTD)** was calculated using the following equation: ATTD = [(nutrient intake – nutrient output in the feces) / nutrient intake] × 100 (Nair et al., 2019b). To account for gut fill, BW of steers was reported on a shrunk basis (BW × 0.96; Chibisa and Beauchemin, 2018).

All data were statistically analyzed by analysis of variance using the MIXED procedure of SAS (version 9.3.1; SAS Inst. Inc., 2012). For the aerobic stability study, the effect of treatment on fermentation parameters and microbial data during AE was assessed using a repeated measures analysis with the effect of treatment (T), days of AE (D), and T × D included in the model. Insulated container (n = 4) was used as the experimental unit. Silo bag (n = 2) was taken as a

random factor. For the digestibility study, a crossover design was used for the analysis of the nutrient composition, DMI, total tract nutrient digestibility, and N balance data. Heifer (period) was taken as a random factor. A repeated measures analysis was carried out for ruminal VFA profile, ruminal pH, and ammonia with the fixed effect of time of sampling and diet × time interaction (DIET × TIME) included in the model. Covariance structure with the lowest Akaike's and Bayesian information criteria value was selected (Littell et al., 1996) for the repeated measures analysis. For the performance study, nutrient composition and performance data were analyzed as a randomized complete block design with treatment as a fixed effect and individual pen as the experimental unit. Block was taken as a random factor. Denominator degrees of freedom were determined using the Kenward–Roger option. Means were separated using Tukey's test. Significant differences and trends were declared at $P < 0.05$ and $0.10 > P > 0.05$, respectively.

Results

Ensiling fermentation characteristics and aerobic stability of whole-crop corn silages

Chemical composition, fermentation products, and microbial population of fresh corn forage and terminal (120 d of ensiling) silages ensiled in silo bags are presented in Table 1. Terminal silage pH did not vary ($P > 0.05$) between treatments. The DM content of fresh corn forage at ensiling averaged 34.90 ± 0.96% (mean ± SD). The DM content of INOC silage was slightly lower ($P = 0.08$) than CON silage. Similarly, the OM content of INOC was lower ($P < 0.001$) than that of CON silage. The CP content averaged 8.66 ± 0.32% (mean ± SD; % of DM basis) across treatments. ADICP content (% of DM and % of CP basis) did not vary between treatments and averaged 0.66 ± 0.07% (mean ± SD) and 7.61 ± 0.71% (mean ± SD), respectively. NDICP content (% of DM and % of CP basis) was greater ($P \leq 0.05$) for INOC than CON silages. There was a tendency ($P = 0.09$) for greater ADF content for INOC than CON, while the NDF content of INOC was greater ($P = 0.02$) than CON in terminal silages. Starch content did not vary between treatments and averaged 23.6 ± 2.31% (mean ± SD), while the WSC content (mg g^{-1} DM) of terminal INOC was lower ($P < 0.001$) than CON silage.

The addition of inoculant increased ($P < 0.001$) the concentrations (mg g^{-1} DM) of AC and total VFA in INOC as compared with CON silage. Lactate concentration (mg g^{-1} DM) did not differ between treatments and averaged 48.6 ± 5.6 mg g^{-1} DM (mean ± SD). Compared with CON, the LA:AC ratio (3.64 vs. 1.85; $P \leq 0.001$) and ethanol concentrations (0.43 vs. 0.15 mg g^{-1} DM; $P < 0.01$) were lower in INOC silage. TB and LAB populations did not vary between treatments and averaged 8.57 ± 0.28 (mean ± SD) and 8.58 ± 0.36 $\log_{10}$ cfu g^{-1} DM (mean ± SD), respectively. The addition of inoculant decreased ($P < 0.001$) yeast population as compared with CON silage. Mold was not detected in terminal silages across treatments.

During AE, there was a T × D interaction for silage pH; concentrations of WSC, AC, and LA; and populations of TB, LAB, yeast, and mold (Figures 1 and 2). After 7 d of AE, the pH of INOC was lower ($P < 0.001$) than CON (Figure 1A). The WSC concentration of INOC was lower ($P \leq 0.04$) at days 0, 3, and 7 than CON, but was similar by day 14 of AE (Figure 1B). Compared with CON, INOC had lower ($P < 0.001$) LA concentration on day 3, but greater ($P < 0.01$) LA concentration after 14 d of AE (Figure 1C). In contrast, AC concentration of INOC was higher ($P < 0.001$) than CON on days 0, 3, and 7, but was similar after 14 d of AE (Figure 1D). Propionate concentrations were below detectable limits for both INOC and CON, while butyrate concentrations remained low (0.004 ± 0.002 mg g^{-1} DM; mean ± SD) in both silages.

The numbers of TB and LAB were lower ($P < 0.001$) for INOC than CON on day 7 but did not differ between silages on other days of AE (Figure 2A and B). Yeast counts were lower ($P < 0.001$) for INOC than CON on days 0, 3, and 7 of AE (Figure 2C). Inoculant lowered mold counts ($P < 0.001$) as compared with CON on day 14 of AE (Figure 2D). The aerobic stability (h) of INOC and CON was 270.8 and 99.2 h, respectively (Figure 3). Moreover, the temperature of INOC was lower ($P < 0.001$) than CON on day 7 of AE.

Rumen metabolites, nutrient digestibility, and N retention

Nutrient composition of diets and the composition of TMR (CON and INOC) used for the nutrient digestibility and growth performance study are presented in Table 2. Diets did not differ ($P > 0.05$) in their chemical composition.

There was no effect of diet or DIET × TIME interaction ($P > 0.05$) for any of the measured ruminal fermentation parameters (Table 3). Ruminal pH did not vary ($P > 0.05$) among heifers and averaged 6.45 ± 0.35 (mean ± SD). Time of sampling impacted ($P \leq 0.01$) all ruminal fermentation parameters, including pH ($P < 0.001$), VFA, acetate:propionate ratio, LA, and ruminal NH_3-N concentrations.

Dry matter intake and intake as a percentage of BW did not ($P > 0.05$) differ among heifers and averaged 10.40 ± 1.22 kg (mean ± SD) and 1.31 ± 0.18 (mean ± SD), respectively (Table 4). ATTD of DM, OM, ADF, and NDF also did not differ ($P > 0.05$) among heifers and averaged (mean ± SD) 69.6 ± 2.1, 71.8 ± 2.2, 50.6 ± 6.1, and 57.6 ± 3.7, respectively. However, there was a tendency for lower CP ($P < 0.07$) and starch ($P < 0.06$) digestibility for heifers fed INOC than CON.

Total fecal (kg DM d^{-1}) and urine (kg d^{-1}) output did not differ ($P > 0.05$) among heifers and averaged 3.14 ± 0.38 kg DM d^{-1} (mean ± SD) and 9.14 ± 1.84 kg d^{-1} (mean ± SD), respectively (Table 5). Total N intake was lower ($P = 0.04$) for heifers fed INOC than CON. Apparent total retained N (g d^{-1}) was lower ($P = 0.02$) for heifers fed INOC than CON. Nevertheless, there was no difference in N efficiency (%) between heifers which averaged 37.2 ± 4.99% (mean ± SD).

Animal growth performance

All steers used in the feedlot experiment were healthy and no mortality or morbidity occurred during the experiment. Initial and final shrunk BW (kg), ADG (kg d^{-1}), and DMI (kg) were similar ($P > 0.05$) among steers and averaged (mean ± SD) 366.3 ± 20.7 kg, 492.5 ± 29.6 kg, 1.50 ± 0.23 kg d^{-1}, and 9.84 ± 0.92 kg, respectively (Table 6). Dry matter intake as a percentage of BW was lower ($P = 0.04$) for steers fed INOC than CON silage. There was a tendency ($P = 0.07$) for greater feed efficiency (G:F) for steers fed INOC than CON. The NE_m and NE_g contents of diets (Mcal kg^{-1} DM) calculated based on growth performance were greater ($P = 0.03$) for INOC than CON.

Discussion

The strain *L. hilgardii* (CNCM I-4785) used in our study was recently isolated from sugar cane (Ávila et al., 2014) and was deposited and patented (European Patent Application

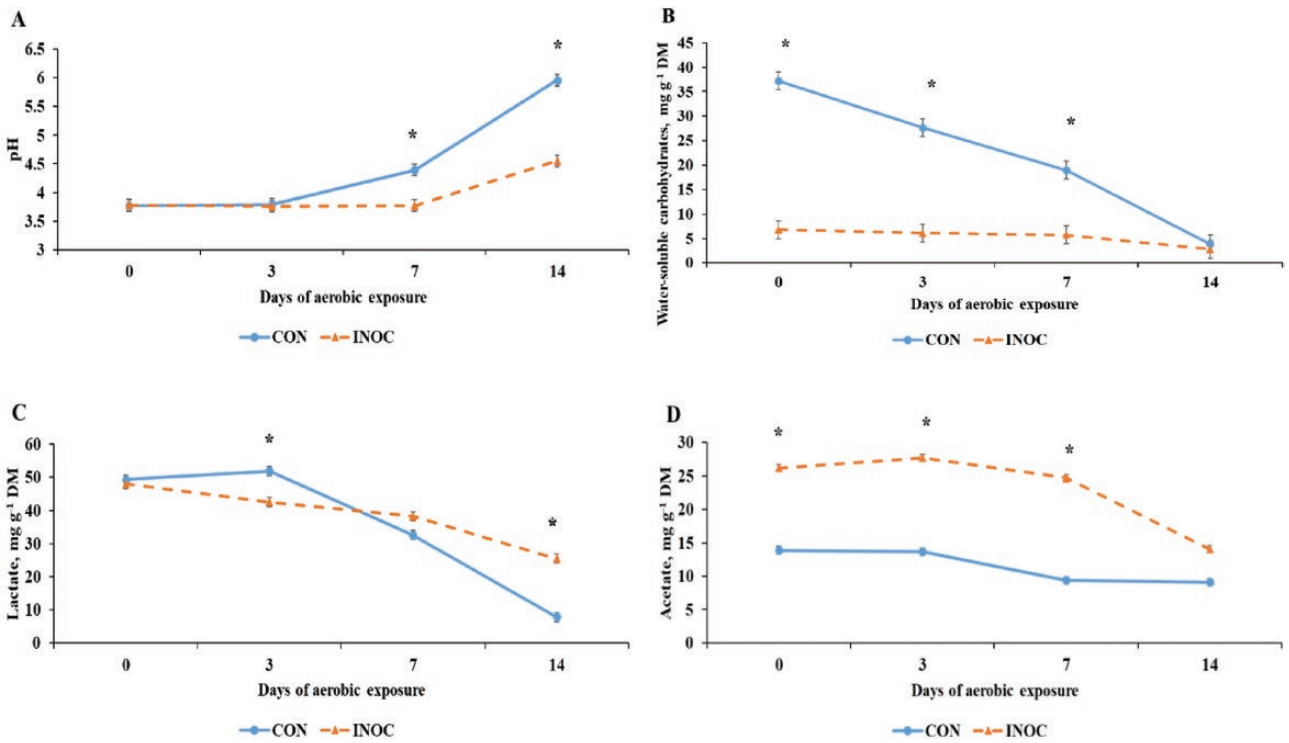

Figure 1. Impact of inoculation of corn silage with LAB inoculant containing 1.5 × 10^5 cfu g^{-1} fresh forage *L. hilgardii* and 1.5 × 10^5 cfu g^{-1} fresh forage *L. buchneri* for a total of 3.0 × 10^5 cfu g^{-1} fresh forage LAB on (A) pH, (B) WSC content, (C) LA, and (D) AC concentrations in terminal silages ensiled in silo bags and during AE (n = 4). (A) *Denotes treatment differences in silage pH in terminal silage and upon AE. pH of INOC was lower ($P < 0.001$) than CON on days 7 and 14 of AE. (B) *Denotes treatment differences in silage WSC content in terminal silage and upon AE. The WSC content of INOC was lower ($P < 0.001$) than CON in terminal silage and on days 3 ($P < 0.001$) and 7 ($P = 0.04$) of AE. (C) *Denotes treatment differences in silage LA concentrations in terminal silage and upon AE. The LA concentration of INOC was lower ($P < 0.001$) than CON on day 3 and greater ($P = 0.01$) than CON on day 14 of AE. (D) *Denotes treatment differences in silage AC concentrations in terminal silage and upon AE. The AC concentration of INOC was greater ($P \leq 0.001$) than CON in terminal silage and on days 3 and 7 of AE.

EP2826385A1) as an inoculant for improving the aerobic stability of silages by Lallemand SAS, 19 rue des Briquetiers, 31702 Blagnac Cedex, France. Ensiling characteristics of this newer strain of obligate heterofermentative LAB has not been evaluated in farm-scale silos. Phylogenetic evaluation and fermentation profiling of *L. hilgardii* revealed that it is closely related to *L. buchneri* as both of them possess the ability to degrade LA to AC and 1, 2-propanediol in anaerobic conditions (Heinl et al., 2012; Carvalho et al., 2014; Drouin et al., 2019). Recent studies evaluating the impact of *L. hilgardii* on ensiling fermentation and aerobic stability of sugar cane and corn silages indicated that these strains resulted in higher concentrations of AC, lower yeast population, and a higher aerobic stability than uninoculated silages (Ávila et al., 2014; Carvalho et al., 2014; Ferrero et al., 2019).

Lactobacillus buchneri is the most common heterofermentative LAB used to improve the aerobic stability of silages (Muck et al., 2018; Ferrero et al., 2019) as it produces AC that possesses fungicidal activity (Reich and Kung, 2010; Addah et al., 2014). However, *L. buchneri* requires ~60 d to produce AC at levels that improve the aerobic stability of silages (Kleinschmit and Kung 2006; Muck et al., 2018). Frequently, producers wish to start to feed out silage from silos after ensiling periods of less than 30 d, making it desirable to have AC produced by heterofermentative LAB earlier during ensiling (Ferrero et al., 2019). Carvalho et al. (2014) reported an increase in AC concentration in sugar cane silages inoculated with *L. hilgardii* as early as day 12 of ensiling relative to uninoculated silages. Similarly, in laboratory silos, Reis et al. (2018) reported an increase in AC concentration in corn silage inoculated with *L. hilgardii* by day 19 of ensiling with improved aerobic stability than uninoculated silage.

In general, combination inoculants contain two or more strains of LAB, typically including homo and heterofermentative LAB to combine the improvements in fermentation efficiency brought about by the homofermentative LAB and aerobic stability conferred by heterofermentative LAB (Muck et al., 2018). Studies evaluating a combination of different obligate heterofermentative LAB on the impact on corn silage fermentation and aerobic stability are limited. Recently, Ferrero et al. (2019) used *L. hilgardii* alone or in combination with another heterofermentative LAB *L. buchneri* to verify the ability of *L. hilgardii* to enhance the aerobic stability of corn silages after a short conservation period and to evaluate potential complementary action of these inoculants. In laboratory silos, these authors reported a relatively greater AC concentration in corn silage inoculated with the combination inoculant compared with that ensiled with either of the inoculants alone or not inoculated. It was also reported that the aerobic stability of corn silage inoculated with the combination inoculant was greater compared with that ensiled with *L. buchneri* alone or not inoculated as early as after 15 d of ensiling, while after 250 d of ensiling, the aerobic stability was greater for corn silage inoculated with the combination inoculant compared with that ensiled with either of the inoculants alone or not inoculated. However, the results were not consistent when studies were carried out in multiple crop years.

The inoculant had no effect on LA production and both silages reached a terminal silage pH that was indicative of a good

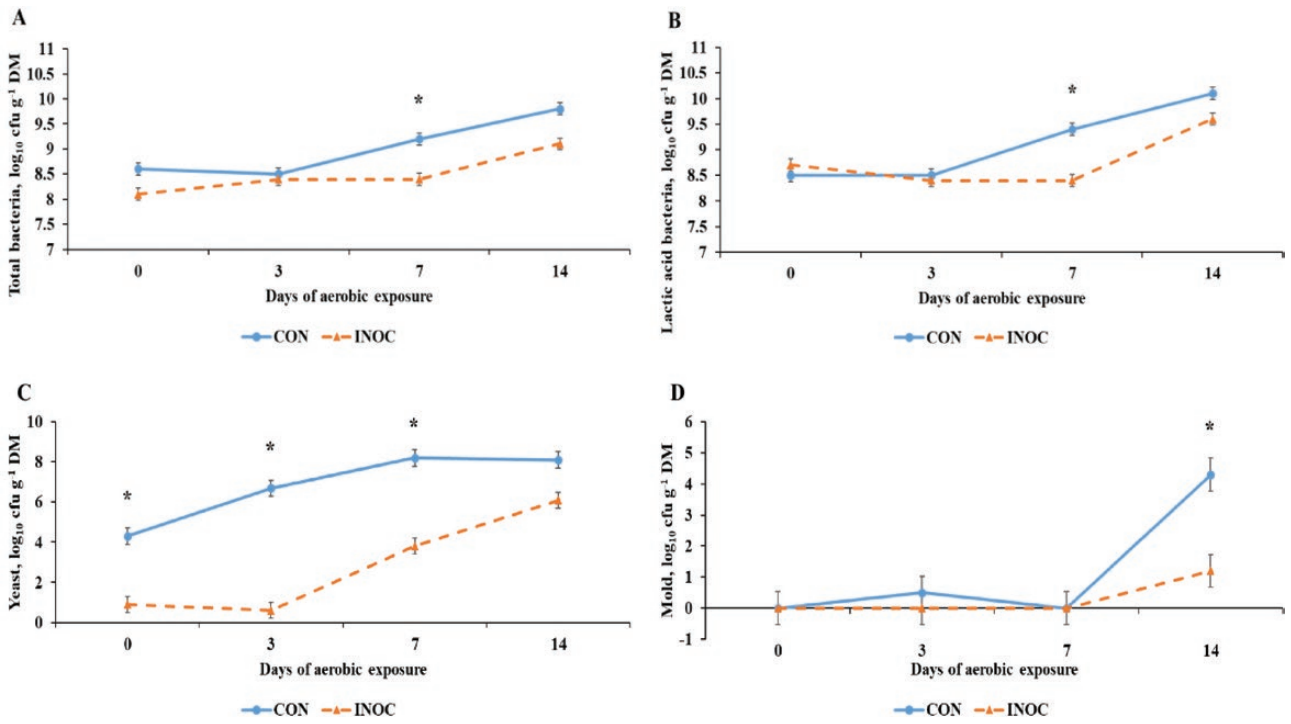

Figure 2. Impact of inoculation of corn silage with LAB inoculant containing 1.5 × 10^5 cfu g^{-1} fresh forage *L. hilgardii* and 1.5 × 10^5 cfu g^{-1} fresh forage *L. buchneri* for a total of 3.0 × 10^5 cfu g^{-1} fresh forage LAB on (A) TB, (B) LAB, (C) yeast, and (D) mold counts in terminal silages ensiled in silo bags and during AE (n = 4). (A) *Denotes treatment differences in silage TB counts in terminal silage and upon AE. The TB counts of INOC were lower (P < 0.001) than CON on day 7 of AE. (B) *Denotes treatment differences in silage LAB counts in terminal silage and upon AE. The LAB counts of INOC were lower (P < 0.001) than CON on day 7 of AE. (C) *Denotes treatment differences in silage yeast counts in terminal silage and upon AE. Yeast counts of INOC were lower (P < 0.001) than CON in terminal silage and on days 3 and 7 of AE. (D) *Denotes treatment differences in silage mold counts in terminal silage and upon AE. Mold counts of INOC were lower (P < 0.001) than CON on day 14 of AE.

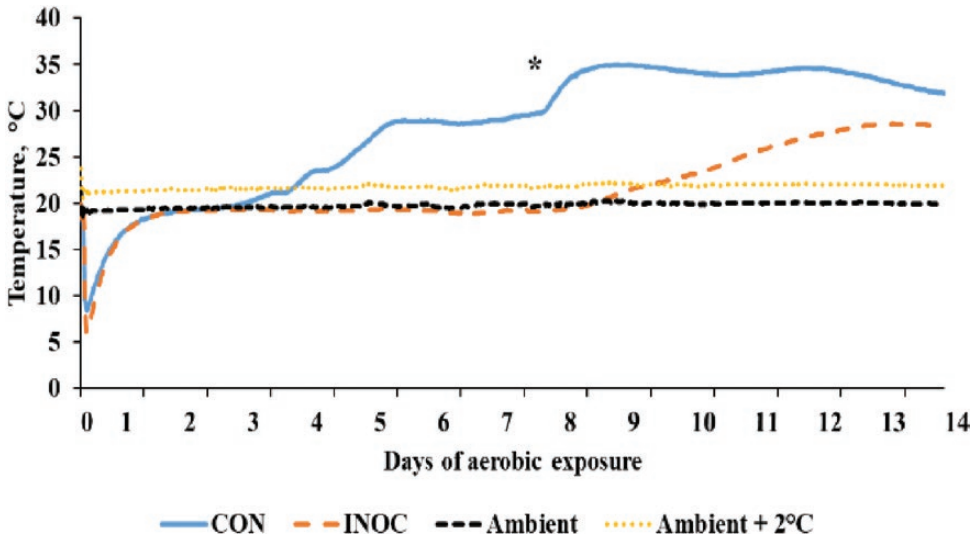

Figure 3. Impact of inoculation of corn silage with LAB inoculant containing 1.5 × 10^5 cfu g^{-1} fresh forage *L. hilgardii* and 1.5 × 10^5 cfu g^{-1} fresh forage *L. buchneri* for a total of 3.0 × 10^5 cfu g^{-1} fresh forage LAB on silage temperature during AE of corn silage ensiled in silo bags (n = 4). *Denotes treatment differences in silage temperature during AE. Silage temperature of INOC was lower (P < 0.001) than CON on day 7 of AE.

quality corn silage (Kung et al., 2018). Production of LA during ensiling decreases silage pH, which suppresses the growth of spoilage microorganisms resulting in stable silage (Rooke and Hatfield, 2003). Similarly, total VFA concentration increased from 1.76 ± 0.14 mg g^{-1} DM (mean ± SD) in fresh forage to 20.1 ± 7.08 mg g^{-1} DM (mean ± SD) in terminal silages. However, total VFA and AC concentration in terminal silage were almost twice as high in INOC than CON. In addition, WSC concentration in INOC silage was more than 5-fold lower than CON. Greater utilization of WSC in silages inoculated with *L. hilgardii* than uninoculated control silages have been reported by others (Carvalho et al., 2014). A decrease in LA concentration with a corresponding

Table 3. Ruminal pH, proportions of VFA, total VFA concentrations, acetate to propionate ratio, and ruminal ammonia concentrations of heifers fed diets containing CON or INOC corn sillage ensiled in silo bags

	Treatments[1]			*P*-value[3]		
Item	CON	INOC	SEM[2]	DIET	TIME	DIET × TIME
Ruminal pH	6.41	6.48	0.058	0.45	< 0.001	0.82
VFA, mol/100 mol						
Acetate (AC)	66.3	67.6	1.12	0.42	< 0.001	0.78
Propionate (PA)	18.6	18.4	0.81	0.84	< 0.001	0.75
Butyrate	11.3	10.2	0.610	0.25	< 0.01	0.92
Isobutyrate	0.84	0.86	0.028	0.72	< 0.001	0.82
Valerate	1.04	1.04	0.046	0.94	< 0.001	0.33
Isovalerate	1.39	1.42	0.101	0.86	< 0.01	0.78
Caproate	0.53	0.46	0.053	0.39	< 0.001	0.58
Total VFA, mM	93.5	92.0	3.78	0.78	< 0.001	0.78
Lactate, mM	0.58	0.24	0.182	0.19	0.01	0.07
AC:PA Ratio	3.72	3.79	0.198	0.80	< 0.001	0.49
Ruminal NH_3-N, mg dL^{-1}	5.40	4.54	0.425	0.17	< 0.01	0.38

[1]CON, control; INOC, inoculated.
[2]SEM, pooled standard error of mean (n = 4).
[3]P-value for the effect of DIET, TIME, and the DIET × TIME interaction.

Table 4. Apparent nutrient digestibility of beef heifers fed diets containing CON or INOC corn sillage ensiled in silo bags

	Treatments[1]			
Item	CON	INOC	SEM[2]	*P*-value
Dry matter intake				
kg d^{-1}	10.5	10.3	0.44	0.45
% of BW	1.32	1.30	0.064	0.52
Apparent nutrient digestibility coefficient, % DM basis				
DM	70.0	69.3	0.77	0.38
OM	72.1	71.5	0.79	0.60
CP	68.3	66.1	0.78	0.07
ADF	51.6	49.6	2.20	0.22
NDF	58.0	57.3	1.35	0.72
Starch	97.8	97.3	0.25	0.06

[1]CON, control; INOC, inoculated.
[2]SEM, pooled standard error of mean (n = 4).

increase in AC concentration reflects the conversion of LA to AC in corn silages inoculated with *L. buchneri,* an observation confirmed by others (Ranjith and Kung, 2000; Drouin et al., 2019; Nair et al., 2019b). Like *L. buchneri*, *L. hilgardii* has been reported to convert LA to form AC in anaerobic conditions (Heinl et al., 2012). However, in our study, both INOC and CON had similar LA concentrations in terminal silages. Moreover, an increase in AC concentration and lower WSC concentration in terminal INOC than CON silages in our study indicate that the main action of the inoculant was to ferment WSC to produce AC during ensiling.

The LA:AC ratio increased from 0.33 ± 0.05 (mean ± SD) in fresh forage to 3.64 ± 0.60 (mean ± SD) in CON and 1.85 ± 0.19 (mean ± SD) in INOC at the end of 120 d of ensiling. The fermentation pattern and the LA:AC ratio of CON and INOC suggest that CON proceeded mainly thorough homolactic fermentation, with a rapid reduction in silage pH by the production of LA and preservation of WSC levels (Addah et al., 2012; Muck et al., 2018). On the other hand, INOC induced a heterolactic fermentation. Ferrero et al. (2019) reported that a LA:AC ratio of >3 is indicative of a dominant homolactic fermentation, while inoculants containing heterofermenters such as *L. buchneri* reduce the LA:AC ratio, ranging from 2.3:1 to 1.3:1 depending on the inoculation dose (Kung, 2010). Total yeast counts decreased from 7.46 ± 0.12 $\log_{10}$ cfu g^{-1} DM (mean ± SD) in fresh forage to 4.32 ± 1.52 $\log_{10}$ cfu g^{-1} DM (mean ± SD) in CON and 0.91 ± 1.66 $\log_{10}$ cfu g^{-1} DM (mean ± SD) in INOC after 120 d of ensiling. Lower yeast counts for INOC corresponded to a greater AC concentration in terminal silage than CON, reflecting its ability to inhibit these spoilage microorganisms (Muck et al., 2018).

Effects of inoculant on the aerobic stability of whole-crop corn silage

Aerobic stability was nearly 3-fold longer for INOC silage than CON, resulting in slower increases in silage pH and higher LA concentrations after 14 d of AE. This response was likely due to lower residual WSC and the higher concentration of AC in INOC inhibiting the growth of yeast. The composition of terminal silage has a major impact on the aerobic stability of silages (Nair et al., 2019a). Greater amounts of residual WSC can serve as a substrate for spoilage microorganisms during AE (Addah et al., 2012). The decrease in WSC concentration of CON from 37.2 mg g^{-1} DM in terminal silage to less than 4 mg g^{-1} DM after 14 d of AE likely indicates its consumption by the spoilage microorganisms. The WSC concentration of INOC was lower than CON in terminal silage and remained low throughout AE. Conversely, AC concentration of INOC was nearly twice as that in CON throughout AE. Moreover, yeast counts of INOC did not reach 10^6 cfu g^{-1} DM until day 14 of AE while that was greater than 10^6 cfu g^{-1} DM for CON by day 3 of AE. Tabacco et al. (2011) found a negative correlation between aerobic stability of silages and yeast counts and Kung (2010) reported that good quality silage should not contain yeast counts above 10^6 cfu g^{-1} DM. Greater aerobic stability of INOC than CON in our study indicate that the fermentation dynamics during ensiling plays a significant role in chemical composition and microbial populations of corn silages with potential impact on stability and nutrient composition during feed out.

Table 5. Nitrogen balance parameters of beef heifers fed CON or INOC corn sillage ensiled in silo bags

Item	Treatments[1]		SEM[2]	P-value
	CON	INOC		
Fecal output, kg DM d^{-1}	3.15	3.14	0.139	0.85
Urine output, kg d^{-1}	9.22	9.06	0.672	0.76
Nitrogen, g d^{-1}				
Total intake N	270.0	248.5	11.98	0.04
Total excreted N	183.6	178.8	9.72	0.27
Fecal N	85.5	84.0	3.80	0.48
% of excreted N	46.6	47.3	1.04	0.64
Urinary N	98.1	94.8	6.35	0.37
% of intake N	36.5	38.0	1.81	0.58
% of digested N	53.6	57.6	3.10	0.37
% of excreted N	53.4	52.7	1.04	0.64
Apparent total retained N	92.6	74.4	4.94	0.02
Retained as a % of intake N	31.8	28.1	2.29	0.28
Retained as a % of digested N	46.4	42.4	3.10	0.37

[1]CON, control; INOC, inoculated.
[2]SEM, pooled standard error of mean (n = 4).

Table 6. Performance of growing beef steers fed CON or INOC corn sillage ensiled in silo bags

Item	Treatments[1]		SEM[2]	P-value
	CON	INOC		
Number of steers	20	20	—	—
Initial shrunk BW[3], kg	366.4	366.3	4.68	0.98
Final shrunk BW[3], kg	490.6	494.3	6.68	0.69
ADG, kg d^{-1}	1.48	1.52	0.053	0.56
DMI, kg	10.1	9.6	0.20	0.15
DMI as % BW	2.34	2.24	0.034	0.04
G:F[4]	0.147	0.159	0.0044	0.07
NE_m[5], Mcal kg^{-1} DM	1.81	1.92	0.032	0.03
NE_g[5], Mcal kg^{-1} DM	1.18	1.27	0.028	0.03

[1]CON, control; INOC, inoculated.
[2]SEM, pooled standard error of mean, n = 20 steers per treatment.
[3]Shrunken BW calculated as 96% of live weight (NRC, 2000).
[4]Calculated as ADG:DMI.
[5]Calculated based on performance (Zinn and Shen, 1998; Zinn et al., 2002).

Digestibility study

Nutrient composition of diets did not differ as the same silage was used for both the digestibility and growth performance studies. Similarly, ruminal pH and fermentation profiles did not differ between diets, likely due to similar DMI of heifers. Moreover, the total tract digestibility of nutrients also corresponded to similar DMI between heifers. The average ruminal pH of heifers was 6.45 ± 0.35 (mean ± SD) which is typical for cattle fed forage-based diets with a similar dietary composition (Nair et al., 2019b). The inoculant had no effect on ruminal pH, thus had minimal impact on ruminal fiber digestibility. Proportions of AC, PA, and total VFA concentration were similar to those reported by Nair et al. (2019b) for heifers fed similar diets. Acetate:propionate ratio of >3.0 is indicative of the predominance of AC production in the rumen for heifers fed forage-based diets (Bauman et al., 1971). Average ruminal NH_3-N concentration of 4.97 ± 2.44 mg^{-1} dL (mean ± SD) for heifers across treatments likely indicates that the ruminal microbial protein synthesis was not negatively impacted as the ruminal NH_3-N concentration was similar to the recommended minimum of 5 mg^{-1} dL required for optimum ruminal microbial activity (Satter and Slyter, 1974).

Greater NDICP concentration for INOC than CON was surprising as both the silages were prepared from the same corn forage. Tendency for lower total tract CP digestibility for heifers fed INOC than CON likely reflects the greater NDICP content of INOC silage. The NDICP is the insoluble fraction of CP associated with the cell wall (Nair et al., 2016), digestibility of which is lower than that of soluble fractions of the CP (Sniffen et al., 1992). It also needs to be pointed out that although starch apparent digestibility of INOC statistically tended to be lower than that of CON diet, the small difference of 0.5% would not be biologically meaningful.

Greater total N intake for heifers fed CON than INOC was likely due to the numerically higher CP content of the CON diet than INOC (16.0 vs. 15.2 %). A higher dietary N content is also associated with an increase in fecal and urinary N excretion (Vasconcelos et al., 2009).

Nitrogen excretion values for heifers in our study were similar to those reported for heifers fed similar diets (Nair et al., 2019b). However, it should be noted that N retention and estimated N efficiency of heifers in the present study were higher than that reported for heifers in the previously cited study. Walter et al. (2012) reported that an apparent total N retention of 48 to 86 g d^{-1} corresponded to gains in excess of 2 kg d^{-1}. However, the ADG of steers fed similar diets in the growth performance study averaged 1.50 ± 0.23 kg d^{-1}. Potential sampling errors including the underestimation of fecal N caused by incomplete sample collection, volatile losses of NH_3-N from the pen, and losses of N during drying of the fecal samples can result in the overestimation of apparent total N retention (Spanghero and Kowalski, 1997). It was also reported that the volatilization of urinary NH_3-N during total collection can result in the overestimation of retained N (Kohn et al., 2005). However, urine was acidified with 4N sulfuric acid during total collection in order to keep the urine pH under 2.0 in the present study.

Effects of inoculant on growth performance of feedlot steers

Lack of improvement in DMI and ADG for steers fed INOC over CON was surprising as INOC had greater stability than CON upon AE and was expected to have improved nutrient composition. Since aerobic deterioration of both CON and INOC was minimal at the time of feeding, the impact of this factor on the growth performance of steers would have been minimal. Moreover, the WSC content of CON was nearly five times greater than that of INOC in terminal silages. The residual WSC can act as substrates for rumen microbes to produce VFA during digestion.

Tendency for a greater feed efficiency for steers fed INOC reflects numerically lower DMI and similar ADG than those fed CON. It should be noted that the total tract digestibility of CP and starch was lower for heifers fed INOC than CON in the concurrent digestibility study. Diets in both the growth performance and digestibility studies contained greater CP content than what was required for growing and mature beef cattle (NASEM, 2016). The heifers weighed nearly twice as steers in our study. The difference in BW and growth requirements likely impacted the growth performance and nutrient utilization of these animals. Moreover, a greater proportion of NDICP in INOC than CON can likely have an impact on the performance of steers fed INOC as this fraction is slowly degraded in the rumen and the majority escapes to the small intestine (Sniffen et al., 1992) as a fraction

of undegradable intake protein (**UIP**) which is then available for absorption. Oney et al. (2019) reported that the UIP content of corn silage is approximately 1% of DM, of which 50% is digestible. These authors also reported that supplementing UIP to growing beef cattle fed corn silage linearly increased the G:F for growing steers. It is likely that the greater availability of UIP for steers fed INOC than CON improved the feed efficiency of these steers. The NE_m and NE_g calculated based on the growth performance parameters indicated that INOC diets had greater NE_m and NE_g content than CON due to numerically greater final shrunk BW, ADG, and a lower DMI.

Conclusions

The results of the present study indicated that inoculation with strains of *L. hilgardii* and *L. buchneri* improved the aerobic stability of corn silage. An increase in AC concentration, lower WSC content, and yeast counts in terminal INOC silage likely played a significant role in improving its aerobic stability. The inoculant did not affect ruminal fermentation, total tract nutrient digestibility, or growth performance of beef cattle; however, steers fed INOC tended to have improved feed efficiency than those fed CON silage. The potential benefits of feeding INOC with greater aerobic stability could likely be more prominent in large commercial feeding operations with a lower silage packing density, wider silo face, and slower silage removal rate as the silage is exposed to air for a prolonged periods under these scenarios.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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