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REGULAR ARTICLES

Effect of total mixed ration silage containing agricultural by-products with the fermented juice of epiphytic lactic acid bacteria on rumen fermentation and nitrogen balance in ewes

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Abstract

The goal of this study was to determine the effects of the fermented juice of epiphytic lactic acid bacteria (FJLB) on the quality of total mixed ration (TMR) silage containing agricultural by-products, its digestibility, rumen fermentation, and nitrogen balance in ewes. TMR was prepared from rice straw, corn stover silage, brewer grain, tofu waste, steam-flaked corn, and a mineral mixture. The treatments consisted of silage additives added to TMR: CON (no silage additive), FJLB, COM (commercial additive), and MIX (FJLB + COM). Four cannulated ewes were assigned to the 4 × 4 Latin square design. The MIX treatment produced a lower ($P < 0.01$) pH than did the CON and FJLB treatments and a higher ($P < 0.01$) lactic acid concentration than did the other treatments. The fiber content in the COM treatment was lower ($P < 0.05$) than that in the other treatments. The FJLB treatment had similar fermentation quality and chemical composition to those of the CON and COM treatments in all parameters observed. Although the silage quality index (Fleig point) was higher in the MIX and COM treatments than in the CON treatment, all silages had good quality. No silage additives affected intake, digestibility, rumen fermentation, or nitrogen balance. In conclusion, the TMR silage prepared from agricultural by-products mixed with wet-type food by-products with or without FJLB added resulted in well-preserved fermentation, and this product might be used as a ruminant feed.

Keywords Agricultural by-product · Feeding value · Nitrogen balance · Silage additive

Introduction

Agricultural by-products are the main feed for ruminants in developing countries. Generally, these feeds have low nutrient content due to high lignin and low crude protein (CP) content (Huyen et al. 2012), resulting in lower intake, digestibility, and animal performance (Khan et al. 2015). Combining agricultural by-product with other sources of non-structural carbohydrates in the form of total mixed ration (TMR) improved its utilization (Yanti and Yayota 2017). In this respect, the preservation method is a key factor due to the limiting harvesting season and relatively high moisture contents of agricultural by-products.

The use of ensiled TMR is a potential method that has shown increasing use in Japan (Miyaji and Matsuyama 2016). Ensiling is a preservation method for moist crops in aerobic conditions, in which the organic acids produced reduce the pH value (Wanapat et al. 2013). To achieve proper fermentation and nutrient preservation, silage additive was added during silage preparation (Abdel-Aziz et al. 2015). The fermented juice of epiphytic lactic acid bacteria (FJLB) as an additive in silage has been known to improve the fermentation of alfalfa (Tao et al. 2017) and guinea grass (Bureenok et al. 2005). FJLB contains multiplied domestic lactic acid bacteria that stimulate lactic acid production (Wang et al. 2009) and is easily and inexpensively prepared in tropic small-scale farms. If FJLB were applied in TMR prepared from the agricultural by-product, this additive would

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result in good fermentation quality silage, as indicated by a low pH value and high lactic acid production. Good quality silage possibly improves animal nutrient intake, digestibility, and ruminal fermentation. An in vitro study by Yahaya et al. (2004) demonstrated that FJLB silage has a higher digestibility of DM and NDF. An in vivo study by Bureenok et al. (2011) found that CP digestibility was improved in ruzigrass treated with FJLB, and the cellulolytic population in the rumen increased. However, there was limited information regarding applying FJLB in TMR silage prepared from agricultural by-product on animal production. We hypothesized that FJLB in TMR silage prepared from crop waste could improve the fermentation quality of the silage and lead to better nutrient assessment in ruminants. Improving the utilization of agricultural by-products will contribute to ruminant production in tropical areas. This study aimed to determine the effect of TMR silage prepared by agricultural by-product with the application of FJLB on nutrient intake, digestibility, rumen fermentation, and nitrogen balance in ewes.

Materials and methods

All animal experimental procedures were approved by the Committee for Animal Research and Welfare of Gifu University (#17035).

TMR was prepared from rice straw, corn stover silage, brewer grain, tofu waste, steam-flaked maize, and a mineral-vitamin mix. Rice straw was harvested at Yanagido Farm, Gifu Field Science Center, Gifu University, and was chopped into 2–3 cm in length prior to ensiling. Corn stover was harvested at the yellow ripe stage and then chopped to 2–3 cm. Thus, corn stover was ensilaged in a polyethylene bag doubled with a flexible container bag (width 650 mm, length 650 mm, height 800 mm) and then stored outdoors for approximately 2 months until mixing with TMR. Wet brewer grain and tofu waste were purchased from a brewer factory and from a food manufacturer in our local region. These two food by-products were preserved in anaerobic conditions until mixing. Steam-flaked maize was purchased from a local feed company (Minorakuren Co, Ltd., Gifu, Japan). Vitamin-mineral mix (NAS DL05-HVE; NASU AGRI SERVICE, Inc., Tokyo, Japan) contained 5,000,000 IU/kg of vitamin A, 1,000,000 IU/kg of vitamin D, 24,000 IU/kg of vitamin E, 150 mg/kg of Co, 8000 mg/kg of Cu, 15,000 mg/kg of Mn, 250 mg/kg of I, 20,000 mg/kg of Zn, 10 mg/kg of Se, and 700 g/kg of Mg. The nutrient content of each material and the proportion in TMR are shown in Table 1 and Table 2, respectively. TMR was formulated to obtain 12.5% of crude protein (CP) and 66.1% of total digestible nutrients (TDN) to meet or exceed the maintenance requirement of sheep according to the National Research Council (2007).

Preparing silage

All the materials were mixed manually, and silage additive was added according to the following treatments: CON (no silage additive added), FJLB, COM (commercial additive: "Si-Master AC"®, Snow Brand Seed Co., Ltd., Sapporo, Japan), and MIX (FJLB + COM). The FJLB was prepared from Italian ryegrass modified from Bureenok et al. (2016). Italian ryegrass and distilled water (1:5 ratio; *w*:*v*) were blended using a food blender for 2 min and then filtered using a doubled layer of cheesecloth. Then, 2% of glucose was added to the filtrate and mixed thoroughly, and then the mixture was incubated in anaerobic conditions at 30 °C for 48 h. FJLB was added at 1% (*v*:*w*) of fresh material of TMR. The treatment with commercial additive followed the company's instructions. The commercial additive was diluted with distilled water (17:1000 ratio; *w*:*v*) and sprayed at 0.1% (*v*:*w*) on the TMR. Distilled water was added in CON, FJLB, and COM treatments to adjust the moisture content, similar to that of the MIX treatment. TMR was packed into a polyethylene bag doubled with a flexible container bag (capacity 100 kg). Silages were made in four replications for each treatment. TMR was fermented for a minimum of 2 months (from 8 June–31 August 2017) outdoors.

Feeding period

Four Suffolk ewes with cannulas attached to the rumen, aged 7.1 ± 2.2 years old and with an initial body weight (BW) 56.1 ± 15.2 kg, were assigned to a 4 × 4 Latin square design. The ewes were housed in metabolic cages individually, with room temperatures ranging from 13.9–33.4 °C. The ewes in this experiment were managed according to the guidelines of the Committee for Animal Research and Welfare of Gifu University.

The TMR feed was offered (2% of BW on a dry matter basis) twice a day (at 09:30 and 14:30 h) in equal amounts and had 10% of refusal feed. The amount of the diets offered to ewes and the orts was recorded daily. Water was provided ad libitum to the ewes. The adaptation period was a total of 8 days, followed by a collection period for 6 days. The body weight was measured at the beginning of the experiment and at the end of each period.

Sample collection

Before adding the silage additives, approximately 500 g of TMR was collected with three replications to assess the quality of the TMR pre-silage. A representative sample of TMR (approx. 500 g) silage was collected for chemical composition just after the silo opened in the beginning of each experimental period analysis. Approximately 100 g of daily feed sample

Table 1 Ingredient composition of material for total mixed ration

Ingredient	Rice straw	Corn stover silage	Brewer grain	Tofu waste	Steam-flaked maize
Dry matter (%)	91.9	19.8	37.24	25.2	91.8
Crude protein (%DM)	3.6	6.9	22.3	27.3	6.9
aNDFom (%DM)	62.9	68.1	61.2	25.2	16.4
ADFom (%DM)	39.3	41.8	23.5	22.2	4.3
Extract ether (%DM)	2.0	1.8	9.8	8.4	3.7
Crude ash (%DM)	12.2	7.3	4.6	4.8	1.2

DM, dry matter; *aNDFom*, neutral detergent fiber with heat stable amylase and exclusive ash; *ADFom*, acid detergent fiber exclusive ash

was collected throughout the sample collection period to correct the DM content for feed intake calculations.

Rumen samples were collected at 0 and 4 h after feeding at the end of each collection period via the rumen cannulas. The rumen sample was filtered using four layers of cheesecloth; then, the pH of the filtrate was measured immediately using a pH meter (MP220; METTLER TOLEDO, Tokyo, Japan). The filtrate was placed in a 2-mL microtube with three replications for each animal and was then centrifuged at 3500×*g* for 10 min. The supernatant was stored at − 20 °C for ammonia (NH_3) and volatile fatty acid (VFA) analyses.

Fecal and urine samples were collected in the morning and evening every day during each collection period. The feces were weighed and mixed, and then 10% of the feces were sampled. Three milliliters of 10 N formaldehyde solution was added to the sample to stop microbial fermentation, and the sample was then stored in a freezer (− 20 °C). At the end of each collection period, all feces from each animal were mixed thoroughly, and 250 g of representative sample was collected. The fecal samples were dried at 60 °C for 48 h to determine the dry matter (DM) content. Dried fecal samples were ground to pass a 1-mm sieve and packed into sealed polyethylene bags until the chemical composition analysis. Then, 100 mL of 20% sulfuric acid was added to the collected urine every day throughout the collection period. Of the total urine, 20% was collected and then stored in a refrigerator. At the end of each collection period, all the urine from each animal was mixed thoroughly, and 50 mL was collected in plastic tubes, with two replications. All the urine was then stored at − 20 °C for nitrogen content analysis.

Table 2 Proportion of material in total mixed ration

Item	Proportion (%DM)
Rice straw	27.0
Corn stover silage	26.0
Brewer grain	13.5
Tofu waste	15.0
Steam-flaked maize	17.0
Mineral mix[a]	1.5

[a] Contained 5,000,000 IU/kg of vitamin A, 1,000,000 IU/kg of vitamin D, 24,000 IU/kg of vitamin E, 150 mg/kg of Co, 8,000 mg/kg of Cu, 15,000 mg/kg of Mn, 250 mg/kg of I, F0 mg/kg of Zn, 10 mg/kg of Se, and 700 g/kg of Mg

Chemical analysis

The chemical composition of TMR silage and feces was analyzed with the same methods. The DM, ash, acid detergent fiber exclusive ash (ADFom), CP, and ether extract (EE) analyses were performed according to the methods of AOAC (2007): protocol numbers 930.15; 942.05; 973.18; 990.03; and 920.39, respectively). Organic matter (OM) was calculated as weight loss through ashing. The neutral detergent fiber assayed with heat stable α-amylase and exclusive ash (aNDFom) was measured according to Van Soest et al. (1991). The nitrogen content in the urine samples was determined by the Kjeldahl method (AOAC 2007).

The fermentation quality of TMR silage was assayed as follows. A representative sample of 50 g fresh matter TMR silage was taken, macerated with 150 mL of distilled water, and stored at 4 °C for 12 h (Bureenok et al. 2016). Then, the extract was filtered using a filter paper (ADVANTEC No. 1, Tokyo, Japan). The filtrate was used to determine the fermentation quality. The pH of the silage was determined by using a pH meter (MP220; METTLER TOLEDO, Tokyo, Japan) immediately after filtration. The volatile fatty acid (VFA) content was measured by applying gas chromatography (GC–14A, Shimadzu, Kyoto, Japan; column: ULBON HR-20M, 0.25 mmI.D. × 30 mL 0.25 μm, flow rate 7.2 mL/min, injection port at 220 °C, column at 200 °C and detector at 220 °C). Lactic acid content was determined using a commercial kit (D-/L-Lactic Acid Assay Kit; Megazyme, Wicklow, Ireland). The NH3-N of silage and rumen fluids was measured by the indophenol method (Weatherburn 1967). As a silage quality index, Fleig points were calculated with the following equation (Denek and Can 2006):

$$\text{Fleig points} = 220 + (2 \times \text{DM\%} - 15) - 40 \times \text{pH}.$$

The supernatants from the rumen samples were thawed at room temperature, and 25% of meta-phosphoric acid at ratio 1:4 (meta-phosphoric:supernatant) was added before being stored again in a freezer (− 20 °C) for at least 8 h to remove protein before analysis. Frozen supernatants were thawed in a refrigerator and centrifuged at 10,000×g for 5 min. The supernatant was placed into new tubes and mixed with internal standard (10 mmol/L crotonic acid) at a 6:1 ratio. One microliter (μm) was injected for gas chromatography (GC–14A, Shimadzu, Kyoto, Japan; column: ULBON HR-20M, 0.25 mmI.D. × 30 mL 0.25 μm; injection port at 250 °C, column at 100 °C, and detector at 250 °C, flow rate 15 mL/min) for VFA analysis.

Statistical analysis

All obtained data were analyzed using R programming 3.3.2 (R development core team 2016). The fermentation quality and chemical composition of TMR silage were analyzed by one-way analysis of variance (ANOVA). The mean of additives treatment was compared using pairwise t tests. Data regarding intake, digestibility, rumen parameters, and nitrogen balance were subjected to ANOVA using the following model: $Yijkl = \mu + Ti + Pj + Ak + eijkl$, where $Yijk$ is observation, μ is the overall means, Ti is the fixed effect treatment feed, Pj is the fixed effect of the period, Ak is the random effect of the animal, and $eijkl$ is the residual error. The means of the additives were compared using pairwise t tests. The Bonferroni correction was used to detect the differences between the means for each data analysis. Differences were considered significant when $P \leq 0.05$ and were showing tendencies when $P < 0.10$.

Results

Fermentation quality and chemical composition of TMR silage

The MIX treatment had a lower pH value than the CON and FJLB treatments ($P < 0.01$; Table 3). The lactic acid concentration in the MIX treatment was highest among the treatments. However, there were no significant differences in pH among the CON, FJLB, and COM treatments. There were also no significant differences in VFA and NH_3–N concentrations among the treatments. Fleig points in the MIX treatment were higher than those in the CON treatment ($P < 0.05$). The nutritive value of TMR silage showed that DM content tended to be lower in CON than in the other treatments ($P = 0.079$). The CP content in all TMR with silage additives tended to be higher than in TMR without silage additive ($P = 0.089$). The ADFom and aNDFom contents of TMR silage were lower in the COM treatment than those in the CON treatment ($P < 0.05$); however, no significant difference was detected among the other treatments ($P > 0.05$). There were no significant differences in EE and OM contents among the treatment groups ($P > 0.05$).

Intake, digestibility, and ruminal fermentation of TMR silages

There was no significant difference in nutrient intake and apparent digestibility ($P > 0.05$) in ewes fed TMR silage with different silage additive treatments (Table 4). The pH in the rumen was 7.1 before feeding and 6.8–6.9 after feeding for all TMR silages (Table 5). The silage additive treatments did not affect ammonia-nitrogen and VFA concentrations in the rumen at 0 and 4 h after feeding. The ewes showed similar N intake, excretion, and retention, regardless of the silage additive treatments in TMR silage (Table 6; $P > 0.05$).

Discussion

Fermentation quality

The MIX treatment in this study was categorized as well-preserved silage because of the pH value was around four (Cao et al. 2010). The pH in the MIX treatment was the lowest among the treatments due to the high lactic acid concentration in the MIX treatment (Table 3). This result suggested that the combination of lactic acid bacteria from FJLB and a commercial additive contributed to greater lactic acid production.

With respect to VFA concentration, there was no significant difference in VFA concentration in all the treatments. The concentration of acetic acid in all treatments was less than 1% of DM silages, although acetic acid contributes to improving aerobic stability by depressing the growth of yeast (Da Silva et al. 2014). Butyric acid was detected both in the control and in the treated silages in the present study. However, the butyric acid concentration in all additive-treated silage was under the acceptable level (0.2% of DM silage; Zhang et al. 2013).

The NH_3–N/TN concentrations in all the treatment silages were less than 12.5%, indicating that all treatment silages contained well-preserved fermentation (Kung Jr. 2010). The NH_3–N in the control silage was not different from the FJLB treatment silage in this study. This result was inconsistent with the study by Wang et al. (2009), who found that FJLB additive in alfalfa silage had a lower NH_3–N content than that of the control. A possible reason for these different results is that

Table 3 Fermentation quality and chemical composition of total mixed ration (TMR) silage prepared from agriculture by-product with different silage additives

Item	Pre-silage	Treatment				P value
		CON	FJLB	COM	MIX	
Fermentation quality						
pH	NA	4.6 ± 0.12[b]	4.5 ± 0.20[b]	4.3 ± 0.09[ab]	4.1 ± 0.13[a]	0.005
Lactic acid (g/kg DM)	NA	13.8 ± 6.5[a]	17.1 ± 4.36[a]	19.0 ± 8.64[a]	37.0 ± 5.78[b]	0.006
VFA (g/kg DM)						
Acetic acid	NA	23.6 ± 6.2	37.9 ± 13.9	29.2 ± 8.2	34.6 ± 14.6	0.812
Propionic acid	NA	2.9 ± 0.74	3.1 ± 0.76	3.6 ± 0.82	2.7 ± 0.31	0.799
Butyric acid	NA	2.7 ± 1.34	0.8 ± 0.20	0.3 ± 0.19	1.1 ± 0.46	0.161
NH_3–N (g/kg TN)	NA	54.3 ± 4.27	59.9 ± 6.73	56.6 ± 7.06	57.9 ± 4.85	0.916
Fleig point	NA	85.4 ± 6.81[a]	96.5 ± 3.56[ab]	108.5 ± 3.15[b]	111.2 ± 2.17[b]	0.004
Chemical composition						
DM	38.1	31.8 ± 2.54	36.6 ± 0.51	37.0 ± 0.42	35.8 ± 0.86	0.079
OM (%DM)	91.4	90.9 ± 0.21	91.1 ± 0.06	91.2 ± 0.05	91.3 ± 0.1	0.180
CP (%DM)	8.0	11.0 ± 0.23	11.4 ± 0.20	11.8 ± 0.10	11.5 ± 0.24	0.089
aNDFom (%DM)	59.0	56.8 ± 0.82[b]	56.2 ± 1.52[ab]	51.6 ± 0.69[a]	52.7 ± 1.27[ab]	0.016
ADFom (%DM)	35.9	34.0 ± 0.53[b]	33.0 ± 0.69[ab]	31.1 ± 0.22[a]	31.7 ± 0.93[ab]	0.030
EE (%DM)	2.8	4.2 ± 0.41	4.2 ± 0.09	4.8 ± 0.23	4.6 ± 0.15	0.244

Values are presented as mean ± standard error. *CON*, control (no additive added); *FJLB*, fermented juice of epiphytic lactic acid bacteria additive; *COM*, commercial silage additive; *MIX*, FJLB + COM; *VFA*, volatile fatty acid; *NH_3–N*, ammonia-nitrogen; *DM*, dry matter; *OM*, organic matter; *CP*, crude protein; *aNDFom*, neutral detergent fiber with heat stable amylase and exclusive ash; *ADFom*, acid detergent fiber exclusive ash; *EE*, ether extract; *NA*, not analyzed. The values with different superscript within the same row are significantly different at 5% level

CON in this study had a lower pH (4.6; Table 3) than that in the other study. Since we used fermented corn stover and tofu waste as ingredients of TMR silage, the original ingredients may contain enough LAB. Thus, CON showed a relatively low pH value and inhibited the further breakdown of protein.

The Fleig point is a method for assessing fermentation quality based on the DM content and pH value of the silage. The Fleig points in MIX and COM treatments were higher than those in the CON treatment. However, according to Ziaei and Molaei (2010), all silage in this study was categorized as very good quality since the value was greater than 85.

DM and CP contents in the silage additive treatment including FJLB tended to be higher than those in the CON treatment ($P = 0.079$), suggesting that FJLB has the ability to inhibit DM losses and CP catabolism. This result is in line with that of previous studies (Denek et al. 2011 and 2012) that found that DM content in FJLB-treated silage was higher than that in un-treated silage. Higher CP content in the FJLB treatment than in the control was also reported in rice straw silage (Jin-ling et al. 2013).

The FJLB treatment in this study showed no clear distinction with CON treatment ($P > 0.05$) in NDFom and ADFom contents. This result is in agreement with some previous studies that showed applying FJLB as silage additive did not affect the NDF and ADF contents of alfalfa silage (Wang et al. 2009) and ruzigrass silage (Bureenok et al. 2011). Structural carbohydrates in NDFom and ADFom are not fermentable substrates for lactic acid bacteria in silage (McDonald et al. 1991). However, studies by Denek et al. (2011 and 2012) found that FJLB treatment had lower NDF and ADF contents than those of un-treated alfalfa silage. Therefore, it is suggested that the lower cell wall content in the FJLB treatment compared to the control in Denek's study was a result of the hydrolysis brought about by the action of some bacteria that are able to breakdown cellulose and hemicelluloses (Wanapat et al. 2013) or by enzymes that are present in FJLB. However, the details of this mechanism need to be further investigated.

Nutrient intake and digestibility

DM, OM, CP, aNDFom, ADFom, and EE intakes were not affected by silage additive treatment in the TMR silage prepared from agricultural by-product, suggesting that all TMR silage in this study has similar palatability. This finding might be explained by the fact that all TMR silages in this study were categorized as well-preserved silage based on the Fleig point. This result was in agreement with that of a previous study (Bureenok et al. 2012), which found that FJLB treatment in ruzigrass silage did not affect total intake in cows.

Table 4 Effect of TMR contained agricultural by-product treated with FJLB on nutrient intake and apparent digestibility of ewes

Item	Treatment				*P* value
	CON	FJLB	COM	MIX	
Intake (g/day/kg BW$^{0.75}$)					
DM	34.2 ± 5.64	39.6 ± 2.30	38 ± 1.83	39.2 ± 4.44	0.393
OM	32.0 ± 5.69	37.3 ± 1.56	34.3 ± 2.73	35.3 ± 3.67	0.129
CP	3.8 ± 0.59	4.5 ± 0.24	4.3 ± 0.23	4.6 ± 0.66	0.347
aNDFom	18.6 ± 2.77	21.7 ± 0.87	20.2 ± 0.85	20.8 ± 2.74	0.612
ADFom	11.5 ± 1.86	13.3 ± 0.44	12.3 ± 0.48	13.2 ± 1.76	0.625
EE	1.1 ± 0.22	1.4 ± 0.23	1.3 ± 0.32	1.5 ± 0.48	0.374
Apparent digestibility (% of intake)					
DM	59.8 ± 2.73	65.6 ± 2.89	55.9 ± 6.37	62.2 ± 4.92	0.371
OM	57.5 ± 1.92	62.9 ± 3.17	53.5 ± 6.07	59.2 ± 4.15	0.584
CP	60.1 ± 4.6	65.8 ± 2.84	57.8 ± 8.52	63.2 ± 2.71	0.505
aNDFom	57.6 ± 2.7	64.8 ± 4.85	49.1 ± 4.29	57.4 ± 7.13	0.101
ADFom	56.8 ± 2.39	62.8 ± 4.30	47.3 ± 5.45	57.2 ± 6.89	0.172
EE	80.5 ± 3.01	83.7 ± 4.47	83.2 ± 5.39	85.2 ± 0.54	0.760

Values are presented as mean ± standard error. *CON*, control (non-additive added); *FJLB*, fermented juice of epiphytic lactic acid bacteria additive; *COM*, commercial silage additive; *MIX*, FJLB + COM; *DM*, dry matter; *OM*, organic matter; *CP*, crude protein; *OM*, organic matter; *aNDFom*, neutral detergent fiber with heat stable amylase and exclusive ash; *ADFom*, acid detergent fiber exclusive ash; *EE*, ether extract

The apparent digestibility of DM, OM, CP, aNDFom, ADFom, and EE was not affected by silage additive treatments. Yahaya et al. (2004) found that FJLB treatment in elephant grass silage increased the DM and NDF digestibility in situ compared to control and acetic acid treatments. Bureenok et al. (2011) found that the addition of FJLB in ruzigrass silage increased the digestibility of CP compared to that of the control. Another study (Takahashi et al. 2005) found that whole crop rice straw treated with FJLB and crushing improved the digestibility of fibrous components compared to the control. This inconsistency with the present study might be explained by the fact that, in the present study, all treated silages were well-preserved, including that of the CON treatment. Wet tofu waste, brewer grain, and corn stover silage as the ingredients of TMR in the present study might contain a sufficient level of LAB, although we did not analyze the LAB in the material. However, the presence of LAB in tofu waste and brewer grain was documented by Tanaka et al. (2001), and LAB in corn stover was also documented by Wang et al. (2017). Thus, we supposed that the doses of FJLB in this present study were not sufficient to have a positive effect on the TMR silage over the effect of the original materials.

Rumen fermentation

The fermentation characteristics in the rumen were not affected by treatment. The pH in all silages was 7.1 before feeding and 6.8–6.9 after feeding and was within the normal range (6.5–7.0) for optimal microbial digestion of fiber and protein (Huyen et al. 2012).

The silage additive treatments did not affect the ammonia-nitrogen concentrations in the rumen. According to Satter and Slyter (1974), the minimum ammonia-nitrogen concentration in the rumen is 2.94 mmol/L to supply the rumen microbes with sufficient N for protein synthesis. All the ruminal ammonia-nitrogen values in the present study were more than the minimum level, indicating that they were sufficient for microbial protein synthesis. This result is in line with that of Bureenok et al. (2016), who found that ammonia-nitrogen concentration was more than the minimum concentration in goats after applying FJLB in stylo legume, guinea grass, and their mixture. The present experiment was also in line with previous studies that applied FJLB in ruzigrass (Bureenok et al. 2011) or napier grass silage (Bureenok et al. 2012), resulting in similar rumen NH_3–N concentrations in cows. In the present study, as the TMR silage has similar fermentation quality, ewes showed similar nutrient intake, which in turn led to a similar result of the rumen NH_3–N concentration.

The silage additive treatments also did not affect VFA concentrations in the rumen, and the total VFA concentrations in the present study were in the normal range (70–130 mmol; Huyen et al. 2012). This result might relate to similar nutrient intakes among the four treatments. Takahashi et al. (2005) reported a similar result; the ruminal VFA concentration in sheep fed with FJLB-treated whole rice straw silage did not differ from the control sheep. In addition, the level of DM intake in the present experiment was too low to influence the

Table 5 Effect of TMR contained agricultural by-product treated with FJLB on rumen fermentation of ewes at 0 and 4 h after feeding

Item	Treatment				*P* value
	CON	FJLB	COM	MIX	
0 h					
pH	7.0 ± 0.16	7.1 ± 0.08	7.1 ± 0.06	7.1 ± 0.05	0.889
NH_3–N (mmol/L)	4.0 ± 0.41	3.7 ± 0.32	3.0 ± 0.53	3.4 ± 1.08	0.691
VFA (mmol/L)					
Acetic acid	62.4 ± 7.68	70.4 ± 18.05	56.6 ± 3.91	49 ± 8.95	0.834
Propionic acid	14.2 ± 1.27	15.1 ± 3.73	12.8 ± 1.48	12 ± 2.81	0.982
Butyric acid	4.8 ± 0.59	5.6 ± 0.85	4.5 ± 0.90	3.7 ± 0.78	0.470
4 h					
pH	6.8 ± 0.15	6.8 ± 0.10	6.9 ± 0.09	6.8 ± 0.09	0.996
NH_3–N (mmol/L)	3.3 ± 0.68	3.5 ± 1.38	3.4 ± 0.65	3.3 ± 1.11	0.983
VFA (mmol/L)					
Acetic acid	74.7 ± 8.64	79.0 ± 4.35	73.9 ± 4.99	62.5 ± 13.42	0.176
Propionic acid	21.3 ± 4.89	18.6 ± 0.83	21.5 ± 2.29	16.1 ± 6.61	0.524
Butyric acid	6.2 ± 1.45	5.7 ± 0.25	6.0 ± 0.86	4.8 ± 1.25	0.585

Values are presented as mean ± standard error. *CON*, control (no additive added); *FJLB*, fermented juice of epiphytic lactic acid bacteria additive; *COM*, commercial silage additive; *MIX*, FJLB + COM; *VFA*, volatile fatty acid; *NH_3–N*, ammonia-nitrogen

process of rumen fermentation (Bureenok et al. 2012). Compared to some studies, DM intake in the present study was lower and thus might not influence the VFA concentration. The study by Yani et al. (2015) reported the DM intake in sheep was 43.8–45.4 g/day/kg $BW^{0.75}$, whereas Ishida et al. (2012) reported that DM intake in sheep was 49.27–50.58 g/day/kg $BW^{0.75}$. In their studies, the effect of treatment on ruminal fermentation was detected as a higher DM intake in sheep.

Finally, sheep showed similar N intake, excretion, and retention, regardless of the treatment. The similar N balance in the present study is due to the same quality of silages, which did not influence rumen microbial protein synthesis or the utilization of amino acids. This result was consistent with the study by Takahashi et al. (2005), in which FJLB was added to whole rice straw, and by Horiguchi and Takahashi (2007), who found that FJLB treatment in green soybean stover did not affect nitrogen intake, fecal or urine nitrogen, or nitrogen retention in sheep. However, Cao et al. (2002) showed improvements in nitrogen retention in dry cows fed a total mixed ration composed of alfalfa silage treated with FJLB, but FJLB did not affect nitrogen intake or the fecal and urinary excretion of nitrogen. These differences might be caused by the different materials used as feed in their studies and the present study.

Conclusion

The FJLB as a silage additive in TMR made from agricultural by-product had similar fermentation quality to that of non-additive TMR silage. If the FJLB was combined with a commercial additive, the fermentation quality was improved. The

Table 6 Effect of TMR contained agricultural by-product treated with FJLB on nitrogen (N) balance of ewes

Item	Treatment				*P* value
	CON	FJLB	COM	MIX	
N balance (g/day/$BW^{0.75}$)					
Intake N	0.63 ± 0.10	0.72 ± 0.03	0.68 ± 0.05	0.73 ± 0.09	0.234
Fecal N	0.24 ± 0.03	0.25 ± 0.02	0.28 ± 0.04	0.26 ± 0.04	0.803
Urinary N	0.09 ± 0.01	0.09 ± 0.03	0.09 ± 0.02	0.08 ± 0.01	0.953
Retention N	0.29 ± 0.07	0.39 ± 0.05	0.31 ± 0.10	0.39 ± 0.06	0.192
Proportion of N retention to N intake (%)	46.70 ± 4.75	53.98 ± 5.47	45.56 ± 11.89	53.42 ± 3.35	0.465

Values are presented as mean ± standard error. *CON*, control (no additive added); *FJLB*, fermented juice of epiphytic lactic acid bacteria additive; *COM*, commercial silage additive; *MIX*, FJLB + COM application of FJLB additive in agricultural by-product silage has no negative effects on nutrient intake, nutrient digestibility, and nitrogen balance in ruminants. The present study suggests that doses of FJLB should be further investigated, although original materials containing a certain amount of lactic acid bacteria contribute to the improvement of the quality of TMR silage prepared from agricultural by-products.

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Compliance with ethical standards

All animal experimental procedures were approved by the Committee for Animal Research and Welfare of Gifu University (#17035).

Conflict of interest The authors declare that they have no conflict of interest.

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References

Abdel-Aziz, N.A., Salem, A.Z.M., El-Adawy M.M., Camacho L.M., Kholif A.E., Elghandour M.M.Y. and Borhami B.E., 2015. Biological treatments as a mean to improve feed utilization in agriculture animals-An overview. Journal of Integrative Agriculture, 14, 534–543.

Association of Official Analytical Chemist, AOAC, 2007. Official methods of analysis of the association of official analytical chemist, 18th Edition. Association of official analytical chemists. Virginia, USA.

Bureenok, S., Namihira, T., Kawamoto, Y. and Nakada, T., 2005. Additive effects of fermented juice of epiphytic lactic acid bacteria on the fermentative quality of guineagrass (*Panicum maximum* Jacq.) silage. Grassland Science, 51, 243–248.

Bureenok, S., Suksombat, W. and Kawamoto, Y., 2011. Effects of the fermented juice of epiphytic lactic acid bacteria (FJLB) and molasses on digestibility and rumen fermentation characteristics of ruzigrass (*Brachiaria ruziziensis*) silages. Livestock Science, 138, 266–271.

Bureenok, S., Yuangklang, C., Vasupen, K., Schonewille, J.T. and Kawamoto, Y., 2012. The effect of additives in napier grass silage on chemical composition, feed intake, nutrient digestibility and rumen fermentation. Asian-Australasian Journal of Animal Sciences, 25, 1248–1254.

Bureenok, S., Sisaath, K., Yuangklang, C., Vasupen, K. and Schonewille, J.T., 2016. Ensiling characteristics of silages of stylo legume (*Stylosanthes guianensis*), guinea grass (*Panicum maximum*) and their mixture, treated with fermented juice of lactic bacteria, and feed intake and digestibility in goats of rations based on these silages. Small Ruminant Research, 134, 84–89.

Cao, L.M., Goto, M., Karita, S., Yamamoto, Y., Mizutani, M., Deguchi, Y., Urakawa, S., Maekawa, Y., Yamamoto, Y. and Masuko, T., 2002. Effect of fermented juice of epiphytic lactic acid bacteria on the fermentation quality of alfalfa (*Madicago sativa* L.) silage and its energy and nitrogen utilization by dry cows. Grassland Science, 48, 227–235.

Cao, Y., Takahashi T., Horiguchi, K. and Yoshida, N., 2010. Effect of adding lactic acid bacteria and molasses on fermentation quality and *in vitro* ruminal digestion of total mixed ration silage prepared with whole crop rice. Grassland Science, 56, 19–25.

Da Silva, N.C., Dos Santos, J.P., Avila, C.L.S., Evangelista, A.R., Casagrande, D.R. and Bernardes, T.F., 2014. Evaluation of the effects of two *Lactobacillus buchneri* strains and sodium benzoate on the characteristics of corn silage in a hot-climate environment. Grassland Science, 60, 169–177.

Denek, N. and Can, A., 2006. Feeding value of wet tomato pomace ensiled with wheat straw and wheat grain for Awassi sheep. Small Ruminant Research, 65, 260–265.

Denek, N., Can, A., Avci, M., Aksu, T. and Durmaz, H., 2011. The effect of molasses-based pre-fermented juice on the fermentation quality of first-cut lucerne silage. Grass and Forage Science, 66, 243–250.

Denek, N., Can, A., Avci, M. and Aksu, T., 2012. The effect of fresh and frozen pre-fermented juice on the fermentation quality of alfalfa silage. Kafkas Universitesi Veteriner Fakultesi Dergisi, 18, 785–790.

Horiguchi, K. and Takahashi, T., 2007. Fermentation quality and nutritive value of green soybean stover silage. Grassland Science, 53, 27–31.

Huyen, N.T., Wanapat, M. and Navanukraw, C., 2012. Effect of mulberry leaf pellet (MUP) supplementation on rumen fermentation and nutrient digestibility in beef cattle fed on rice straw-based diets. Animal Feed Science and Technology, 175, 8–15.

Ishida, K., Yani, S., Kitagawa, M., Oishi, K., Hirooka, H. and Kumagai, H., 2012. Effects of adding food by-products mainly including noodle waste to total mixed ration silage on fermentation quality, feed intake, digestibility, nitrogen utilization and ruminal fermentation in wethers. Animal Science Journal, 83, 735–742.

Jin-ling, H., Li-ke, W. and Si-fa, D., 2013. Effects of previously fermented juice on nutritive value and fermentative quality of rice straw silage. Journal of Northeast Agricultural University, 20, 48–52.

Khan, N.A., Hussain, S., Ahmad, N., Alam, S., Bezabhi, M., Hendriks, W.H., Yu P. and Cone J.W., 2015. Improving the feeding value of straws with *Pleurotus ostreatus*. Animal Production Science, 55, 241–245.

Kung, L. Jr., 2010. Understanding the biology of silage preservation to maximize quality and protect the environment. Proceedings, California Alfalfa & Forage Symposium and Corn/Cereal Silage Conference, Visalia, CA.

McDonald, P., Henderson, A.R. and Heron, S.J.E., 1991. The Biochemistry of Silage, Second Ed. Chalombe Publications, Marlow, Buckinghamshire, 1–340.

Miyaji, M. and Matsuyama, H., 2016. Lactation and digestion in dairy cows fed ensiled total mixed ration containing steam-flaked or ground rice grain. Animal Science Journal, 87, 767–774.

National Research Council of the National Academies (NRC), 2007. Nutrient requirements of small ruminants sheep, goats, cervids, and new world camelids. (The National Academic Press, Washington D.C. 1–362).

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Satter, L.D. and Slyter, L.L., 1974. Effect of ammonia concentration on rumen microbial protein production in vitro. British Journal of Nutrition, 32, 199–208.

Takahashi, T., Horiguchi, K. and Goto, M., 2005. Effect of crushing unhulled rice and the addition of fermented juice of epiphytic lactic acid bacteria on the fermentation quality of whole crop rice silage and its digestibility and rumen fermentation status in sheep. Animal Science Journal, 76, 353–358.

Tanaka, O., Akiyama, F., Yamada, A., Ando, S., Uegaki, R., Kobayashi, R. and Kume, T., 2001. Effect of gamma radiation on microflora and fermentation quality of silages containing tofu cake and brewer's grain. Grassland Science, 47, 274–282. (In Japanese with English abstract)

Tao, L., Zhou H., Zhang, N., Si, B., Tu, Y., Ma, T., and Diao, Q. 2017. Effects of different source additives and wilt conditions on the pH value, aerobic stability, and carbohydrate and protein fractions of alfalfa silage. Animal Science Journal, 88, 99–106.

Van Soest, P.J., Rovertson, J.B. and Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583–3597.

Wanapat, M., Kang, S., Khejornsart, P. and Pilajun, R., 2013. Improvement of whole crop rice silage nutritive value and rumen degradability by molasses and urea supplementation. Tropical Animal Health and Production, 45, 1777–1781.

Wang, J., Wang, J.Q., Zhou, H. and Feng, T., 2009. Effects of addition of previously fermented juice prepared from alfalfa on fermentation quality and protein degradation of alfalfa silage. Animal Feed Science and Technology, 151, 280–290.

Wang, S., Yuan, X., Dong, Z., Li, J., Shao, T. 2017. Effect of ensiling corn stover with legume herbages in different proportions on fermentation characteristics, nutritive quality and digestibility on the Tibetan Plateau. Grassland Science 63(4):236–244

Weatherburn, M.W., 1967. Phenol-Hypochlorite reaction for determination of ammonia. Analytical Chemistry, 39, 971–974.

Yahaya, M.S., Goto, M., Yimiti, W., Smerjai, B. and Kawamoto, Y., 2004. Evaluation of fermentation quality of a tropical and temperate forage crops ensiled with additives of fermented juice of epiphytic lactic acid bacteria (FJLB). Asia-Australasian Journal of Animal Science, 17, 942–946.

Yani, S., Ishida, K., Goda, S., Azumai, S., Murakami, T., Kitagawa, M., Okano, K., Oishi, K., Hirooka, H. and Kumagai, H., 2015. Effects of utilization of local food by-products as total mixed ration silage materials on fermentation quality and intake, digestibility, rumen condition and nitrogen availability in sheep. Animal Science Journal, 86, 174–180.

Yanti, Y. and Yayota, M., 2017. Agricultural by-products as feed for ruminants in tropical area: nutritive value and mitigating methane emission. Reviews in Agricultural Science, 5, 65–76.

Zhang, X.Q., Jin, Y. M., Zhang, Y.J., Yu, Z. and Yan, W.H., 2013. Silage quality and preservation of *Urtica cannabina* ensiled alone and with additive treatment. Grass and Forage Science, 69, 405–414.

Ziaei, N. and Molaei, S., 2010. Evaluation of nutrient digestibility of wet potato pomace ensiled with wheat straw compared to alfafa hay in Kermani sheep. Journal of Animal and Veterinary Advances, 9, 771–773.