Utilization and development of liquid brewer's yeast mixed with cassava pulp for cattle feed

Sukanya Kamphayae

Laboratory of Animal Husbandry Resources

A dissertation presented to the Office of Graduated School of Agriculture

Kyoto University

In partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

January 2017

Utilization and development of liquid brewer's yeast mixed with cassava pulp for cattle feed

Doctoral thesis

Sukanya Kamphayae, 2017

Khon Kaen Animal Nutrition Research and Development Center, Khon Kaen, Thailand

Thesis supervisor

Associate Professor Hajime Kumagai

Laboratory of Animal Husbandry Resources

Graduate School of agriculture, Kyoto University

Utilization and development of liquid brewer's yeast mixed with cassava pulp for cattle feed

Sukanya Kamphayae

Khon Kaen Animal Nutrition Research and Development Center, Khon Kaen, Thailand

ABSTARCT

Four mixtures of fresh liquid brewer's yeast (LBY) and cassava pulp (CVP) were made (LBY0, LBY10, LBY20 and LBY30 for LBY:CVP at 0:100, 10:90, 20:80 and 30:70, respectively) on a fresh matter basis, in 500 g in plastic bags and stored at 30 to 32 °C. After storage, the bags were opened weekly from weeks 0 to 4. The contents of crude protein (CP), ether extract (EE) increased, whereas all other components decreased, in proportion to LBY inclusion ($P < 0.01$). The dry matter (DM) and organic matter (OM) contents gradually decreased in weeks 3 and 4 ($P < 0.05$), while EE contents were lowest in week 0. The pH, ammonia nitrogen per total nitrogen (NH_3-N/TN) and V-score in each mixture and storage period demonstrated superior fermentation quality (pH ≤ 4.2, NH_3-N/TN ≤ 12.5% and V-score > 90%). The pH increased and NH_3-N/TN decreased, with proportionate increases of LBY, whereas the pH decreased and NH_3-N/TN increased, as the storage periods were extended ($P < 0.01$). Although *in vitro* gas production (IVGP) decreased in proportion to the amount of LBY inclusion ($P < 0.01$), *in vitro* organic matter digestibility (IVOMD) was unaffected by the mixture ratios. The highest IVGP and IVOMD were observed in week 0 ($P < 0.01$). The inclusion of LBY (as high as 30%) into CVP improves the chemical composition of the mixture, thereby increasing the CP content, while decreasing IVGP, without decreasing fermentation quality and IVOMD. In addition, a preservation period of up to four weeks can guarantee superior fermentation quality in all types of mixtures. Therefore, the limiting use of cassava pulp as a feed ingredient, given its low nutritional value, is recommended by improving feed quality with the inclusion of liquid brewer's yeast.

Four mixtures of LBY, CVP and rice straw (RS) were made, i.e. 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 DM, OM, CP, 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 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 increase of LBY inclusion. 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 period.

Sixteen 13.8±1.6 months of Holstein crossbred heifers weighing 209.9±22.7 kg were randomly allocated to four feeding treatments, which were 0:0:100 (RS), 0:70:30 (0%LBY), 20:50:30 (20%LBY) and 50:20:30 (50%LBY), respectively, for LBY:CVP:RS on a fresh matter basis. The heifers were offered conventional concentrate at 1.5% initial body weight daily and fed the treatment diets ad libitum. Average daily gain and feed intake were not significantly different among the treatments. The heifers fed 50%LBY had the highest CP intake and digestibility ($P < 0.05$). The ruminal pH did not differ significantly among treatments, while NH_3-N was the highest ($P < 0.05$) in 50%LBY. Total volatile fatty acid

(VFA) concentrations and the molar proportion of each VFA in rumen fluid were not significantly different among the treatments. Blood urea nitrogen concentrations of 50%LBY were the highest among the treatments ($P < 0.05$). The results indicated that 50%LBY improved CP digestibility.

Key words: cassava pulp, chemical composition, dairy heifer, fermentation, liquid brewer's yeast, rice straw

CONTENTS

LIST OF FIGURES

Figure Page

LIST OF TABLES

Table Page

ACKNOWLEDGEMENT

I wish to express sincere gratitude to the Agricultural Research Development Agency (Public Organization), Thailand, for their financial support.

I would like to gratefully thank and an deeply indebted to Associate Professor Dr. Hajime Kumagai, Graduated School of Agriculture, Kyoto University, my supervisor for advice, encouragement and valuable suggestions for the study and writing of this thesis.

I would like to sincerely thank Mr.Supachai Udchachon, director of Khon Kaen Animal Nutrition Research and Development Center, who pushed and encouraged me to the Ph.D. study program.

I would like to sincerely thank Associate Professor Dr. Pornchai Lowilai, Assistant Professor Dr. Smerjai Bureenok, Miss Wanna Angthong, Mrs. Ramphrai Narmseelee, Miss Patima Butcha, Mr. Supakit Sunato, Miss Ornvimol Kaeokliang, Assistant Professor Dr. Khanitta Ruangwittayanusorn, Dr. Sayan Subepang, Dr. Chatchai Kaewpila and Assistant Professor Dr. Anusorn Cherdthong for their valuable comments and suggestion.

I would like to sincerely thank all of the technical staffs of Khon Kaen Animal Nutrition Research and Development Center (especially, Mr.Paitoon Wangnan and Mr.Kongklai Ngamkiew), Faculty of Agriculture (Khon Kaen University), dairy farmers (members of Khon Kaen Dairy Cooperative), Khon Kaen Brewery Co.,Ltd, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima, Sakon Nakhon Animal Nutrition Development Station and Veterinary Research and Development Center (Upper Northeastern Region), who supported the research implements and laboratory analysis.

I would like to sincerely thank all of the general service officer of Agricultural Research Development Agency (Public Organization), Department of Livestock Development (Bureau of Animal Nutrition Development, Personnel Division, Finance Division, Division of International Livestock Cooperation and Bureau of Legal Affairs), who helps for arranged many documents process.

I would like to also thank you and really appreciate to the current and graduated students of Laboratory of Animal Husbandry Resources, in addition to Kubo-san for many helps and advice during I stay there.

I am especially appreciative of my parents (Mr. Manoo and Mrs. Wilawan Hachanon) for their continuing encouragements and also my husband, daughter and son who always give me heartfelt love during my Ph.D. study.

With best regards,

Sukanya Kamphayae

Laboratory of Animal Husbandry Resources,

Division of Applied Biosciences, Graduated School of Agriculture,

Kyoto University

January, 2017

CHAPTER 1

General Introduction

1.1 Background

In Northeast Thailand, most of dairy animals are raised by small-scale farmers, 128,030 dairy cattle are kept by 4,048 families according to the report of Department of Livestock Development in 2015 (DLD 2015). Which encounters a long dry season (6-7 months) resulting in scarcity of feeds, especially high protein feed resources (Wanapat *et al.* 2000). The majority of small dairy farmers have been using self-mixed concentrate in varying proportions of their total feed concentrate use. However, ingredients of commercial concentrate such as soy bean meal are expensive. As the result, feed cost is inevitably high representing more than 70% of the total production costs, resulting in lower income (Lapar *et al*. 2005). Feeding management is also needed to effectively bring down the production cost. In the situations where commercial concentrate is expensive, farmers should be capable of formulating their own feeds based on available farm resources and their economical viability (Wanapat 1999). Thus the cheap by-products obtained from local industries have often been utilized by small farmers living around the factories. Agricultural and industrial by-products are economical alternatives for feeding livestock, with relatively lower prices than those of commercial concentrate.

However, major limitation in using cheap by-products for livestock feeding is the variability in their composition and high moisture contents. To improve fermentation quality and to reduce effluent losses of high moisture silage, the addition of dry absorbent to the mixture feed might be necessary. 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 2008a).

1.2 The importance of by-products from beer and starch factories as animal feed

Beer is a millennial alcoholic beverage that allows consumers to taste different types and styles depending on how the production process is conducted and/or raw materials which are used (Santos *et al.* 2014). In the manufacture of beer, various by-products are generating, such as brewers' grains and brewers dried yeast, which have been used for animal feed (Aliyu & Bala 2011; Levic *et al.* 2010).

Liquid brewer's yeast (LBY) is obtained by removal of yeast after fermentation in the beer brewing process and most studies involving brewer's yeast have used the dried product. However, recent concern over the energy cost of drying surplus yeast or disposing as a waste, and possible potential as an alternate source of protein has prompted an investigation into the feasibility of using the raw slurry as a feed stuff, particularly in mixed rations for ruminants (Steckley *et al.* 1978). Recently, small farmers in Thailand have approached the factories of beer manufactures to obtain LBY for dairy cattle feeding. One of the factories in Northeast Thailand has a beer production capacity of 700 million liters per year (KKB 2015). They generate about 1,000 m^3 LBY per month and about 40% of LBY is received by farmers within the vicinity of the factories.

Cassava (*Manihot esculenta* Crantz) is also known as 'tapioca', 'manioc', 'mandioca' or 'yucca' in many countries. Cassava is grown in tropical countries in Africa, Asia and Latin America, with 60% of the world's cassava production coming from Nigeria, Thailand, Indonesia, Brazil and the Democratic Republic of Congo (FAO 2012). Cassava could become the raw material base for an array of processed food products that will effectively increase demand for cassava and contribute to agricultural transformation and economic growth in developing countries. Cassava root can be used to produce cassava chips, cassava pellets and cassava starch, which are in high demand throughout the world. Cassava pulp (CVP) is produced as a by-product of starch manufacturing and is a major biomass resource in animal feedstock. In 2013, approximately 15 million tons of fresh cassava roots were extracted from the fields in Northeast Thailand (OAE 2014). Approximately 10 to 15% of the exhausted CVP was utilized as livestock feed (Yimmongkol 2009).

1.3 The utilization and development of LBY and CVP as animal feed

The utilization of agro-industrial by-products may be economically worthwhile, since conventional feedstuffs are often expensive. Liquid brewer's yeast, contains 10-14% DM and 40-50% CP on a DM basis, which is available for small dairy farms around brewer's factories. Compare with basic diets, supplementary LBY improved dry matter intake resulting more rapid gains, but did not affect production response in lactating dairy cattle (Grieve 1979). Furthermore, supplementary LBY (6-12% DM) in complete rations for lactating cows did not affect milk yield, composition or quality (Steckley *et al*. 1979b).

Cassava pulp, contains 17-19% DM (Jintanawit *et al*. 2006) and is low in CP content at 2.4% in DM (Kosoom *et al*. 2009), which has high levels of starch and cellulose at approximately 60 and 20% on a DM basis, respectively (Kosugi *et al*. 2009). Several studies have been conducted to enrich the CP content of cassava pulp through various treatments in solid-state fermentation, such as yeast culturing (Kaewwongsa *et al*. 2011) and microorganic fermentation (Chumpawadee & Soychuta 2009; Thongkratoke *et al*. 2010; Vorachinda *et al*. 2011).

The use of LBY, a liquid state fermentation product, mixed with CVP offers farmers enriched protein at lower overall feed costs. Since both LBY and CVP have high moisture contents, the livestock feed must be stored in bunkers after mixing, in preparation for transportation to farmers in the distance, 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). Ensiling can be considered as a method to use wet by-products more effectively (Kayouli & Lee 1999). If wet by-products containing high moisture were ensiled as TMR silage with dry feeds, the risk of effluent production would be minimized and the feeding time could be reduced (Imai 2000). Moreover, TMR silage can stabilize rumen function and avoid self-selection by animals (Coppock *et al*. 1981), also has high DM intake and digestibility in ruminants (Shioya 2008). Rice straw (RS) is the main crop-residue, which farmers in Asian countries usually store as ruminant feed. Feeding with rice straw alone does not provide enough nutrients to the ruminants for maintaining high production levels (Sarnklong *et al*. 2010), due to its low nutritive value with low level of protein, high levels of fiber and lignin, and low DM digestibility (Wanapat *et al*. 2009).

Well-grown dairy heifers play an important role in the future success of all dairy farms. Dairy heifers represent a large expense of resources, especially feed is the largest cost associated with heifer production (Gabler *et al*. 2000). Overall management of dairy heifers must be handled in a manner that yields the best quality heifer, with a high potential to be productive, and at minimal cost to the farm and the environment. Dairy nutrition is essentially as simple as understanding the nutrient requirements of growing ruminants at various stages of growth and combining various feed ingredients to meet those needs in a cost-effective manner. High quality feed must be followed to achieve maximum performance from growing dairy heifers. Thus providing dairy heifers with high quality roughage produces high daily gain, which contributes to their early starts of heat, mating and milk production. To improve the productivity of ruminant animals, making TMR silage, by mixing RS with LBY and CVP, can be developed in areas where beer and starch manufacturing factories are located.

1.4 Objectives of the study

By-products from beer and starch factories, mainly as LBY and CVP, respectively, can be utilized by mixing themselves or mixed with rice straw as TMR for animal feed. The suitable ratios and storage periods, however, would affect to feed intake, digestibility, rumen fermentation and productivity of animals. Therefore, the objectives of the present study were as follows:

1. to evaluate the chemical composition, fermentation quality and *in vitro* ruminal fermentation of various ratios and storage periods of LBY mixed with CVP.

2. to evaluate the effect of LBY addition on chemical composition and fermentation quality of mixture of LBY and CVP with rice straw in different ratios during preservation periods as a pilot study for TMR feeding for ruminants.

3. to evaluate the effects of the mixture of LBY and CVP with rice straw on feed intake, digestibility, rumen fermentation and growth of dairy heifers.

CHAPTER 2

Effects of different ratios and storage periods of liquid brewer's yeast mixed with cassava pulp on chemical composition, fermentation quality and *in vitro* ruminal fermentation

2.1 Introduction

The lack of a high quality diet to feed livestock has become a very urgent problem for small farmers in developing countries. A solution using a relatively high amount of commercial concentrate would be possible, if the farmers had access to such a concentrate. However, feedstuff concentrates are expensive, as most contain protein sources, such as soybean meal. Cheap by-products (and waste products) obtained from local industries have often been utilized by small farmers, living around the factories. Specific by-products obtained from the production of beer, such as brewers' grains and brewers dried yeast, have been used in animal feed (Aliyu & Bala 2011; Levic *et al*. 2010). *Saccharomyces cerevisiae* is the prevalent budding yeast microbe involved in brewing (Manzano *et al*. 2005) and is considered to be a generally accepted alternative by-product for livestock feed, due to its high nutritional value (Bruning & Yokoyama 1987). Liquid brewer's yeast (LBY), obtained by removal of yeast after fermentation in the beer brewing process, contains at least 32% crude protein (CP) on a dry matter (DM) basis (Linton 1977). Compare to basic diets, supplementing LBY improved dry matter intake and resulted in more rapid gains, and there was no difference between yeast- and soybean- supplemented rations (Grieve 1979). Furthermore, supplementary LBY (6 to 12% DM) in complete rations for lactating cows did not affect milk yield, composition or quality (Steckley et al. 1979b).

Consequently, small farmers in Thailand have approached the factories of beer manufactures to obtain LBY for dairy cattle feeding. One such factory in Northeast Thailand has a beer production capacity of 700 million liters per year (KKB 2015). It is thought that they generate about 1,000 m^3 LBY per month and about 40% of this amount is received by farmers within the vicinity (unconfirmed estimate). Cassava (*Manihot esculenta* Crantz.) pulp (CVP) is produced as a by-product of starch manufacturing, and is a major biomass resource in animal feedstock. In 2013, approximately 15 million tons of fresh cassava roots were extracted from the fields in Northeast Thailand (OAE 2014). Approximately 10 to 15% of the resulting cassava pulp was utilized as livestock feed (Yimmongkol 2009), and contained 17 to 19% DM (Jintanawit *et al*. 2006), as well as high rates of starch and cellulose [approximately 60 and 20% on a DM basis, respectively (Kosugi *et al*. 2009)]. However the CP content, 2.4% DM, was relatively low (Kosoom *et al*. 2009).

Several studies have been conducted to enrich the CP content of cassava pulp through various treatments in solid-state fermentation, such as yeast culturing (Kaewwongsa *et al*. 2011) and microorganic fermentation (Chumpawadee & Soychuta 2009; Thongkratoke *et al*. 2010; Vorachinda *et al*. 2011). The use of LBY, a liquid state fermentation product mixed with CVP, offers farmers enriched protein at lower overall feed costs. Since both LBY and CVP have high moisture contents, the livestock feed must be stored in bunkers after mixing, in preparation for transportation to distant farmers. Therefore, maintaining an optimum ratio of LBY and CVP during the necessary storage periods is critical for its practical use in farms. This study aims to evaluate the chemical composition, fermentation quality and *in vitro* ruminal fermentation of various ratios and storage periods of LBY mixed with CVP.

2.2 Materials and methods

2.2.1 Sample preparation

Experiments were conducted at Khon Kaen Animal Nutrition Research and Development Center, in May 2013. Fresh LBY and CVP, obtained from a brewery and a starch factory, respectively in the Khon Kaen Province, were collected in four mixtures (LBY0, LBY10, LBY20 and LBY30), in LBY:CVP ratios of 0:100, 10:90, 20:80 and 30:70, respectively, on a fresh matter basis. Twenty composites of 500 g were made from each mixture and packed into 15 x 23 cm plastic bags. The bags were sealed using a vacuum sealer and stored at room temperature (30 to 32 °C). Four bags from each mixture were opened for analysis at 0, 1, 2, 3 and 4 weeks after storage.

2.2.2 Chemical analyses

A 50 g sample from each composite was homogenized with 100 ml of distilled water and stored overnight in a refrigerator, at 4°C. The homogenate was then filtered through Whatman no.5 filter paper for pH, ammonia nitrogen (NH_3-N), lactic acid (LA) and volatile fatty acid (VFA) determination. The pH was measured by pH meter (D-22; Horiba, Kyoto, Japan) and the NH_3-N content was determined using a steam distillation technique (Society of Utilization of Self Supplied Feeds 2009). The remaining sample mixture was oven dried at 60°C for 48 h. The dried samples were ground to pass through a 1 mm screen for subsequent analyses of DM (on oven drying procedure at 135°C for 2 h), CP, ether extract (EE) and crude ash (CA), according to the Association of Official Analytical Chemists (AOAC 2000). The organic matter (OM) was calculated as lost weight 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). LA and VFA concentrations were determined by using high performance liquid chromatography (LC-20A; Shimadzu, Kyoto, Japan), using a Nova-pak C18 steel column: 3.9 x 300 mm, according to the methods of Cai (2004). The flow rate was 0.5 ml/min and the wavelength of UV detector was 210 nm. To assess the quality of the mixture, the V-scores were calculated from the NH_3-N/TN and VFA concentrations (Society of Utilization of Self Supplied Feeds 2009). According to the scoring criteria, the samples were divided into three ranks: superior (81 to 100), good (60 to 80) and bad (<60) (Yang *et al.* 2014).

2.2.3 *In vitro* gas production analyses

Buffered mineral solution (Menke & Steingass 1988) was prepared and placed in a water bath at 39°C under continuous CO_2 flushing. Rumen fluid was collected from two fistulated Brahman crossbred cattle, prior to their morning feeding. The dried and ground samples, were weighted (200 mg each) and inserted into two 100 ml calibrated glass syringes. The samples were incubated *in vitro* with the buffered rumen fluid (30 ml each) in calibrated glass syringes, duplicated for each sample. The ratio of rumen fluid to buffered mineral solution was 1:2. Gas production volume was recorded at 6, 12, 24 and 48 h of incubation and gas production of the incubated mixtures was calculated for mean blank value over a 24 h period (Menke & Steingass 1988), through the following equations.

GP = (V24 - V0 - GP0)/ Weight in mg DM

where GP = gas production (ml/mg DM), V0 = volume at 0 h (ml), V24 = volume at 24 h (ml) and GP0 = the mean blank value at 0 h (ml).

Organic matter digestibility (OMD) and metabolizable energy (ME) were estimated (Menke & Steingass 1988), in the 24 h gas production period, through the following equations.

OMD (%) = 9.00 + 0.9991 GP + 0.0595 XP + 0.0181 XA

ME (MJ/kg DM) = 1.06 + 0.1570 GP + 0.0084 XP + 0.0220 XE - 0.0081 XA

where GP = gas production at 24 h (ml/200 mg DM), XP = CP content (g/kg DM), XE = EE content (g/kg DM) and XA = crude ash content (g/kg DM)

2.2.4 Statistical analyses

Data were analyzed with repeated measures, using the PROC MIXED procedure of SAS (SAS Institute Inc. 1996), through the following model: $Yijk = \mu + Mi + Sj + (MS)ij + eijk$, where $Yijk$ is the observed value, μ is overall mean, Mi is the effect associated with the mixture ratios, Sj is the effect associated with the storage periods, $(MS)ij$ is the interaction effect between mixture ratios and storage period and $eijk$ is residual (s).

The orthogonal polynomial contrast examined linear and quadratic responses to the LBY mixture ratios and storage periods and the Tukey-Kramer test was used to detect the differences between the means for each data analysis (Kramer 1956). Correlation coefficients among parameters from chemical composition, gas production, OMD and ME were calculated through Pearson's correlation. Significances were declared at $P < 0.05$.

2.3 Results

2.3.1 Chemical composition of the mixtures of LBY and CVP

The chemical compositions of fresh LBY and CVP prior to mixing are shown in Table 2.1. The pH, and DM, OM, CP and EE contents of the LBY were 5.3, and 12.0, 92.0, 48.3 and 0.7% on a DM basis, respectively. The NDFom, ADFom and ADL contents were undetected in the LBY. The CVP displayed pH, and DM, OM, CP, EE, NDFom, ADFom and ADL contents at 3.7, and 18.6, 96.8, 2.9, 0.4, 36.6, 24.5 and 4.9%, respectively.

Changes within the chemical composition of the four mixture ratios and five storage periods are shown in Table 2.2. Among the mixture ratio means, the DM, OM, CP, EE, NDFom, ADFom and ADL of the four mixtures ranged 16.5 to 18.0, 95.7 to 96.6, 3.2 to 14.1, 0.4 to 0.5, 32.0 to 36.2, 21.1 to 24.8 and 3.8 to 4.9%, respectively. The results were dependent on the differences in chemical composition and mixture ratio within the LBY and CVP. Increasing the level of LBY linearly increased CP and EE contents, and decreased other chemical compositions ($P < 0.01$). For the means of storage period, the DM and OM contents linearly and quadratically decreased, while the EE and ADFom contents increased linearly, as the storage periods progressed ($P \leq 0.01$). The interaction effects between mixture ratios and storage periods on DM, OM, CP, EE and ADFom are identified in Table 2.

2.3.2 Fermentation quality in the LBY and CVP mixtures

Table 2.3 shows the changes in pH, NH_3-N/TN, LA and VFA concentrations of the mixture during the storage period ($P \leq 0.01$). All parameters were quadratically affected by the mixture ratios, and acute increases in pH, acetic and butyric contents, as well as decreases in NH_3-N/TN, LA contents and V-scores were observed in the progression from LBY0 to LBY10. More specifically, the quadratically increases of acetic and propionic acids and the linear decrease in V-scores were more severe in the later preservation periods. The interaction effects between the mixture ratios and storage periods were observed in pH, LA, acetic, propionic and butyric acid contents, and V-score ($P < 0.01$), (Fig.2.1).

2.3.3 Gas production, estimated OMD, ME and their relationship with chemical composition

Gas production from the mixture of LBY and CVP with different mixture ratios, storage periods and post incubation parameters, described as OMD and ME, are shown in Table 2.4. Comparing the mixture ratio means, the gas production of LBY0 and LBY10 differed only slightly, yet were higher than that of LBY20 and LBY30 ($P < 0.01$). The gas production at 48 h decreased quadratically with the inclusion of LBY. As indicated within the storage period means, gas production decreased as the storage period increased, especially within weeks 0 to 1. The interaction effects between the mixture ratios and storage periods were observed in the 6, 24 and 48 h (Table 2.4). Among the four mixture ratio means, estimated OMD and ME ranged from 77.4 to 79.7% and 11.4 to 11.8 MJ/kgDM, respectively, each scoring lowest in LBY20 (P < 0.05). Figure 2.2 provides the interaction effects of the mixture ratios and storage periods for both OMD and ME (P < 0.01).

The correlation coefficients between chemical compositions, OMD, ME and *in vitro* gas production are shown in Table 2.5. DM and OM contents were positively correlated at 6 h (P < 0.05), 12 and 24 h (P < 0.01) and 48 h (P < 0.05). NDFom and non-fiber carbohydrate (NFC) contents were positively correlated at 12, 24 and 48 h (P < 0.01). The NH_3-N/TN contents at 6 h incubation were also positively correlated (P < 0.01). Contrastingly, the CP content was negatively correlated at 12 h (P < 0.05), and at 24 and 48 h (P < 0.01). EE content was also negatively correlated at 6 h (P < 0.05), 12 and 24 h (P < 0.01) and 48 h (P < 0.05). OMD and ME did not significantly correlate with the chemical composition.

2.4 Discussion

2.4.1 Chemical composition and fermentation characteristics

Comparatively, the LBY in this experiment had lower DM and CP contents than the compositions within the study conducted by Steckley *et al*. (1979a), (14.4 and 53.0%), yet fell within the same range of those within the study conducted by Grieve (1979), (10.8 to 13.4 and 43.1 to 51.4%). The CVP was highly acidic, and the pH (3.66) was lower than that reported by Jintanawit *et al*. (2006), (3.89). These variations may be a result of different materials, plant locations and processing. However, the CP, EE, NDFom and ADFom contents of CVP in this study were similar to the sample means of a report of three starch factories in Northeast Thailand, by Kosoom *et al*. (2009), at 2.4, 0.4, 40.1 and 25.3% on a DM basis, respectively.

In this study, CP content increased ($P < 0.01$) with the amount of LBY inclusion in the mixture from 3 to 14%, with the 0-30% proportion of LBY (Table 2.2), which attributed to a higher CP content in LBY than in CVP. The linear declines of DM, NDFom, ADFom and ADL contents from LBY10 to LBY30 were caused by the decreases in the proportions of CVP, which contained a higher ratio of DM and fiber contents, than LBY.

Through prolonged storage periods, the contents of DM and OM in the mixture declined in contrast with the increase in CP content, which agreed with the findings of Adeyemi *et al*. (2007). The decline of DM and OM may have been due to the generation of moisture and loss of fermentation substrates, such as NFC.

The changes in fermentation characteristics indicate that LBY0 had the lowest pH, given the strong acidic content of CVP, due to the starch extraction process (Yimmongkol 2009). While NH_3-N/TN ranked the least amount of content within the mixture, it was highest in LBY0, which may have been due to the relatively lower CP content in LBY0. Lounglawan *et al*. (2011) reported a CVP which an NFC as high as 25% on a DM basis. In the present study, the CVP's NFC content was estimated as 56.8% on a DM basis, through the equation NFC=100-(CP+EE+NDFom+CA). The higher LA content may have been a result of the higher NFC content in CVP as a substrate for the fermentation of LA.

During preservation, the pH of each treatment decreased from weeks 0 to 4. These results coincide with the study of Jintawanit *et al*. (2006), which found that the pH of CVP continuously decreased from 3.89 to 3.40 during 28 days of ensiling. The NH_3-N/TN, as well as the acetic and propionic acid contents, gradually increased during the preservation of all LBY levels. However, the NH_3-N/TN content increased only slightly, at 2.8 to 5.9%. According to Umana *et al*. (1991), an NH_3-N content of less than 10% of total nitrogen has been identified as a characteristic of well-preserved silage. Previous research on ensiling wet by-products have also indicated low propionic acid contents and very small (if any) quantities of butyric acid (Nishino *et al*. 2003; Wang & Nishino 2008b). The V-score means in all mixture ratios and storage periods were higher than 90%, which suggested that the mixture had superior fermentation quality in all preservation periods (Yang *et al*. 2014).

2.4.2 Gas production, estimated OMD, ME and their relationship with chemical composition

The gas production technique is widely used for evaluating nutritive values in ruminant feeds. In this study, each mixture had high GP early in the fermentation period, which may have been due to its low fiber content (Table 2.4). The highest GP of LBY0 and LBY10 (reduced with the inclusion of LBY) may also be explained by the low fiber content. However, GP reduced with increased CP content, as well as with decreased DM and NDFom contents (Table 2.4). These same results were initially found in the study of Dung *et al*. (2014), which suggested that GP reduced with increased CP levels. The estimated ME values of the four mixture ratios, ranging from 11.4 to 11.7 MJ/kgDM ($P < 0.05$), were higher than those reported in the studies of Nitipot *et al*. (2009), (10.89 MJ/kgDM) and Suksombat *et al*. (2006), (10.04 MJ/kgDM).

The gas production parameters suggest differences in nutritive values that are closely related to their chemical composition (Cerrillo & Juarez 2004). In this study, we found strong negative correlations between GP and the contents of CP and EE (Table 2.5), consistent with the study of Getachew *et al*. (2004). While, the contents of DM, OM, NDFom and NFC were positively correlated with GP, it is likely that NDFom and NFC in the mixture were highly fermentable, demonstrated by low-NDF fall-grown oat forages (Coblentz *et al*. 2013). The NH_3-N/TN content was positively correlated with GP in the first 6 h of incubation, which might have reflected the synchronizing fermentable nitrogen with carbohydrates, present in early fermentation. The OMD and ME, however, did not significantly correlated with any chemical composition.

2.5 Conclusion

The inclusion of LBY (up to 30%) into CVP can significantly improve the chemical composition of a feed mixture. A remarkable increase in CP content with decreased IVGP was achieved, with acceptable fermentation quality and without decreasing IVOMD. In addition, we determined that a preservation period of up to four weeks could assure superior fermentation quality in all types of mixtures. Therefore, a low nutritive value of livestock feed, due to the limitation of CVP as a feed ingredient, can be instantly improved by the inclusion of LBY.

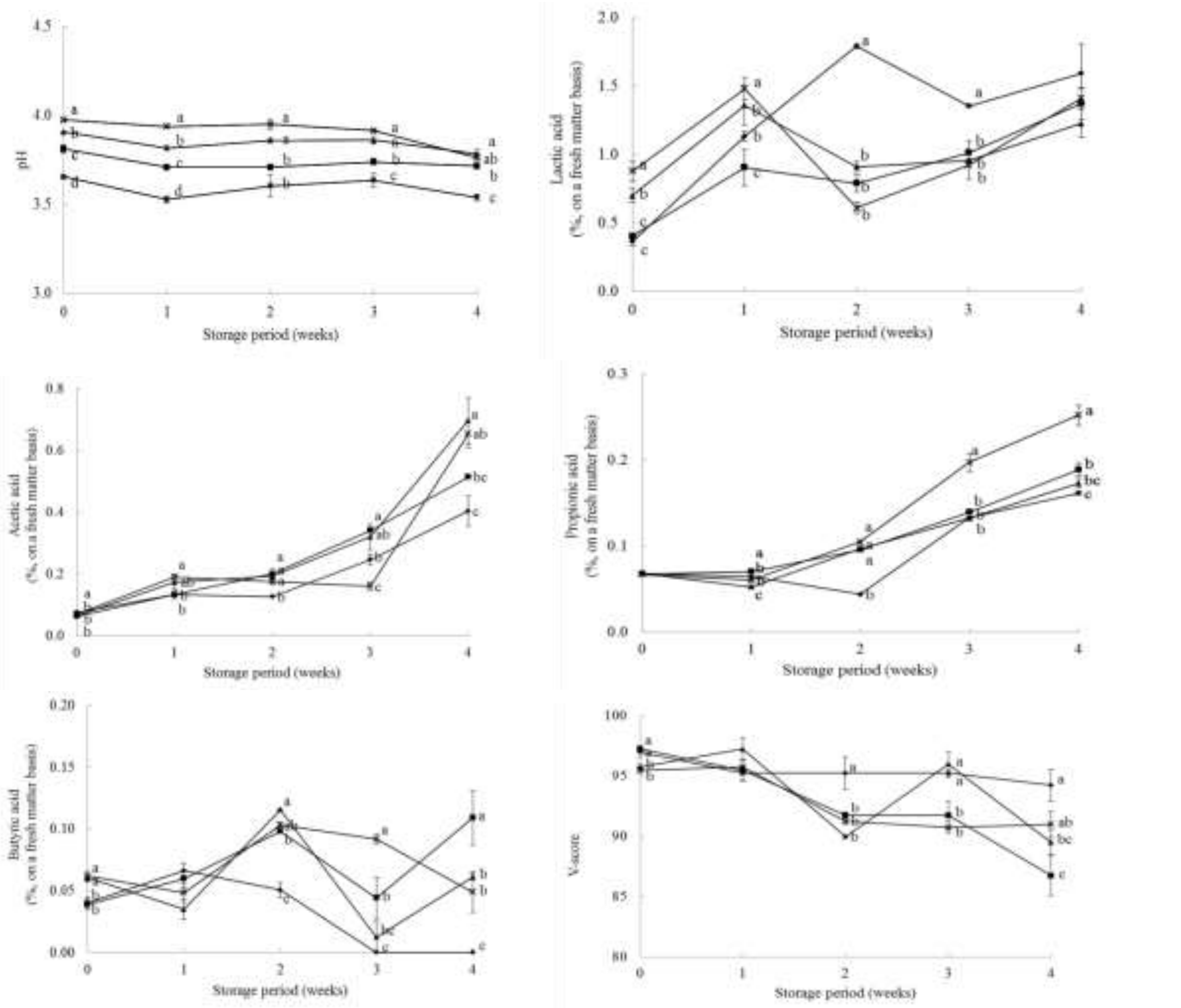

Figure 2.1 pH, lactic acid, acetic acid, propionic acid, butyric acid and V-score showing interaction effects between mixture ratio and storage period
Bars are standard error of the means. Symbols: ♦, LBY0; ■, LBY10; ▲, LBY20; ×, LBY30. LBY0, 10, 20 and 30, mixture ratios of LBY:CVP at 0:100, 10:90, 20:80 and 30:70, respectively, on a fresh matter basis. LBY, liquid brewer's yeast; CVP, cassava pulp. Different letters indicate a significant difference between treatments in each storage period ($P < 0.05$).

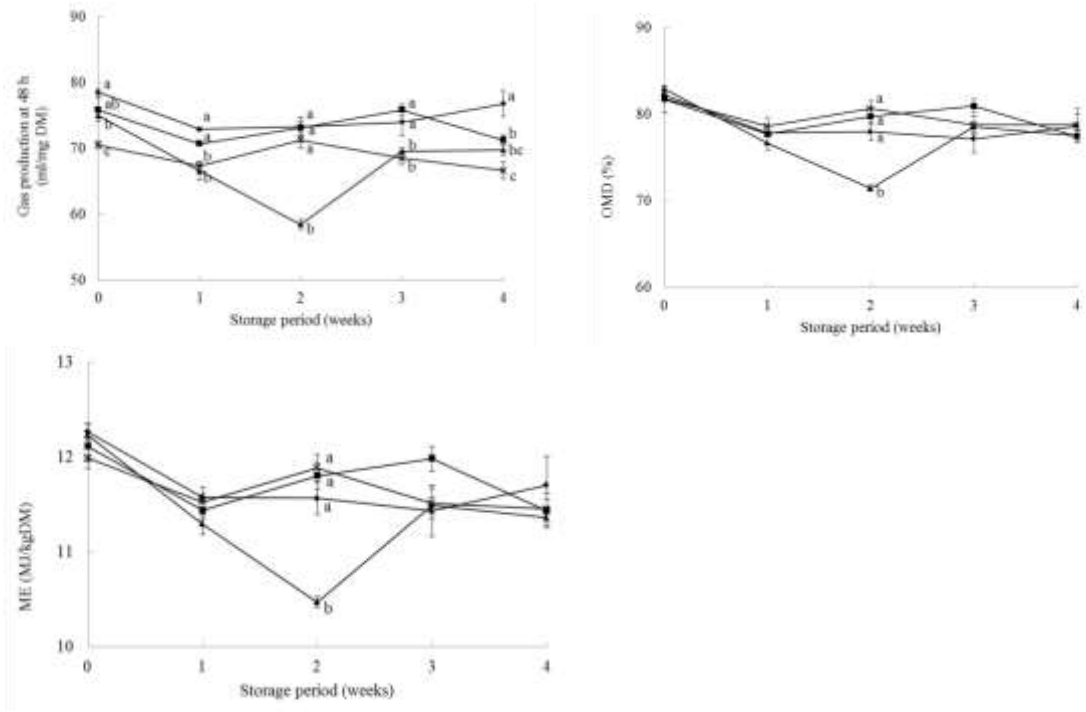

Figure 2.2 Gas production at 48 h, organic matter digestibility (OMD) and metabolizable energy (ME) showing interaction effects between mixture ratio and storage period
Bars are standard error of the means. Symbols: ♦, LBY0; ■, LBY10; ▲, LBY20; ×, LBY30. LBY0, 10, 20 and 30, mixture ratios of LBY:CVP at 0:100, 10:90, 20:80 and 30:70, respectively, on a fresh matter basis. LBY, liquid brewer's yeast; CVP, cassava pulp. Different letters indicate a significant difference between treatments in each storage period (*P* < 0.05).

Table 2.1 pH and chemical composition of experimental diets

Item	pH	DM	OM	CP	EE	NDFom	ADFom	ADL
		%	--------------------		% of dry matter			--------------------
Liquid brewer's yeast	5.3	12.0	92.0	48.3	0.7	ND	ND	ND
Cassava pulp	3.7	18.6	96.8	2.9	0.4	36.6	24.5	4.9

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 2.2 Chemical composition of mixture of liquid brewer's yeast (LBY) and cassava pulp (CVP) at different ratios and storage periods (%)

Item	DM	OM‡	CP‡	EE‡	NDFom‡	ADFom‡	ADL‡
Mixture ratio							
LBY0†	18.0^{a}	96.6^{a}	3.2^{d}	0.37^{c}	36.2^{a}	24.8^{a}	4.9^{a}
LBY10†	17.4^{b}	96.3^{b}	6.8^{c}	0.40^{bc}	35.1^{b}	23.5^{b}	4.5^{b}
LBY20†	16.8^{c}	96.1^{c}	10.5^{b}	0.44^{b}	33.8^{c}	22.2^{c}	4.2^{c}
LBY30†	16.5^{d}	95.7^{d}	14.1^{a}	0.49^{a}	32.0^{d}	21.1^{d}	3.8^{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	0.99	0.84	0.28	0.14	0.87	0.32
Storage period (week)							
0	17.4^{a}	96.3^{a}	8.6^{bc}	0.39^{b}	34.8^{a}	22.8^{b}	4.5
1	17.4^{a}	96.3^{a}	8.4^{c}	0.42^{ab}	33.4^{b}	22.2^{c}	4.2
2	17.5^{a}	96.4^{a}	8.7^{ab}	0.43^{ab}	34.6^{a}	22.8^{b}	4.3
3	16.9^{b}	96.0^{b}	8.9^{a}	0.43^{ab}	34.6^{a}	23.8^{a}	4.5
4	16.8^{b}	95.9^{b}	8.6^{bc}	0.45^{a}	34.0^{ab}	22.9^{b}	4.3
P-value							
Storage period (S)	<0.01	<0.01	<0.01	0.02	<0.01	<0.01	0.12
Linear	<0.01	<0.01	0.01	<0.01	0.63	<0.01	0.44
Quadratic	<0.01	0.01	0.09	0.56	0.72	0.75	0.54
P-value							
Interaction (M x S)	0.04	<0.01	<0.01	0.05	0.40	0.04	0.58
SEM	0.199	0.111	0.151	0.027	0.450	0.294	0.162

$^{a\text{-}d}$Means with different superscripts within columns significantly differed ($P < 0.05$). †LBY0, 10, 20 and 30, mixture ratios of LBY:CVP at 0:100, 10:90, 20:80 and 30:70, respectively, on a fresh matter basis. ‡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.

Table 2.3 Fermentation quality of mixture of liquid brewer's yeast (LBY) and cassava pulp (CVP) at different ratios and storage periods

Item	pH	NH_3-N/TN ‡	LA ‡	AA ‡	PA ‡	BA ‡	V-score
Mixture ratio							
LBY0†	3.59^{d}	5.22^{a}	1.25^{a}	0.20^{b}	0.09^{d}	0.03^{b}	95.4^{a}
LBY10†	3.74^{c}	4.54^{b}	0.90^{c}	0.25^{a}	0.11^{b}	0.07^{a}	92.6^{b}
LBY20†	3.84^{b}	4.03^{b}	1.03^{bc}	0.29^{a}	0.10^{c}	0.06^{a}	93.7^{b}
LBY30†	3.91^{a}	4.04^{b}	1.06^{b}	0.25^{a}	0.14^{a}	0.07^{a}	92.9^{b}
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	<0.01	0.01	<0.01	0.01	0.01	<0.01	0.01
Storage period (week)							
0	3.84^{a}	2.75^{d}	0.59^{d}	0.07^{d}	0.07^{d}	0.05^{bc}	96.4^{a}
1	3.75^{b}	3.67^{c}	1.22^{b}	0.16^{c}	0.06^{e}	0.05^{bc}	95.9^{a}
2	3.78^{b}	4.56^{b}	1.03^{c}	0.17^{c}	0.09^{c}	0.09^{a}	92.1^{b}
3	3.79^{b}	5.92^{a}	1.06^{bc}	0.27^{b}	0.15^{b}	0.04^{c}	93.4^{b}
4	3.70^{c}	5.38^{a}	1.40^{a}	0.57^{a}	0.19^{a}	0.06^{b}	90.4^{c}
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	0.72	<0.01
Quadratic	0.66	<0.01	<0.01	<0.01	<0.01	<0.01	1.00
P-value							
Interaction (M x S)	<0.01	0.30	<0.01	<0.01	<0.01	<0.01	<0.01
SEM	0.023	0.301	0.088	0.027	0.004	0.009	0.796

$^{a\text{-}e}$Means with different superscripts within columns significantly differed (P < 0.05). †LBY0, 10, 20 and 30, mixture ratios of LBY:CVP at 0:100, 10:90, 20:80 and 30:70, respectively, on a fresh matter basis. ‡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 and BA, n- and i-butyric acid. SEM, standard error of the means.

Table 2.4 Gas production, organic matter digestibility and metabolizable energy of mixture of liquid brewer's yeast (LBY) and cassava pulp (CVP) at different ratios and storage periods

Item	Gas production (ml/mgDM)				OMD (%)	ME (MJ/kgDM)
	6 h	12 h	24 h	48 h		
Mixture ratio						
LBY0†	33.6^{a}	56.0^{a}	67.3^{a}	75.1^{a}	78.8^{ab}	11.7^{a}
LBY10†	33.5^{a}	55.2^{a}	65.8^{a}	73.4^{a}	79.5^{a}	11.8^{a}
LBY20†	32.4^{b}	52.0^{b}	61.5^{b}	67.9^{b}	77.4^{b}	11.4^{b}
LBY30†	32.4^{b}	51.3^{b}	61.6^{b}	68.9^{b}	79.7^{a}	11.7^{a}
P-value						
Mixture ratio (M)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Linear	<0.01	<0.01	<0.01	<0.01	0.71	0.12
Quadratic	0.09	0.81	0.06	<0.01	0.08	0.06
Storage period (week)						
0	36.4^{a}	57.7^{a}	67.4^{a}	75.0^{a}	82.2^{a}	12.2^{a}
1	33.1^{b}	53.8^{b}	63.0^{b}	69.4^{c}	77.7^{b}	11.5^{b}
2	32.8^{b}	53.5^{bc}	62.7^{b}	69.1^{c}	77.5^{b}	11.4^{b}
3	30.6^{c}	52.0^{cd}	63.9^{b}	72.0^{b}	78.8^{b}	11.6^{b}
4	30.2^{c}	51.1^{d}	63.3^{b}	71.2^{bc}	78.1^{b}	11.5^{b}
P-value						
Storage period (S)	<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
Quadratic	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
P-value						
Interaction (M x S)	<0.01	0.05	<0.01	<0.01	<0.01	<0.01
SEM	0.547	0.773	0.859	0.994	0.876	0.138

$^{a\text{-}d}$Means with different superscripts within columns significantly differed (P < 0.05). †LBY0, 10, 20 and 30, mixture ratios of LBY:CVP at 0:100, 10:90, 20:80 and 30:70, respectively, on a fresh matter basis. OMD, organic matter digestibility; ME, metabolizable energy. SEM, standard error of the means.

Table 2.5 Correlation coefficients (r) between chemical compositions, *in vitro* gas production, organic matter digestibility and metabolizable energy

Item	Gas production (ml/mgDM)				OMD	ME
	6 h	12 h	24 h	48 h	(%)	(MJ/kgDM)
DM	0.45*	0.72**	0.60**	0.46*	0.00	0.17
OM	0.51*	0.66**	0.57**	0.47*	-0.02	0.15
CP	-0.30	-0.62*	-0.70**	-0.63**	0.01	-0.16
EE	-0.54*	-0.70**	-0.65**	-0.55*	-0.14	-0.26
NDFom	0.36	0.65**	0.74**	0.69**	0.11	0.27
NFC	0.29	0.59**	0.64**	0.55*	-0.08	0.09
NH_3-N/TN	0.57**	-0.36	0.01	0.12	-0.32	-0.27

DM, dry matter; OM, organic matter: CP, crude protein; EE, ether extract; NDFom, neutral detergent fiber expressed exclusive of residual ash; NFC, non fiber carbohydrate (100-(CP+EE+NDF+crude ash)); NH_3-N/TN, ammonia nitrogen/total nitrogen. OMD, organic matter digestibility; ME, metabolizable energy. *, $P < 0.05$; **, $P < 0.01$.

CHAPTER 3

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

3.1 Introduction

Feeding costs for small dairy farmers in Northeast Thailand represent more than 70% of the total costs (Lapar *et al*. 2005). There is a growing concern with increasing prices of imported feed ingredients, e.g., 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 (*Manihot esculenta* 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 15 million tons 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 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 with a 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 liters per year (KKB 2015). It is estimated that about 1,000 m^3 LBY per month was produced, and 40% of this amount would be sent to the farmers around the beer factory (personal 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 brewer's factories.

The mixture of CVP and LBY is, however, 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 rice straw does not provide enough nutrients to the ruminant animal to maintain high production level (Sarnklong *et al*. 2010) due to its low nutritive value with low level 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 reduced the silage DM loss during the ensiling and improved the 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 2008a). 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 of 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 mixture of LBY and CVP with rice straw in different ratios during preservation periods as a pilot study for TMR feeding for ruminants.

3.2 Materials and methods

3.2.1 The mixture preparation

The experiment was conducted in Khon Kaen Animal Nutrition Research and Development Center, Khon Kaen, Thailand in December 2013. Rice straw was obtained from a paddy field in Khon Kaen Province. The straw was chopped into 5-10 cm pieces by a chaff cutter and mixed manually. Fresh CVP and LBY were obtained from a 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 x 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.

3.2.2 Chemical analyses

Fifty gram sample 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 (LA) 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 VFA concentrations were determined by gas chromatography (Shimadzu GC2014, Shimadzu Corp., Kyoto, Japan) using a 25 m x 0.53 mm capillary column (BP21 0.5 P/N 054474, SGE Analytical Science Pty Ltd., 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 NH_3-N /TN (A%: A' = 50 if A ≦ 5, A' = 60 - 2A if 5 < A < 10), B' from acetic acid plus propionic acid concentrations (B%: B' = 10 if B≦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 3 ranks: superior (81-100), good (60-80) and bad (<60) (Yang *et al.* 2014). The remained 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 hours), crude protein (CP), ether extract (EE) and crude ash were analyzed according to the standards of 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).

3.2.3 Statistical analyses

The data were analyzed by the GLM procedure of SAS (1996) using the following mathematical model: $Yijk = \mu + Mi + Sj + (MS)ij + eijk$, where μ is overall mean, Mi is effect associated with the mixture ratios, Sj is effect associated with storage period, $(MS)ij$ is interaction effect between mixture ratios and storage periods and $eijk$ 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 $P < 0.05$.

3.3 Results

3.3.1 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 3.1. 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.

3.3.2 Chemical composition of the mixtures

Changes in the chemical composition of four mixture ratios and five storage periods are shown in Table 3.2. The mixture ratio influenced all the chemical composition ($P < 0.01$). The DM, OM, CP, EE, NDFom and ADFom contents were 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. For the means of storage period from week 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.3.1).

3.3.3 Fermentation quality of the mixtures

Table 3.3 shows the changes of pH, NH_3-N/TN, lactic acid, VFA concentrations and V-score of the mixture during storage period. 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 4 mixture ratios, the pH and NH_3-N/TN contents were 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 were 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 ($P < 0.01$). 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 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 3.3, Fig.3.2). The increase of NH_3-N/TN and acetic acid contents during the preservation were more acute as increase of LBY inclusion. Propionic acid and butyric acid contents were 0.01% or lower in each mixture and storage period. Among the storage period, V-score ranged from 88.6 to 100.0, week 0 had the highest V-score followed by week 1, 2, 4 and 8 ($P < 0.05$). The decrease of V-score during the preservation was more drastic as increase of LBY inclusion.

3.4 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 condition 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 3.3). 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, similarly with 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 Cao *et al*. (2011) who found the sharply decreased in pH of vegetable residue silage after ensiled. While, pH kept relatively stable at 4.2-4.3 from week 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 initial of ensiling (Fig. 3.2). 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 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 period 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 was activated and was able to produce acetic acid from lactic acid when the lack of substrate such as water soluble carbohydrates in material under anaerobic conditions (Lindgren *et al*. 1990; Oude Elferink *et al*. 2001; Wang & Nishino 2008a). 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 increase of LBY inclusion (Fig. 3.2). This might have been influenced by the increasing of soluble protein fraction and increasing of moisture content of the mixture caused by the 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 86 and 83, respectively, which were in a good rank of the criteria (Yang *et al*. 2014).

3.5 Conclusion

In conclusion, the present study indicates that addition of LBY into the CVP and RS can improve the chemical composition of the TMR mixture, and 50%LBY is the better ratio for highest nutritive value i.e. 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.

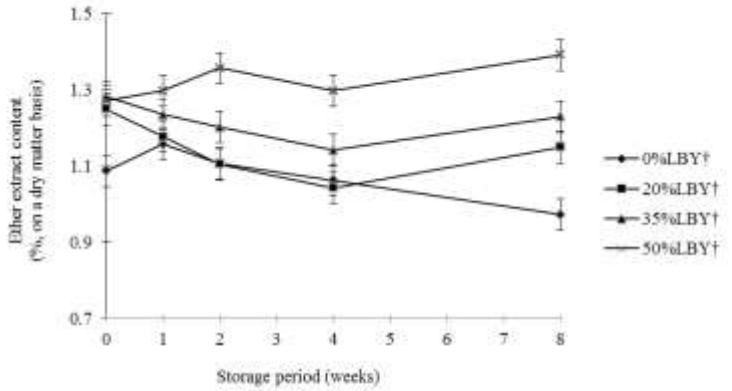

Figure 3.1 Ether extract contents showing interaction effect between mixture ratio and storage period

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.

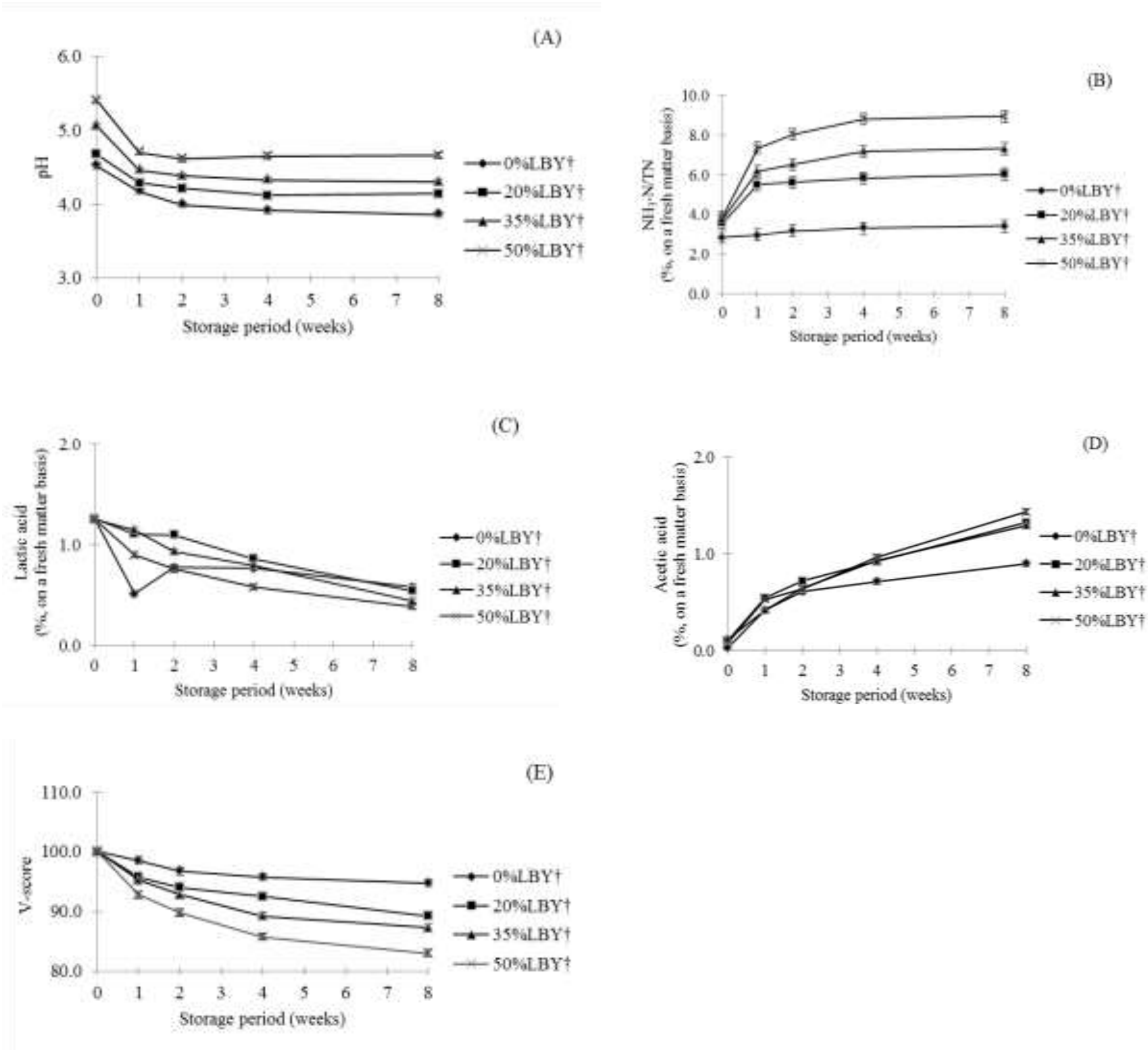

Figure 3.2 (A) pH, (B) NH_3-N/TN, (C) Lactic acid, (D) Acetic acid and (E)V-score showing interaction effect between mixture ratio and storage period.

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.

Table 3.1 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 3.2 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 x 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

[a-d]Means with different superscripts within columns significantly differed ($P < 0.05$). †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.

Table 3.3 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^{c}	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 (week)							
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 x 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

$^{a\text{-}e}$Means with different superscripts within columns significantly differed (P < 0.05). †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 and BA, n- and i-butyric acid. SEM, standard error of the means.

CHAPTER 4

Effects of different ratios for mixture of liquid brewer's yeast and cassava pulp with rice straw upon the daily gain, feed intake, digestibility, ruminal condition and blood metabolites of growing dairy heifers

4.1 Introduction

Recently, cassava pulp (CVP), a cheap by-product of starch manufacturing, has gained increased interest for use as an energy source in cattle feed. It contains high levels of starch and cellulose at approximately 60 and 20% on a dry matter (DM) basis, respectively (Kosugi *et al*. 2009), however, the crude protein (CP) content, 1.4 to 2.5% DM, was relatively low (Chauynarong *et al*. 2015). One strategy attempted by small dairy farms around brewery factories is the use of liquid brewer's yeast (LBY), containing 12 % DM and 48.3 % CP on a DM basis (Kamphayae *et al*. 2016b), as a protein source by ensiling it with CVP for animal feed. However, the high moisture content of the mixture is a major factor influencing silage fermentation. To improve fermentation quality and reduce any effluent losses of high moisture silage, the addition of dry absorbent to the mixture of LBY and CVP might be necessary. Rice straw (RS) is the main crop residue, which farmers in Asian countries usually store as ruminant feed. Feeding rice straw alone does not provide enough nutrients for high production levels (Sarnklong *et al*. 2010), due to its low nutritive value (Peripolli *et al*. 2016). To improve the productivity of ruminant animals, making total mixed ration (TMR) silage, by mixing RS with LBY and CVP, can be developed in areas where starch and beer manufacturing factories are located. The addition of LBY to CVP and RS can improve the chemical composition of the TMR mixture, with 50%LBY providing the highest CP content. From the perspective of fermentation quality, the mixtures can be fed to animals during 8 weeks of storage (Kamphayae *et al*. 2016a). Providing dairy heifers with high quality roughage produces high daily gain, which contributes to their early starts of heat, mating and milk production. The objective of this study was to evaluate the quality of diet on feed intake, digestibility, rumen fermentation and growth of dairy heifers.

4.2 Materials and methods

4.2.1 Feed Preparation, animals and experimental design

The experiment was conducted at Khon Kaen Animal Nutrition Research and Development Center over 10 weeks from June to September 2014. The first 2 weeks consisted of a preliminary period and the remaining 8 weeks was the main period for experimentation. Sixteen 13.8±1.6 months of Holstein crossbred heifers weighing 209.9±22.7 kg in body weight (BW), were randomly allocated to four feeding treatments with four replications. Ratios of LBY, CVP and RS in each test feed were 0:0:100 (RS), 0:70:30 (0%LBY), 20:50:30 (20%LBY) and 50:20:30 (50%LBY) on a fresh matter basis. The mixtures were mixed well by feed-mixing machine and packed into bag silos (400 kg) 2 to 4 weeks before feeding. All heifers were individually housed in pens and fed the diets. The test feeds were fed ad libitum, allowing for 10% refusal of feeding amount. The heifers were offered conventional concentrate (CC) at 1.5 % initial BW in two equal portions at 07:30 and 16:30 hours. The CC (g/kg) consisted of 451 cassava chip, 140 rice bran, 130 soybean meal, 120 ground corn meal, 120 oil palm kernel cake, 20 urea, 5 salt, 1 sulfur, 8 dicalcium phosphate and 5 mineral premix, for which total digestible nutrient (TDN) and CP contents were 75.3 and 18.2%, respectively, on a DM basis. The heifers had ad libitum access to water and mineral block throughout the experiment.

4.2.2 Data collection, sampling procedures and chemical analyses

The samples of test feed mixtures were taken when the bag silos were opened. A 50 g sample was homogenized with 100 ml of distilled water and stored overnight in a refrigerator at 4°C. The homogenate was then filtered through Whatman No.5 filter paper for pH, ammonia nitrogen (NH_3-N), lactic acid (LA), and volatile fatty acid (VFA) determination. The pH was measured by pH meter (D-22; Horiba, Kyoto, Japan), and the NH_3-N content was determined using a steam distillation technique (Society of Utilization of Self Supplied Feeds 2009). Lactic acid and VFA concentrations were determined by high performance liquid chromatography (HPLC) (LC-20A; Shimadzu, Kyoto, Japan), using a Nova-pak C18 steel column: 3.9 x 300 mm, flow rate was 0.5 ml/min and the wavelength of the UV detector was 210 nm.

Feed intake was measured by the amount of feed supplied and refused daily. The BW of heifers was measured every 2 weeks during the experiment, with diets and refusals also sampled. Fecal samples were collected by grab sampling during the last 5 days for analysis of acid-insoluble ash (AIA). The AIA was used to estimate the digestibility of nutrients (Van Keulen & Young 1977).

The samples of CC, test feeds, feed refusals and feces were oven dried at 60°C for 48 h. The dried samples were ground to pass through a 1 mm screen for subsequent analyses of DM, CP, ether extract (EE) and crude ash (CA), according to AOAC (2000). The organic matter (OM) was calculated as lost weight through ashing. Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by the procedure of Van Soest *et al.* (1991). TDN content was estimated using the following equation: TDN = (OM content x OM digestibility) + (EE content x EE digestibility) (Society of Utilization of Self Supplied Feeds 2009).

At the end of the experiment, rumen fluid and blood samples were collected at 0 and 4 h post feeding. Approximately 200 ml of rumen fluid was taken from the middle part of the rumen using a stomach tube connected to a vacuum pump at the sampling time. Each sample was measured immediately for pH using a pH meter (D-22; Horiba, Kyoto, Japan) and then filtered through four layers of cheesecloth. After adding 5 ml of 6 N HCl solution to 50 ml of the filtered rumen fluid, the mixture was centrifuged at 3,000 × *g* for 10 min. Supernatant was then used for NH_3-N and VFA analyses. The NH_3-N contents were measured by Kjeldahl methods (AOAC 2000) and VFA were determined by HPLC (LC-20A; Shimadzu, Kyoto, Japan) using a Nova-pak C18 steel column: 3.9 x 300 mm, flow rate was 1 ml/min and the wavelength of the UV detector was 210 nm. Approximately 10 ml of blood was sampled from the jugular vein using EDTA-containing tubes, with plasma separated by centrifugation at 500 x *g* for 10 min.

The concentrations of blood urea nitrogen (BUN), glucose and total cholesterol were determined using a reflotron test strip (Roche reflotron). Total protein concentrations were determined by a refractometer (PUR, Japan). All procedures were approved by the Ethical Principles for the Use of Animals for Scientific Purposes of the National Research Council of Thailand.

4.2.3 Statistical analyses

The data were analyzed by the GLM procedure of SAS (SAS 1996) using the following mathematical models: $Yij = \mu + Mi + \varepsilon ij$, where Yij = the observation, μ = overall means, Mi = effect of treatment and εij = residual error. The Tukey-Kramer test was used to detect the differences between the means (Kramer 1956). Significance was declared at $P < 0.05$.

4.3 Results

4.3.1 Chemical composition of experimental diets

Dry matter contents of CVP and LBY were 16.5 and 11.7%, respectively. The OM, CP, EE, NDF, ADF and ADL contents of CVP used for the mixture were 96.9, 2.9, 0.3, 39.4, 29.3 and 4.2%, respectively, on a DM basis. The OM, CP and EE contents in LBY were 91.3, 50.9 and 0.5%, respectively, on a DM basis. Fibers, i.e. NDF, ADF and ADL, were not detected in LBY. Table 4.1 shows the chemical composition of the experimental diets and fermentation characteristics of the mixtures. The DM content of the mixtures ranged from 35.3 to 40.3%. The DM, OM, NDF, ADF and ADL contents in the mixtures were decreased linearly with the proportion of LBY in the mixtures contrasting with the increase of CP and EE contents. The pH value, NH_3-N/TN and LA contents of the mixtures ranged from 3.6 to 3.9, 3.0 to 4.8% and 2.4 to 2.7%, respectively, on a fresh matter basis. Butyric acid content was not detected as the level of 0.0% on a fresh matter basis.

4.3.2 Weight gain, voluntary feed intake and digestibility

The weight gained, average daily gain (ADG), feed intake and apparent digestibility are presented in Table 4.2. The 56-day weight gained and ADG were not significantly different among treatments. For the intake of dietary treatment in this study, the test feed mixtures, conventional concentrate and total DM intake did not significantly differ, while 50%LBY had the highest CP intake compared to other treatments. RS had the highest intake of NDF and ADF ($P < 0.05$).

DM, OM and CP digestibility ranged from 64.9 to 71.2, 67.9 to 73.6 and 58.3 to 76.1%, respectively. 50%LBY had the highest values of CP digestibility among treatments (*P*

< 0.05). NDF digestibility ranged from 57.6 to 63.5% and the digestibility of 50%LBY was higher than those of 0%LBY and 20%LBY ($P < 0.05$). The TDN of the diet ranged from 61.9 to 68.6%, which did not differ significantly among treatments.

4.3.3 Ruminal condition

The parameters of ruminal condition are presented in Table 4.3. Ruminal pH values did not significantly differ among treatments, while NH_3-N in 50%LBY was higher than in other treatments 4 h after feeding ($P < 0.05$). Although total VFA concentrations and molar portions of VFA were not significantly different, C2:C3 ratio of 50%LBY was higher than those of 0%LBY and 20%LBY 4 h after feeding ($P < 0.05$).

4.3.4 Blood metabolites

The concentrations of BUN, glucose, total cholesterol and total protein in plasma samples are presented in Table 4.4. The BUN concentrations, at 0 and 4 h after feeding, ranged from 9.1 to 13.2 and 10.3 to 15.4 mg/dL, respectively, where 50%LBY provided the highest levels at both 0 and 4 h after feeding ($P < 0.05$). Blood glucose, blood cholesterol and total protein concentrations did not differ significantly among dietary treatments, either at 0 or 4 h after feeding.

4.4 Discussion

Xu *et al*. (2007) indicated that ensiling wet by-products containing high moisture with dry feeds as TMR is a suitable method to minimize the risk of effluent. In this study, effluent from silos and damage from mold were not observed visually in any of the bags silos.

The CP content of the mixture increased, while fiber content decreased in proportion to LBY inclusion. The increase of CP content 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, however, resulted in an increase of NH_3-N content when fermentation was prolonged (Mahanna & Chase 2003). As for fermentation quality, the mixture of 0%LBY, 20%LBY and 50%LBY were judged good (pH<4.2) and excellent (NH_3-N/TN<12.5%) according to the classification by McDonald & Whittenbury (1973). Butyric acid concentration was not detected, as shown in Table 1. Fermentation quality was likely to sustain the feed intake of the mixture.

The treatment of 50%LBY had the highest CP intake compared to the other treatments. Similar with the study by Moallem *et al*. (2011) who reported that the intake of high dietary crude protein diet was higher than low and moderate crude protein diet in dairy heifers. While RS had the highest intake of NDF and ADF ($P < 0.05$). This might be resulted by its chemical composition of feed.

The rumen NH_3-N concentration is a limiting factor for rumen microorganisms and affects the digestion of feed (Perdock 1987). Mutsvangwa *et al*. (2016) reported that ruminal NH_3-N concentration tended to be greater in cows fed the high-CP diet compared with the low-CP diet. The minimal level of NH_3-N in rumen is 3 mg/dL to allow proper microbial protein synthesis. Ruminal NH_3-N content higher than 5 to 10 mg/dL increases ammonia absorption from the rumen wall and results in loss of nitrogen (Matsumoto 2004). This study found that ruminal NH_3-N concentration before feeding of RS and 0%LBY was less than 3 mg/dL. However, after 4 h of feeding, all dietary treatments except for 0%LBY had ruminal NH_3-N concentrations higher than 3 mg/dL, with the highest level observed in 50%LBY at 5.8 mg/dL.

The concentration of BUN was determined to investigate the relationship between ruminal NH_3-N and protein utilization (Khampa *et al.* 2010; Mapato *et al.* 2010). The concentration of BUN at 0 and 4 h after feeding was highest in 50%LBY. This study revealed that the concentration of BUN was highly correlated with CP intake and digestibility, reflecting the level of NH_3-N production in the rumen. Huntington *et al.* (2001) indicated that the amount and degradability of dietary protein affects urea metabolism, as evidenced by changes in the concentrations of plasma urea nitrogen. Hassan & Saeed (2012) found that BUN concentration was significantly increased due to increasing level of dietary protein. However, the increased CP intake and digestibility did not improve daily gain of heifers in the present study. Blood glucose, blood cholesterol and total protein concentrations did not differ significantly among dietary treatments, either at 0 or 4 h after feeding.

4.5 Conclusion

The addition of LBY to CVP and RS can increase CP and decrease fiber content in proportion to LBY inclusion, without decreasing fermentation quality. Therefore, the inclusion of LBY up to 50% can improve CP digestibility, while the daily gain of each treatment is similar. The present results suggested that the effect of decreasing of RS and increasing of non-structural carbohydrate in the mixture should be investigated in further studies for ruminant feeding trial.

Table 4.1 Chemical composition and fermentation quality of conventional concentrate and test feeds

Item	CC	Test feeds				SEM
		RS	0%LBY	20%LBY	50%LBY	
Chemical composition, % of DM						
DM	87.6	89.7^{a}	40.3^{b}	37.6^{c}	35.3^{d}	0.38
OM	93.6	88.7^{d}	91.3^{a}	90.4^{b}	89.5^{c}	0.14
CP	18.2	3.4^{c}	3.7^{c}	7.1^{b}	12.3^{a}	0.20
EE	5.3	0.9^{b}	0.7^{c}	0.9^{b}	1.2^{a}	0.02
NDFom	20.9	74.0^{a}	63.5^{b}	62.1^{bc}	60.3^{c}	0.35
ADFom	10.9	50.5^{a}	43.3^{b}	42.3^{b}	41.6^{b}	0.49
ADL	2.2	4.3^{ab}	4.7^{a}	4.4^{ab}	4.0^{b}	0.12
Fermentation quality						
pH	-	-	3.6^{b}	3.6^{b}	3.9^{a}	0.04
NH_3-N/TN	-	-	3.0^{b}	3.5^{b}	4.8^{a}	0.12
Lactic acid	-	-	2.4^{b}	2.7^{a}	2.4^{ab}	0.05
Acetic acid	-	-	0.7^{c}	0.9^{b}	1.1^{a}	0.01
Propionic acid	-	-	0.2	0.2	0.2	0.02
Butyric acid	-	-	0.0	0.0	0.0	0.00

$^{a-d}$Means with different superscripts within rows significantly differed (P < 0.05). CC, conventional concentrate (g/kg) contains 451 cassava chip, 140 rice bran, 130 soybean meal, 120 ground corn meal, 120 oil palm kernel cake, 20 urea, 5 salt, 1 sulfur, 8 dicalcium phosphate and 5 mineral premix; RS, Rice straw; 0%LBY, 20%LBY and 50%LBY, mixture ratio of liquid brewer's yeast:cassava pulp:RS at 0:70:30, 20:50:30 and 50:20:30, respectively, on a fresh matter basis. 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; ADL, acid detergent lignin and NH_3-N/TN, ammonia nitrogen/total nitrogen. SEM, standard error of the means.

Table 4.2 Effect of dietary treatment on weight gain, average daily gain, intake and apparent digestibility in growing heifers

Item	Treatments				SEM
	RS	0%LBY	20%LBY	50%LBY	
Animal number	4	4	4	4	-
Initial wt, kg	211.5	207.8	212.5	207.8	-
Final wt, kg	292.3	274.8	282.3	274.8	15.04
56-d wt gained, kg	66.8	53.8	56.8	56.8	5.60
Daily gain, kg/d	1.19	0.96	1.02	1.01	0.10
Dry matter intake (DM g/kgBW$^{0.75}$/d)					
Feed					
Test feed	61.3	57.9	54.8	52.7	3.00
Concentrate	46.9	47.1	47.4	46.9	0.74
Total	108.2	105.0	102.2	99.6	3.21
Chemical compositions, % of DM					
CP	10.6^{c}	10.7^{c}	12.5^{b}	15.0^{a}	0.34
NDF	55.2^{a}	47.8^{b}	43.9^{b}	41.6^{b}	1.97
ADF	36.1^{a}	30.2^{b}	28.3^{b}	27.0^{b}	1.34
Apparent digestibility (%)					
DM	64.9^{b}	66.2^{b}	67.3^{ab}	71.2^{a}	1.12
OM	67.9^{b}	68.5^{b}	69.4^{ab}	73.6^{a}	1.15
CP	63.4^{bc}	58.3^{c}	66.8^{b}	76.1^{a}	1.36
NDF	60.7^{ab}	57.6^{b}	58.6^{b}	63.5^{a}	1.16
ADF	50.4	49.1	50.5	54.9	1.66
TDN	61.9	64.0	64.8	68.6	1.69

$^{a\text{-}c}$Means with different superscripts within rows significantly differed (P < 0.05). RS, Rice straw; 0%LBY, 20%LBY and 50%LBY, mixture ratio of liquid brewer's yeast:cassava pulp:RS at 0:70:30, 20:50:30 and 50:20:30, respectively, on a fresh matter basis; BW, body weight; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber and TDN, total digestible nutrients = (OM content x OM digestibility) + (EE content x EE digestibility). SEM, standard error of the means.

Table 4.3 Effect of dietary treatment on ruminal pH, ammonia nitrogen (NH_3-N) and volatile fatty acid (VFA) concentrations in growing heifers

Item	Treatments				SEM
	RS	0%LBY	20%LBY	50%LBY	
Ruminal pH					
0 h	7.1	7.0	7.0	7.0	0.03
4 h	6.9	6.7	6.8	6.8	0.08
NH_3-N, mg/dL					
0 h	2.7^{ab}	2.3^{b}	3.0^{ab}	3.6^{a}	0.27
4 h	3.8^{bc}	2.9^{c}	4.8^{b}	5.8^{a}	0.27
Total VFA, mM/L					
0 h	67.0	64.2	67.3	65.2	4.30
4 h	68.8	81.0	74.7	74.3	4.66
Molar portion of VFA, mol/100 mol					
Acetic acid (C_2), %					
0 h	70.5	69.0	70.8	70.1	0.64
4 h	68.6	68.6	67.4	70.1	0.62
Propionic acid (C_3), %					
0 h	19.0	19.7	18.0	18.5	0.69
4 h	20.0	21.4	21.3	18.9	0.61
Butyric acid (C_4), %					
0 h	10.5	11.3	11.3	11.4	0.42
4 h	11.4	10.0	11.3	10.9	0.70
C_2 : C_3 ratio					
0 h	3.7	3.5	4.0	3.8	0.17
4 h	3.4^{ab}	3.2^{b}	3.2^{b}	3.7^{a}	0.12

[a-c]Means with different superscripts within rows significantly differed ($P < 0.05$). RS, Rice straw; 0%LBY, 20%LBY and 50%LBY, mixture ratio of liquid brewer's yeast:cassava pulp:RS at 0:70:30, 20:50:30 and 50:20:30, respectively, on a fresh matter basis. SEM, standard error of the means.

Table 4.4 Effect of dietary treatment on blood urea nitrogen, blood glucose, blood cholesterol and total protein in growing heifers

Item	Treatments				SEM
	RS	0%LBY	20%LBY	50%LBY	
Blood urea nitrogen, mg/dL					
0 h	9.2^{b}	9.1^{b}	10.0^{b}	13.2^{a}	0.66
4 h	11.3^{b}	10.3^{b}	10.9^{b}	15.4^{a}	0.95
Blood glucose, mg/dL					
0 h	87.8	97.1	98.2	100.2	6.32
4 h	97.2	100.4	106.6	109.2	4.91
Blood cholesterol, mg/dL					
0 h	122.5	109.8	105.0	116.3	5.55
4 h	129.3	116.5	114.8	115.8	4.91
Total protein, g/dL					
0 h	6.0	6.2	6.5	6.6	0.19
4 h	6.3	6.3	6.4	6.1	0.20

$^{a\text{-}b}$Means with different superscripts within rows significantly differed ($P < 0.05$). RS, Rice straw; 0%LBY, 20%LBY and 50%LBY, mixture ratio of liquid brewer's yeast:cassava pulp:RS at 0:70:30, 20:50:30 and 50:20:30, respectively, on a fresh matter basis; SEM, standard error of the mean.

CHAPTER 5

General Conclusion

In Northeast Thailand, most of dairy animals are raised by small-scale farmers, which encounters a long dry season (6-7 months), resulting in scarcity of high quality diet to feed livestock. Moreover, farmers tend to use a relatively high level of concentrate which are expensive, as the result of high total feed cost of production. It is therefore essential to reduce the cost of feeding by utilizing food by-products, with relatively lower prices than those of commercial concentrate. Finding the cheap by-products obtained from local industries such as LBY and CVP can reduce the cost and economical alternatives for feeding livestock by small farmers. The mixture of CVP and LBY is, however, high in moisture, which is also a major factor influencing fermentation quality during the preservation. To improve the fermentation quality and reduce effluent losses of high moisture silage, addition of dry absorbent might be suitable to minimize the risk of effluent and preservation problems. Rice straw is the main crop residue and widely use as ruminant feed in Asian countries, adding RS as absorbent to high moisture silage can improve the fermentation quality.

TMR silage made by mixing wet by-products with roughage is practical to minimize the risk of effluent production and to avoid self-selection of feeds by animals. Therefore, studying optimum mixing ratios and storage periods is necessary for the practical use in farms. Dairy heifers play an important role in the future success of all dairy farms. Overall management of dairy heifers must be handled in a manner that yields the best quality of heifers with a high potential to be productive, and at minimal cost to the farm and the environment. Dairy nutrition is essentially as simple as understanding the nutrient requirements of growing ruminants at various stages of growth and combining various feed ingredients to meet those needs in a cost-effective manner. Of course, high quality feed must be followed to achieve maximum performance from growing dairy heifers. Providing the animals with those TMR produces high productivity of ruminant animals, which can be developed in areas where beer and starch manufacturing factories are located. From the experimental results, the following conclusion was made.

In Chapter 2, therefore, maintaining an optimum ratio of LBY and CVP during the necessary storage periods is critical for its practical use in farms. To evaluate the chemical composition, fermentation quality and *in vitro* ruminal fermentation of various ratios and storage periods of LBY mixed with CVP. The mixtures of fresh LBY and CVP were made (LBY:CVP were 0:100, 10:90, 20:80 and 30:70) on a fresh matter basis. After storage, the mixtures were opened for analysis at 0, 1, 2, 3 and 4 weeks after storage. The inclusion of LBY (up to 30%) into CVP can significantly improve the chemical composition of a feed mixture. A remarkable increase in CP content with decreased IVGP was achieved, with acceptable fermentation quality and without decreasing IVOMD. In addition, a preservation period of up to 4 weeks could assure superior fermentation quality in all types of mixtures. Therefore, a low nutritive value of livestock feed, due to the limitation of CVP as a feed ingredient, can be instantly improved by the inclusion of LBY.

In Chapter 3, the mixture of CVP and LBY is, however, high in moisture, which is also a major factor influencing fermentation quality. 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. To evaluate the effect of LBY addition on chemical composition and fermentation quality of mixture of LBY and CVP with rice straw in different ratios during preservation periods as a pilot study for TMR feeding for ruminants. The mixtures of LBY, CVP and RS were made (LBY:CVP:RS were 0:70:30, 20:50:30, 35:35:30 and 50:20:30) on fresh matter basis. Four bags from each mixture were opened at 0, 1, 2, 4 and 8 weeks after storage. The addition of LBY into the CVP and RS can improve the chemical composition of the TMR mixture, and 50%LBY is the better ratio for highest nutritive value i.e. 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.

In Chapter 4, to improve the productivity of ruminant animals, making total mixed ration (TMR) silage, by mixing RS with LBY and CVP, can be developed in areas where starch and beer manufacturing factories are located. Providing dairy heifers with high quality roughage produces high daily gain, which contributes to their early starts of heat, mating and milk production. Therefore, to evaluate the effects of the mixture of LBY and CVP with RS on feed intake, digestibility, rumen fermentation and growth of dairy heifers. The experiment was conducted at Khon Kaen Animal Nutrition Research and Development Center over 10 weeks. The first 2 weeks consisted of a preliminary period and the remaining 8 weeks was the main period for experimentation. Sixteen 13.8±1.6 months of Holstein crossbred heifers weighing 209.9±22.7 kg in body weight (BW), were randomly allocated to four feeding treatments with four replications. Ratios of LBY, CVP and RS in each test feed were 0:0:100, 0:70:30, 20:50:30 and 50:20:30, respectively for LBY:CVP:RS on a fresh matter basis, with were offered conventional concentrate at 1.5% initial body weight daily and fed the treatment diets ad libitum. Therefore, the inclusion of LBY up to 50% can improve CP digestibility, while the daily gain and feed intake were not significantly different among the treatments. The ruminal pH did not differ significantly among treatments, while NH_3-N and BUN were highest in 50%LBY.

GENERAL SUMMARY

Feed is the primary cost associated with growing replacement heifers and producing milk, so cheaper feeds that offer the potential to lower feed cost and improve the bottom line are worth considering. In addition to lowering feed costs, commodity or by-product from various food processing industries can be used as animal feed. Wet by-product, such as LBY and CVP can be utilized by mixing themselves or mixed with dry absorbent (rice straw) with optimal proportion and storage period for dairy cattle feed. Therefore, ensiling wet by-products with dry feeds as a TMR is a suitable method to minimize the risk of effluent and conserve nutritive value of feed. Feeding a TMR helps a dairy heifers achieve maximum performance, which accomplished by feeding a nutritionally balanced ration, allowing cows to consume as close to their actual requirements. This would be especially beneficial for small farmers in dairy production, which located near the beer and starch manufacturing factories. Hence, three studies were carried out to evaluate of beer and starch by-products as feed for dairy heifers.

1. Effects of different ratios and storage periods of liquid brewer's yeast mixed with cassava pulp on chemical composition, fermentation quality and *in vitro* ruminal fermentation

The 500 g of each mixture were ensiled in plastic bags and stored at 30 to 32 °C. After storage, the bags were opened for analysis at 0, 1, 2, 3 and 4 weeks after storage. A 50 g sample from each composite was homogenized with 100 ml of distilled water and stored overnight in a refrigerator, at 4°C. The homogenate was then filtered through Whatman no.5 filter paper for pH, NH_3-N, LA and VFA determination. The remaining sample mixtures were analyzed for DM, EE, CA, OM, NDFom, ADFom and ADL. To assess the quality of the mixture, the V-scores were calculated from the NH_3-N/TN and VFA concentrations. For gas production analyses, the samples were incubated with the buffered rumen fluid. Gas

production volume was recorded at 6, 12, 24 and 48 h of incubation and gas production of the incubated mixtures was calculated through the following equations.
GP = (V24 - V0 - GP0)/ Weight in mg DM

OMD and ME were estimated in the 24 h gas production period, through the following equations.

OMD (%) = 9.00 + 0.9991 GP + 0.0595 XP + 0.0181 XA

ME (MJ/kg DM) = 1.06 + 0.1570 GP + 0.0084 XP + 0.0220 XE - 0.0081 XA

The pH, and DM, OM, CP and EE contents of the LBY were 5.3, and 12.0, 92.0, 48.3 and 0.7% on a DM basis, respectively. The NDFom, ADFom and ADL contents were undetected in the LBY. The CVP displayed pH, and DM, OM, CP, EE, NDFom, ADFom and ADL contents at 3.7, and 18.6, 96.8, 2.9, 0.4, 36.6, 24.5 and 4.9%, respectively.

Among the mixture ratio means, the DM, OM, CP, EE, NDFom, ADFom and ADL of the four mixtures ranged 16.5 to 18.0, 95.7 to 96.6, 3.2 to 14.1, 0.4 to 0.5, 32.0 to 36.2, 21.1 to 24.8 and 3.8 to 4.9%, respectively. Increasing the level of LBY linearly increased CP and EE contents, and decreased other chemical compositions ($P < 0.01$). For the means of storage period, the DM and OM contents linearly and quadratically decreased, while the EE and ADFom contents increased linearly, as the storage periods progressed ($P \leq 0.01$). The interaction effects between mixture ratios and storage periods on DM, OM, CP, EE and ADFom were found.

The fermentation quality in the LBY and CVP mixtures were shows the changes in pH, NH_3-N/TN, LA and VFA concentrations of the mixture during the storage period ($P \leq 0.01$). All parameters were quadratically affected by the mixture ratios, and acute increases in pH, acetic and butyric contents, as well as decreases in NH_3-N/TN, LA contents and V-scores were observed in the progression from LBY0 to LBY10. More specifically, the quadratically increases of acetic and propionic acids and the linear decrease in V-scores were more severe in the later preservation periods. The interaction effects between the mixture ratios and storage periods were observed in pH, LA, acetic, propionic and butyric acid contents, and V-score ($P < 0.01$).

Gas production from the mixture of LBY and CVP with different mixture ratios, storage periods and post incubation parameters, described as OMD and ME, are shown in Table 2.4. Comparing the mixture ratio means, the gas production of LBY0 and LBY10 differed only slightly, yet were higher than that of LBY20 and LBY30 ($P < 0.01$). The gas production at 48 h decreased quadratically with the inclusion of LBY. As indicated within the storage period means, gas production decreased as the storage period increased, especially within weeks 0 to 1. The interaction effects between the mixture ratios and storage periods were observed in the 6, 24 and 48 h. Among the four mixture ratio means, estimated OMD and ME ranged from 77.4 to 79.7% and 11.4 to 11.8 MJ/kgDM, respectively, each scoring lowest in LBY20 ($P < 0.05$). Provides the interaction effects of the mixture ratios and storage periods for both OMD and ME ($P < 0.01$).

The correlation coefficients between chemical compositions, OMD, ME and gas production were shown DM and OM contents with positively correlated at 6 h ($P < 0.05$), 12 and 24 h ($P < 0.01$) and 48 h ($P < 0.05$). NDFom and NFC contents were positively correlated at 12, 24 and 48 h ($P < 0.01$). The NH_3-N/TN contents at 6 h incubation were also positively correlated ($P < 0.01$). Contrastingly, the CP content was negatively correlated at 12 h ($P < 0.05$), and at 24 and 48 h ($P < 0.01$). EE content was also negatively correlated at 6 h ($P < 0.05$), 12 and 24 h ($P < 0.01$) and 48 h ($P < 0.05$). OMD and ME did not significantly correlate with the chemical composition.

2. 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

This study aimed to evaluate the effect of LBY addition on chemical composition and fermentation quality of mixture of LBY and CVP with rice straw in different ratios during preservation periods as a pilot study for TMR feeding for ruminants. 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 composites of 500 g were made from each mixture and packed into 25 x 35 cm plastic bags. Four bags from each mixture were opened at 0, 1, 2, 4 and 8 weeks after storage.

During preservation mold was not found visually in all the mixtures. 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.

The mixture ratio influenced all the chemical composition ($P < 0.01$). The DM, OM, CP, EE, NDFom and ADFom contents were 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. For the means of storage period from week 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.

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

3. Effects of different ratios for mixture of liquid brewer's yeast and cassava pulp with rice straw upon the daily gain, feed intake, digestibility, ruminal condition and blood metabolites of growing dairy heifers

The experiment was conducted at Khon Kaen Animal Nutrition Research and Development Center. The first 2 weeks consisted of a preliminary period and the remaining 8 weeks was the main period for experimentation. Sixteen 13.8±1.6 months of Holstein crossbred heifers weighing 209.9±22.7 kg in body weight, were randomly allocated to four feeding treatments with four replications. Ratios of LBY, CVP and RS in each test feed were 0:0:100 (RS), 0:70:30 (0%LBY), 20:50:30 (20%LBY) and 50:20:30 (50%LBY) on a fresh matter basis. The mixtures were mixed well by feed-mixing machine and packed into bag silos (400 kg) 2 to 4 weeks before feeding. The heifers were offered conventional concentrate at 1.5% initial body weight daily and fed the treatment diets *ad libitum*. The CC (g/kg) consisted of 451 cassava chip, 140 rice bran, 130 soybean meal, 120 ground corn meal, 120 oil palm kernel cake, 20 urea, 5 salt, 1 sulfur, 8 dicalcium phosphate and 5 mineral premix, for which total digestible nutrient and CP contents were 75.3 and 18.2%, respectively, on a DM basis. At the end of the experiment, rumen fluid and blood samples were collected at 0 and 4 h post feeding.

The 56-day weight gained and ADG were not significantly different among treatments. The test feed mixtures, conventional concentrate and total DM intake did not significantly differ, while 50%LBY had the highest CP intake compared to other treatments. The RS had the highest intake of NDF and ADF ($P < 0.05$).

The digestibility of DM, OM and CP ranged from 64.9 to 71.2, 67.9 to 73.6 and 58.3

to 76.1%, respectively. The 50%LBY had the highest values of CP digestibility among treatments ($P < 0.05$). Digestibility of NDF ranged from 57.6 to 63.5% and the digestibility of 50%LBY was higher than those of 0%LBY and 20%LBY ($P < 0.05$). The TDN of the diet ranged from 61.9 to 68.6%, which did not differ significantly among treatments.

Ruminal pH values did not significantly differ among treatments, while NH_3-N in 50%LBY was higher than in other treatments 4 h after feeding ($P < 0.05$). Although total VFA concentrations and molar portions of VFA were not significantly different, C2:C3 ratio of 50%LBY was higher than those of 0%LBY and 20%LBY 4 h after feeding ($P < 0.05$).

The BUN concentrations, at 0 and 4 h after feeding, ranged from 9.1 to 13.2 and 10.3 to 15.4 mg/dL, respectively, where 50%LBY provided the highest levels at both 0 and 4 h after feeding ($P < 0.05$). Blood glucose, blood cholesterol and total protein concentrations did not differ significantly among dietary treatments, either at 0 or 4 h after feeding.

The addition of LBY to CVP and RS can increase CP and decrease fiber content in proportion to LBY inclusion, without decreasing fermentation quality. Therefore, the inclusion of LBY up to 50% can improve CP digestibility, while the daily gain of each treatment is similar. The results suggested that the effect of decreasing of RS and increasing of non-structural carbohydrate in the mixture should be investigated in further studies for ruminant feeding trial.

REFERENCES

REFERENCES

Association of Official Analytical Chemists (AOAC). 2000. Official Methods of Analysis, 17th edn. Association of Official Analytical Chemists, Arlington, VA.

Adeyemi OA, Eruvbetine D, Oguntona T, Dipeolu MA, Agunbiade JA. 2007. Enhancing the nutritional value of whole cassava root meal by rumen filtrate fermentation. *Archivos de zootecnia* **56**: 261-264.

Aliyu S, Bala M. 2011. Brewer's spent grain: A review of its potentials and applications. *African Journal of Biotechnology* **10**: 324-331.

Bouqué CV, Fiems LO. 1988. Vegetable by-products of agro-industrial origin. *Livestock Production Science* **19**: 97-135.

Bruning CL, Yokoyama MT. 1987. Characteristics of live and killed brewer's yeast slurries and intoxication by intraruminal administration to cattle. *Journal of Animal Science* **66**:585-591.

Cai Y. 2004. Methods for feed evaluation of forages: Silage Analyses. In Japan Society of Grassland Science (ed.): Field and Laboratory Methods for Grassland Science. Japan Livestock Technology Association, Tokyo, pp. 279-283.

Cao Y, Cai Y, Takahashi T, Yoshida N, Tohno M, Uegaki R, Nonaka K, Terada. 2011. Effect of lactic acid bacteria inoculant and beet pulp addition on fermentation characteristics and *in vitro* ruminal digestion of vegetable residue silage. *Journal of Dairy Science* **94**: 3902-3912.

Cerrillo MA, Juarez RAS. 2004. *In vitro* gas production parameters in cacti and tree species commonly consumed by grazing goats in a semi arid region of North Mexico. *Livestock Research for Rural Development* **16**: 4-9.

Chumpawadee S, Soychuta S. 2009. Nutrient enrichment of cassava starch industry by-product using rumen microorganism as inoculums source. *Pakistan Jounal of Nutrition* **8**: 1380-1382.

Coblentz WK, Nellis SE, Hoffman PC, Hall MB, Weimer PJ, Esser NM, Bertram MG. 2013. Unique interrelationships between fiber composition, water-soluble carbohydrates, and gas production for fall-grown oat forages. *Journal of Dairy Science* **96**:7195-7209.

Coppock CE, Bath DL, Harris B. 1981. From feeding to feeding systems. *Journal of Dairy Science* **64**: 1230–1249.

Chauynarong N, Bhuiyan MM, Kanto U, Iji PA. 2015. Variation in nutrient composition of cassava pulp and its effects on *in vitro* digestibility. *Asian Journal of Poultry Science* **9**: 203-212.

Department of Livestock Development (DLD). 2015. Report. [homepage on the Internet]. Information and Communication Technology Center, Bangkok; [cited 26 September 2016]. Available from URL

http://ict.dld.go.th/th2/images/stories/stat_web/yearly/2558/3.milkcow_region.pdf

Dung DV, Shang W, Yao W. 2014. Effect of crude protein levels in concentrate and concentrate levels in diet on *in vitro* fermentation. *Asian-Australasian Journal of Animal Sciences* **27**:797-805.

Food and Agriculture Organization of the United Nations (FAO). 2012. FAOSTAT. [cited 20 August 2016]. Available from URL http:// http://faostat.fao.org/site/339/default.aspx

Gabler MT, Tozer PR, Heinrichs AJ. 2000. Development of a cost analysis spreadsheet for calculating the costs to raise a replacement dairy heifer. *Journal of Dairy Science* **83**:1104-1109.

Getachew G, Robinson PH, DePeters EJ, Taylor SJ. 2004. Relationships between chemical composition, dry matter degradation and *in vitro* gas production of several ruminant feeds. *Animal Feed Science and Technology* **111**: 57-71.

Grieve DG. 1979. Feed intake and growth of cattle fed liquid brewer's yeast. *Canadian Journal of Animal Science* **59**: 89-94.

Hassan SA, Saeed AA. 2012. Effect of feeding different levels of dietary protein with high or low rumen degradable:undegradable dietary nitrogenon awassi lambs performance 3-selected biochemical parameters. *Kahramanmaras Sutcu Imam University Journal of Natural Sciences* **15**: 36-45.

Higginbotham GE, Torabi M, Huber JT. 1989. Influence of dietary protein concentration and degradability on performance of lactation cows during hot environmental temperatures. *Journal of Dairy Science* **72**: 2554-2564.

Huntington GE, Poore M, Hopkins B, Spears J. 2001. Effect of ruminal protein degradability on growth and N metabolism in growing beef steers. *Journal of Animal Science* **79**:533-541.

Imai A. 2000. Silage making and utilization of high moisture by-products. *Japanese Journal of Grassland Science* **47**:307-310.

Jintanawit W, Juttupornpong S, Markranit R, Srimongcholngam S, Viwatwongwana N. 2006. A study of changing of population of lactic acid bacteria and yeast during ensilaging of cassava pulp. Proceedings of the 44th Kasetsart university annual conference, Kasetsart, 30 January - 2 February, 2006. pp.131-137.

Kaewwongsa W, Traiyakun S, Yuangklang C, Wachirapakorn C, Paengkoum P . 2011. Protein enrichment of cassava pulp fermentation by *Saccharomyces cerevisiae*. *Journal of Animal and Veterinary Advances* **10**:2434-2440.

Kamphayae S, Kumagai H, Angthong W, Narmseelee R, Bureenok S. 2016a. Effects of different ratios and storage periods of liquid brewer's yeast mixed with cassava pulp on chemical composition, fermentation quality and in vitro ruminal fermentation. *Asian-Australasian Journal of Animal Sciences*. doi:10.5713/ajas.16.0218.

Kamphayae S, Kumagai H, Bureenok S, Narmseelee R, Butcha P, 2016b. 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. *Animal Science Journal*. doi:10.1111/asj.12682.

Khampa S, Chuelong S, Kosonkittiumporn S, Khejornsart P. 2010. Manipulation of yeast fermented cassava chip supplementation in dairy heifer raised under tropical condition. *Pakistan Journal of Nutrition* **9**:950-954.

Khon Kaen Brewery (KKB). 2015. History. [homepage on the Internet]. Khon Kaen Brewery Co, Ltd, Khon Kaen; [cited 25 February 2015]. Available from URL

http:// khonkaen brewery.co.th/ index.php?route=about/about

Kosoom W, Charoenwattanasakun N, Ruangpanit Y, Rattanatabtimtong S, Attamangkune S. 2009. Physical, chemical and biological properties of cassava pulp. Proceedings of the 47th Kasetsart University Annual Conference, Kasetsart, 17-20 March, 2009. pp.117-124.

Kosugi A, Kondo A, Ueda M, Murata Y, Vaithanomsat P, Thanapase W, Arai T, Mori Y. 2009. Production of ethanol from cassava pulp via fermentation with a surface-engineered yeast strain displaying glucoamylase. *Renewable Energy* **34**:1354-1358.

Kramer CW. 1956. Extension of multiple range tests to group means with unequal numbers of replications. *Biometrics* **12**: 307-310.

Lapar MLA, Garcia A, Aditto S, Suriya P. 2005. Measuring cost efficiency in small holder dairy: empirical evidence from northeast Thailand. [homepage on the Internet]. American Agricultural Economics Association, 2005 Annual meeting, July 24-27, Providence, RI; [cited 2 December 2014]. Available from URL http://ageconsearch. umn.edu/bitstream/19514/1/sp05la07.pdf

Levic J, Djuragic O, Sredanovic S. 2010. Use of new feed from brewer by-products for breeding layers. *Romanian Biotechnological Letters* **15**: 5559-5565.

Lindgren SE, Axelsson LT, Mcfeeters RF. 1990. Anaerobic L-lactate degradation by Lactobacillus plantarum. *FEMS Microbiology Letters* **66**: 209-214.

Linton JH. 1977. Nutritional evaluation of brewery by-products. Review of nutrition papers from Brewers Feed Conference. *Feedstuffs* **51**: 8.

Lounglawan P, Khungaew M, Suksombat W. 2011. Silage production from cassava peel and cassava pulp as energy source in cattle diets. *Journal of Animal and Veterinary Advances* **10**:1007-1011.

Mahanna B, Chase LE. 2003. Practical applications and solutions to silage problems. In: Buxton DR, Muck RE, Harrison JH (eds.), Silage Science and Technology, pp. 251-304. Madison, WI: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America.

Manzano M, Giusto C, Bartolomeoli I, Buiatti S, Comi G. 2005. Microbiological analyses of dry and slurry yeasts for brewing. *Journal of Institute of Brewing* **111**: 203-208.

Mapato C, Wanapat M, Cherdthong A. 2010. Effects of urea treatment of straw and dietary level of vegetable oil on lactating dairy cows. *Tropical Animal Health and Production* **42**:1635-1642.

Matsumoto M. 2004. Dietary protein degradation and microbial protein synthesis. In: Onodera R (eds), A World of Rumen. pp. 271-279. Nobunkyo, Tokyo. (In Japanese)

McDonald P, Henderson AR, Heron SJE. 1991. The Biochemistry of Silage 2nd edn.

Chalcombe Publications, Marlow, Bucks, UK.

McDonald P, Whittenbury R. 1973. The ensilage process. In: Butler GW, Bailey RW (eds), Chemistry and Biochemistry of Herbage, Vol. 3, pp. 33-60. Academic Press, London and New York.

Menke KH, Steingass H. 1988. Estimation of energetic feed value obtained from chemical analysis and *in vitro* gas production. *Animal Research and Development* **28**: 7-55.

Moallem U, Blanck R, Leer H, Livshitz L, Zachut M, Arieli A, 2011. Effects of high dietary crude protein on the characteristics of preovulatory follicles in dairy heifers. *Journal of Dairy Science* **94**: 785-792.

Moore KA. 2015. Effects of brewer's yeast on growth performance and carcass traits of Angus-influenced steer. [homepage on the Internet]. College of Agriculture, California State University, CA; [cited 2 August 2015]. Available from URL http://www20.csueastbay.edu/programs/csr/files/docs/2014%20CSU%20Award%20Winner%20Summaries/KatherineMoore-2014%20Summary.pdf

Muck RE, Moser LE, Pitt RE. 2003. Postharvest factors affecting ensiling. In: Buxton DR, Muck RE, Harrison JH (eds), Silage Science and Technology. pp. 51-304. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI.

Mutsvangwa T, Davies KL, McKinnon JJ, Christensen DA. 2016. Effects of dietary crude protein and rumen-degradable protein concentrations on urea recycling, nitrogen balance, omasal nutrient flow, and milk production in dairy cows. *Journal of Dairy Science* **99**: 6298-6310.

National Research Council (NRC). 2001. Nutrient Requirements of Dairy Cattle7thedn. National Academy Press. Washington, DC.

Nishino N, Harada H, Sakaguchi E. 2003. Evaluation of fermentation and aerobic stability of wet brewers grains ensiled alone or in combination with various feeds as a total mixed ration. *Journal of the Science of Food and Agriculture* **83**:557-563.

Nitipot P, Nishida K, Chaithiang R, Pattarajinda V, Sommart K. 2009. Metabolizable energy evaluation of pangola grass hay, cassava chip, cassava pulp and brewery waste in Thai native cattle. [cited 5 November 2014]. Available from URL http://lib248.kku.ac.th/ annaul- conference/work/pdf/ AG%2052-12-p55-57.pdf.

Office of Agricultural Economics (OAE). 2014. Agricultural production data. [homepage on the Internet]. Office of Agricultural Economics, Bangkok; [cited 5 November 2014]. Available from URL http://www.oae.go.th/download/prcai/DryCrop/ cassava.pdf

Oude Elferink SJWH, Krooneman J, Gottschal JC, Spoelstra SF, Faber F, Driehuis F. 2001. Anaerobic conversion of lactic acid to acetic acid and 1,2-propanediol by Lactobacillus buchneri. *Applied and Environmental Microbiology* **67**: 125-132.

Perdock HB. 1987. Ammoniated rice straw as a feed for growing cattle. Ph.D. thesis, University of New England, Armidale, Australia.

Peripolli V, Barcellos JOJ, Prates ER, McManus C, Silva LP, Stella LA, Costa Jr JBG, Lopes RB. 2016. Nutritional value of baled rice straw for ruminant feed. *Revista Brasileira de Zootecnia* **45**: 392-399.

Porter MG, Murray RS. 2001. The volatility of components of grass silage on oven drying and the inter-relationship between dry-matter content estimated by different analytical methods. *Grass and Forage Science* **56**: 405-411.

Santos M, Moretzsohn M, Camporese S. 2014. Solid wastes in brewing process: A review. *Journal of Brewing and Distilling* **5**: 1-9.

Sarnklong C, Cone JW, Pellikann W, Hendriks WH. 2010. Utilization of rice straw and different treatments to improve its feed value for ruminants: A review. *Asian-Australasian Journal of Animal Sciences* **23**: 680-692.

Shioya S. 2008. Future prospects of TMR center based on self-supplying feed. *Japanese Journal of Grassland Science* **54**: 178–181.

Society of Utilization of Self Supplied Feeds. 2009. The Guidebook for Quality Evaluation of Forage, 3th edn. Japan Grassland Agriculture and Forage Seed Association, Tokyo. (In Japanese)

Steckley JD, Grieve DG, Macleod GK, Moran ET Jr. 1979a. Brewer's Yeast Slurry, I. Composition as affected by length of storage, temperature, and chemical treatment. *Journal of Dairy Science* **62**: 941-946.

Steckley JD, Grieve DG, Macleod GK, Moran ET Jr. 1979b. Brewer's Yeast Slurry. II. A source of supplementary protein for lactating dairy cattle. *Journal of Dairy Science* **62**: 947-953.

Suksombat W, Lounglawan P, Noosen P. 2006. Energy and protein evaluation of five feedstuffs used in diet in which cassava pulp as main energy source for lactating dairy cow. *Suranaree Journal of Science and Technology* **14**:99-107.

Thongkratoke R, Khempaka S, Molee W. 2010. Protein enrichment of cassava pulp using microorganisms fermentation techniques for use as an alternative animal feedstuff. *Journal of Animal and Veterinary Advances* **9**: 2859-2862.

Umana R, Staples CR, Bates CB, Wilcox CJ, Mahanna WC. 1991. Effects of a microbial inoculant and (or) sugarcane molasses on the fermentation, aerobic stability, and digestibility of bermuda grass ensiled at two moisture contents. *Journal of Animal Science* **69**: 4588-4601.

Van Keulen J, Young BA. 1977. Evaluation of acid-insoluble ash as a marker in ruminant digestibility. *Journal of Animal Science* **44**: 282-287.

Van Soest PJ, Robertson JB, Lewis AB. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* **74**: 3583 - 3597.

Vorachinda R, Bunyatratchata W, Suriyagamon S, Kanchani N, Butsapuni S, Masi C. 2011. Improvement of protein content in cassava pulp by fungi fermentation. The 3rd International Conference on Sustainable Animal Agriculture for Developing Countries (SAADC2011) July 26-29, 2011 Nakhon Ratchasima, Thailand. pp. 307 -311.

Wanapat M. (1999). Feeding of ruminant in the tropics based on local feed resources. Khon Kaen Publishing, Khon Kaen, Thailand.

Wanapat M, Pimpa O, Petlum A, Wachirapakorn C, Yuangklang C. 2000. Participation scheme of smallholder dairy farmers in the Northeast Thailand on improving feeding system. *Asian-Australasian Journal of Animal Sciences* **13**: 830-836.

Wanapat M, Polyorach S, Boonnop K, Mapato C, Cherdthong A. 2009. Effects of treating rice straw with urea or urea and calcium hydroxide upon intake, digestibility, rumen fermentation and milk yield of dairy cows. *Livestock Science* **125**: 238-243.

Wang F, Nishino N. 2008a. Ensiling of soybean curd residue and wet brewer grains with or without other feeds as a total mixed ration. *Journal of Dairy Science* **91**: 2380-2387.

Wang F, Nishino N. 2008b. Resistance to aerobic deterioration of total mixed ration silage: Effect of ration formulation, air infiltration and storage period on fermentation characteristics and aerobic stability. *Journal of the Science of Food and Agriculture* **88**:133-140.

Wang RR, Wang HL, Liu X, Xu CC. 2011. Effects of different additives on fermentation characteristics and protein degradation of green tea grounds silage. *Asian-Australasian*

Journal of Animal Sciences **24**: 616-622.

Xu C, Cai Y, Moriya N, Ogawa M. 2007. Nutritive value for ruminants of green tea grounds as a replacement of brewers' grains in totally mixed ration silage. *Animal Feed Science and Technology* **138**: 228-238.

Yahaya MS, Kawai M, Takahashi J, Matsuoka S. 2002.The effects of different moisture content and ensiling time on silo degradation of structural carbohydrate of Orchardgrass. *Asian-Australasian Journal of Animal Sciences* **15**: 213-217.

Yang XL, Li JL, Yu Z, Yan YH, Zhang XQ, Yu W, Ma X, Huang LK Peng Y. 2014. Influence of moisture content on the silage quality of Lolium multiflorum. *Journal of Animal and Veterinary Advances* **13**: 702-705.

Yimmongkol S. 2009. Research and development projects on improvement of the potential use of dried cassava pulp and cassava leaf meal in concentrate of feedlot cattle. Ph.D. thesis, Kasetsart University, Thailand.

Zhang WW, Zhang YG, Liu Z. 2012. Effect of different absorbents on fermentation quality of wet potato pulp. *Journal of Animal and Veterinary Advances* **11**: 4230-4235.