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Fermentation quality and *in vitro* methane production of sorghum silage prepared with cellulase and lactic acid bacteria

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Objective: The effects of lactic acid bacteria (LAB) and cellulase enzyme on fermentation quality, microorganism population, chemical composition and *in vitro* gas production of sorghum silages were studied.
Methods: Commercial inoculant *Lactobacillus plantarum* Chikuso 1 (CH), local selected strain *Lactobacillus casei* (*L. casei*) TH 14 and *Acremonium* cellulase (AC) were used as additives in sorghum silage preparation.
Results: Prior to ensiling Sorghum contained 10^4 LAB and 10^6 cfu/g fresh matter coliform bacteria. The chemical compositions of sorghum was 26.6% dry matter (DM), 5.2% crude protein (CP), and 69.7% DM for neutral detergent fiber. At 30 days of fermentation after ensiling, the LAB counts increased to a dominant population; the coliform bacteria and molds decreased to below detectable level. All sorghum silages were good quality with a low pH (<3.5) and high lactic acid content (>66.9 g/kg DM). When silage was inoculated with TH14, the pH value was significantly ($p<0.05$) lower and the CP content significantly ($p<0.05$) higher compared to control, CH and AC-treatments. The ratio of *in vitro* methane production to total gas production and DM in TH 14 and TH 14+AC treatments were significantly ($p<0.05$) reduced compared with other treatments while *in vitro* dry matter digestibility and gas production did not differ among treatments.
Conclusion: The results confirmed that *L. casei* TH14 could improve sorghum silage fermentation, inhibit protein degradation and decrease methane production.

Keywords: Cellulase; Lactic Acid Bacteria; Methane Production; Sorghum Silage

INTRODUCTION

Native grasses, forage straw and food by-products are the major feed resources for ruminants in tropical developing countries including Thailand [1]. The most important constraint for ruminant production in the tropics is shortage of feed in terms of quality, especially in the dry season [1-3]. When ruminants cannot be fed high quality roughage this results in low milk and beef production. In recent years, many varieties of forage crops with an ability to tolerate hot weather and drought conditions have been developed, and their adaptability to various cultivation conditions, nutritive value and productivity have also been studied [1]. Normally, forage crops grow well in the rainy season with a high dry matter (DM) yield [4,5]. Thus, they should be conserved in the rainy season to supply feed for ruminants during the dry season. Silage is considered to be one of the most effective feeds for animal production to cover shortage in the tropical dry season [6]. Sorghum (*Sorghum bicolor*), a warm season tropical grass, is well adapted to a wide range of soil types and is tolerant to waterlogging. It usually requires less water than other forage crops such as maize, and produces higher yield in hotter areas such as Africa and Asia [7-10]. Sorghum is a very important worldwide forage crop and widely used for silage making [11]. It is one of the main tropical forage crops ensiled to provide feed for milk and meat production of ruminants [12].

Sorghum has a high growth rate, is drought tolerant and is high in water-soluble carbohydrate (WSC) [8,9]; it is also high in fiber which decreases nutrient utilization in animals [13]. Silage additives such as bacteria inoculants and cellulase enzymes have played an important role in improving silage quality and nutrient digestibility [14]. Previous studies reported that lactic acid bacteria (LAB) inoculation enhanced the ensiling process by promoting conversion of WSC to lactic acid [15,16]. Addition of cellulase to ensiling materials can improve fiber degradation thus increasing WSC to produce lactic acid [17-20]. Cellulase treated sorghum straw, corn, and *Leymus chinensis* silages also showed an increase in *in vitro* neutral detergent fiber digestibility [17,21,22]. The application of LAB inoculant combined with cellulase improves silage fermentation and *in vitro* digestibility [22-25]. Many researchers have studied inhibition of methane production by ruminants to help address global climate change [26-28]. Inhibition of methane production is normally accompanied by an increase in propionate production, which utilises hydrogen and lactic acid [29,30]. The addition of LAB and cellulase may contribute to high lactic acid content and low pH during silage fermentation, indicating that when this high-quality silage is fed to ruminants, their methane production may be reduced.

However, there is very limited information available on sorghum silage fermentation and *in vitro* methane production when treated with microbiological additives and cellulase enzyme in the tropics. The objectives of this study were to determine the effects of LAB, cellulase enzyme and their combination on silage fermentation and *in vitro* gas production of tropical sorghum silage.

MATERIALS AND METHODS

Sorghum material and silage preparation

This experiment was conducted at Khon Kaen University (KKU), Khon Kaen Province, Thailand, from October 2014 to February 2015. Sorghum (*Sorghum bicolor* cv. IS 23585) was grown in an area of 400 m^2 at the experimental farm, Faculty of Agriculture, KKU. The plots were plowed twice and harrowed once. Before the second plowing, the soil was fertilized with fermented cattle manure at a rate of 40 t/ha, dolomite [$CaMg(CO_3)_2$] at 3,125 kg/ha and phosphorus at 57.5 kg P/ha. Sorghum was sown at seeding rate of 25 kg/ha. At day 7 after emergence, seedlings were thinned to allow a spacing of 1 plant per 50×10 cm and weeded at 2 weeks after emergence (WAE). At 2, 4, and 8 WAE, sorghum was fertilized with urea at a rate of 200 kg N/ha and potassium was applied at 50 kg K/ha at 2 and 4 WAE.

Sorghum was harvested at milky growth stage by hand-sickle at 15 cm above ground level at 11 WAE on 20 December 2014. After harvesting, the sorghum was chopped immediately into 1 cm lengths using a forage chopper (Supachai, Kanchanaburi, Thailand). A locally selected strain, *Lactobacillus casei* (*L. casei*) TH14 [6], a commercial inoculant Chikuso 1 (CH, *Lactobacillus plantarum*, Snow Brand Seed Co., Ltd, Sapporo, Japan) and a commercial cellulase enzyme (AC, *Acremonium cellulase*, Meiji Seika Pharma Co., Ltd, Tokyo, Japan) were used as silage additives. Strain TH 14 was isolated from sweet corn (*Zea mays* L.) stover silage; it has previously been shown to improve silage quality in the tropics [6]. Strains CH and TH14 were incubated in Lactobacilli de Man, Rogosa, Sharpe (MRS) broth (Difco, Laboratories, Detroit, MI, USA) overnight. After incubation, the optical density of the suspension at 620 nm was adjusted with sterile 0.85% NaCl solution to 0.42. The LAB inoculum size was 1 mL of suspension/kg as 1.0×10^5 colony forming unit (cfu)/g fresh matter (FM). Cellulase was added at 0.01% of FM. The experiment was set out in a completely randomized design with three replications. Silage treatments were control, CH, TH14, AC, CH+AC, TH14+AC. A synthetic silo laminated from nylon and polyethylene (Hiryu KN type, Asahikasei, Tokyo, Japan) and vacuum sealer (SQ–303, Asahi Kasei Pax Corp., Tokyo, Japan) were used for silage preparation [31]. All silos were kept at room temperature (25°C to 37°C) and were opened at 30 days after fermentation. Fermentation quality, microbial population, and chemical composition were analyzed.

Microorganism analysis of fresh sorghum and silages

The microorganism count on fresh sorghum and silage samples at 30 days after fermentation was done using the plate count method [32] and reported as colony forming unit per gram of fresh matter (cfu/g FM). Fresh chopped sorghum and silages (10 g each) were shaken well by hand with 90 mL sterilized distilled water, and serially diluted at 10^{-1} to 10^{-5} in sterilized distilled water. Twenty μL from each dilution was spread on agar plates. LAB numbers were counted on Lactobacilli MRS agar (Difco Laboratories, USA) after incubating in an anaerobic jar (A-110, Sugiyamagen Co., Ltd., Tokyo, Japan) at 30°C for 48 h. Coliform bacteria numbers were counted on blue light broth agar (Nissui-Seiyaku Co., Ltd., Tokyo, Japan) after incubating at 30°C for 48 h. Aerobic bacteria and bacilli numbers were counted on nutrient agar (Difco, USA), yeasts and mold numbers were counted on potato dextrose agar (Nissui-Seiyaku, Japan) after incubating at 30°C for 48 h. In this experiment, mold was counted at 48 h of incubation. Yeasts were distinguished from molds or bacteria by colony appearance and cell morphology observation.

Chemical composition analysis of fresh sorghum and silages

Fermentation products of the silages were analyzed from cold water extracts as described by [33]. Silage (10 g FM) was homogenized with 90 mL of sterilized distilled water [31]. The pH value was measured by a glass electrode pH meter (FiveGo; Mettler Toledo, Greifensee, Switzerland), and ammonia nitrogen content was analyzed using a spectrophotometer (UV/VIS Spectrometer, PG Instruments Ltd., London, UK) [34]. Lactic acid buffering capacity (LBC) was determined by titrating with 0.1 M HCl (to

reduce pH from initial pH to pH 3) and then titrated with 0.1 M NaOH from pH 3 to pH 6 [19]. The organic acid content and water soluble carbohydrate (WSC) were measured by high performance liquid chromatograghy methods, [31].

Samples of fresh sorghum and silage at 30 days after fermentation were dried in a forced air oven at 60°C for 48 h, and ground to pass a 1 mm mesh screen for chemical composition analyses using procedures of [35] viz. 934.01 for DM, 942.05 for organic matter (OM), 976.05 for crude protein (CP) and 920.39 for ether extract (EE). Based on the procedure described by [36], the neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by a fiber analyzer (ANKOM 200, ANKOM Technology, New York, USA. Acid detergent lignin (ADL) by the procedures of [37]. Gross energy (GE) using an automatic adiabatic bomb calorimeter (AC 500, LECO, St. Joseph, MI, USA).

In vitro gas production technique

Ruminal fermentation of sorghum silage samples was conducted using an *in vitro* gas production technique [38]. Rumen fluid was collected from three Thai native beef cattle (*Bos indicus*) bulls by a stomach tube sucker before morning feeding, and filtered through 4 layers of cheesecloth into pre-warmed (40°C) thermo bottles, and transported directly to laboratory within 15 minutes of collection. Rumen medium preparations containing buffer solution (730 mL), macro mineral solution (365 mL), micro mineral solution (0.23 mL), resazurine solution (1 mL), reduction solution (60 mL), and distilled water (1,095 mL) were mixed with rumen fluid (660 mL) and flushed with carbon dioxide gas to produce an oxygen-free system. Zero point five g of ground silage was put into 50 mL serum bottles (3 replications per sample). The serum bottles were closed with a rubber stopper and an aluminum seal cap. Forty mL of rumen medium was injected into each sample bottle using a 60 mL syringe (Nipro Thailand corporation, Ltd., Phra Nakhon Si Ayutthaya, Thailand) with an 18 gauge×1.5 inch needle (Nipro Corporation, Osaka, Japan). All samples were incubated in a water bath at 39°C for 24 h, and swirled by hand at 2 h intervals. Two blanks of 40 mL of rumen medium per bottle were also incubated. Gas production was measured using a 25 mL calibrated glass syringe and summarized as total gas production according to [39]. The gas from each 2 hour interval was transferred from the glass syringe into a gas bag (GL science Inc., Tokyo, Japan) for methane (CH_4) production analysis. After 24 h of incubation, gas samples in the gas bag were analyzed for methane concentration by a gas chromatogram (GC8A, Shimadzu Corp., Tokyo, Japan) equipped with a stainless steel pack column (molecular sieve 13×30/60 mesh, Alltech Associates Inc., Deerfield, IL, USA). Each incubated bottle was opened and filtered through a glass filter crucible (20501, GmbH, Hattert, Germany), dried at 100°C in a forced air oven for 24 h and weighed for *in vitro* dry mater digestibility (IVDMD) determination. The dried residues were ashed at 550°C for 3 h for *in vitro* organic matter digestibility (IVOMD) calculation.

Statistical analysis

Data for microorganism counts, fermentation products, chemical composition, *in vitro* digestibility, gas production and methane production of the 30-day silages were analyzed using a completely randomized design. The analysis of variance procedure of SAS version 6.12 (SAS Institute Inc., Cary, NC, USA) was used for the analysis and the statistical model is as follows:

$$Y_{ij} = \mu+\tau_i+\varepsilon_{ij}$$

Where Y_{ij} = observation; μ = overall mean, τ_i = treatment effect, and ε_{ij} = error. The treatment mean differences were determined by Duncan's New Multiple Range Test (DMRT) at p = 0.05 [40].

Ethics of animal experimentation

The use of an animal procedure in this study was approved by the Animal Ethics Committee, KKU, based on the Ethic of Animal Experimentation of National Research Council, Thailand, Record No. AEKKU 15/2558, Reference No. 0514.1.75/6.

RESULTS

Microorganism counts and chemical composition of fresh sorghum and silages

Microorganism counts of fresh chopped sorghum prior to ensiling and its silages at 30 days after fermentation are shown in Table 1. The microorganism numbers of fresh sorghum were 10^4 for LAB, 10^6 for coliform bacteria, 10^6 for aerobic bacteria, 10^6 for yeasts and 10^5 cfu/g FM for molds. The counts in sorghum silages were 10^5 to10^7 for LAB, 10^3 to 10^5 for aerobic bacteria, and not detectable (ND) to 10^5 cfu/g FM for yeasts. Coliform bacteria and molds were not detected in all silages. The highest LAB counts were found in TH14-inoculated silages. LAB counts sig-

Table 1. Microorganism counts of sorghum prior to ensiling and its silages at 30 days after fermentation

Items	Microorganism (cfu/g FM)				
	LAB	Coliform bacteria	Aerobic bacteria	Yeasts	Molds
Sorghum material	2.5×10^{4c}*	1.4×10^{6}	5.1×10^{6a}	3.3×10^{6a}	1.0×10^{5}
Silage					
Control	1.9×10^{5c}	ND	5.2×10^{5b}	5.1×10^{5b}	ND
CH	1.5×10^{5c}	ND	7.0×10^{4b}	8.0×10^{3b}	ND
TH14	5.3×10^{7a}	ND	5.0×10^{3b}	ND	ND
AC	1.5×10^{5c}	ND	3.0×10^{3b}	ND	ND
AC+CH	2.2×10^{6c}	ND	1.3×10^{4b}	ND	ND
AC+TH14	1.6×10^{7b}	ND	1.7×10^{4b}	ND	ND
SEM	2.97	0.05	0.23	4.16	0.03
p-value	<0.001	<0.001	<0.001	<0.001	<0.001

cfu, colony-forming unit; FM, fresh matter; LAB, lactic acid bacteria; ND, not detected; CH, *Lactobacillus plantarum*; TH14, *Lactobacillus casei*; AC, *acremonium cellulase*; SEM, standard error of the mean.

[a-c] * Means in the same column followed by different letters differ (p<0.05).

nificantly increased and coliform bacteria and molds significantly decreased in the silages compared to fresh sorghum.

Chemical composition of sorghum prior to ensiling and its silages are shown in Table 2. The DM of sorghum prior to ensiling was 26.6% DM, and OM, CP, EE, NDF, ADF, and ADL were 96.7, 5.2, 1.5, 69.7, 43.5, and 4.6% DM, respectively. The GE content, LBC, and WSC of sorghum were 4.5 kcal/g, 579.8 meq/kg DM and 35.5 g/kg DM, respectively.

The DM contents of silages were significantly lower than the fresh sorghum. The silage in control treatment had a CP content significantly lower than other treatments except for AC and AC+TH 14 treated silages. OM, EE, NDF, ADF, ADL, and GE of the fresh sorghum were not significantly different from the silages.

Fermentation quality of sorghum silage

Fermentation quality of sorghum silages at 30 days of ensiling are shown in Table 3. All silages were well preserved with low pH values (<3.5) and high lactic acid contents (>66.9 g/kg DM). Butyric acid contents were below the detected level (<0.01 g/kg DM) in all silages. Ammonia nitrogen contents were <0.40 g/kg DM. AC treated silage had a significantly lower lactic acid content than other treatments except control and AC+CH treated silages. The lowest acetic acid contents were found in CH and AC+CH treatments. Propionic and butyric acids could not be detected in all silages. When silage was inoculated with TH14, the pH was significantly (p<0.05) lower compared to control, CH and AC- treatments.

In vitro digestibility and methane production of sorghum silage

The IVDMD, IVOMD, total gas production (GP), and methane production at 24 h incubation of sorghum silages are shown in Table 4. The IVDMD, IVOMD, and GP of all silages ranged from 497.9 to 517.5 g/kg, 560.1 to 577.1 g/kg, and 67.9 to 85.0 L/kg of DM, respectively. These data were not significantly different among control, LAB and cellulase treatments. The ratio of methane production to GP, DM, IVDMD, IVOMD, and GE in TH 14 treatment was significantly (p<0.05) reduced cf. other treatments.

Table 3. Fermentation quality of sorghum silages at 30 days after fermentation

Items	pH	Lactic acid	Acetic acid	Propionic acid	Butyric acid	Ammonia nitrogen
		g/kg DM				
Control	3.54[a]*	72.84[ab]	14.25[b]	ND	ND	0.40
CH	3.49[ab]	79.92[a]	8.54[c]	ND	ND	0.28
TH14	3.43[c]	81.85[a]	18.08[a]	ND	ND	0.35
AC	3.51[a]	66.90[b]	15.96[ab]	ND	ND	0.38
AC+CH	3.44[bc]	74.28[ab]	10.69[c]	ND	ND	0.32
AC+TH14	3.39[c]	81.20[a]	18.50[a]	ND	ND	0.35
SEM	0.019	3.755	1.181	0.000	0.000	0.031
p-value	<0.001	0.044	<0.001	0.000	0.000	0.083

DM, dry matter; ND, not detected; CH, *Lactobacillus plantarum*; TH14, *Lactobacillus casei*; AC, acremonium cellulase; SEM, standard error of the mean.
[a-c] * Means in the same column followed by different letters differ (p<0.05).

DISCUSSION

Silage has now become an increasingly important source of animal feed in the tropics in both dry and rainy seasons [6]. Epiphytic LAB is commonly found living in association with plant material and dairy products. Some studies have reported LAB as the dominant microbial population on forage crops contributing to silage fermentation [31,41,42]. When epiphytic LAB reaches at least 10^5 cfu/g FM as the dominant population, silage is usually preserved well [31,43]. As shown in Table 1, LAB count in fresh sorghum was 10^4 cfu/g FM; however, coliform bacteria, aerobic bacteria and yeasts were higher (10^6 cfu/g FM) and dominated

Table 2. Chemical composition, LBC, WSC, and GE of sorghum material prior to ensiling and its silages at 30 days after fermentation

Items	DM %	OM	CP	EE	NDF	ADF	ADL	GE (kcal/g)
		% DM						
Sorghum material	26.56[a]*	96.68	5.20[ab]	1.52	69.71	43.48	4.56	4.47
Silage								
Control	22.37[bc]	96.62	4.59[c]	1.45	69.46	43.50	4.74	4.50
CH	21.67[c]	96.51	5.29[a]	1.57	69.35	43.57	4.75	4.48
TH14	22.02[bc]	96.66	5.12[ab]	1.47	69.37	43.38	4.66	4.49
AC	22.57[b]	96.68	4.94[abc]	1.56	68.88	44.33	4.81	4.43
AC+CH	21.89[bc]	96.71	5.02[ab]	1.48	69.96	44.93	4.68	4.47
AC+TH14	22.24[bc]	96.73	4.87[bc]	1.43	68.82	43.42	4.62	4.51
SEM	0.275	0.072	0.135	0.106	0.618	0.616	0.075	0.027
p-value	<0.001	0.296	0.015	0.911	0.731	0.345	0.188	0.297

LBC, lactic acid buffering capacity; WSC, water soluble carbohydrate; GE, gross energy; DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; GE, gross energy; CH, *Lactobacillus plantarum*; TH14, *Lactobacillus casei*; AC, acremonium cellulase; SEM, standard error of the mean.
[a-c] * Means in the same column followed by different letters differ (p<0.05).
Sorghum material: LBC = 579.82±10.7 meq/kg DM; WSC = 35.47±0.2 g/kg DM.

Table 4. IVDMD, IVOMD, GP, and methane production at 24 hour of sorghum silage after 30 days of fermentation

Items	IVDMD g/kg	IVOMD g/kg	GP L/kg DM	Methane production					
				mL/L GP	L/kg DM	L/kg OM	L/kg IVDMD	L/kg IVOMD	% GE
Control	510.31	571.50	84.36	157.17[a]*	12.29[a]	12.72[a]	23.48[a]	21.65[a]	2.58[a]
CH	517.47	575.47	76.53	147.75[a]	12.34[a]	12.78[a]	25.61[a]	23.71[a]	2.61[a]
TH14	511.52	571.89	77.79	86.98[c]	6.40[c]	6.63[b]	12.19[b]	12.03[b]	1.35[c]
AC	510.72	577.12	85.03	145.50[ab]	14.30[a]	13.85[a]	24.51[a]	22.69[a]	2.86[a]
AC+CH	497.86	560.13	67.89	167.25[a]	12.49[a]	12.91[a]	23.95[a]	22.10[a]	2.64[a]
AC+TH14	507.77	575.32	81.70	124.03[b]	10.08[b]	8.49[b]	24.51[a]	22.69[a]	2.11[b]
SEM	6.272	9.679	4.128	5.913	0.712	0.848	0.994	0.865	0.184
p-value	0.296	0.7412	0.154	0.001	<0.001	<0.001	<0.001	<0.001	<0.001

IVDMD, *in vitro* dry matter digestibility; IVOMD, *in vitro* organic matter digestibility; GP, gas production; DM, dry matter; OM, organic matter; GE, gross energy; CH, *Lactobacillus plantarum*;TH14, *Lactobacillus casei*; AC, acremonium cellulase; SEM, standard error of the mean.
[a-c] * Means in the same column followed by different letters differ ($p < 0.05$).

LAB. This suggests that the numbers of microbes should be controlled during silage fermentation by using LAB inoculants [31].

In this study, at 30 days after fermentation, the LAB counts increased as a dominant population, and the coliform bacteria and molds decreased to below a detectable level. All sorghum silages were good quality with a relatively low pH (<3.5) and high lactic acid content (>66.9 g/kg DM). The most plausible explanation lies in the physiological properties of LAB and the chemical composition in sorghum that contained a high level of WSC (35.5% DM) for LAB to produce higher lactic acid. Some tropical isolates were homofermentative LAB which could grow well <pH 4.0 in silage [6]. During silage fermentation, the LAB could grow vigorously in the early stage of ensiling and thus ferment WSC to produce lactate, reducing the pH value of the silage to less than 4.0 [6]. When silage was inoculated with TH14, the highest LAB counts (10^7 cfu/g FM) and the lowest number of aerobic bacteria (10^3 cfu/g FM) were found, pH significantly ($p<0.05$) decreased while lactic acid tended to increase when compared with control, and significantly increased when compared with AC treatment. These results clearly indicate that the TH14 strain used in this study was a homofermentative LAB which produced higher lactic acid content and may grow at lower pH than epiphytic LAB or other inoculant strains as reported by [6]. When sufficient quantity of WSC is present in sorghum, cellulase is unlikely to improve silage fermentation. When the silage was inoculated with TH14, the CP content was significantly ($p<0.05$) higher than control, CH and AC-treatments. Our findings indicate that addition of TH 14 results in beneficial effects by promoting the propagation of LAB. Thus, the pH decreases sharply inhibiting the growth of Clostridia, as well as decreasing CP loss [22,23]. Clostridia usually produce ammonia nitrogen from decomposed protein in the silage materials [17].

Therefore, inoculation with TH14 may result in beneficial effects by promoting the propagation of LAB, inhibiting the growth of aerobic bacteria and improving fermentation quality of sorghum silage.

Xing et al and Ebrahimi et al [17,25] reported that addition of cellulase enzyme and cellulase plus LAB resulted in a decrease of pH, increase in both lactic acid content and IVDMD in sorghum straw and oil palm frond. In the present study, the cellulase or cellulase plus LAB did not promote silage fermentation and fiber degradation. This could be attributed to the fibrolytic enzyme activity depending on both the temperature and pH conditions [44]. The optimum temperature and pH for cellulase activity were 39°C to 50°C and 5.0 to 6.5, respectively [45-47]. However, in the present study we would not expect temperature to affect enzyme activity because silos were kept at room temperature (25°C to 37°C). The sharp decrease in pH from 5.1 to below 4.0 within 3 days after fermentation in all silages (data not shown) could have led to an inhibition of cellulase activity. In addition, high WSC in sorghum could be a source of energy for rapid propagation of LAB, thus producing lactic acid early in the fermentation process leading to relatively high lactic acid contents of control and cellulose plus LAB treatments. The present work agreed with [48] who found that a complex of cellulase, hemicellulase and xylanase enzymes did not significantly decrease NDF and ADF contents in the IS 23585 sorghum cultivar.

Filya et al and Weinberg et al [49,50] reported that LAB inoculants affected the *in vitro* digestibility of wheat, corn and alfalfa silage after 48 h incubation. In the present study, CH and TH14 inoculants did not increase the silage IVDMD after 24 h *in vitro* incubation but TH14 significantly decreased methane production leading to a decrease in the energy loss of the feed. We cannot fully explain the mechanism of these effects, but there are some known mechanisms for the conversion of lactic or pyruvic acid to propionic acid [51]. When lactic acid is secondarily fermented in the rumen by lactate-utilizing bacteria such as *Megasphaera elsdenii*, *Selenomonas ruminantium*, *Fusobacterium necrophorum*, and *Veillonella parvula*, propionate is generally produced [52,53]. This can reduce methanogenesis because electrons are used during propionate formation. If hydrogen is then used to convert lactic acid to propionic acid in the rumen [29], the hydrogen will decrease, which in turn will inhibit the conversion of hydrogen and CO_2 to methane. Cao et al [28] reported that sheep fed higher

lactic acid content fermented total mixed ration had higher ruminal propionic acid at 2 h after feeding than those fed the control diet with lower lactic acid content. Therefore, we suspect that TH14-inoculated silage with high lactic acid content may have led to higher propionic acid production and reduced methane production accordingly. However, without monitoring emissions from the diet itself, it is impossible to make any overall conclusions about the effect of methane emissions on the environment.

The results confirmed that the local selected strain *L. casei* TH14 could significantly improve silage quality and result in inhibited protein degradation and decreased methane production.

CONCLUSION

All sorghum silages treated with LAB and cellulase enzyme were good quality. When sufficient WSC is present in sorghum, cellulase is unlikely to improve silage fermentation and fiber degradation. The local selected strain *L. casei* TH14 can improve silage fermentation and decrease methane production.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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