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

Waroon Khota¹, Suradej Pholsen¹*, David Higgs², and Yimin Cai³*

**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 m² 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 (CO₃)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 1.0×10⁵ 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, Asahi Kasei, 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 to 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, Tokyo, 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 chromatography 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) 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 (IVOMD) 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; \( \mu \) = overall mean, \( \tau_i \) = treatment effect, and \( \varepsilon_{ij} \) = 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 \( 10^5 \) for LAB, \( 10^6 \) for coliform bacteria, \( 10^6 \) for aerobic bacteria, \( 10^5 \) for yeasts and \( 10^5 \) cfu/g FM for molds. The counts in sorghum silages were \( 10^3 \) to \( 10^5 \) for LAB, \( 10^1 \) to \( 10^3 \) 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                                            | LAB, \( 10^5 \)                            | Coliform bacteria, \( 10^6 \) | Aerobic bacteria, \( 10^6 \) | Yeasts, \( 10^5 \) |
| Sorghum material Silage                         |                                   |                                   |                                   |                                   |
| Control                                         | 1.9 \( 10^6 \) ND                      | 5.2 \( 10^6 \) ND                 | 5.1 \( 10^6 \) ND                 | ND                                 |
| TH14                                            | 5.3 \( 10^6 \) ND                      | 5.0 \( 10^6 \) ND                 | ND                                 | ND                                 |
| AC                                              | 1.5 \( 10^5 \) ND                      | 3.0 \( 10^5 \) ND                 | ND                                 | ND                                 |
| AC+CH                                           | 2.2 \( 10^6 \) ND                      | 1.3 \( 10^6 \) ND                 | ND                                 | ND                                 |
| AC+TH14                                         | 1.6 \( 10^5 \) ND                      | 1.7 \( 10^5 \) ND                 | ND                                 | ND                                 |
| SEM                                             | 2.97                                  | 0.05                               | 0.23                               | 0.16                               |
| p-value                                          | <0.001                                | <0.001                            | <0.001                            | <0.001                             |

**Means in the same column followed by different letters differ (p<0.05).**

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.
significantly 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+TH14 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 TH14 treatment was significantly (p<0.05) reduced cf. other treatments.

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 10^9 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 10^7 cfu/g FM; however, coliform bacteria, aerobic bacteria and yeasts were higher (10^8 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) |
|---------------------|-------|-------|-------|-------|-------|-------|-------|-------------|
| Sorghum material    |       |       |       |       |       |       |       |             |
| Silage              | 26.56a | 96.68 | 5.20a  | 1.52  | 69.71 | 43.48 | 4.56  | 4.47        |
| Control             | 22.37b | 96.62 | 4.59c  | 1.45  | 69.46 | 43.50 | 4.74  | 4.50        |
| CH                  | 21.67b | 96.51 | 5.29c  | 1.57  | 69.35 | 43.57 | 4.75  | 4.48        |
| TH14                | 22.02b | 96.66 | 5.12b  | 1.47  | 69.37 | 43.38 | 4.66  | 4.49        |
| AC                  | 22.57b | 96.68 | 4.94ac | 1.56  | 68.88 | 44.33 | 4.81  | 4.43        |
| AC+CH               | 21.89b | 96.71 | 5.02ac | 1.48  | 69.96 | 44.93 | 4.68  | 4.47        |
| AC+TH14             | 22.24b | 96.73 | 4.82bc | 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.

* * * Means in the same column followed by different letters differ (p<0.05).

Sorghum material: LBC = 579.8 ± 10.7 meq/kg DM; WSC = 35.47 ± 0.2 g/kg DM.

---

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

| Items       | pH | Lactic acid | Acetic acid | Propionic acid | Butyric acid | Ammonia nitrogen |
|-------------|----|-------------|-------------|---------------|--------------|-----------------|
| Control     | 3.54** | 72.84** | 14.25 | ND | ND | 0.40 |
| CH          | 3.49b  | 79.92  | 8.54b | ND | ND | 0.28 |
| TH14        | 3.43c  | 81.85c | 18.08c | ND | ND | 0.35 |
| AC          | 3.51a  | 66.90a | 15.96a | ND | ND | 0.38 |
| AC+CH       | 3.44b  | 74.28b | 10.69b | ND | ND | 0.32 |
| AC+TH14     | 3.39a  | 81.20a | 18.50a | 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.

* * * 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 (10⁵ cfu/g FM) and the lowest number of aerobic bacteria (10⁴ 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 cellulase 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 secondary 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 CO₂ to methane. Cao et al [28] reported that sheep fed higher

### 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 |
|-------|--------------|--------------|--------------|-------------------|
|       | L/kg DM      | L/kg OM      | L/kg IVMD    | % GE              |
| Control | 510.31       | 571.50       | 84.36        | 157.17ab         |
| CH     | 517.47       | 575.47       | 76.53        | 147.75ab         |
| TH14   | 511.52       | 571.89       | 77.79        | 86.98c           |
| AC     | 510.72       | 577.12       | 85.03        | 145.50c          |
| AC+CH  | 497.86       | 560.13       | 67.89        | 167.25c          |
| AC+TH14| 507.77       | 575.32       | 81.70        | 124.03b          |
| SEM    | 6.272        | 9.679        | 4.128        | 5.913            |
| p-value | 0.296        | 0.7412       | 0.154        | 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; SEM, standard error of the mean.

* Means in the same column followed by different letters differ (p<0.05).
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.

**ACKNOWLEDGMENTS**

This study was supported by Thailand Government Grants for KKU, National Research Council, Thailand, and the Project “The Establishment of the Sustainable and Independent Farm Household Economy in the Rural Areas of Indo-China”, Japan International Research Center for Agricultural Sciences (JIRCAS), Japan.

We thank Meiji Seika Pharma Co., Ltd., Tokyo, Japan for providing the commercial cellulose and Snow Brand Seed Co., Ltd, Sapporo, Japan for providing the commercial LAB inoculants.

**REFERENCES**

1. Phaikaew C, Poathong S, Nakamanee G. Important role of improved pastures in the development of dairy farms in Thailand. In: Kaligis D, Moog F, editors. 7th meeting of the regional working group on grazing and feed resources, forage development in southeast asia: strategies and impacts. Manado, Indonesia. p. 27-53.
2. Khan M, Peters K, Uddin M. Feeding Strategy for improving dairy cattle productivity in small holder farm in Bangladesh. Bangladesh J Anim Sci 2012;38:67-85.
3. Mutibvu T, Maburutse B, Mbiriri D, Kashangura M. Constraints and opportunities for increased livestock production in communal areas: a case study of Simbe, Zimbabwe. Livest Res Rural Dev 2012;24: Article #165.
4. Hare M, Tatsapong P, Phengphet S. Herbage yield and quality of *Brachiaria* cultivars, *Paspalum atratum* and *Panicum maximum* in north-east Thailand. Trop Grasslands 2009;43:65-72.
5. Tudsri S, Jorgensen ST, Riddach P, Pookpakdi A. Effect of cutting height and dry season closing date on yield and quality of five napier grass cultivars in Thailand. Trop Grasslands 2002;36:248-52.
6. Pholsen S, Khota W, Pang H, Higgs D, Cai Y. Characterization and application of lactic acid bacteria for tropical silage preparation. Anim Sci J 2016;87:1202-11.
7. Calabró S, Tudisco R, Grossi M, et al. *In vitro* fermentation characteristics of corn and sorghum silages. Ital J Anim Sci 2010;9:559-62.
8. Qu H, Liu XB, Dong CF, Lu XY, Shen YX. Field performance and nutritive value of sweet sorghum in eastern China. Field Crops Res 2014;157:84-8.
9. Wu X, Staggenborg S, Propheter JL, et al. Features of sweet sorghum juice and their performance in ethanol fermentation. Ind Crops Prod 2010;31:164-70.
10. Zhang SJ, Chaudhry AS, Osman A, et al. Associative effects of ensiling mixtures of sweet sorghum and alfalfa on nutritive value, fermentation and methane characteristics. Anim Feed Sci Technol 2015;206:29-38.
11. Cândido EP, Pimenta Filho EC, Gonzaga Neto S, et al. Sorghum silage production system in Cariri, Paraíba. Rev Bras Zootec 2014;43:336-42.
12. Muhammad A, Brandon B. A scoping review on sorghum silage quality enhancement with advanced ensiling operations. J Adv Bot Zool 2015;3:1-5.
13. Aydin G, Grant RJ, O’Rear J. Brown midrib sorghum in diets for lactating dairy cows. J Dairy Sci 1999; 82:2127-35.
14. Muck RE. Silage microbiology and its control through additives. Rev Bras Zootec 2010;39:183-91.
15. Filyla I. 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 2003;86:3575-81.
16. Kozelev IK, Iliev F, Hristov AN, Zaman S, McAllister TA. Effect of fibrolytic enzymes and an inoculant on *in vitro* degradability and gas production of low-dry matter alfalfa silage. J Sci Food Agric 2008; 88:2568-75.
17. Xing L, Chen LJ, Han LJ. The effect of an inoculant and enzymes on fermentation and nutritive value of sorghum straw silages. Bioresour Technol 2009;100:488-91.
18. Nkosi BD, Vadlani PV, Brijwani K, Nanjunda A, Meeske R. Effects of bacterial inoculants and an enzyme on the fermentation quality and aerobic stability of ensiled whole-crop sweet sorghum. S Afr J Anim Sci 2012;42:232-40.
19. McDonald P, Henderson A, Heron S. The biochemistry of silage. Marlow, UK: Chalcombe Publications; 1991.
20. Eun J-S, Beauchemin KA. Relationship between enzymic activities and *in vitro* degradation of alfalfa hay and corn silage. Anim Feed Sci Technol 2008;145:53-67.
21. Phakachoed N, Sukombat W, Colombatto D, Beauchemin KA. Use of fibrolytic enzymes additives to enhance *in vitro* ruminal fermentation of corn silage. Livest Sci 2013;157:100-12.
22. Tian J, Yu Y, Yu Z, et al. Effects of lactic acid bacteria inoculants and cellulase on fermentation quality and *in vitro* digestibility of *Leymus*.
chinesis silage. Grassl Sci 2014;60:199-205.
23. Nadeau EMG, Russell JR, Buxton DR. Intake, digestibility, and composition of orchardgrass and alfalfa silages treated with cellulase, inoculant, and formic acid fed to lambs. J Anim Sci 2000;78:2980-9.
24. Queiroz OCM, Arriola KG, Daniel JLP, Adesogan AT. Effects of 8 chemical and bacterial additives on the quality of corn silage. J Dairy Sci 2013;96:5836-43.
25. Ebrahimim R, Rajion MA, YongMeng G, et al. The effects of adding lactic acid bacteria and cellulase in oil palm (Elaines guineensis Jacq.) frond silages on fermentation quality, chemical composition and in vitro digestibility. Ital J Anim Sci 2014;13:557-62.
26. Van Nevel CJ, Demeyer DI. Feed additives and other interventions for decreasing methane emissions. In: Walace RJ, Chesson A, editors. Biotech­ nology in animal feeds & animal feeding. New York, USA: VCH Publishers Inc.; 1995. p. 329-49.
27. McGinn SM, Beauchemin KA, Coates T, Colombatto D. Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. J Anim Sci 2004;82:3346-56.
28. Cao Y, Takahashi T, Horiguchi K, Yoshida N, Cai Y. Methane emissions from sheep fed fermented or non-fermented total mixed ration containing whole-crop rice and rice bran. Anim Feed Sci Technol 2010; 157:72-8.
29. Mos AR, Jouany J-P, Newbold J. Methane production by ruminants: its contribution to global warming. Ann Zoottech 2000;49:231-53.
30. Wolin MJ. Interactions between the bacterial species of the rumen. In: McDonald IW, Warner ACI, editors. Digestion and metabolism in the ruminant. Armidale, Australia: University of New England Publ. Unit; 1975. p. 134-48.
31. Cai Y, Benno Y, Ogawa M, Kumai S. Effect of applying lactic acid bacteria isolated from forage crops on fermentation characteristics and aerobic deterioration of silage. J Dairy Sci 1999;82:520-6.
32. Kozaki M, Uchimura T, Okada S. Experimental Manual for lactic bacteria isolated from forage crops on fermentation characteristics and aerobic deterioration of silage. J Dairy Sci 2013;26:1304-12.
33. Ni K, Wang Y, Pang H, Cai Y. Effect of cellulase and lactic acid bacteria on fermentation quality and chemical composition of wheat straw silage. Anim J Plant Sci 2014;5:1877-84.
34. Cao Y, Cai Y, Takahashi T, et al. Effect of lactic acid bacteria inoculant and beet pulp addition on fermentation characteristics and in vitro ruminal digestion of vegetable residue silage. J Dairy Sci 2011; 94: 3902-12.
35. Colombatto D, Mould FL, Bhat MK, Phipps RH, Owen E. In vitro evaluation of fibrolytic enzymes as additives for maize (Zea mays L.) silage. Anim Feed Sci Technol 2004;111:111-28.
36. Chung Y-H, Zhou M, Holtshausen L, et al. A fibrolytic enzyme additive for lactating Holstein cow diets: ruminal fermentation, rumen microbial populations, and enteric methane emissions. J Dairy Sci 2012; 95:1419-27.
37. Lowe SE, Theodorou MK, Trinci AP. Cellulases and xylanase of an anaerobic rumen fungus grown on wheat straw, wheat straw holocellulose, cellulose, and xylan. Appl Environ Microbiol 1987;53:1216-23.
38. Kung L, Cohen MA, Rode LM, Treacher RJ. The effect of fibrolytic enzymes sprayed onto forages and fed in a total mixed ratio to lactating dairy cows. J Dairy Sci 2002;85:2396-402.
39. Sawmonghua P, Higgs D, Pholsen S. Effects of fibrolytic enzyme additive on silage quality of IS 23585 forage sorghum cultivar plus cavalcade hay and cassava chip. In: Graduate research conference. Khom Khaen, Thailand; 2014. p. 632-43.
40. Filya I, Muck RE, Contreras-Govea FE. Inoculant effects on alfalfa silage: fermentation products and nutritive value. J Dairy Sci 2007;90:5108-14.
41. Weinberg ZG, Schatz O, Chen Y, et al. Effect of lactic acid bacteria inoculants on in vitro digestibility of wheat and corn silages. J Dairy Sci 2007;90:4754-62.
42. Leng R. Formation and production of volatile fatty acids in the rumen. In: Phillipson A, editor. Physiology of digestion and metabolism in the ruminant. Newcastle upon Tyne, England: Oriel Press; 1970. p. 406-21.
43. Dawson KA, Rasmussen MA, Allison MJ. Digestive disorders and nutritional toxicity. In: Hobson PN, Stewart CS, editors. The rumen microbial ecosystem. 2nd ed. London, England: Blackie Academic and Professional; 1997. p. 633-60.
44. Russell JB, Wallace RJ. Energy-yielding and energy-consuming reactions. In: Hobson PN, Stewart CS, editors. The rumen microbial ecosystem. 2nd ed. London, England: Blackie Academic and Professional; 1997. p. 246-82.