USE OF COCULTURE FIBROLYTIC *RUMINOCOCCUS ALBUS* KU-F152 AND NON-FIBROLYTIC *SELENOMONAS RUMINANTIUM* S137 FOR IMPROVING FIBER DIGESTIBILITY AND NUTRITION VALUES OF RICE STRAW AND PARA GRASS IN *IN VITRO* RUMINAL FERMENTATION

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ABSTRACT

Ruminococcus albus represents a significant proportion of culturable rumen bacteria and contribute to fiber degradation and various substrates utilization in the rumen. This study was to investigate the interaction between fibrolytic *R. albus* KU-F152 and non-fibrolytic *S. ruminantium* S137 on the improvement of fiber digestibility and fermentation products of rice straw and para grass. In the present study, dry matter (DM) and neutral detergent fiber (NDF) digestion, ammonia-nitrogen (NH_3-N) and volatile fatty acids (VFA) products were examined in the basal medium and mixed rumen microflora using rice straw and papa grass as substrates for 72 h incubation. The data analysis was used to 2×5 factorial in completely randomized design. The results showed that coculture of *R. albus* KU-F152 with *S. ruminantium* S137 had higher DM and NDF digestibility of rice straw and para grass compared with monoculture ($P<0.01$). In addition, coculture of *R. albus* KU-F152 with *S. ruminantium* S137 showed significantly higher NH_3-N concentration difference for all the fiber sources than *R. albus* KU-F152. Bacterial monocultures significantly lowered acetate production ($P<0.01$) and no differences were found ($P>0.05$) in VFA concentrations between rice straw and para grass of basal medium and mixed rumen microflora. This finding suggests that the combination of *R. albus* KU-F152 with *S. ruminantium* S137 can improve fiber digestibility and increase the fermentation product. However, further studies are required to develop and apply coculture of fibrolytic *R. albus* KU-F152 with non-fibrolytic *S. ruminantium* S137 in *in vivo* study.

Keywords: coculture, *Ruminococcus albus* KU-F152, *Selenomonas ruminantium* S137, fiber digestibility, fermentation products, buffaloes, *Bubalus bubalis*

INTRODUCTION

Rumen microbial is important for

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forage digestion and fermentation in the rumen of ruminant. The rumen microbial ecosystem consists of bacteria, protozoa, fungi, and archaea (Hespell *et al*., 1997; Koike and Kobayashi, 2009). More than 90% of microbial in the rumen was active for plant fiber digestion (Van Soest, 2006). Nutritive interactions including hydrogen transfer and cross-feeding of fermentation products, derived from fiber degradation, are important to maintain fibrolytic activity (Flint, 1997). The fibrolytic rumen bacteria such as *Fibrobacter succinogenes*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Butyrivibrio fibrisolvens* and *Prevotella ruminicola* play a major role in ruminal fiber digestion (Weimer, 1996). Wanapat (2000) reported that buffaloes, could utilize fiber with higher efficiency and fiber digestibility than cattle, probably because buffaloes have a higher population of cellulolytic bacteria (*R. albus*) to recycle nitrogen compared with bovine rumen.

Ruminococcus albus KU-F152 is a fibrolytic rumen bacterium, which was isolated from buffalo rumen (Poonko, 2014). It belongs to a family of anaerobic gram-positive cocci, which produces acetate, succinate, lactate, ethanol, hydrogen, and carbon dioxide from cellobiose. Therefore, *R. albus* KU-F152 is considered important for fiber degradation in the buffalo rumen (Shi and Weimer, 1996). Previous studies have demonstrated fibrolytic interaction of various bacterial combinations such as *R. flavefaciens* or *F. succinogenes* with non-fibrolytic *S. ruminantium* or *P. ruminicola* (Fondevila and Dehority, 1996; Sawanon and Kobayashi, 2006). They found interesting data about non-fibrolytic bacteria and fibrolytic bacteria relationship in fiber degradation in the rumen, as non-fibrolytic bacteria species can activate fibrolytic bacteria through nutritive interactions including hydrogen transfer or cross-feeding of degradation and fermentation products derived from plant fiber (Flint, 1997; Koike *et al*., 2012; Kudo *et al*., 1987). On the other hand, the fiber-associated bacterial community consists of not only fibrolytic species but also non-fibrolytic species and fiber degradation would be accelerated by interactions between these fibrolytic and non-fibrolytic bacteria (Brulc *et al*., 2009). Wolin *et al*. (1997) reported that fibrolytic bacteria such as *F. succinogenes*, *R. flavefaciens* and *R. albus* produced succinate during fiber digestion; however succinate does not accumulate in the rumen because non-fibrolytic bacteria such as *S. ruminantium* converts succinate (succinate-decarboxylation) into propionate.

Selenomonas ruminantium S137 is a non-fibrolytic species gram-negative rumen bacterium isolated from sheep rumen (Sawanon and Kobayashi, 2006). Studies of Avicel digestion and associated acid production by *F. succinogenes* and its coculture with *S. ruminantium* isolates showed that Avicel digestibility was increased highest by *S. ruminantium* S137 when compared with another *S. ruminantium* isolate (28.1% for *F. succinogenes* monoculture vs. 34.7% for the coculture). In addition, *S. ruminantium* S137 ferments carbohydrates mainly to lactate, propionate, acetate, and carbon dioxide. This strains decarboxylates succinate to propionate and CO_2 in the rumen (Sawanon *et al*., 2011).

Rice straw is the most important agricultural by-product for cattle in Thailand. However, its application in livestock is limited by relatively lower crude protein (CP) and nutrients digestibility, which is caused by the high lignification and silicification (Sarnklong *et al*., 2010). The slow and limited ruminal degradation is the main deficiency of rice straw and thus affects bacterial population in the rumen (Van Soest, 2006).

Para grass (*Brachiaria mutica*) is a common forage type in Thailand that contains a higher CP content than rice straw. It has been utilized extensively for grazing or cutting and it can be preserved as hay for feeding cattle during dry seasons (Phaikaew *et al*., 1997). However, farmers use rice straw as the main forage for ruminant feed, because there are limited lands available for grazing ruminants in the region (Sarnklong *et al*., 2010). Therefore, this *in vitro* study was to investigate the interaction between fibrolytic *R. albus* KU-F152 and non-fibrolytic *S. ruminantium* S137 on improvement fiber digestibility and fermentation products of rice straw and para grass.

MATERIALS AND METHODS

Bacteria strains and medium

R. albus KU-F152 as a fibrolytic rumen bacterium was isolated from the rumen of buffalo (Poonko, 2014). *R. albus* type strain 7 was received from Professor Yasuo Kobayashi, research faculty of Agriculture, Hokkaido University, Japan. *S. ruminantium* S137 as a non-fibrolytic rumen bacterium was isolated from the rumen of sheep (Sawanon and Kobayashi, 2006) and both of *R. albus* KU-F152, *R. albus* type strain 7 and *S. ruminantium* S137 were used in this study.

Basal medium for fermentation studies was prepared anaerobically for maintaining bacteria which contains glucose and cellobiose 0.2% (w/v). One hundred ml of basal medium was prepared, following the composition: 7.5 ml of mineral solution I (0.6 g of K_2HPO_4 to 100 ml of distilled water) and mineral solution II (1.2 g of NaCl, 1.2 g of $(NH_4)_2SO_4$, 0.6 g of KH_2PO_4, 1.2 g of $CaCl_2$, 0.25 g of $MgSO_4 \cdot 7H_2O$ and 100 ml of distilled water), 0.1 ml of 0.1% resazurin, 0.1 g of L-cysteine-HCl·H_2O, 0.2 g of bactopeptone, 0.12 g of yeast extract, 0.1 g of glucose, 0.1 g of cellobiose, 30 ml of clarified rumen fluid, 50 ml of distilled water, and adjust the pH to 6.8 with 1 N NaOH before add 5 ml of 8% Na_2CO_3 (Sarnklong *et al*., 2010).

Mixed rumen microflora was prepared in this study; containing rumen fluid of Brahman crossbred cattle was diluted at 1:1 ratio in McDougall's Buffer (per liter supplemented with 9.8 g of $NaHCO_3$, 2.44 g of Na_2HPO_4, 0.57 g of KCl, 0.47 g of NaCl, 0.12 g of $MgSO_4 \cdot 7H_2O$, 0.16 g of $CaCl_2 \cdot H_2O$) in an anaerobic chamber (McDougall, 1948).

Fibers, innocula and incubation conditions

Rice straw was collected from local farms near the Kamphaeng Saen City, Nakorn Pathom province, zone of central Thailand and Para grass (*Brachiaria mutica*) was collected from the farm in Kasetsart University, Kamphaeng Saen Campus, Nakorn Pathom province, Thailand. All samples were used for the measurement of fiber digestibility. Rice straw and para grass were chopped into 3 to 5 cm lengths and air-dried in an oven at 60°C for 2 days. The fibers, ground by hammer mill, were passed through a 1 mm screen and stored at room temperature until analysis. The chemical compositions of rice straw and para grass are presented in Table 1.

In vitro digestibility was determined from forage samples using filter bags 'ANKOM F57'. The bag was 50x55 mm, made from polyester/ polyethylene extruded filaments in a three dimensional matrix claimed to retain particles >25 microns (ANKOM Technology, Macedon, New York, USA). It was pre-rinsed in acetone (3 to 5 minutes) and completely air dried at 100°C for 5 h in order to remove surfactant that inhibits microbial

Table 1. Chemical composition of rice straw and para grass in experiment.

Item	Fiber sources	
	Rice straw	Para grass
Chemical composition (% DM basis)		
Moisture	4.63	7.66
DM	95.37	92.34
CP	5.48	12.97
CF	31.06	30.21
EE	1.89	0.85
Ash	11.61	11.04
Ca	0.36	0.65
P	0.08	0.27
NFE	45.33	37.27
TDN	48.85	50.38
NDF	66.83	68.84
ADF	42.24	44.20

DM, dry matter; CP, crude protein; CF, crude fiber; EE, ether extract; Ca, calcium; P, phosphorus; NFE, nitrogen free extract; TDN, total digestible nutrients; NDF, neutral detergent fiber; ADF, acid detergent fiber.

digestion (Lattimer *et al*., 2007). After drying, the weight of filter bags was recorded. Samples of rice straw and para grass were weighed with air-dried sample (1 g) added to filter bags. The bags were sealed by sealing machine (Model: PFS-300, 220V, 50 Hz, 400W, Guangdong, China) and transferred to a forced-air oven at 100°C for 24 h, after which the weight was recorded. Three replicates of the filter bags for each forage were placed in each of the bottle containing medium.

R. albus KU-F152 was grown at 38°C for 24 h in basal medium containing 0.2% (w/v) rice straw as the carbon source. The bacteria was subcultured ten times consecutively with basal medium, after ten passages the culture was centrifuged (1000×g, 4°C, 5 minutes) for separating the rice straw particles and collecting supernatant. The supernatant was centrifuged (3000×g, 4°C, 10 minutes) to collect bacteria pellets and the pellet to suspend in anaerobic dilution solution (Bryant and Burkey, 1959) to adjust OD_{660} at 0.2 for used as inoculate.

S. ruminantium S137 was grown at 38°C for 4 h in basal medium containing 0.2% (w/v) glucose as a carbon source. *S. ruminantium* S137 was subcultured three times consecutively with basal medium. After three passages, the culture was centrifuged to collect the bacteria pellet. The bacteria pellet was suspended in anaerobic dilution solution to optical density at OD_{660} at 0.2 to be used for inoculation (Sawanon and Kobayashi, 2006). The preparations for inoculums (3 ml for monoculture and 1.5 ml for coculture of *R. albus* KU-F152 and *S. ruminantium* S137) was added

to 300 ml of basal medium and mixed rumen microflora (three filter bags for each of forage) and were placed in each of the bottles under anaerobic condition. Two test bottles of forage for respective monoculture and coculture were incubated at 38°C for 72 h. The bottles of media without inoculum were used as a blank and treated in the same manner.

Measurement of DM digestion and metabolites

After 72 h incubation, the cultures had their pH measured immediately by a portable pH meter (Oakton pH Testr 30, USA), and were centrifuged (4000×g, 4°C for 10 minutes) to collect the supernatant for the measurement of ammonia-nitrogen (NH_3-N) using spectrophotometer (Thermo Scientific, Helios Zeta ultraviolet-visible (UV-VIS) model, USA). Volatile fatty acid (VFA) was determined by gas chromatograph (TRACE™ 1300, Thermo Scientific, China). Filter bags were washed and transferred to be air dried at 100°C for 48 h before being weighed and analyzed for apparent DM digestibility and neutral detergent fiber (NDF). NDF was determined according to the methods of Goering and Van Soest (1970).

Statistical analyses

The data (n=5) on DM and NDF digestibility, VFA, NH_3-N and pH were subjected to one-way analysis of variance. When the effect of fiber source or culture was detected, differences between fiber sources or culture were evaluated by Duncan's new multiple range test using R version 3.2.3 software (R Team., 2015). A 2×5 factorial in completely randomized design was applied to evaluate pairwise comparisons of fiber sources or culture types. Statistical significance was declared at $P<0.05$.

RESULTS AND DISCUSSION

Dry matter digestibility

The results of dry matter (DM) digestion of rice straw and para grass by *R. albus* KU-F152 and *S. ruminantium* S137 in basal medium and mixed rumen micoflora are shown in Figure 1. For DM digestion of monocultures of *R. albus* KU-F152, *S. ruminantium* S137 and *R. albus* type strain 7 of rice straw in basal medium (0.22, 18.19 and 17.14%, respectively) and para grass (0.25, 23.52, and 20.84%, respectively), the results showed that the amount of rice straw and para grass digested in bacteria of monoculture was lower than that in coculture. In addition, non-fibrolytic *S. ruminantium* S137 bacteria digested less fiber content compared with other bacteria species ($P<0.01$). For coculture of *R. albus* KU-F152 with *S. ruminantium* S137, DM digestion was significantly higher in basal medium and mixed rumen microflora ($P<0.01$).However, DM digestion in the culture was greater for para grass compared with rice straw ($P<0.01$), and the combination of *R. albus* KU-F152 with *S. ruminantium* S137 had significantly higher digestibility ($P<0.01$) when compared with the coculture of *R. albus* type strain 7 with *S. ruminantium* S137. Sawanon and Kobayashi (2006) reported that the combination of fibrolytic and non-fibrolytic bacteria increased fiber digestion. Although the effect of fiber digestibility depends on the selection of non-fibolytic bacterial strain, there was evidence that *S. ruminantium* S137 had higher fiber digestion when combined with *R. flavefaciens* because *S. ruminantium* S137 had high efficiency in utilization of cellodextrins and succinate. Accordingly, Fondevila and Dehority (1996) reported that when a non-fibrolytic *P. ruminicola* strain was cocultured with *F. succinogenes* or *R. flavefaciens*,

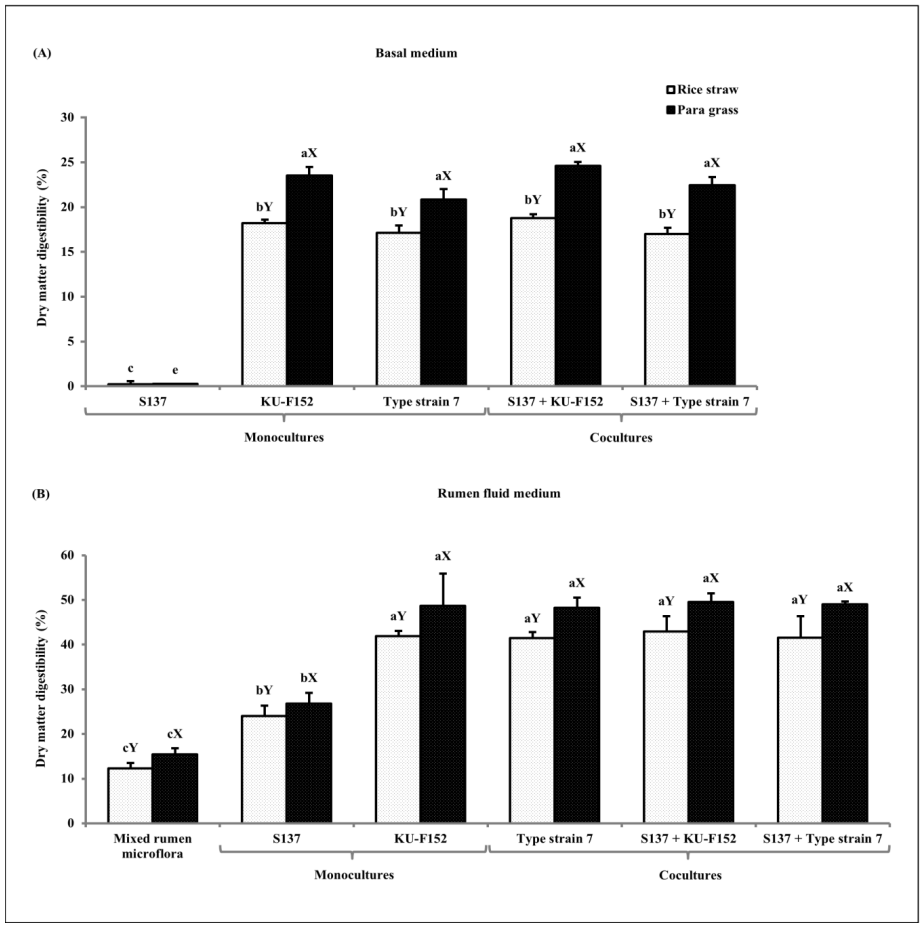

Figure 1. Effect of inoculated fibrolytic *R. albus* KU-F152, *R. albus* type strain 7 and non-fybrolytic *S. ruminantium* S137 on DM digestibility in basal medium (A) and mixed rumen microflora (B) *in vitro*. Data are shown as mean±S.E (n=5). $^{a\text{-}e}$Means followed by different letters indicate differences with significance between bacterial strains ($P<0.05$). $^{X\text{-}Y}$Means followed by different letters indicate differences with significance between rice straw and para grass ($P<0.05$).

fiber digestion was improved when compared with the fibrolytic species alone. Consistent with that reported by Koike *et al*. (2012) they suggested that the consumption of D-lactate and succinate by *S. ruminantium* S137 could improve the growth of strains R-25 and *F. succinogenes* S85, resulting in increased digestion in the triculture.

The digestibility of NDF of *R. albus* KU-F152 and *S. ruminantium* S137 in monoculture or coculture was shown in Figure 2. Because NDF digestion was an important characteristic to ruminants, the assessment of digestibility of forages give us more understanding of digest potential. A significant difference between monoculture and coculture was observed for NDF ($P<0.01$). In the coculture of *R. albus* KU-F152 with *S. ruminantium* S137, NDF digestibility was significantly higher than that of monoculture in both cultures ($P<0.01$). The NDF digestibility was found higher for para grass in both monoculture (3.78, 26.08, 24.62, 29.08, and 28.98% for S137, KU-F152, type strain 7, S137+KU-F152 and

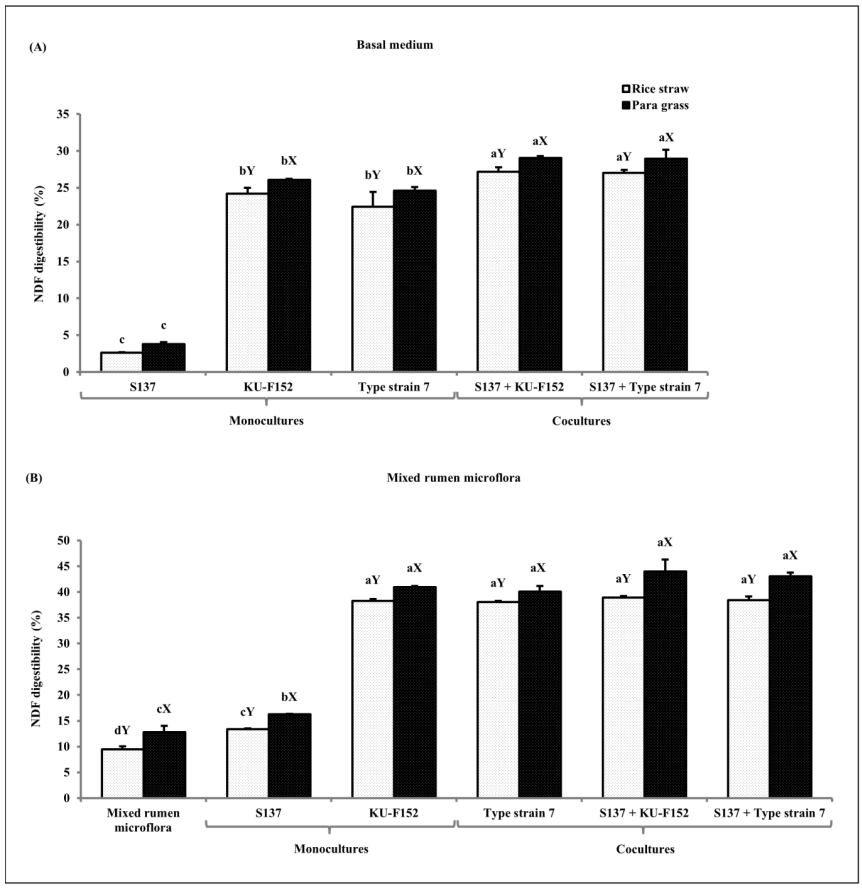

Figure 2. Effect of inoculate fibrolytic *R. albus* KU-F152, *R. albus* type strain 7 and non-fybrolytic *S. ruminantium* S137 on NDF digestibility in basal medium (A) and mixed rumen microflora (B) *in vitro*. Data are shown as mean±S.E (n=5). $^{a-d}$Means followed by different letters indicate between significant differences between bacterial strains ($P<0.05$). $^{X-Y}$Means followed by different letters indicate differences with significance between rice straw and para grass ($P<0.05$).

S137+type strain 7, respectively) and coculture (12.78, 16.29, 40.98, 40.08, 43.99, and 43.06% for mixed rumen microflora, S137, KU-F152, Type strain 7, S137+KU-F152 and S137+Type strain 7, respectively) compared with that in rice straw ($P<0.01$). Therefore, monoculture of *R. albus* KU-F152 gave higher NDF digestibility from forages than other species and also found that in the rice straw there was low NDF digestibility because it had high crude fiber content (31.06%) shown in the Table 1. While digestibility of NDF depends on pH value, it was found that the delay in NDF digestion increased pH value. On the other hand, when pH value decreased, NDF digestion also decreased (Weimer, 1996).

Fermentation parameters

Characteristics of fermentation including pH, NH_3N and VFA in the basal medium and mixed rumen microflora were measured for 72 h. The effects of *R. albus* KU-F152 and *S. ruminantium* S137 on the pH values of basal medium and mixed

rumen microflora fermentation shown in the Figure 3. The *R. albus* KU-F152 and *S. ruminantium* S137 were both cultures with the same initial pH 6.8. After 72 h incubation, the pH value of basal medium decreased from 6.8 to 5.8. On the other hand, the pH of the mixed rumen microflora decreased only by 0.21. In addition, there was no significant difference in the pH values between rice straw and para grass ($P<0.05$) (Figure 3). In the study of Roger *et al*. (1990) the *R. flavefaciens* addition to fiber showed it to remain stable between pH 6.0 and 7.0. This was not similar to *F. succinogenes* bacteria pH, which increased approximately 4.5 to 6.0 in basal medium. Yet, the addition of *R. albus* resulted in the pH decreasing from 5.5 to 6.0 in basal medium as confirmed in this study. On the other hand, effects of pH on the combination of fibrolytic bacteria (*F. succinogenes*, *R. flavefaciens* and *R. albus*) to rice straw was clearly inhibited when the pH was lower than 6.0 (Sung *et al*., 2007). Thus, the results of this study indicated that even modest declines in pH could have a negative impact on ruminal fiber digestion and fermentation.

Ammonia-nitrogen (NH_3-N) in basal medium and mixed rumen microflora culture was assessed to provide information about the

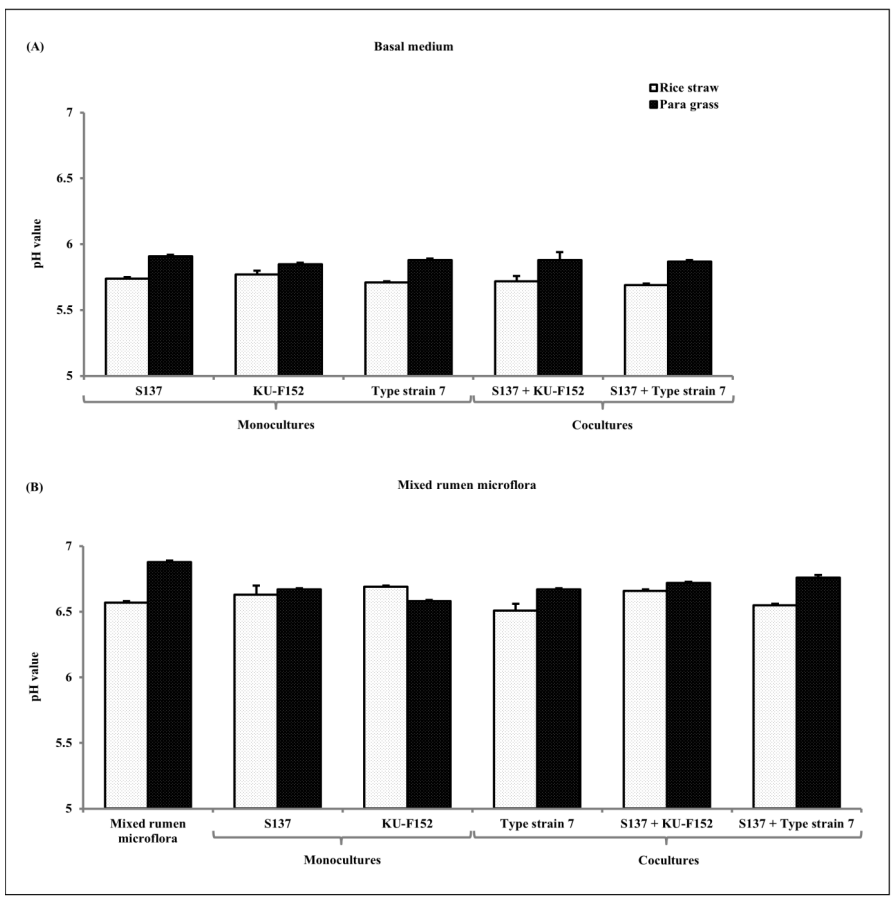

Figure 3. Effect of inoculated fibrolytic *R. albus* KU-F152, *R. albus* type strain 7 and non-fybrolytic *S. ruminantium* S137 on pH value in basal medium (A) and mixed rumen microflora (B) *in vitro*. Data are shown as mean±S.E. (n=5).

combination between fibolytic and non-fibrolytic bacteria in the rice straw and papa grass as substrates. Concentrations of NH_3-N were lowered (P<0.01) in non-fibrolytic of *S. ruminantium* S137 when compared with fibrolytic bacterial *R. albus* KU-F152 and *R. albus* type strain 7 (Figure 4). Coculture of *R. albus* KU-F152 with *S. ruminantium* S137 was found NH_3-N highest (34.38 mg/l) in mixed rumen microflora. The level of the NH_3-N was not significantly different between types of forages (P<0.05) in basal medium, yet in mixed rumen microflora, NH_3-N in para grass was higher than that in rice straw of fibrolytic bacteria (*R. albus* KU-F152 and *R. albus* type strain 7) and coculture with *S. ruminantium* S137 with significant difference (P<0.01). High consumption of DM intake may affect fermentation and concentrations of NH_3-N. Normally, the optimal of NH_3-N in rumen is 20 to 50 mg/l (Newbold and Rust, 1992). Also, in this study, the average concentrations of

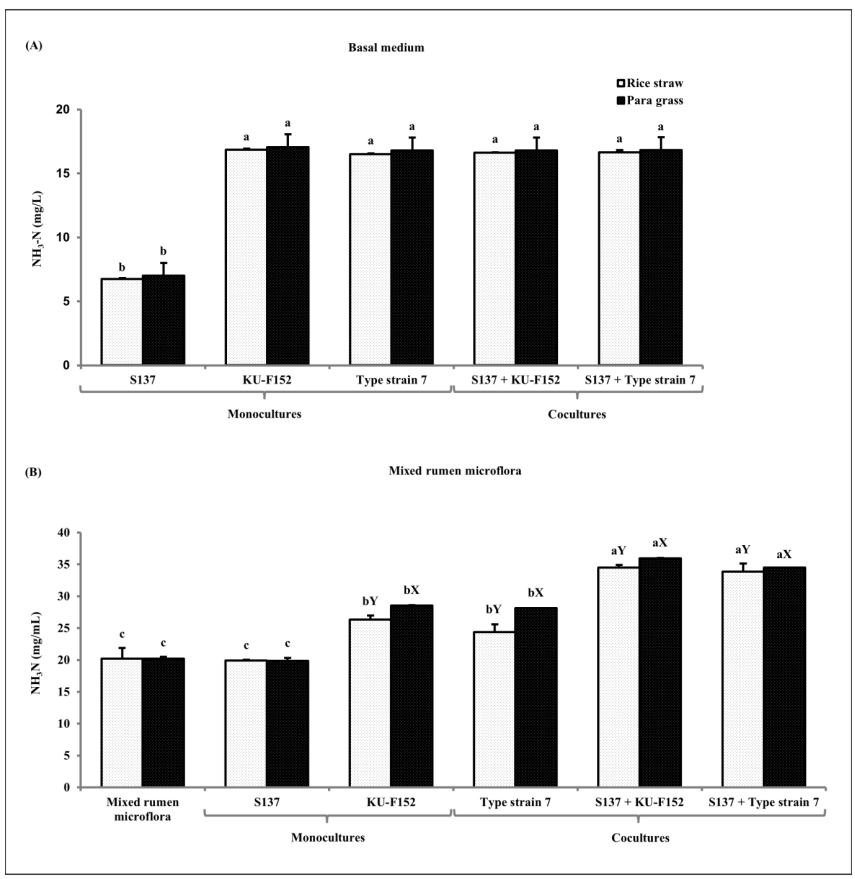

Figure 4. Effect of inoculated fibrolytic *R. albus* KU-F152, *R. albus* type strain 7 and non-fybrolytic *S. ruminantium* S137 to concentrate ratio of ammonia-nitrogen (NH_3-N) in basal medium (A) and mixed rumen microflora (B) *in vitro*. Data are shown as mean±S.E. (n=5). $^{a-d}$Means followed by different letters indicate between differences with significance between bacterial strains (P< 0.05). $^{X-Y}$Means followed by different letters indicate differences with significance between rice straw and para grass (P< 0.05).

NH_3-N was 7 to 17 mg/l in basal medium and 20 to 36 mg/l in mixed rumen microflora after 72 h of incubation. The combinations of coculture of *R. albus* KU-F152 with *S. ruminantium* S137 resulted in higher concentrations of NH_3-N on para grass when compared with rice straw in mixed rumen microflora (Figure 4). The result could be due to para grass structure and crude fiber digestion from degradation by the fermentation of *R. albus* KU-F152 with *S. ruminantium* S137.

The volatile fatty acid (VFA) concentrations in basal medium and mixed rumen micoflora are shown in Table 2 and 3. There were no differences ($P>0.05$) in total of VFA concentrations or acetate (C2), propionate (C3), butyrate (C4), iso-valerate (iso-C5), valerate (C5) and acetate to propionate (C2:C3) ratio in rice straw and para grass. However, coculture of *R. albus* KU-F152 with *S. ruminantium* S137 significantly increased total VFA, acetate and propionate products ($P<0.01$). This difference did not have any effect on the acetate to propionate (C2:C3) ratio. Nevertheless, concentration of propionate was higher in monoculture of *S. ruminantium* S137 when compared with the control group of mixed rumen microflora. Accordingly, previous research has reported that *S. ruminantium* S137 had highest propionate production in the coculture with *R. flavefaciens*. The combinations of *S. ruminantium* S137 and *F. succinogenes* showed activity of decarboxylating succinate to produce propionate. This *S. ruminantium* demonstrated the ability to utilize cellodextrin and it could make a good partner with cellodextrin producers such as *F. succinogenes* (Sawanon and Kobayashi, 2006). In addition, the sources of inoculums had no effect on total VFA from rice straw and para grass, thus demonstrated that ruminal fibrolytic bacterial could not certainly adapt to low pH (Russell and Wilson, 1996).

CONCLUSIONS

We have investigated the interaction between fibrolytic *R. albus* KU-F152 and non-fibrolytic *S. ruminantium* S137 on fiber digestibility and fermentation products of rice straw and para grass. These results suggest that there is a synergistic relationship between fibrolytic *R. albus* KU-F152, *R. albus* type strain 7 and non-fibrolytic *S. ruminantium* S137 as enhancement of fiber digestion were increase VFA production, especially propionate production and improvement in fiber digestion. A close association between fibrolytic bacteria *R. albus* KU-F152 and non-fibrolytic *S. ruminantium* S137 can enhance the adhesion or ingress of the fibrolytic bacteria into the plan cell. Further studies are required to develop and apply coculture of fibrolytic *R. albus* KU-F152 with non-fibrolytic *S. ruminantium* S137 for improving fiber digestibility and fermentation production in *in vivo* study.

Conflict of interest

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

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Table 2. Effect of inoculate fibrolytic *R. albus* KU-F152, *R. albus* type strain 7 and non-fybrolytic *S. ruminantium* S137 to concentrate ratio on volatile fatty acid (VFA) in basal medium (n=5).

Item	Roughage	Bacteria strain					SD	S.E.M	P-value
		S.137	KU-F152	Type strain 7	S.137+KU-F152	S.137+Type strain 7			
VFA (mM/L)									
Total VFA	Rice straw	9.38^{c}	25.39^{bY}	24.30^{bY}	29.83^{aY}	28.11^{aY}	0.29	0.38	<0.01
	Para grass	10.97^{c}	29.48^{bX}	29.13^{bX}	32.43^{aX}	31.87^{aX}	0.21	0.28	<0.01
	P-value	0.31	0.02	0.03	0.03	0.03			
Acetate (C2)	Rice straw	4.0^{c}	12.92^{b}	12.91^{b}	14.99^{a}	14.53^{ab}	0.39	0.28	<0.01
	Para grass	5.3^{d}	13.40^{bc}	13.00^{c}	15.96^{a}	15.50^{ab}	0.44	0.40	<0.01
	P-value	0.24	0.87	0.99	0.64	0.20			
Propionate (C3)	Rice straw	3.6^{b}	8.85^{a}	8.48^{a}	9.66^{a}	9.30^{a}	0.35	0.30	<0.01
	Para grass	3.12^{c}	8.42^{b}	8.14^{b}	11.06^{a}	10.51^{a}	0.27	0.18	<0.01
	P-value	0.54	0.47	0.94	0.50	0.32			
Butyrate (C4)	Rice straw	0.88	2.35^{a}	2.06	2.65	2.61	0.45	0.27	0.13
	Para grass	0.91^{b}	2.54^{a}	2.50^{a}	3.12^{a}	3.02^{a}	0.27	0.18	0.02
	P-value	0.39	0.76	0.31	0.74	0.62			
Valerate (C5)	Rice straw	0.14^{c}	0.35^{ab}	0.32^{b}	0.44^{a}	0.37^{ab}	0.02	0.01	<0.01
	Para grass	0.18	0.37	0.27	0.33	0.32	0.04	0.02	0.15
	P-value	0.22	0.91	0.62	0.54	0.73			
Iso-valerate (I-C5)	Rice straw	0.09^{c}	0.19^{b}	0.19^{b}	0.25^{a}	0.25^{a}	0.01	0.01	<0.01
	Para grass	0.10	0.31	0.29	0.29	0.33	0.04	2.23	0.10
	P-value	0.87	0.21	0.43	0.68	0.48			
C2:C3	Rice straw	1.13	1.46	1.52	1.55	1.56	0.17	0.05	0.13
	Para grass	1.72	1.60	1.59	1.44	1.47	0.11	0.06	0.46
	P-value	0.07	0.51	0.50	0.34	0.20			

[a-c]Means with different superscripts within the same row are significantly different (P<0.05).

[X-Y]Means with different superscripts within the same column are significantly different (P<0.05).

Table 3. Effect of inoculate fibrolytic *R. albus* KU-F152, *R. albus* type strain 7 and non-fybrolytic *S. ruminantium* S137 to concentrate ratio on volatile fatty acid (VFA) in mixed rumen microflora (n=5).

Item	Roughage	Mixed rumen microflora	Bacteria strain					SD	S.E.M	P-value
			S.137	KU-F152	Type strain 7	S.137+KU-F152	S.137+Type strain 7			
VFA (mM/L)										
Total VFA	Rice straw	21.07^{c}	22.69^{c}	52.95^{b}	50.76^{b}	59.62^{a}	59.41^{a}	1.69	0.41	<0.01
	Para grass	22.16^{d}	23.05^{d}	53.60^{b}	50.92^{c}	63.85^{a}	63.22^{ab}	2.68	1.65	<0.01
	P-value	0.88	0.76	0.35	0.22	0.65	0.18			
Acetate (C2)	Rice straw	9.28^{b}	10.62^{b}	33.33^{a}	31.68^{a}	37.10^{a}	36.86^{a}	2.20	1.28	<0.01
	Para grass	10.60^{b}	11.11^{b}	30.95^{a}	29.40^{a}	38.78^{a}	38.12^{a}	2.69	1.52	<0.01
	P-value	0.77	0.88	0.38	0.47	0.70	0.76			
Propionate (C3)	Rice straw	5.33^{c}	6.01^{c}	10.78^{b}	10.69^{b}	13.17^{a}	13.16^{a}	0.34	0.23	<0.01
	Para grass	5.88^{c}	6.10^{c}	12.86^{b}	12.31^{b}	14.53^{a}	14.23^{a}	0.43	0.21	<0.01
	P-value	0.20	0.79	0.41	0.40	0.10	0.21			
Butyrate (C4)	Rice straw	3.47^{b}	3.33^{b}	5.69^{a}	5.42^{a}	6.43^{a}	6.14^{a}	0.26	0.20	<0.01
	Para grass	2.94^{b}	3.53^{b}	6.53^{a}	6.18^{a}	7.47^{a}	7.39^{a}	0.58	0.31	<0.01
	P-value	0.36	0.95	0.74	0.44	0.34	0.20			
Valerate (C5)	Rice straw	0.46^{b}	0.44^{b}	0.60^{abY}	0.53^{abY}	0.67^{aY}	0.66^{aY}	0.04	0.02	0.06
	Para grass	0.57^{b}	0.53^{b}	0.85^{aX}	0.88^{aX}	0.98^{aX}	0.97^{aX}	0.04	0.04	0.02
	P-value	0.61	0.23	0.12	0.10	0.14	0.20			
Iso-valerate (I-C5)	Rice straw	0.25^{d}	0.22^{d}	0.35^{c}	0.37^{bc}	0.43^{a}	0.42^{ab}	0.01	0.01	<0.01
	Para grass	0.26^{b}	0.22^{b}	0.39^{a}	0.36^{a}	0.44^{a}	0.43^{a}	0.02	0.02	<0.01
	P-value	0.90	0.97	0.36	0.80	0.24	0.31			
C2:C3	Rice straw	1.65^{c}	1.72^{c}	3.05^{a}	2.65^{b}	2.88^{ab}	2.43^{b}	0.54	0.14	<0.01
	Para grass	1.80^{b}	1.83^{b}	2.41^{ab}	2.39^{ab}	2.67^{a}	2.68^{a}	0.36	0.04	0.05
	P-value	0.57	0.07	0.17	0.06	0.63	0.60			

$^{a-d}$Means with different superscripts within the same row are significantly different (P<0.05).

$^{X-Y}$Means with different superscripts within the same column are significantly different (P<0.05).

providing *Ruminococcus albus* type strain 7 for the research.

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