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ORIGINAL ARTICLE

Effects of graded levels of liquid brewer's yeast on chemical composition and fermentation quality in cassava pulp and rice straw-based total mixed ration silage

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ABSTRACT

This study aimed to evaluate the effect of liquid brewer's yeast (LBY) addition on chemical composition and fermentation quality of mixture of LBY and cassava pulp (CVP) with rice straw (RS) in different ratios during preservation periods. Four mixtures of LBY, CVP and RS were made, that is mixture ratio of LBY : CVP : RS of 0% LBY, 20% LBY, 35% LBY and 50% LBY were 0:70:30, 20:50:30, 35:35:30 and 50:20:30 as fresh matter, respectively. The bags were opened at weeks 0, 1, 2, 4 and 8 after storage. The contents of dry matter, organic matter, crude protein (CP), ether extract (EE), neutral detergent fiber and acid detergent fiber ranged 36.4-40.0, 88.9-90.8, 4.0-12.0, 1.1-1.3, 58.8-61.6 and 37.6-40.0, respectively, and the contents of CP and EE increased and the other components decreased in proportion to LBY inclusion ($P < 0.01$). 50% LBY had the highest ($P < 0.05$) pH (4.81) and ammonia nitrogen per total nitrogen (NH_3-N/TN) (7.40%) and the lowest V-score (90.3). Propionic and butyric acid contents were 0.01% or lower in each mixture and storage period. There were rapid pH decrease and NH_3-N/TN increase during the first week of the storage period. The increases of NH_3-N/TN and acetic acid content and decreases of pH, lactic acid content and V-score during the preservation were more drastic as LBY inclusion increased. Although higher proportion of LBY produced higher CP and lower fiber contents in the mixture, attention should be paid for the reduction of fermentation quality during longer storage periods.

Key words: *cassava pulp, chemical composition, fermentation quality, liquid brewer's yeast, rice straw.*

INTRODUCTION

Feeding costs for small dairy farmers in northeast Thailand represent more than 70% of total costs (Lapar *et al.* 2005). There is a growing concern with increasing prices of imported feed ingredients, for example soybean meal and maize grain, causing cost of production to continuously rise under current productivity levels. It is therefore essential to reduce the cost of feeding by utilizing food by-products. Agricultural and industrial by-products are economical alternatives for feeding livestock, with relatively lower prices than those of commercial concentrate. Cassava (*Manihotesculenta* Crantz.) pulp (CVP), produced in large amounts as a by-product of starch manufacturing, is a major biomass resource for animal feeding. In 2013, approximately 13.5 million tonnes of fresh cassava roots were produced from the fields in northeast Thailand (OAE 2014), and 10-15% of cassava pulp is produced as a by-product from the cassava root in starch manufacturing (Yimmongkol 2009). It contains a high moisture content of approximately 80% (Jintanawit *et al.* 2006), starch and cellulose approximately of 60 and 20% on a dry matter (DM) basis, respectively (Kosugi *et al.* 2009), but low in crude protein (CP) content at 2.4% on a DM basis (Kosoom *et al.* 2009). Liquid brewer's yeast (LBY) is obtained by removal of yeast after fermentation during brewing for beer. *Saccharomyces cerevisiae* is the prevalent yeast species involved in brewing (Manzano *et al.* 2005). One of the major factories in northeast Thailand has beer production capacity of 700 million L/year (KKB 2015). It is estimated that about 1000 m^3 LBY per month is produced, and 40% of this amount would be sent to the farmers around the beer factory (personal

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communication). To increase protein content, CVP can be mixed with LBY, containing 10-14% DM and 40-50% CP on a DM basis (Grieve 1979), which is available for small dairy farms around brewers' factories.

However, the mixture of CVP and LBY is high in moisture, which is also a major factor influencing fermentation quality and losses of DM, water soluble carbohydrate (WSC), hemicellulose and cellulose during the preservation (Yahaya *et al*. 2002). To improve the fermentation quality and reduce effluent losses of high moisture silage, addition of dry absorbent to the mixture of LBY and CVP might be suitable to minimize the risk of effluent and preservation problems. Rice straw (RS) is the main crop residue which farmers usually store as ruminant feed in Asian countries. The feeding of only RS does not provide enough nutrients to the ruminant animal to maintain high production levels (Sarnklong *et al*. 2010) due to its low nutritive value with low levels of protein, high fiber and lignin contents and low DM digestibility (Wanapat *et al*. 2009). On the other hand, adding RS as absorbent to potato pulp silage could prevent effluent losses and reduce silage DM loss during ensiling and improve fermentation quality (Zhang *et al*. 2012).

Total mixed ration (TMR) silage made by mixing wet by-products with roughage is in practice at dairy farms. This helps to omit the time of mixing before feeding, minimizes the risk of effluent production and avoids self-selection of feeds by animals (Wang & Nishino 2008). Thus, making TMR silage mixing RS with LBY and CVP can be developed in areas where starch and beer manufacturing factories are located. However, information on the optimal ratio of LBY and CVP with rice straw has not been studied yet. This study aimed to evaluate the effect of LBY addition on chemical composition and fermentation quality of in the mixture of LBY and CVP with RS in different ratios during preservation periods as a pilot study for TMR feeding for ruminants.

MATERIALS AND METHODS

Mixture preparation

The experiment was conducted in Khon Kaen Animal Nutrition Research and Development Center, Khon Kaen, Thailand in December 2013. RS was obtained from a paddy field in Khon Kaen Province. The straw was chopped into 5-10 cm pieces by a chaff cutter and manually mixed. Fresh CVP and LBY were obtained from starch and brewer factories located in Khon Kaen Province, respectively. Four mixtures with different levels of LBY were prepared. Ratios of LBY, CVP and RS in each mixture were 0:70:30 (0% LBY), 20:50:30 (20% LBY), 35:35:30 (35% LBY) and 50:20:30 (50% LBY) as fresh matter, respectively. The RS content as 30% aims to increase DM content of the mixture of LBY and CVP up to 40% and also increase nutritive value of RS for ruminant feed. Twenty composites of 500 g were made from each mixture and packed into 25 × 35 cm plastic bags. The bags were sealed using a vacuum sealer and stored at room temperature (30-32°C). Four bags from each mixture were opened at 0, 1, 2, 4 and 8 weeks after storage and offered for the following analyses.

Chemical analyses

Fifty gram samples from each composite of each sample was homogenized with 100 mL of distilled water and stored overnight at 4°C in a refrigerator. Then the homogenate was filtered through Whatman no.5 filter paper and the filtrate was used for pH, ammonia nitrogen (NH_3-N), lactic acid and volatile fatty acid (VFA) determination. The pH was directly measured by using a pH meter (D-22; Horiba, Kyoto, Japan). The NH_3-N content was determined using a steam distillation technique (Society of Utilization of Self Supplied Feeds 2009). Lactic acid and volatile fatty acid concentrations were determined by gas chromatography (Shimadzu GC2014, Shimadzu Corp., Kyoto, Japan) using a 25 m × 0.53 mm capillary column (BP21 0.5 P/N 054474, SGE Analytical Science Pty Ltd., Melbourne, Victoria, Australia) according to the method of Porter and Murray (2001). The flow rate of nitrogen as the carrier gas was 30 mL/min, injection temperature was 220°C and the temperature of the column and detector was 85°C. To assess the quality of the mixture, V-score was calculated from the NH_3-N/TN, acetic, propionic and butyric acid concentrations (Society of Utilization of Self Supplied Feeds 2009) using the following formula: A′ + B′ + C′, where A′ from VBN-N/TN (A%: $A' = 50$ if $A \leqq 5$, $A' = 60 - 2A$ if $5 < A < 10$), B′ from acetic acid plus propionic acid concentrations (B%: $B' = 10$ if $B \leqq 0.2$, $B' = (150 - 100B)/13$ if $0.2 < B < 1.5$) and C′ from butyric acid concentrations (C%: $C' = 40$ if $C = 0$, $C' = 40 - 80C$ if $0 < C < 0.5$). According to the scoring criteria, the samples were divided into three ranks: superior (81-100), good (60-80) and bad (<60) (Yang *et al*. 2014). The remaining mixing samples were oven dried at 60°C for 48 h. The dried samples were ground to pass through a 1 mm screen for the subsequent analyses. The contents of DM (on oven drying procedure at 135°C for 2 h), CP, ether extract (EE) and crude ash were analyzed according to the standards of the Association of Official Analytical Chemists (AOAC 2000; 930.15, 976.05, 920.39 and 942.05, respectively). The organic matter (OM) was calculated as weight loss through ashing. Neutral detergent fiber (NDFom) and acid detergent fiber (ADFom), expressed exclusive of residual ash without amylase treatment, and acid detergent lignin (ADL) were determined according to the methods of Van Soest *et al*. (1991).

Statistical analysis

The data were analyzed by the GLM procedure of SAS (1996) using the following mathematical model: $Y_{ijk} =$

$\mu + M_i + S_j + (MS)_{ij} + e_{ijk}$, where μ is overall mean, M_i is effect associated with the mixture ratios, S_j is effect associated with storage period, $(MS)_{ij}$ is interaction effect between mixture ratios and storage periods and e_{ijk} is residuals. Orthogonal polynomial contrast was used to examine the linear and quadratic responses of the mixture ratios of LBY and storage periods. The Tukey-Kramer test was used to detect the differences between the means for each data analysis (Kramer 1956). Significance was declared at $P < 0.05$.

RESULTS

Chemical composition of RS, LBY and CVP

During preservation mold was not found visually in all the mixtures. The chemical composition of RS, LBY and CVP are shown in Table 1. The RS had a low CP and high NDFom and ADFom contents showing 3.5, 75.5 and 45.8%, respectively, on a DM basis. The LBY had moderate acidity at pH 5.7 and high CP content at 53.9% on a DM basis, while NDFom, ADFom and ADL were not detected. The CVP had high acidity at pH 3.6 and low CP content at 3.0% on a DM basis with high NDFom and ADFom contents showing 35.8 and 25.7%, respectively.

Chemical composition of the mixtures

Changes in the chemical composition of four mixture ratios and five storage periods are shown in Table 2. The mixture ratio influenced all the chemical compositions ($P < 0.01$). The DM, OM, CP, EE, NDFom and ADFom contents ranged 36.4-40.0, 88.9-90.8, 4.0-12.0, 1.1-1.3, 58.8-61.6 and 37.6-40.0, respectively. Among the mixture ratio, the 0% LBY had the highest DM, OM, NDFom, ADFom and ADL contents followed by 20% LBY, 35% LBY and 50% LBY ($P < 0.05$). The 50% LBY had the highest ($P < 0.05$) CP and EE contents followed by 35% LBY, 20% LBY and 0% LBY.

Table 1 pH and chemical composition of experimental diets

Item	pH	DM	OM	CP	EE	NDFom	ADFom	ADL
		%	------------------------ % of dry matter ------------------------					
Liquid brewer's yeast	5.73	12.0	92.0	53.9	0.40	ND	ND	ND
Cassava pulp	3.63	20.0	91.3	3.0	0.26	35.8	25.7	5.1
Rice straw	-	88.8	87.9	3.5	1.33	75.5	45.8	3.4

DM, dry matter; OM, organic matter; CP, crude protein; EE, ether extract; NDFom, neutral detergent fiber expressed exclusive of residual ash; ADFom, acid detergent fiber expressed exclusive of residual ash and ADL, acid detergent lignin. ND, not detected.

Table 2 Chemical composition of mixture of liquid brewer's yeast (LBY) and cassava pulp (CVP) with rice straw (RS) at different mixture ratios and storage periods

Item	DM	OM[‡]	CP[‡]	EE[‡]	NDFom[‡]	ADFom[‡]	ADL[‡]
Mixture ratio							
0% LBY[†]	40.0[a]	90.8[a]	4.0[d]	1.08[c]	61.6[a]	40.0[a]	4.4[a]
20% LBY[†]	39.0[ab]	90.0[b]	6.8[c]	1.15[c]	61.0[ab]	39.3[a]	4.1[b]
35% LBY[†]	38.2[b]	89.6[b]	9.2[b]	1.22[b]	60.1[b]	38.2[b]	3.8[c]
50% LBY[†]	36.4[c]	88.9[c]	12.0[a]	1.32[a]	58.8[c]	37.6[b]	3.6[d]
P-value							
Mixture ratio (M)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Quadratic	0.14	0.37	0.46	0.32	0.30	0.74	0.59
Storage period (week)							
0	39.9[a]	90.0	7.9	1.22[a]	60.6	38.8	4.0
1	38.9[ab]	89.9	8.0	1.22[ab]	60.5	38.5	4.0
2	38.0[bc]	89.7	8.1	1.19[ab]	59.7	38.6	3.9
4	37.8[bc]	89.9	8.1	1.14[b]	60.5	39.0	4.0
8	37.4[c]	89.8	8.0	1.19[ab]	60.6	38.9	4.0
P-value							
Storage period (S)	<0.01	0.24	0.61	<0.05	0.19	0.61	0.73
Linear	<0.01	0.19	0.49	<0.05	0.86	0.40	0.94
Quadratic	0.15	0.16	0.14	0.31	0.08	0.48	0.86
P-value							
Interaction (M × S)	0.99	0.23	0.74	<0.05	0.95	0.59	0.94
SEM	0.64	0.21	0.15	0.041	0.64	0.53	0.11

[†]Mixture ratio of LBY : CVP : RS of 0% LBY, 20% LBY, 35% LBY and 50% LBY were 0:70:30, 20:50:30, 35:35:30 and 50:20:30 as fresh matter, respectively. [‡]Values were expressed as percentage of dry matter. OM, organic matter; CP, crude protein; EE, ether extract; NDFom, neutral detergent fiber expressed exclusive of residual ash; ADFom, acid detergent fiber expressed exclusive of residual ash and ADL, acid detergent lignin. SEM, standard error of the means. [a–d]Means with different superscripts within columns significantly differed ($P < 0.05$).

For the means of storage period from weeks 0 to 8, week 0 had the highest DM content, which linearly decreased ($P < 0.01$) as the storage period prolonged. Although the storage period influenced the EE content ($P < 0.05$), interaction effect between mixture ratios and storage periods was observed ($P < 0.05$). The 50% LBY maintained higher EE content during the storage period (Fig. 1).

Fermentation quality of the mixtures

Table 3 shows the changes of pH, NH_3-N/TN, lactic acid, VFA concentrations and V-score of the mixture during storage periods. The mixture ratio and storage period influenced pH, NH_3-N/TN, contents of lactic and acetic acids, and V-score ($P < 0.01$). Among the means of four mixture ratios, the pH and NH_3-N/TN contents ranged 4.1-4.8 and 3.2-7.4%, respectively, and the 50% LBY had the highest pH and NH_3-N/TN contents followed by 35% LBY, 20% LBY and 0% LBY ($P < 0.05$). The pH and NH_3-N/TN increased linearly and quadratically with the LBY content ($P < 0.01$). Lactic acid contents ranged between 0.8 and 1.0% for the mixtures. The 0% LBY had the lower acetic acid concentration than the other mixtures ($P < 0.05$) and increased as the amount of LBY inclusion increased ($P < 0.01$). The 0% LBY had the highest V-score followed by 20% LBY, 35% LBY and 50% LBY ($P < 0.05$). There was a rapid decrease in pH during the first week, from 4.9 to 4.4, highest in week 0 ($P < 0.05$) then followed by a slow decline until week 8, while week 0 had the lowest NH_3-N/TN ($P < 0.05$) at 3.5% and increased for the duration of

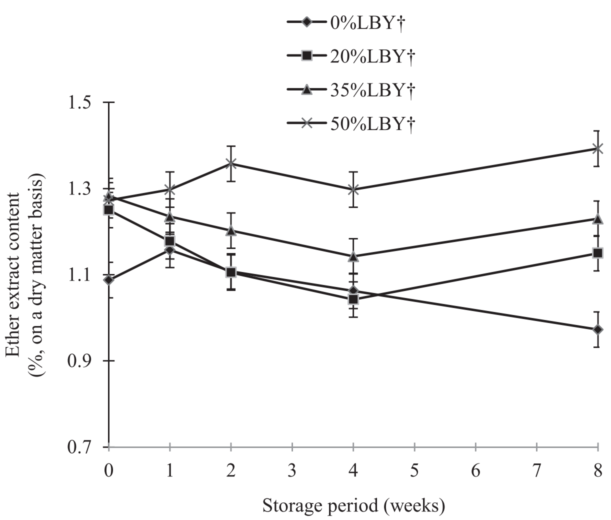

Figure 1 Ether extract contents showing interaction effect between mixture ratio and storage period. Bars are standard errors of the means. †Mixture ratio of LBY : CVP : RS of 0% LBY, 20% LBY, 35% LBY and 50% LBY were 0:70:30, 20:50:30, 35:35:30 and 50:20:30 as fresh matter, respectively. LBY, liquid brewer's yeast; CVP, cassava pulp; RS, rice straw

the storage period. The decline of pH and increase of NH_3-N/TN were linearly and quadratically affected by the storage period ($P < 0.01$). The highest ($P < 0.05$) lactic acid content was found in week 0 at 1.3% and lowest in week 8 at 0.5% ($P < 0.05$), which contrasted with acetic acid content: lowest in week 0 at 0.1% and highest in week 8 at 1.2% ($P < 0.05$).

Interaction effects between mixture ratios and storage periods on pH, NH_3-N/TN and lactic and acetic acid contents and V-score were identified ($P < 0.01$, Table 3, Fig. 2). The increase of NH_3-N/TN and acetic acid contents during the preservation were more acute as LBY inclusion increased. Propionic acid and butyric acid contents were 0.01% or lower in each mixture and storage period. Among the storage periods, V-score ranged from 88.6 to 100.0; week 0 had the highest V-score followed by weeks 1, 2, 4 and 8 ($P < 0.05$). The decrease of V-score during the preservation was more drastic as LBY inclusion increased.

DISCUSSION

In this study, a higher proportion of LBY in the mixture contained higher CP concentration while NDFom, ADFom and ADL concentrations were lower in the mixture (Table 2). Thus the low protein content of CVP and RS could be improved by mixing with LBY to 6.8, 9.2 and 12.0% of CP contents for 20% LBY, 35% LBY and 50% LBY, respectively. Nitipot *et al.* (2009) and Moore (2015) examined that chemical composition and digestibility of RS, LBY and CVP. The chemical compositions of the diets were similar to those used in the present study and the TDN contents were 44.0, 78.0 and 71.5 for RS, LBY and CVP, respectively. TDN contents in the mixture could be estimated as 63.3, 64.6, 65.5 and 66.5 for 0% LBY, 20% LBY, 35% LBY and 50% LBY, respectively, according to the TDN content of each diet examined by Nitipot *et al.* (2009) and Moore (2015). The diets containing 12% CP and 66.5% TDN should be suitable for dairy cattle producing 10 kg/day milk (NRC 2001). Thus feeding 50% LBY for dairy cattle can sustain minimum milk production in local conditions in northeast Thailand. The pH is considered as an important factor that influences the extent of fermentation and silage quality, since low pH ensures that the silage is retained in a stable form (Wang *et al.* 2011). In the present study, pH of all the mixture ratio means increased ($P < 0.01$) with increasing LBY mixture ratio (Table 3). This might have been due to the higher pH value of LBY. During the storage, pH was the highest at the start of fermentation (week 0) and decreased ($P < 0.01$) with the storage periods, similar to the study of Wang *et al.* (2011) who found that all tested treatments rapidly decreased pH within the first 7 days ensiling of green tea grounds silage, and that of Cao *et al.* (2011) who found sharply decreased pH of vegetable

Table 3 Fermentation quality of mixture of liquid brewer's yeast (LBY) and cassava pulp (CVP) with rice straw (RS) at different mixture ratios and storage periods

Item	pH	NH_3-N/TN[‡]	LA[‡]	AA[‡]	PA[‡]	BA[‡]	V-score
Mixture ratio							
0% LBY[†]	4.09[d]	3.15[d]	0.78[a]	0.53[b]	0.00	0.01	97.2[a]
20% LBY[†]	4.29[c]	5.32[c]	0.97[a]	0.72[a]	0.00	0.01	94.3[b]
35% LBY[†]	4.51[b]	6.19[b]	0.91[b]	0.68[a]	0.00	0.00	92.9[c]
50% LBY[†]	4.81[a]	7.40[a]	0.77[c]	0.73[a]	0.00	0.01	90.3[d]
P-value							
Mixture ratio (M)	<0.01	<0.01	<0.01	<0.01	0.04	0.02	<0.01
Linear	<0.01	<0.01	0.25	<0.01	0.12	0.23	<0.01
Quadratic	<0.01	<0.01	<0.01	<0.01	0.06	0.22	0.71
Storage period (weeks)							
0	4.92[a]	3.50[c]	1.25[a]	0.08[e]	0.00	0.01	100.0[a]
1	4.41[b]	5.50[b]	0.91[b]	0.47[d]	0.00	0.01	95.6[b]
2	4.30[c]	5.84[ab]	0.89[b]	0.65[c]	0.00	0.00	93.3[c]
4	4.26[c]	6.29[a]	0.75[c]	0.88[b]	0.00	0.01	90.8[d]
8	4.24[c]	6.43[a]	0.48[d]	1.23[a]	0.01	0.00	88.6[e]
P-value							
Storage period (S)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linear	<0.01	<0.01	<0.01	<0.01	<0.01	0.94	<0.01
Quadratic	<0.01	<0.01	0.68	0.65	0.16	0.90	<0.01
P-value							
Interaction (M × S)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
SEM	0.038	0.299	0.030	0.030	0.001	0.001	0.61

[†]Mixture ratio of LBY : CVP : RS of 0% LBY, 20% LBY, 35% LBY and 50% LBY were 0:70:30, 20:50:30, 35:35:30 and 50:20:30 as fresh matter, respectively. [‡]Values were expressed as percentage of fresh matter. NH_3-N/TN, ammonia nitrogen/total nitrogen (%); LA, lactic acid; AA, acetic acid; PA, propionic acid; BA, n- and i-butyric acid. SEM, standard error of the means. [a–e]Means with different superscripts within columns significantly differed ($P < 0.05$).

residue silage after ensiling, while pH kept relatively stable at 4.2-4.3 from weeks 2 to 8, in the present study. The NH_3-N/TN in silage reflects the degree of protein degradation and it should contain less than 10% of N in well preserved silages (McDonald *et al.* 1991). The significant differences in NH_3-N/TN of all mixtures were detected but the value did not exceeded 10% in the present study.

Lactic acid contents of all the mixtures were similar at the initiation of ensiling (Fig. 2). Therefore, addition of LBY with CVP and RS did not affect the lactic acid content of the material mixture. A reduction of lactic acid content which coupled with an increase of acetic acid content was observed when the storage period was prolonged and acetic acid was the major acid product in all the mixtures. This might have been suggested that heterofermentation dominated in the mixtures. Thus, the low pH values of all the mixtures in longer preservation periods were caused by acetic acid content instead of lactic acid content which had lower acid dissociation constant (pKa) compared with acetic acid and other VFAs (Muck *et al.* 2003). Some lactic acid bacteria, such as *Lactobacillus plantarum* or *Lactobacillus buchneri*, were activated and were able to produce acetic acid from lactic acid when there was a lack of substrate such as water soluble carbohydrates in material under anaerobic conditions (Lindgren *et al.* 1990; Oude Elferink *et al.* 2001; Wang & Nishino 2008). Propionic and butyric acid concentrations were less than 0.01%, which indicated that these acid producing bacteria might not have multiplied during the preservation. According to the V-score criteria, all the mixtures had superior quality.

The increase of NH_3-N/TN during the preservation was more drastic as LBY inclusion increased (Fig. 2). This might have been influenced by the increasing of soluble protein fraction and increasing of moisture content of the mixture caused by LBY inclusion. Steckley *et al.* (1979) reported that nitrogen solubility in brewer's yeast slurry was 40% in total nitrogen and quadratically increased during 35 days of anaerobic preservation at 30°C. Higher moisture in silage material resulted in increase of NH_3-N content when fermentation is prolonged (Mahanna & Chase 2003). Although the V-score of 50% LBY was lower than that of 0% LBY in 4 and 8 weeks after preservation, showing scores of 86 and 83, respectively, these were still good rankings (Yang *et al.* 2014).

In conclusion, the present study indicates that addition of LBY into CVP and RS can improve the chemical composition of the TMR mixture, and 50% LBY is the better ratio for highest nutritive value, that is higher CP content. In addition, all mixture ratios can be fed to animals during 8 weeks of storage, from the view point of fermentation quality. Further studies are needed to evaluate the palatability of the mixture and effects of the feeding on the performance of ruminants.

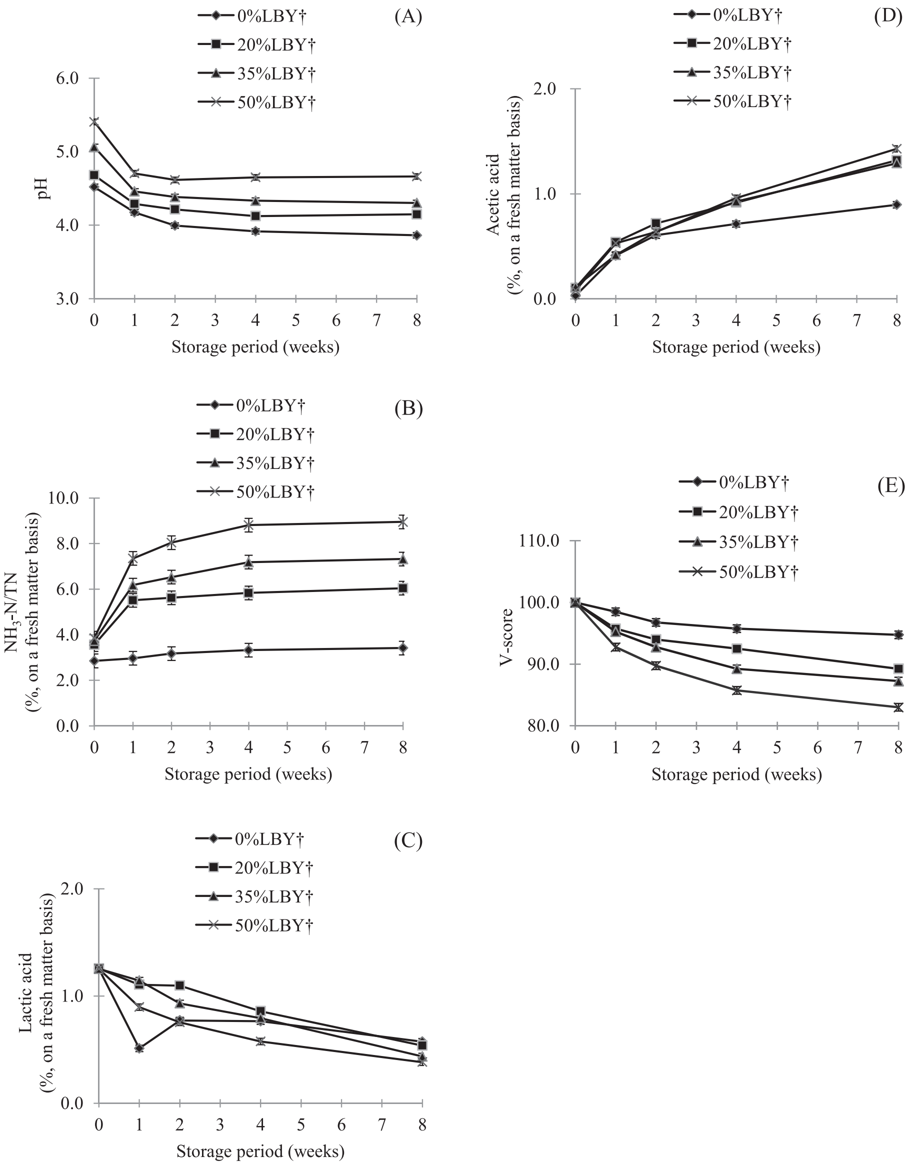

Figure 2 (A) pH, (B) NH_3-N/TN, (C) lactic acid, (D) acetic acid and (E) V-score showing interaction effect between mixture ratio and storage period. Bars are standard errors of the means. †Mixture ratio of LBY : CVP : RS of 0% LBY, 20% LBY, 35% LBY and 50% LBY were 0:70:30, 20:50:30, 35:35:30 and 50:20:30 as fresh matter, respectively. LBY, liquid brewer's yeast; CVP, cassava pulp; RS, rice straw

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