Effects of inoculant application on fermentation quality and rumen digestibility of high moisture sorghum-sudangrass silage

Dimas Hand Vidhya Paradhipta, Young Ho Joo, Hyuk Jun Lee, Seong Shin Lee, Dong Hyeon Kim, Jong Duk Kim, and Sam Churl Kim

Division of Applied Life Science (BK21Plus, Insti. of Agric. & Life Sci.), Gyeongsang National University, Jinju, South Korea; Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia; Division of Animal Husbandry, Yonam College, Cheonan, South Korea

ABSTRACT
This study estimated the effects of a new inoculant producing antifungal and esterase activity on quality of high moisture sorghum-sudangrass (SS) silage with two different hybrids (SX-17 and Speed-up). The SS hybrids were chopped, treated without an inoculant (CON) and with an inoculant containing Lactobacillus plantarum R48-27 and Lactobacillus buchneri R4-26 at ratio 1:1 (INO), then ensiled into 20-L mini silo in quadruplicate for 60 days. After ensiling, silage was placed under aerobic condition for 8 days to estimate yeast and mold. The INO silages had higher (P < 0.05) dry matter, crude protein, neutral detergent fibre, and acid detergent fibre than those of CON silages. The INO silages also had higher (P < 0.05) pH and acetate, but lower (P < 0.05) ammonia-N, butyrate, and lactate to acetate ratio than those of CON silages. Applied INO in both hybrids had lower (P < 0.05) yeast after 4–8 days of aerobic exposure than CON. In rumen, INO silages had higher (P < 0.05) in vitro dry matter digestibility, pH, ammonia-N, and acetate than those of CON silages. In conclusion, the new inoculant application improved not only fermentation quality, but also rumen digestibility of high moisture SS silage.

1. Introduction
In the warm season, annual grass such as sorghum-sudangrass (SS; Sorghum bicolor x S. bicolor var. sudanense) is one of the potential forages that could be applied in year-round crop systems. The SS has greater biomass production with high tolerance to environmental stress, especially in high temperature during the summer season (Venuto and Kindinger 2008). It could be an alternative forage for corn silage, which provides a suitable nutrient for dairy cows (Dann et al. 2008; Gurbuz et al. 2008). However, SS forage usually wilts long before reaching the ideal moisture content due to their stalk, which could reduce the silage quality (McDonald et al. 1991; Lim et al. 2009). Our preliminary study (Data unpublished) also had shown that SS forages still contained high moisture (>75%) after 3 days of the wilting process in the field. Generally, ensiling forage with high moisture content usually produces high butyrate concentration, which is associated with silage quality and aerobic deterioration (McDonald et al. 1991; Danner et al. 2003; Vissers et al. 2007). Microbial inoculant application on forages will stimulate organic acid production, followed by decrease of pH that can inhibit undesirable microbes and reduce nutrient loss during ensiling (McDonald et al. 1991). Moreover, Weinberg et al. (2007) reported that the digestibility of wheat and corn silages improved by applications of microbial inoculants. And, it had been reported that the applications of combo inoculants containing homo and heterofermentative lactic acid bacteria (LAB) could improve the quality of silage as well as aerobic stability (Filya 2003; Huisden et al. 2009; Queiroz et al. 2012; Joo et al. 2018). Several studies have shown that inoculants had various effects on silages by forage hybrids (Andrae et al. 2001; Beck et al. 2007; Kim et al. 2018). The SX-17 and Speed-up hybrids reported that it had high growth rate and dry matter (DM) yield (Zahid et al. 2002).

In our previous study, Lactobacillus plantarum R48-27 and Lactobacillus buchneri R4-26 were isolated from rye silage and confirmed by plate assay to produce the fibrinolytic enzymes and antifungal substances, respectively (Kim et al. 2017). However, the effects of these new inoculants on aerobic deterioration and nutrient digestibility of SS silage did not confirm yet. The application of these inoculants on high moisture SS silage possibly inhibits the growth of undesirable microbes by antifungal substances as well as the improvement of digestibility by fibrinolytic enzymes. Therefore, this study estimated the effects of new inoculants on fermentation quality, aerobic deterioration, and rumen digestibility of high moisture SS silages.

2. Materials and methods
2.1. Silage production and sampling
Two SS hybrids, SX-17 (SX, Monsanto, USA) and Speed-up (SU, Hanong Bio Industry Corp, Jeju, South Korea), were grown at Jinju city, Gyeongsangnam-do, South Korea (latitude 35.2°N and longitude 128.1°W). Two hundred kilograms of SS forages

CONTACT Sam Churl Kim, kimschu@gnu.ac.kr Division of Applied Life Science (BK21Plus, Insti. of Agric. & Life Sci.), Gyeongsang National University, Jinju 660-701, South Korea
© 2019 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group
This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
harvested at the late heading stage from four plots, respectively, and then wilted for 24 h. The mean DM concentration of both hybrids was 23.1% at the end of wilting. The wilted SS forage chopped to 3–5 cm length separately and ensiled into 20 L bucket silo (4 kg) in quadruplicate for 60 days, following: silages applied 1% distilled water in fresh forage (CON); and silages applied combo inoculant containing Lactobacillus plantarum R48-27 and Lactobacillus buchneri R4-26 at 2 × 10⁵ of fresh forage at a ratio of 1:1 (INO). The SS forage (500 g) and silage (1 kg) were sub-sampled for chemical compositions and in vitro rumen digestibility. Additionally, silages were also sub-sampled for fermentation indices (20 g) and aerobic deterioration (1 kg). The sub-sampled silage for aerobic deterioration was located in a polystyrene box (25 cm diameter × 45 cm depth) and covered with two layers of cheesecloth for 8 days at room temperature (10°C) as described by Kang et al. (2009). On 1, 2, 3, 4, 6, and 8 days of aerobic exposure, 50 g of silages were sub-sampled to measure the changes of yeast and mold.

2.2. Chemical composition

The sub-sampled (500 g) forage and silage were dried at 65°C for 48 h and ground to pass 1-mm screen using a cutting mill (Shinmyung Electric Co., Ltd, Gimpo, South Korea). The DM concentration was analyzed using a forced-air dry oven at 105°C for 24 h. Crude ash (CA) was determined with a muffle furnace at 550°C for 5 h. Crude protein (CP) and ether extract (EE) were measured by the producers of Kjeldahl (method number 984.13; AOAC 1995) and the Soxhlet (method number 920.39; AOAC 1995), respectively. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined using an Ankom 200 fibre analyzer (Ankom Technology, Macedon, NY, USA) following the method of Van Soest et al. (1991).

2.3. Fermentation indices

Twenty grams of silage were blended with 200 mL of sterile ultrapure water for 30 sec and filtered through two layers of cheesecloth for silage extraction. The analyses of pH, ammonia-N, lactate, volatile fatty acid (VFA), and microbial counts used silage extraction. The pH was measured by a pH metre (SevenEasy, Mettler Toledo, Greifensee, Switzerland). Ammonia-N was determined using a colorimetric method described by Chaney and Marbach (1962). The silage extraction was centrifuged at 5,645 × g for 15 min and collected the supernatant for lactate and VFA analyses. The concentrations of lactate and VFA were determined using HPLC (L-2200, Hitachi, Tokyo, Japan) fitted with a UV detector (L-2400, Hitachi, Tokyo, Japan) and a column (Metacarb 87H; Varian, Palo Alto, CA, USA) described by Muck and Dickerson (1988).

2.4. Microbial counts

Microbial counts were determined using silage extractions (first dilution), which were continued into several dilutions (10⁻⁵–10⁻⁷). The injection of silage extraction was in triplicate in selective agar medium as described by Kim et al. (2017) and Joo et al. (2018). The LAB count used lactobacilli MRS agar media (MRS; Difco, Detroit, MI, USA) and yeast and mold counts used potato dextrose agar (PDA; Difco, Detroit, MI, USA). The MRS agar plates were put on a CO₂ incubator (Thermo Scientific, Waltham, MA, USA) at 30°C for 24 h, while PDA plates were put on an aerobic incubator at 30°C for 72 h in an aerobic incubator (Johnsam Corp., Boocheon, South Korea). Visible colonies from the plates were calculated and the number of colonies forming units (cfu) was per gram of silage. The microbiological data was transformed to log10.

2.5. In vitro rumen digestibility

Animal Care and Ethics Committee of Gyeongsang National University, Jinju city, Gyeongsangnam-do, South Korea, approved the animal procedure for the cannulated cow in the present study. Rumen fluid collected before morning feeding from two non-pregnant cannulated Hanwoo heifers, which had been fed rice straw hay and grains mixed at 8:2 ratio. The collected rumen fluid was composited and then filtered via two layers of cheesecloth. In vitro medium was prepared by mixing rumen fluid with Van soest medium at 1:2 ratio. A ground silage sample (0.5 g) and in vitro medium (40 mL) were placed into the incubation bottle at triplicate with two blanks. Then, the incubation bottle was filled with CO₂ to reach anaerobic condition (Tilley and Terry 1963). All incubation bottles were placed into an anaerobic incubator (Thermo Scientific, Waltham, MA, USA) at 39°C for 72 h. After incubation, the bottle was opened and transferred to 50 mL conical tube to separate the residue and supernatant (in vitro medium) through centrifugation at 2,568 × g for 15 min (Supra 21k, Hanil Electric Corporation, Gimpo, South Korea, with rotor A505-6C No.6). The residue was used to measure in vitro digestibility of DM (IVDMD) and NDF (IVNDFD), while the supernatant was used to measure the rumen fermentation characteristics such as pH, ammonia-N, and VFA using the same protocol as described before.

2.6. Statistical analysis

The experiment was conducted by a 2 (hybrid; SX vs. SU) × 2 (inoculant; CON vs. INO) factorial design with four replicates per treatment. All data was analyzed using general linear model (GLM) procedure of Statistical Analysis System (SAS), version 9.3 package programme to test the effects of hybrid, inoculant, and its interaction (hybrid × inoculant). The model was Yijk = μ + αi + βj + (αβ)ij + εijk, where Yijk = response variable, μ = overall mean, αi = the effect of hybrid treatment, βj = the effect of inoculant treatment, (αβ)ij = the interaction effect of hybrid and inoculant, εijk = error term. The significant differences were declared at P < 0.05.

3. Results

The mean concentrations of DM, CP, NDF, ADF, IVDMD, and IVNDFD across SS hybrids were 23.1, 11.5, 67.4, 37.6, 57.9, and 53.4%, respectively (Table 1).
The SU silage had higher concentrations of DM ($P = 0.002$; 19.8 vs. 17.9%) and ammonia-N concentration ($P = 0.004$; 4.31 vs. 4.38) and acetate concentration ($P = 0.001$; 0.91 vs. 0.69%) than CON silage, but lower concentrations of ADF ($P = 0.044$; 0.07 vs. 0.08%) and NDF ($P < 0.001$; 12.5 vs. 11.4%). The SU silage resulted in higher pH ($P < 0.001$; 4.41 vs. 4.29) and acetate concentration ($P = 0.001$; 0.91 vs. 0.69%) than CON silage, but lower concentrations of ammonia-N ($P = 0.004$; 0.07 vs. 0.08%), lactate ($P = 0.045$; 1.81 vs. 1.98%), and butyrate ($P = 0.047$; 0.75 vs. 0.85%), and lactate to acetate ratio ($P < 0.001$; 2.00 vs. 2.90). The interaction effect between hybrid and inoculant was found on acetate concentration ($P = 0.006$; 2.00 vs. 2.90). The SU silage had higher concentrations of DM ($P = 0.002$; 19.8 vs. 17.9%), EE ($P = 0.002$; 3.27 vs. 2.58%), and CA ($P = 0.006$; 9.76 vs. 9.11%), and NDF ($P < 0.020$; 68.8 vs. 68.5%) than SX silage (Table 2). Application of INO in both hybrids resulted in higher concentrations of DM ($P = 0.002$; 19.7 vs. 17.9%), CP ($P < 0.001$; 12.5 vs. 11.4%), NDF ($P < 0.010$; 68.8 vs. 68.5%), and ADF ($P = 0.046$; 40.1 vs. 39.1%) than CON.

The SU silage resulted in lower pH ($P = 0.001$; 4.31 vs. 4.38) and ammonia-N concentration ($P = 0.004$; 0.07 vs. 0.08%; Table 3). On the other side, the application of inoculant in SX silage resulted in higher pH ($P < 0.001$; 4.41 vs. 4.29) and acetate concentration ($P = 0.001$; 0.91 vs. 0.69%) than CON silage, but lower concentrations of ammonia-N ($P = 0.004$; 0.07 vs. 0.08%), lactate ($P = 0.045$; 1.81 vs. 1.98%), and butyrate ($P = 0.047$; 0.75 vs. 0.85%), and lactate to acetate ratio ($P < 0.001$; 2.00 vs. 2.90). The interaction effect between hybrid and inoculant was found on acetate concentration ($P = 0.032$) which application of inoculant in SU hybrid had 0.2% higher than in SX hybrid. Applications of hybrid and inoculant did not affect LAB count. The INO silage had higher ($P = 0.025$; 7.68 vs. 7.54 log$_{10}$ cfu/g) yeast count than CON silage, while application of the hybrid did not present the effect. However, molds were not detect in all silages.

Application of INO in SX silage resulted in higher yeast count ($P = 0.015$) than CON only on day 1 of aerobic exposure (Table 4). However on 4, 6, and 8 days of aerobic exposure, both hybrids inoculated with INO had lower ($P < 0.05$) yeast count than CON. Yeast count was not affected by hybrid during aerobic exposure, except on 8 days, which SX hybrid was higher ($P = 0.039$; 7.96 vs. 7.93 log$_{10}$ cfu/g) than SU hybrid. Also an interaction effect between hybrid and inoculant was detected ($P = 0.010$) on yeast count on day 8 of aerobic exposure, while yeast count only decreased in SU silage by inoculant application.

The SU silage had higher IVDMD ($P < 0.001$; 55.9 vs. 51.0%) and lower ammonia-N concentration ($P = 0.001$; 32.5 vs. 38.9 mg/dL) than SX silage (Table 5). The INO silage had higher IVDMD ($P = 0.003$; 55.0 vs. 51.9%), pH ($P < 0.001$; 6.39 vs. 6.28), concentrations of ammonia-N ($P = 0.003$; 39.6 vs. 31.8 mg/dL), acetate ($P = 0.014$; 54.8 vs. 54.0%), and valerate ($P = 0.012$; 3.37 vs. 3.14%), and acetate to propionate ratio ($P = 0.009$; 2.41 vs. 2.28%) than CON silage. However, INO silage had a lower concentration of propionate ($P = 0.015$; 22.8 vs. 23.7%) than CON silage.

### Table 1. Chemical compositions and in vitro digestibility of sorghum-sudangrass forages before ensiling (% DM).

| Item                    | SX      | SU      |
|-------------------------|---------|---------|
| Dry matter              | 22.8    | 23.3    |
| Crude protein           | 11.8    | 11.2    |
| Ether extract           | 2.80    | 3.41    |
| Crude ash               | 8.64    | 9.13    |
| Neutral detergent fibre | 67.3    | 67.5    |
| Acid detergent fibre    | 37.3    | 37.9    |
| IVDMD                   | 57.4    | 58.4    |
| IVNDFD                  | 52.9    | 53.8    |

SX, SX-17 sorghum-sudangrass hybrid; SU, Speed-up sorghum-sudangrass hybrid.

**4. Discussion**

The chemical compositions of SX and SU forages were in the expected range (Beck et al. 2007; Dann et al. 2008). After 60 days of ensiling, SU silage presented higher concentrations of DM, EE, CA, and NDF than SX silage. Generally, these results could occur by chemical compositions of those forages (Johnson et al. 2002; Kim et al. 2018). The higher DM and CP concentrations by INO application might indicate a lower nutrient loss during ensiling. The previous study also reported that an applied combo inoculant consisting of *Lactobacillus plantarum* and *Lactobacillus buchneri* produced higher DM concentration than untreated silage (Driehuis et al. 2001; Filya 2003). In addition, the application of inoculant could reduce proteolysis caused by rapid acidification during ensiling (Winters et al. 2000). These results could support higher CP concentration of INO silage than CON silage in the present study. Huisden et al. (2009) reported that inoculant application led to increases of NDF concentration due to the higher degradation of non-structural carbohydrates. Similarly in the present study, NDF and ADF concentrations were slightly higher in INO silage than in CON silage. Additionally, this result also might be supported partially by higher microbial counts in INO silage than in CON silage (Table 3).

In general, the forages containing high CP concentration produce a high ammonia-N concentration in the silages by proteolysis during the ensiling period (McDonald et al. 1991; Kim et al. 2018). The SX forage had a slightly higher CP concentration (11.8 vs. 11.2%) than SU forage. This might have influenced higher ammonia-N concentration in SX silage than in SU silage, which also could lead to higher pH in SX silage.

Inoculant applications on silages lead to lower pH and ammonia-N concentration due to the stimulation of organic acid, which also could inhibit the growth of undesirable microbes (McDonald et al. 1991; Filya 2003). However,

### Table 2. Effects of new inoculant on the chemical composition of high moisture sorghum-sudangrass silages ensiled for 60 days (% DM).

| Item                    | CON      | INO      | SEM      | H     | I     | H*I    |
|-------------------------|----------|----------|----------|-------|-------|--------|
| SX                      |          |          |          |       |       |        |
| Dry matter              | 17.2     | 18.5     | 20.9     | 0.819 | 0.002 | 0.002  |
| Crude protein           | 11.5     | 12.5     | 11.3     | 12.4  | 0.176 | <0.001 |
| Ether extract           | 2.50     | 2.66     | 3.03     | 3.51  | 0.340 | 0.002  |
| Crude ash               | 9.14     | 9.08     | 9.52     | 10.0  | 0.385 | 0.006  |
| Neutral detergent fibre | 68.5     | 68.6     | 68.6     | 68.9  | 0.121 | 0.020  |
| Acid detergent fibre    | 39.5     | 40.4     | 38.6     | 39.7  | 0.872 | 0.046  |

SX, SX-17 sorghum-sudangrass hybrid; SU, Speed-up sorghum-sudangrass hybrid; CON, silage treated without inoculant; INO, silage inoculated with a combination of *Lactobacillus plantarum* R48-27 and *Lactobacillus buchneri* R4-26 at 2 × 10⁵ of fresh forage.

H, hybrid effect; I, inoculant effect; H*I, interaction between hybrid and inoculant effect.
application of heterofermentative LAB had shown an increase of pH due to the conversion of lactate into acetate and propionate (Elferink et al. 2001; Danner et al. 2003; Joo et al. 2018). In addition, it could increase yeast count without a negative effect on aerobic stability of silage (Oliveira et al. 2017). The results of fermentation indices in the present study were in agreement with those previous studies, which were higher in pH, acetate concentration, and yeast count, and lower in ammonia-N, lactate and butyrate concentrations by new inoculant application. Additionally, the effects of new inoculants in the present study also supported our previous study that had demonstrated fibrinolytic and antifungal effects (Kim et al. 2017). As an interaction effect between hybrid and inoculant, application of INO increased acetate concentration greater in SU silage than in SX silage. The previous studies have also reported an interaction effect between hybrid and inoculant on acetate concentration (Kang et al. 2009; Kim et al. 2018).

The presence of butyrate in the present study might be due to the high moisture of SS forage (McDonald et al. 1991). However, the new inoculant application in the present study proved a decrease of butyrate concentration, which is an indicator of silage fermentation quality and feed value for ruminant (McDonald et al. 1991; Danner et al. 2003; Vissers et al. 2007; Nkosi and Meeske 2010; Dolci et al. 2011). Yeast is especially an initiator of aerobic deterioration in silage, followed by mold growth (Danner et al. 2003; Dolci et al. 2011). Application of heterofermentative LAB had reported inhibiting the growth of yeast and mold in feedout phase due to the presence of antifungal substances such as acetate, propionate and so on (Danner et al. 2003; Kim et al. 2017). The previous studies also had reported that application of combo inoculant containing homofermentative LAB and Lactobacillus buchneri improved aerobic stability of silages (Filya 2003; Huisden et al. 2009; Queiroz et al. 2012; Joo et al. 2018). Although yeast count (7.93 vs. 7.54 log10 cfu/g) of INO silage was higher than CON silage on 0 d of aerobic exposure in the present study, the opposite was observed (7.79 vs. 7.91 log10 cfu/g) from 4 days of aerobic exposure. Moreover, the increase of yeast count from 1 to 8 days of aerobic exposure was higher in CON silage than in INO silage (0.46 vs. 0.18 log10 cfu/g), which indicated CON silage spoiled rapidly compared to INO silage. This result occurred by higher acetate concentration of INO silage in the present study (Table 3). The result of aerobic deterioration in the present study also supported the previous study that reported improvement on aerobic stability by application of antifungal inoculant (Kleinschmit et al. 2005). The effects of Lactobacillus buchneri R4-26 on aerobic deterioration of SS silage in the present study also

### Table 3. Effect of new inoculant on fermentation indices and microbial counts of high moisture sorghum-sudangrass silages ensiled for 60 days.

| Item                  | SXa | SU            | SEM  | H   | I   | H*I |
|-----------------------|-----|---------------|------|-----|-----|-----|
| Fermentation indices  |     |               |      |     |     |     |
| pH                    | 4.32| 4.44          | 4.25 | 4.37| 0.026|      |
| Ammonia-N, % of DM    | 0.08| 0.07          | 0.07 | 0.06| 0.002|      |
| Lactate, % of DM      | 2.08| 1.81          | 1.89 | 1.81| 0.098|      |
| Acetate, % of DM      | 0.69| 0.84          | 0.68 | 1.01| 0.069|      |
| Butyrate, % of DM     | 0.89| 0.73          | 0.81 | 0.76| 0.073|      |
| Lactate: Acetate ratio| 3.01| 2.22          | 2.78 | 1.79| 0.085|      |
| Microbial counts, log10 cfu/g |    |               |      |     |     |     |
| Lactic acid bacteria  | 7.81| 7.91          | 7.82 | 7.94| 0.017|      |
| Yeast                 | 7.48| 7.65          | 7.59 | 7.71| 0.107|      |
| Mold                  | ND  | ND            | ND   | ND  | –   | –   |

†SX, SX-17 sorghum-sudangrass hybrid; SU, Speed-up sorghum-sudangrass hybrid; CON, silage treated without inoculant; INO, silage inoculated with a combination of Lactobacillus plantarum R48-27 and Lactobacillus buchneri R4-26 at 2 × 107 of fresh forage. 

‡H, hybrid effect; I, inoculant effect; H*I, interaction between hybrid and inoculant effect. 

Not detected.

### Table 4. Effect of new inoculant on yeast and mold count of high moisture sorghum-sudangrass silages during aerobic exposure (log10 cfu/g).

| Item | Aerobic days | SXa | SU | Contrastb |
|------|--------------|-----|----|-----------|
|      |              | CON | INO |          | H   | I   | H*I |
| Yeast| 1             | 7.48| 7.66| 7.61| 7.74| 0.110|      |
|      | 2             | 7.74| 7.69| 7.62| 7.74| 0.105|      |
|      | 4             | 7.92| 7.81| 7.89| 7.76| 0.100|      |
|      | 6             | 7.96| 7.82| 8.02| 7.79| 0.107|      |
|      | 8             | 7.97| 7.94| 8.03| 7.82| 0.045|      |
| Mold | 1             | ND  | ND  | ND  | ND  | –    | –   |
|      | 2             | ND  | ND  | ND  | ND  | –    | –   |
|      | 3             | ND  | ND  | ND  | ND  | –    | –   |
|      | 4             | ND  | ND  | ND  | ND  | –    | –   |
|      | 6             | ND  | ND  | ND  | ND  | –    | –   |
|      | 8             | ND  | ND  | ND  | ND  | –    | –   |

†SX, SX-17 sorghum-sudangrass hybrid; SU, Speed-up sorghum-sudangrass hybrid; CON, silage treated without inoculant; INO, silage inoculated with a combination of Lactobacillus plantarum R48-27 and Lactobacillus buchneri R4-26 at 2 × 107 of fresh forage.

‡H, hybrid effect; I, inoculant effect; H*I, interaction between hybrid and inoculant effect. 

Not detected.
Table 5. Effect of new inoculant on in vitro rumen digestibility and fermentation characteristic of high moisture sorghum-sudangrass silages after incubated for 72 h.

| Item                        | SX     | SX  | CON  | CON  | INO  | INO  | SEM  | H    | I    | H*I  |
|-----------------------------|--------|-----|------|------|------|------|------|------|------|------|------|
| IVDMDc, % of DM             | 49.3   | 52.6| 54.4 | 57.3 | 57.3 | 1.355| <.001| 0.003| 0.772|
| IVNDFD, % of DM             | 56.4   | 56.8| 57.0 | 58.0 | 0.754| 0.065| 0.147| 0.533|
| pH                          | 6.27   | 6.42| 6.28 | 6.35 | 0.031| 0.055| <.001| 0.056|
| Ammonia-N, mg/dL            | 33.8   | 43.9| 29.8 | 35.2 | 3.472| 0.001| <.001| 0.484|
| Total VFA, mM/L             | 73.8   | 73.8| 74.4 | 73.9 | 0.001| 0.001| 0.835| 0.831|
| Acetate, % of molar         | 53.8   | 54.8| 54.1 | 54.8 | 0.574| 0.607| 0.014| 0.599|
| Propionate, % of molar      | 23.9   | 22.8| 23.4 | 22.7 | 0.633| 0.463| 0.015| 0.512|
| Iso-butyrate, % of molar    | 1.98   | 1.97| 2.04 | 2.03 | 0.065| 0.113| 0.769| 0.943|
| Butyrate, % of molar        | 12.3   | 11.8| 12.4 | 12.0 | 0.435| 0.456| 0.082| 0.831|
| Iso-valerate, % of molar    | 4.95   | 5.2 | 4.83 | 5.12 | 0.260| 0.380| 0.075| 0.902|
| Valerate, % of molar        | 3.06   | 3.42| 3.22 | 3.32 | 0.156| 0.808| 0.012| 0.136|
| Acetate:Propionate ratio    | 2.25   | 2.41| 2.31 | 2.41 | 0.081| 0.600| 0.009| 0.528|

SX, SX-17 sorghum-sudangrass hybrid; SU, Speed-up sorghum-sudangrass hybrid; CON, silage treated without inoculant; INO, silage inoculated with a combination of Lactobacillus plantarum R48-27 and Lactobacillus buchneri R4-26 at 2 × 10^7 for fresh forage.

Disclosure statement
No potential conflict of interest was reported by the authors.

5. Conclusion
The present study concluded that applied SU silage presented higher rumen digestibility than SX silage. Applications of the new inoculant in high moisture sorghum-sudangrass might help to improve not only the silage quality by inhibitions of undesirable substances (butyrate and yeast), but also rumen digestibility.

References
Andrae JG, Hubt CW, Pritchard GT, Kennington LR, Harrison JH, Kezar W, Mahanna W. 2001. Effect of hybrid, maturity, and mechanical processing of corn silage on intake and digestibility by beef cattle. J Anim Sci. 79:2268–2275. doi:10.2527/2001.7992268x.
AOAC. 1995. Official methods of analysis, 16th ed., Washington, DC, USA: Association of Official Analytical Chemists.
Arriola KG, Kim SC, Staples CR, Adesogan AT. 2011. Effect of fibrolytic enzyme application to low- and high-concentrate diets on the performance of lactating dairy cattle. J Dairy Sci. 94:832–841. doi:10.3168/jds.2010-3424.
Beck AP, Hutchison S, Gunter SA, Losi TC, Steward CB, Capps PK, Philips JM. 2007. Chemical composition and in situ dry matter and fiber disappearance of sorghum x sudangrass hybrid. J Anim Sci. 85:545–553. doi:10.2527/2006-292.
Chaney AL, Marbach EP. 1962. Modified reagents for determination of urea and ammonia. Clin Chem. 8:13–132.
Dann HM, Grant RJ, Cotanch KW, Thomas ED, Ballard CS, Rice R. 2008. Comparison of brown midrib sorghum-sudangrass with corn silage on lactational performance and nutrient digestibility in Holstein dairy cows. J Dairy Sci. 91:663–672. doi:10.3168/jds.2007-0521.
Danner H, Holzer M, Mayrhuber E, Braun R. 2003. Acetic acid increases stabili-
ity of silage under aerobic condition. Appl Environ Microbiol. 69:562–567. doi:10.1128/AEM.69.3.555-1.125.1.125-132.2001.
Dolci P, Tabacco E, Cocolin L, Borreani G. 2011. Microbial dynamics during anaerobic exposure of corn silage stored under oxygen barrier or polyethylene films. Appl Environ Microbiol. 77:7499–7507. doi:10.1128/AEM.05050-11.
Driehuis F, Oude Elferink SJWH, Vinkelaar PGV. 2001. Fermentation characteristics and aerobic stability of grass silage inoculated with Lactobacillus buchneri, with or without homfermentative lactic acid bacteria. Grass Forage Sci. 56:330–343. doi:10.1046/j.1365-2494.2001.00282.x.
Elferink SJWH, Kroonenman J, Gottschal JC, Spoelstra SF, Faber F, Driehuis F. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by Lactobacillus buchneri. Appl Environ Microbiol. 67:125–132. doi:10.1128/AEM.67.1.125-132.2001.
Filya I 2003. The effect of Lactobacillus buchneri and Lactobacillus plantarum on the fermentation, aerobic stability, and ruminal degradability of low dry matter corn and sorghum silages. J Dairy Sci. 86:3575–3581. doi:10.3168/jds.S0022-0302(03)73963-0.
Gurbuz Y, Kaplan M, Davies DR. 2008. Effects of condensed tannin content on digestibility and determination of nutritive value of some selected sorghum:sudangrass hybrids. J Anim Sci. 86:3575–3581. doi:10.3168/jds.S0022-0302(03)73963-0.

Funding
This research was performed with the support of 'Cooperative Research Program for Agriculture Science & Technology Development (Project No. PJ013869022019)' Rural Development Administration, South Korea.
native legumes species. J Anim Vet Adv. 7:854–862. doi:javava.2008.854.862.

Hobson PN, Stewart CS. 1997. The rumen microbial ecosystem, 2nd ed. London, UK: Blackie Academic and Professional. doi:10.1007/978-94-009-1453-7.

Huisden CM, Adesogan AT, Kim SC, Oosanya T. 2009. Effect of applying molasses or inoculants containing homofermentative or heterofermentative bacteria at two rates on the fermentation and aerobic stability of corn silage. J Dairy Sci. 92:690–697. doi:10.3168/jds.2008-1546.

Johnson LM, Harrison JH, Davidson D, Robutti JL, Swift M, Mahnna WC, Shinners K. 2002. Corn silage management: effects of hybrid, maturity, and mechanical processing on chemical and physical characteristics. J Dairy Sci. 85:833–853. doi:10.3168/jds.s0022-0302(02)74143-X.

Joo YH, Kim DH, Parhadipta DHV, Lee HJ, Amanullah SM, Kim SB, Chang JS, Kim SC. 2018. Effect of microbial inoculants on fermentation quality and aerobic stability of sweet potato vines silage. Asian-Australas J Anim Sci. 31:1897–1902. doi:10.5713/ajas.18.0264.

Kang TW, Adesogan AT, Kim SC, Lee SS. 2009. Effects of an esterase-producing inoculant on fermentation, aerobic stability, and neutral detergent fiber digestibility of corn silage. J Dairy Sci. 92:732–738. doi:10.3168/jds.2007-0780.

Kim DH, Amanullah SM, Lee HJ, Joo YH, Han OK, Adesogan AT, Kim SC. 2018. Effects of hybrid and bacterial inoculation on fermentation quality and fatty acid profile of barley silage. Anim Sci J. 89:140–148. doi:10.1111/asj.12923.

Kim HS, Han OK, Kim SC, Kim MJ, Kwak YS. 2017. Screening and investigation Lactobacillus spp. to improve Secale cereale silage quality. Anim Sci J. 88:1538–1546. doi:10.1111/asj.12781.

Kleinschmit DH, Schmidt RJ, Kung Jr L. 2005. The effects of various antifungal additives on fermentation and aerobic stability of corn silage. J Dairy Sci. 88:2130–2139. doi:10.3168/jds.s0022-0302(05)72889-7.

Lim HJ, Kim JD, Lee HJ, Jeon KH, Yang KY, Kwon CH, Yoon SH. 2009. Effect of pre-wilting on the forage quality of organic sorghum-sudangrass silage. Korean J Organic Agric. 17:519–527.

McDonald P, Henderson AR, Heron SJE. 1991. The biochemistry of silage, 2nd ed. Bucks, UK: Chalcombe Publ. doi:10.1017/S0021859600067162.

Muck RE, Dickerson JT. 1988. Storage temperature effects on proteolysis in alfalfa silage. Trans ASAE. 31:1005–1009. doi:10.13031/2013.30813.

Nkosi DB, Meeke R. 2010. Effects of ensiling totally mixed potato hash ration with or without a heterofermentative bacterial inoculant on silage fermentation, aerobic stability, growth performance and digestibility. Anim Feed Sci Technol. 161:38–48. doi:10.1016/j.anifeedsci.2010.07.015.

Oliveira AD, Weinberg ZG, Ogunsade IM, Cervante AAP, Arnola KG, Jiang Y, Kim DH, Li X, Gonçalves MCM, Vyas D, Adesogan AT. 2017. Meta-analysis of effects of inoculation with homogenous and facultative heterofermentative lactic acid bacteria on silage fermentation, aerobic stability, and the performance of dairy cows. J Dairy Sci. 100:4587–4603. doi:10.3168/jds.2016-11815.

Queiroz OCM, Adesogan AT, Arriola KG, Queiroz MFS. 2012. Effect of dual-purpose inoculant on the quality and nutrient losses from corn silage produced in farm-scale silos. J Dairy Sci. 95:3354–3362. doi:10.3168/jds.2011-5207.

Romero JJ, Zarate MA, Adesogan AT. 2015. Effect of the dose of exogenous fibrilolytic enzyme preparations on preigestive fiber hydrolysis, ruminal fermentation, and in vitro digestibility of bermudagrass haylage. J Dairy Sci. 98:406–417. doi:10.3168/jds.2014-8285.

Sutton JD, Dhanoa MS, Morant SV, France J, Napper DJ, Schuller E. 2003. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. J Dairy Sci. 86:3620–3633. doi:10.3168/jds.s0022-0302(03)73968-x.

Tilley JMA, Terry RA. 1963. A two-stage technique for the in vitro digestion of forage crops. J Br Grassl Soc. 18:104–111. doi:10.1111/j.1365-2494.1963.tb00335.x.

Van Soest PJ, Robertson JB, Lewis BA. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J Dairy Sci. 74:3583–3597. doi:10.3168/jds.s0022-0302(91)78551-2.

Venuto B, Kindinger B. 2008. Forage and biomass feedstock production from hybrid forage sorghum and sorghum-sudangrass hybrids. Grassl Sci. 54:189–196. doi:10.1111/j.1744-697X.2008.00123.x.

Vissers MMM, Driehuis F, Te Gijt I, van Dijk H, Robutti JL. 2007. Concentration of butyrate acid bacteria spores in silage and relationships with aerobic deterioration. J Dairy Sci. 90:928–936. doi:10.3168/jds.s0022-0302(07)71576-x.

Weinberg ZG, Shatz O, Chen Y, Yosef E, Nikbahat M, Ben-Ghedalia D, Miron J. 2007. Effect of lactic acid bacteria inoculants on in vitro digestibility of wheat and corn silages. J Dairy Sci. 90:4754–4762. doi:10.3168/jds.2007-0176.

Winters AL, Cockburn JE, Dhanoa MS, Merry RJ. 2000. Effects of lactic acid bacteria in inoculants on changes in amino acid composition during ensilage of sterile and sterile ryegrass. J Appl Microbiol. 89:442–451. doi:10.1046/j.1365-2672.2000.01133.x.

Zahid MS, Mufti MU, Shaheeq S, Qamar IA, Haqpani AM. 2002. Performance of sorghum-sudangrass hybrids. Pak J Agr Sci. 17:255–260.