Studies on utilization of by-products for ruminant feeds in tropical Asia

Takashi Sakai

Laboratory of Animal Husbandry Resources

A dissertation presented to the Office of Graduate School of Agriculture

Kyoto University

In partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

January 2017

Studies on utilization of by-products for ruminant feeds in tropical Asia

Doctoral thesis

Takashi Sakai, 2017

Laboratory of Animal Husbandry Resources,

Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University

Kitashirakara-Oiwakecho, Sakyo-ku, Kyoto, Japan

Thesis supervisor

Prof. Dr. Hiroyuki Hirooka
Laboratory of Animal Husbandry Resources
Graduate School of Agriculture, Kyoto University

Studies on utilization of by-products for ruminant feeds in tropical Asia

Takashi Sakai
Laboratory of Animal Husbandry Resources,
Division of Applied Biosciences, Graduate School of Agriculture, Kyoto University

ABSTRACT

In the tropical Asia, the demand for utilizing by-products for animal feeds is increasing in order to reduce feed cost of the livestock farmers and to mitigate the environmental impact from the industrial activities. To investigate the effect of by-product feedstuffs on nutritional availability and productivity of livestock animals, three experiments were conducted in two tropical Asian areas.

In Tarai, Nepal, *in vivo* digestibility experiment using Nepal native goats and lactation experiment using Murrah buffaloes were conducted to evaluate the effect of TMR silage including brewers grain as replacement for concentrate. The TMR silage, which had higher neutral and acid detergent fiber contents and the digestibility than concentrate (P<0.05) and similar crude protein and total digestible nutrient contents with concentrate, were used for the lactation experiment. Although no significant difference in milk yield was observed between concentrate feeding and TMR silage feeding, milk yield was increased when substitution rate of TMR silage was twice as much amount (P<0.05).

In Khon Kean, Thailand, four steers of Thai native cattle were used to determine an adequate level of supplementation of desalted mother liquor (DML) based on digestibility of nutrients, nitrogen balance, blood metabolites and ruminal fermentation. A 4x4 Latin square design experiment was conducted by adding different amounts of DML to three experimental diets (T1: 1.1%, T2: 2.2%, T3: 3.4% sodium chloride concentration with supplementary DML on a dry matter (DM) basis) and comparing their effects with those of a control diet (C) containing 1.0% commercial salt on a DM basis. The result showed that T3 achieved the lowest nitrogen retention (P<0.05), followed by C, T2 and T1. The ratios of energy retention to gross energy were higher in T1 and T3 than T2, and that in C was the lowest (P<0.05).

Key words: tropical Asia, animal nutrition, by-product feedstuffs, Murrah buffalo, Thai native cattle,

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PUBLICATIONS

Chapters 2 and 3 are the peer reviewed version of the following articles, respectively:

Sakai T, Devkota NR, Oishi K, Hirooka H, Kumagai H. 2015.

> Evaluation of total mixed ration silage with brewers grains for dairy buffalo in Tarai, Nepal. *Animal Science Journal*.

Sakai T, Angthong W, Takeda M, Oishi K, Hirooka H, Kumagai H. 2016.

> Studies on supplementary desalted mother liquor on digestibility of nutrients, ruminal fermentation, and energy and nitrogen balance in Thai Native cattle. *Animal Science Journal*.

, which have been published in final forms at
http://onlinelibrary.wiley.com/doi/10.1111/asj.12374/full and, respectively

These articles may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

ACKNOWLEDGEMENTS

The author wishes to acknowledge sincere gratitude to Professor Dr. Hiroyuki Hirooka of Kyoto University, for guiding me in the study and unreserved commitment to bring me to the completion of this thesis, and to my supervisor, Associate Professor Dr. Hajime Kumagai of Kyoto University and Assistant Professor Dr. Kazato Oishi for their elaborated guidance, considerable encouragement and invaluable discussion that make my research of great achievement and my study life unforgettable.

Acknowledgements are extended to Professor Dr. Naba R. Devkota of Tribhuvan University, for helpful discussions in Nepal, and to Ms. Wanna Angthong of Khon Kaen Animal Nutrition Research and Development Center, Mr. Motoharu Takeda of International Production and Technology Center, Ajinomoto Co., Dr. Tomoyuki Suzuki of Crop, Livestock and Environment Division, Japan International Research Center for Agricultural Science, for enabling me to conduct research in Thailand.

I also gratefully acknowledge the work of past and present members of the Laboratory of Animal Science Resources for their encouragements and friendship.

Finally, I am filled with gratitude to my family for their endless love, understanding, support, encouragement and sacrifice throughout my study.

Takashi Sakai

January, 2017

CHAPTER 1

General introduction

1.1 Livestock production in tropical Asia

In tropical Asian countries, cattle are well known as sources of income, saving, traditional food, social status, religion, culture, labor and their compost, and therefore cattle production becomes an important sector of agriculture. At present, Asia has 491.0 million head of domestic cattle and 14.3 million ton of beef product in 2013 (FAOSTAT 2013). Over the last 10 years (from 2004 to 2013), increasing growth rate of the cattle population was 10.3% (445 to 491 million heads) and beef production was 27.2% (11.2 to 14.3 million tons). The trend of growth of cattle production in East Asia was faster, while in other parts of Asia such as Central, South, Southeast and West Asia, cattle meat production have maintained stable long-term growth from 2004 to 2013. Hence, cattle industry in most of Asian countries is now facing to improve the growth rate, reproduction rate, milk production, carcass composition and meat quality, and to reduce costs of production (Wagenhoffer 2007; Devendra 2008).

On the other hand, buffalo and small ruminants are also very important livestock animals in these countries. Although cattle population in the South and Southeast Asia is about one thirds of the world’s population, almost all buffalo (97%) and half of all sheep and goats in the world are managed only in these areas (Table 1.1). In particular, due to religious constraints for cattle, a large number of buffaloes are traditionally managed as important livestock in Hinduism countries in these areas. Although meat and milk production of cattle were accounted for 6.0% and 14.9% of the global output, those of buffalo were occupied 89.2% and 97.3% of the global production, respectively (Table 1.2). There are two types of local buffaloes, namely swamp buffaloes or water buffaloes and river buffaloes. Swamp buffaloes are popular as draft animals whereas river buffaloes are predominantly used for milk production (Thu & Udén 2000). Draft power and meat from the river buffaloes are also important but not yet fully exploited (Gill 1998).

Table 1.1 Livestock population in Asia (million heads)

Region/sub-region	Cattle	Buffaloes	Goats	Sheep	Pigs	Poultry	Ducks
World total	1,474.5	194.5	1,011.3	1,195.6	985.7	21,409.7	1,132.0
Asia	491.0	188.8	580.7	539.3	590.5	11,923.5	986.5
South Asia	269.2	151.6	293.2	153.3	11.3	2,841.7	76.5
Nepal	7.2	5.2	10.2	0.8	1.2	48.1	0.4
Southeast Asia	48.4	13.2	31.1	17.5	75.4	3,015.3	230.7
Thailand	4.9	1.0	0.4	0.0	7.6	267.0	15.2

Source, FAOSTAT (2013).

Table 1.2 Meat and milk production of cattle and buffaloes in Asia

	Total production (million tons)				Per capita production (kg)			
	Cattle		Buffalo		Cattle		Buffalo	
Region/sub-region	Meat	Milk	Meat	Milk	Meat	Milk	Meat	Milk
World total	64.7	656.0	3.7	114.0	9.0	91.3	0.5	15.9
Asia	14.5	190.2	3.3	110.9	3.4	44.2	0.8	25.8
South Asia	2.4	93.2	2.6	107.3	1.3	52.5	1.5	60.4
Nepal	0.05	0.53	0.17	1.2	1.9	19.1	6.2	42.0
Southeast Asia	1.5	4.7	0.3	0.4	2.5	7.6	0.5	0.7
Thailand	0.16	1.1	0.03	ND	2.4	15.8	0.4	ND

Source, FAOSTAT (2014).

1.2 Utilization of by-products

There is increasing demand for reducing the pollution arising from industrial activities due to environmental concerns, and most of countries are trying to adapt to this situation by modifying their processes to enable the residue to be recycled. Therefore, large companies are now changing the concept of residues: from wastes to raw materials for other process (Mussatto *et al*. 2006).

The most important process to utilize the residues is to use them as by-product feedstuffs for livestock. Most by-product feedstuffs are produced from various processing industries, such as commercial crops, food or beverage processing industry and fiber industry. By-product feedstuffs can support growth and lactation of livestock and result in the production of human edible food: milk and meat (Mirzaei & Maheri 2008). Consequently, by-product feedstuffs are becoming increasingly more important in food and fiber production systems because they are available for use as livestock feeds at competitive prices relative to other commodities (Grasser et al. 1995).

Many by-products have a big potential value as animal feedstuffs. In especial, ruminants have the unique ability to utilize fiber. Because of their rumen physiological adaptation, ruminants can utilize inexpensive by-product feedstuffs to meet their requirements for maintenance, growth, reproduction and milk production (Mirzaei & Maheri 2008). Although the nutrient values of most by-product feedstuffs are very variable, they can be raised through the effective processing. Consequently, utilizing by-product can solve the issues of competition between human and animal nutrition, and possibly reduce costs and environmental impact (Westendorf 2000).

1.3 Chemical composition and digestibility of by-products

There are several studies reported on the use of nutrient-rich by-products in the ruminant diet such as, brewers grain, green tea by-product, potato pulp silage, distillers by-product and soybean hulls (Wang *et al*. 2008a; Kondo *et al*. 2007; Aibibula *et al*. 2007; Ham *et al*. 1994; Cavani *et al*. 1990; Ipharraguerre & Clark 2003). However, there are two fundamental problems for the use of such by-product feedstuffs in animal production systems: quantitative and qualitative problems. As some raw materials can be used for different production processes, the available amount of the various by-products is difficult to estimate and it is even more difficult to assess the quantity used as animal feed (Mirzaei & Maheri 2008). As for the qualitative problem, these by-products as animal feeds have several problems, such as the high moisture content, nutritional imbalance, poor preservation and poor intake (Xu *et al*. 2007, Eruden *et al*. 2005). When the raw material feedstuffs such as fresh brewers grain, or fruit pomace or marc, are utilized as animal feeds, the specialized storage facilities such as a commodity shed or a pit (for wet feeds) should be needed for their preservation.

Table 1.3 and 1.4 show the mean chemical composition of some by-products (DM basis) and the apparent digestibility coefficients for ruminants, respectively. Even though they are the same origins of by-products, the chemical characteristics and the digestibility are differed as the changing their forms. In relation to the energy value of by-products for ruminants, authors demonstrated that the digestibility of by-products depends on the composition of the basic diet (Bampidis & Robinson 2006; Denek & Can 2006; Pirmohammadi *et al*. 2007a,b).

Table 1.3 Chemical composition of some agro-industrial by-products (% DM)

By-products	DM (%)	CP	EE	CF	NDF	ADF	Lig	Reference
Apple pomace								Ammerman *et al*. (1963); Alibes *et al*. (1983); Gasa *et al*. (1989); Rodrigues *et al*. (2008); Mirzaei & Maheri (2008).
Fresh	19.6	4.9	3.8	17.7	30.1	25.2	6.4	
Ensiled	18.7	7.2	6.7	22.6	39.2	30.2	9.3	
Brewers grain								Mussatto *et al*. (2006); Batajoo & Shaver (1998); Kyuma *et al*. (2009).
Wet	27.7	27.3	8.2	16.0	60.2	21.3		
Dried	93.1	28.2	7.1	17.2	54.5	20.1	4.6	
Silage	22.6	22.1	6.8	-	43.6	17.4	5.2	
Orange pulp								Bampidis & Robinson (2006); Ensminger & Macedo *et al*. (2007); Bath (1981).
Wet	24.9	6.1	3.4	11.9	36.4	31.3	-	
Dried	89.3	8.1	6.3	11.1	43.0	34.2	-	
Silage	21.0	7.3	9.7	-	-	20.0	-	
Grape marc								Pirmohammadi (2007a,b); Zalikarenab *et al*. (2007); Mirzaei & Maheri (2008).
Dried	49.7	13.2	-	-	50.4	-	-	
Silage	22.5	14.4	-	-	69.3	-	-	

DM, dry matter; CP, crude protein; EE, ether extract; CF, crude fiber; NDF, neutral detergent fibre; ADF, acid detergent fiber; Lig, lignin (Mirzaei & Maheri 2008).

Table 1.4 Digestibility of some agro-industrial by-products (%)

By-products	Animal	Digestibility DM	OM	CP	NDF	Reference
Apple pomace						Gasa *et al*. (1989); Rodrigues *et al*. (2008); Mirzaei & Maheri (2008).
Fresh	Ewes	-	89.5			
Ensiled	Cows	-	77.8			
Brewers grain						Johnson *et al*. (1987); Mussatto *et al*. (2006); Batajoo *et al*. (1998); Kyuma *et al*. (2009); Matsuyama *et al*. (2004).
Wet	Cattle			73.2		
Dried	Cows	43.2	-	48.9	30.1	
Silage	Cattle	69.2	82.2	81.6	54.1	
Orange pulp						Bampidis & Robinson (2006); Macedo *et al*. (2007); Sunvold *et al*. (1995); O'Mara *et al*. (1999).
Wet	Withers	-	85.4	48.2	68.3	
Wet	Steers	-	82.6	42.2	69.0	
Dried	Cows	62.9	73.0	-	22.7	
Silage	Sheep	64.5	52.0	46.3	-	
Grape marc						Pirmohammadi *et al*. (2007a,b); Zalikarenab *et al*. (2007); Mirzaei & Maheri (2008).
Dried	Buffaloes	34.5	24.7	21.8	18.6	
Silage	Buffaloes	28.5	19.5	-	-	

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre.

1.4 Practice in total mixed ration (TMR) silage feeding to ruminants

Among the problems to use by-products, as indicated in the above sub-chapter, the preservation problem is quite important to be solved. The agro-industrial by-products such as fresh apple pomace, wet brewers grain and wet tomato pulp are readily damaged due to their high moisture content. Although these local food by-products have preferable values of nutrients, they are difficult to incorporate into a commercial feeding program because of the preservation problem (Westendorf 2000). Ensiling can be considered to be more effective method to use wet by-products (Kayouli & Lee 1999). However, if high moisture by-products are ensiled without any additives, substantial financial resources are wasted on transporting water (Wang & Nishino 2008b). Therefore, the practice of mixed high moisture by-products with dry feeds to prepare a low moisture total mixed ration (TMR) is a suitable method to minimize the risk of effluent (Xu *et al*. 2007). In addition, unpalatable by-products could possibly be incorporated into a TMR if their odors and flavors were altered by silage fermentation (Xu *et al*. 2007; Cao *et al*. 2009).

Several studies were reported on the use of TMR feeding to ruminants worldwide. According to the literature that can be confirmed so far, the first practice of TMR feeding was introduced by Coppock *et al*. (1981) who reported that farmers are encouraged to feed TMR to animal for stabilizing the microbial function and improve the energy and protein utilization in the rumen. Huuskonen *et al*. (2008) reported that barley fiber was good energy supplement when used in combination with good quality silage or with TMR for growing dairy bulls, and suggested that the performance of growing dairy-breed bulls did not change when 50% of the barley grain concentrates was replaced with barley fiber. Chumpawadee and Pimpa (2009) conducted an experiment to verify the effect of fodder tree, as fiber sources in TMR on feed intake, nutrient digestibility, chewing behavior and ruminal fermentation in beef cattle, and suggested that the fodder tree has positive effect on feed intake and chewing behavior. Therefore, fodder trees can be used as fiber sources in TMR, especially when acute shortage of conventional fiber sources. Moya *et al*. (2011) reported the heifers fed the TMR had a greater total VFA concentration in the rumen after feeding than heifers fed free-choice diets. These differences may have resulted from the greater proportion of barley grain consumed by the animals fed the TMR. The ruminal pH and blood profiles of heifers were similar in TMR, corn silage with dried distiller grains and barley grain with dried distiller grains. They suggested that cattle can effectively self-select diets without increasing the risk of subclinical acidosis and still maintain similar levels of growth and feed efficiency compared with TMR.

1.5 Characteristics of desalted mother liquor (DML)

Desalted mother liquor (DML) is one of the seasoning by-products produced by a seasoning company in Thailand (Table 1.5). In the process of obtaining DML, nucleic acid fermentation broth is produced by culturing nucleic acid-producing microorganisms in a culture medium containing various kinds of carbohydrates such as molasses, tapioca, corn and saccharide sources, and various kinds of ammonia nitrogen raw materials such as ammonia and ammonium sulfate. Then crude crystals containing nucleic acid are removed from the fermentation broth by an appropriate extraction procedure. The nucleic acid is sold as the seasoning; the microorganisms are removed from the liquid by-product (mother liquor), and then the mother liquor is desalted to yield the DML. The resulting DML thus contains non-protein nitrogen from nucleic-acid-related compounds such as inosine, guanosine monophosphate and guanosine monophosphate, which might be utilized in the feeding of animals as nitrogen supplements.

Table 1.5 Chemical composition of DML

DM (%)	TN* (%)	pH	NaCl* (%)	CP* (%)	HxR* (%)	GR* (%)	IMP* (%)	GMP* (%)	IDP* (%)	GDP* (%)
25.20	4.37	7.35	60.30	25.50	1.07	0.08	9.83	7.94	4.92	0.21

*On a DM basis. TN, total protein; CP, crude protein; HxR, inosine; GR, guanosine; IMP, inosine guanosine monophosphate; GMP guanosine monophosphate; IDP, inosine diphosphate; GDP, guanosine diphosphate.

1.6 Objective

The objective of this thesis was to evaluate the nutrient values and availabilities of agro-industry by-products in tropical Asia. For this objective, the results from three experiments were provided in the following chapters. In Chapter 2, to evaluate utilization of TMR silage with brewers grain as replacement for concentrate, *in vivo* digestibility experiment using Nepal native goats and lactation experiment using Murrah buffaloes were conducted in Tarai, Nepal. In Chapter 3, four Thai native steers were used to determine an adequate level of supplementary DML on the moderate ingredients for Thai native cattle by taking measurement of the digestibility of nutrients, nitrogen balance, blood metabolites and ruminal fermentation in Khon Kean, Thailand. In addition, a respiration trial was also conducted in order to obtain more precise information of the diets mixed the novel ingredients on energy metabolism. In Chapter 4, we discussed about utilization of brewers grain for TMR silage, nutrient utilization in buffaloes, seasoning by-products for animal feed additives.

CHAPTER 2

Evaluation of total mixed ration silage with brewers grains for dairy buffalo in Tarai, Nepal

2.1 Introduction

Most of the dairy farms in south Asian countries are small scale with scarce farm land for cultivation of grasses. Silage making is also not a traditional way for animal feeds in these areas. In Nepal, the scarcity and low quality of feed resource are the main constraint against the increase in animal production during the fodder-shortage period (Hayashi *et al*. 2007) and feed resources and their efficient utilization are therefore considered as the first limiting factor in improving animal productivity.

Brewers grains consist of extracted residues of grains appearing in the brewing process. The crude protein (CP) content of wet brewers grain is approximately 28% on a dry matter (DM) basis, making it a suitable replacement for soybean meal in diets of lactating cows in mid-lactation (Johnson *et al*. 1987). Murdock *et al*. (1981) reported that cows fed wet brewers grain to replace concentrate up to 30% of DM as a protein supplement achieved similar levels of milk production in early and mid-lactation. Wet brewers grains were also effective as a concentrate during hot, humid weather with no decrease in DM intake or milk yield (MY) when fed at 30% DM (West *et al*. 1994).

In Nepal, buffaloes are important multipurpose livestock and contribute approximately 67% of the domestic total MY in 2010 (FAO 2012). Murrah buffaloes are traditionally kept in semi-intensive systems and fed mainly natural grasses, rice straw, crop residues and a small amount of concentrates in Nepal. Alexiev (1998) reported that the 305-day MY of Murrah buffaloes ranged from 1645 kg to 2130 kg, and the elite of the same breed yielded over 3000 kg in Europe. Patro and Bhat (1979) reported that the mean 300-day MY of Murrah ranged from 1573 kg to 1964 kg in India. On the other hand, approximately 46 085 tonnes of beer are produced annually in Nepal

(FAO 2012) and brewers grain represented approximately 20 kg per 100 L of beer produced (Mussatto *et al*. 2006). Numerically 9217 tonnes of brewer's grain are produced annually in Nepal and most of them are incinerated or buried in landfill. Therefore, there is the possibility to use the wasted brewer's grain as an effective alternative feed by mixing balanced diets.

However, especially in developing countries, feeding of brewers grain is generally limited to near the factories because of the costs of transportation. Furthermore, although the farmers provide by-products for the animals to cope with the decreased grass supply, the amount of nutrient intake is lower than that in the pasture-sufficient period. Thus, the overall dairy productivity of buffaloes declines during the pasture-decreasing and fodder-shortage periods due to the lower nutrient intake. Moreover, mixing brewer's grain into total mixed ration (TMR) silage is thought to be an effective way for improving the productivity for small-scale farmers to preserve animal feeding in pasture-decreasing and fodder-shortage periods. Nevertheless, there has been a lack of information available on the nutritive attributes of feeding TMR silage with brewer's grain for lactating buffaloes.

The objective of the present study was, therefore, to evaluate the effects of TMR silage, including brewer's grain as replacement for concentrate on feed intake and milk production in middle-to-late lactation buffaloes in Nepal.

2.2 Materials and Methods

The studies were conducted at the Livestock Farm of the Institute of Agriculture and Animal Science (IAAS), Rampur Campus, Tribhuvan University, Chitwan, Nepal, from February to July 2010. The animals used in the experiment were managed according to the guidelines of the Kyoto University and IAAS Animal Ethics Committees.

Feed preparation

The conventional concentrate feed was self-mixed using available feed resources in Chitwan District using wheat bran, rice bran, maize flour, mustard oil cake, salt and vitamin and mineral mixture (Minovit Forte™; Pearl Chemicals, Calcutta, India). The TMR silage was formulated by adding brewer’s grain to the conventional concentrate feed as a replacement of rice bran and wheat bran, and rice straw was used for adjusting moisture content. The TMR silage was formulated to provide the same CP content of the conventional concentrate based on the chemical composition of ingredients given in NARO (2010). These ingredients were manually mixed and ensiled in plastic bottles (60 L) and then stored for 1 month until the experiment. The proportions of ingredients in the concentrate mixture and TMR silage are shown in Table 2.1.

Table 2.1 Proportions of ingredients in conventional concentrate and the total mixed ration (TMR) silage (% on a dry matter basis)

	Diet	
Ingredients	Concentrate	TMR silage
Wheat bran	34.3	-
Rice bran	19.8	-
Maize flour	20.7	32.1
Mustard oil cake	21.3	11.2
Brewers grain	-	29.3
Rice straw	-	23.4
Salt	1.9	2.0
Vitamin and mineral mixture†	1.9	2.0

†Eeach 1kg contains Vitamin A: 750000 IU, D3: 75000 IU, E: 300 mg, Copper: 4.2 g, Magnesium: 6.5 g, Iodine: 350 mg, Zinc: 9.6 g, Potassium: 150 g, Calcium: 250 g, Phosphorus: 127.5 g, DL-Methionine: 1.929 g and L-Lysine: 4.4 g.

Animals, treatment and experimental design in buffalo experiment

Six multiparous and non-pregnant lactating Murrah buffaloes were selected based on the similar days of lactation, daily MY, age, parity and body weight (BW). However, two buffaloes were excluded from the experiment due to their milk supply drying up during the experiment. Consequently, four multiparous (three to six parities) buffaloes (403 ± 54 kg BW and 193 ± 53 days in milk (mean ± SD)) were assigned in a 3 × 3 Latin square design. The buffaloes were allocated in individual pens in a roofed shed. Each pen was equipped with a separate feedbox for feed and water supplies. Prior to the start of this experiment, an adaptation period was set by giving the animals a basal diet, rice straw *ad libitum* with the conventional concentrate, for 7 days. The feeding experiment for buffaloes started at the end of February 2010 and lasted for 63 days. An adaptation period (14 days) was followed by a main period (7 days).

The treatment were control (CTL) in which the animals were fed conventional concentrate at 0.6% of BW on a DM basis, and T1 and T2 in which the animals were fed TMR silage at 0.6% and 1.2% of BW on a DM basis, respectively, with rice straw *ad libitum*. The feed was given at 06.00 and 16.00 hours. The daily rice straw was fed in the amounts based on the previous day's intake, allowing for a 10% refusal. The feed intake was calculated as the difference between the amount of feed supplied and refused by the animals during the 7-day main periods. The animals had free access to water. Samples of the feed supplied and refused by the animals were weighed and the representative samples, 100 g approximately, were collected and dried in a forced draught oven at 60°C for 48 h to measure the dry weight.

The supplied feed samples in each main period were dried, gathered and mixed together as the ratio of daily amount, ground to pass through a 1 mm screen and kept in sealed plastic bags for subsequent chemical analyses. The BW of buffaloes was measured prior to the afternoon feeding at the beginning of the adaptation and the end of each period and the BW change was calculated. The buffaloes were milked manually once daily starting at 06.00 hours and the MY was determined using a weighing scale. The 100 mL representative milk samples were taken to determine the milk composition every day during the 7-day main period.

***In vivo* digestibility experiment using goats**

Digestibility of each diet was determined from a trial using three male goats of indigenous breeds (13.1 ± 2.1 kg (mean ± SD)). The goats were individually housed in metabolic cages. One term of the experiment had 14 days with a 9-day adaptation period and a 5-day sample collection period. The experimental treatments consisted of

C and T. In the C and T treatments, the conventional concentrate feed and TMR silage were combined with rice straw, respectively, at a 1:1 ratio on a DM basis. The goats were given the diets at 2% of BW on a DM basis in two equal portions provided at 10.00 and 17.00 hours and had free access to water.

During the sample collection period, feed residue and feces were obtained and weighed daily before the morning feeding. These samples were respectively mixed per goat in each treatment. All samples of diets, residue and feces were dried at 60°C for 48 h and ground with a Wiley mill to pass a 1 mm screen for chemical analyses. Urine was collected into vessels containing 20% sulfuric acid on a volume basis to prevent the loss of ammonia-nitrogen (N). The volume of urine was measured daily, and 50 mL representative samples were collected. The urine samples were mixed per goat per treatment according to the original excretion ratio and stored in the freezer until analysis. The digestibility of each experimental diet was calculated by subtracting the digestibility of rice straw according to the indirect method as described by NARO (2010). TDN was calculated by the formula: TDN = digestible CP + digestible ether extract (EE) × 2.25 + digestible crude fiber + digestible nitrogen free extract (NFE) and NFE was calculated as follows: NFE = 100 – CP – crude fiber – EE – crude ash (NARO 2010). The value of calculated TDN in this experiment was used in the buffalo experiment.

On the following day after the collection period, ruminal fluid was collected via the rumen cannula of each goat at 0, 1, 4 and 7 h after the morning feeding. The ruminal fluid was filtered through four layers of gauze, and immediately analyzed for ruminal pH using a hand-held glass electrode pH meter (F-72; HORIBA, Kyoto, Japan).

Chemical analyses

The samples of feed supplied and refused by the animals, and the fecal samples of goats were analyzed for DM, CP, EE, crude fiber and crude ash (methods 934.01, 988.05, 920.39, 962.09 and 942.05, respectively) according to the Association of Official Analytical Chemists (AOAC 2000). Neutral detergent fiber expressed exclusive of residual ash (NDFom) and acid detergent fiber expressed exclusive of residual ash (ADFom) were analyzed according to Van Soest *et al* (1991). Samples of urine were analyzed for urinary nitrogen using the Kjeldahl procedure described by the AOAC (2000). The intake of DM, CP, NDFom and ADFom in the buffaloes and goat experiments, and digestibility of each chemical component in the goat experiment were calculated. The representatives of 6 plastic bottle silos were selected to measure the fermentation characteristics. Wet 50g TMR silage was homogenized with 200 mL of distilled water (Cai *et al*. 1999) and the pH was measured using the handheld glass electrode pH meter. Lactic acid was determined according to Barnett (1951). Ammonia-N was determined by the microdiffusion method (Conway 1962).

The milk samples were determined for specific gravity and concentrations of fat on the same day of collection. The specific gravity was measured with a lactometer calibrated at 15.6°C. A correction to be applied to a lactometer reading for other temperatures was followed according to the correction table as suggested by Prasad *et al*. (1999). The Gerber method (Pearson 1976) was used to determine the milk fat concentration. The 7% fat corrected milk yield (FCMY) was derived from the report of Tyrrell and Reid (1965) as the formula of FCMY (kg) = 0.28 × milk yield (kg) + 10.34 × milk fat (kg). The solids-not-fat (SNF) concentration in milk was calculated by the formula of SNF (%) = CLR/4 + milk fat (%)/5 + 0.14 based on the specific gravity and fat content in milk, where CLR is the corrected lactometer reading (Prasad *et al.* 1999). The content of total solids (TS) in milk was derived by the addition of milk fat and SNF concentrations.

Statistical analysis

Data on chemical composition of diets, and intake, digestibility were analyzed using GLM procedure of Statistical Analysis System (SAS 1998). Data on intake, BW change, milk production and milk production per nutrient intake according to the treatments were analyzed using the MIXED procedure of SAS (1998). The statistical model was $Y_{ijk} = \mu + P_i + T_j + A_k + e_{ijk}$, where Y_{ijk} is the observed value, μ is the overall mean, P_i is the fixed effect with the experimental period, T_j is the fixed effect with treatment, and A_k is the random effect of animal and e_{ijk} is the residuals. Differences amongst means were considered statistically significant at $P < 0.05$ and differences at $0.05 < P < 0.15$ were accepted as showing tendencies toward significance.

2.3 Results

Chemical composition and fermentation characteristics of experimental diets

Table 2.2 shows the chemical composition and fermentation characteristics of the experimental diets. The concentrations of CP and EE in TMR silage were similar to those in the conventional concentrate. On the other hand, the TMR silage contained higher NDFom and ADFom contents than the concentrate ($P < 0.05$). The pH value, lactic acid content and ammonia-N/total N were 4.1, 3.5% and 4.1%, respectively.

Table 2.2 Chemical composition and fermentation characteristics of experimental diets

Item	Diet			SEM
	Concentrate	TMR silage	RS	
Chemical composition (%)				
Dry matter	88.2[b]	46.5[a]	93.0[b]	0.8
Crude protein†	18.4[b]	17.6[b]	5.1[a]	0.2
Ether extract†	6.6[b]	5.2[b]	2.4[a]	0.3
NDFom†	27.5[a]	40.0[b]	66.3[c]	0.6
ADFom†	13.7[a]	21.2[b]	42.5[c]	0.5
Crude ash†	7.9	8.1	7.0	0.3
Fermentation characteristics				
pH	−	4.1	−	−
Lactic acid (%)‡	−	3.5	−	−
Ammonia-N/total N (%)‡	−	4.1	−	−

Means in the same row with different superscripts differ significantly ($P < 0.05$). †On a dry matter basis. ‡ On a fresh matter basis. NDFom, neutral detergent fiber expressed exclusive of residual ash; ADFom, acid detergent fiber expressed exclusive of residual ash; N, nitrogen; TMR, total mixed ration; RS, rice straw; SEM, standard error of means.

In vivo **digestibility of experimental diets using goats**

Table 2.3 shows the DM, CP, NDFom and ADFom intake and apparent digestibility in each treatment in goats. The DM, CP, NDFom and ADFom intakes did not significantly differ among C and T, but the NDFom and ADFom intake were numerically higher for treatment T than C. No significant difference of apparent digestibility of DM, CP, NDFom and ADFom between the treatments of T and C was observed.

Table 2.3 Effect of feeding the experimental diets on intake and apparent digestibility in goats

Item	Treatment†		SEM
	C	T	
Intake (g/day)			
Dry matter	266.6	255.0	5.1
Crude protein	30.9	28.8	0.6
NDFom	123.6	138.1	3.1
ADFom	73.8	80.6	1.8
Apparent digestibility (%)			
Dry matter	62.8	65.1	0.6
Crude protein	67.6	68.8	0.5
NDFom	57.7	63.6	0.6
ADFom	43.4	47.7	0.9

†C and T: fed conventional concentrate and TMR silage with rice straw by 1:1 on a DM basis, respectively. NDFom, neutral detergent fiber expressed exclusive of residual ash; ADFom, acid detergent fiber expressed exclusive of residual ash; SEM, standard error of means.

The digestibility of DM, CP, NDFom and ADFom in concentrate and TMR silage estimated by subtracting the digestibility of rice straw using the indirect method and TDN contents are given in Table 2.4. The DM and CP digestibility was similar between the diets, while NDFom and ADFom digestibility for TMR silage was higher than the concentrate ($P < 0.05$). No significant difference of TDN was observed between the concentrate and the TMR silage

Table 2.4 Estimated digestibility and TDN of diets fed to goats (%)†

	Diet		
Item	Concentrate	TMR silage	SEM
Digestibility			
Dry matter	75.9	80.6	1.3
Crude protein	72.6	74.3	0.6
NDFom	82.2[a]	88.6[b]	1.0
ADFom	49.4[a]	52.4[b]	1.8
TDN	72.1	75.2	2.1

Means in the same row with different superscripts differ significantly (P < 0.05). †Estimated by the indirect method. NDFom, neutral detergent fiber expressed exclusive of residual ash; ADFom, acid detergent fiber expressed exclusive of residual ash; TDN, total digestible nutrient (Digestible crude protein + digestible ether extract * 2.25 + digestible crude fiber + digestible nitrogen free extract); SEM, standard error of means.

Feed intake and productivity of buffalo

The average BW, BW change and daily feed intake of buffaloes are presented in Table 2.5. The BW change increased from -0.18 kg/day in CTL and -0.17 kg/day in T1 to 0.42 kg/day in T2 (P < 0.05). Although there was no significant difference of rice straw intake among the treatments, the DM intake of concentrate and total feed was higher in T2 than in CTL and T1 (P < 0.05). The CP intake and TDN intake also changed with the similar trends to the variation of TMR silage intake (P < 0.05 for CP intake and P = 0.08 for TDN intake). Although ADFom intake had no significant difference among the treatments, there was significant difference for NDFom intake between C and T2 (P < 0.05).

Table 2.5 Body weight and daily feed intake of each dietary treatment of buffaloes

	Treatment			
Item	CTL	T1	T2	SEM
BW (kg)	406	397	408	6
BW change (kg/day)	-0.18[a]	-0.17[a]	0.42[b]	0.32
DM intake (kg/day)				
Rice straw	7.8	8.2	7.7	0.7
Concentrate	2.5	-	-	-
TMR silage	-	2.6	5.1	-
Total	10.3[a]	10.8[a]	12.8[b]	0.8
Crude protein intake (g/day)	762[a]	764[a]	1193[b]	38
NDFom intake (kg/day)	5.9[a]	6.5[ab]	7.1[b]	0.8
ADFom intake (kg/day)	3.8	4.0	4.4	0.5
TDN intake (kg/day)	5.2	5.3	6.9	0.4

Means in the same row with different superscripts differ significantly ($P < 0.05$). BW, body weight; DM, dry matter; NDFom, neutral detergent fiber expressed exclusive of residual ash; ADFom, acid detergent fiber expressed exclusive of residual ash; TDN, total digestible nutrient; CTL, fed concentrate at 0.6%; T1, fed TMR at 0.6%; T2, fed TMR at 1.2% of BW; SEM, standard error of means.

The daily MY and milk composition of buffaloes are presented in Table 2.6. Although no significant difference of MY and FCMY was observed between CTL and T1, they were increased as the substitution rate of TMR silage increased from T1 to T2 (from 2.65 to 3.33 kg/day for MY and from 2.28 to 3.13 kg/day for FCMY, $P < 0.05$). Although the composition and yield of fat, SNF and TS, did not differ significantly among the treatments, the yields of milk fat and total solid tended to increase in T2 compared to C and T1 ($P = 0.12$).

Table 2.6 Milk yield, milk composition and production of milk components in buffaloes

Item	Treatment CTL	T1	T2	SEM
MY (kg/day)	2.75[a]	2.65[a]	3.33[b]	0.29
FCMY (kg/day)	2.40[ab]	2.28[a]	3.13[b]	0.29
Composition (%)				
Fat	6.3	5.8	6.4	0.1
SNF	8.7	8.9	9.1	0.1
TS	14.7	14.9	15.1	0.2
Yield (g/day)				
Fat	174.1	152.7	212.2	20.1
SNF	229.4	258.2	256.5	25.7
TS	404.3	366.3	453.1	43.6

Means in the same row with different superscripts differ significantly ($P < 0.05$). MY, milk yield; FCMY, 7% fat corrected milk yield; SNF, solids-not-fat; TS, total solids; CTL, concentrate at 0.6%; T1, TMR at 0.6%; T2, TMR at 1.2% of BW; SEM, standard error of means.

The milk production per nutrient intake of buffaloes in relation to TMR silage feeding level and concentration is presented in Table 2.7. No significant differences of MY/DM intake, FCMY/DM intake, MY/TDN intake and FCMY/TDN intake were observed among the treatments.

Table 2.7 Milk production per nutrient intake of buffaloes (%)

	Treatment			
Item	CTL	T1	T2	SEM
MY/DM intake	26.4	24.5	25.6	1.4
FCMY/DM intake	23.3	21.5	24.5	1.7
MY/TDN intake	52.7	49.5	47.8	2.4
FCMY/TDN intake	46.2	43.0	45.4	3.1

Means in the same row with different superscripts differ significantly ($P < 0.05$). MY, milk yield (kg/day); DM intake, dry matter intake (kg/day); FCMY, 7% fat corrected milk yield (kg/day); TDN intake, total digestible nutrient intake (kg/day); CTL, fed concentrate at 0.6%; T1, fed TMR at 0.6%; T2, fed TMR at 1.2% of BW; SEM, standard error of means.

2.4 Discussion

Chemical composition, fermentation characteristics and digestibility of the TMR silage

McDonald and Whittenbury (1973) reported that a target pH for an adequate preservation of ensiled feeds is less than 4. In the present study, the pH of the TMR silage was close to pH 4. This agreed with several studies that also found pH close to 4 when distillers grain or brewers grain were ensiled as all or a large portion of the ensiled feed (Abrams *et al.* 1983; Wang & Nishino 2008). In addition, fermentation characteristics of TMR silage (pH ≦ 4.2 and ammonia-N/total N ≦ 12.5%) were suggested as satisfactory preservation (Carpemtero *et al.* 1969).

The fermentation quality of TMR silage had no negative effect on the feed intake for goats (Table 2.3) and buffaloes (Table 2.5).

The TMR silage used in this study had similar CP contents to the concentrate and had higher NDFom and ADFom concentrations than the concentrate (Table 2.2). The higher fiber contents were likely due to the high brewers grain and rice straw contents (Table 2.1) in the ingredients, which were rich in fiber contents. The TMR silage in the present study included 29.3% brewers grain which contains high levels of cellulose (Mussatto *et al.* 2006). The NDFom and ADFom contents in brewers grain in the present study were 52.9% and 25.3%, respectively, which were similar to those described in the previous report (NRC 2001; 47.1% and 23.1%, respectively). The large differences between NDFom and ADFom contents in brewers grain were due to high hemicellulose contents, which is rapidly degraded in the rumen. Similar results were reported by McCarthy *et al.* (1990); ensiled wet brewers' grains-corn mixture containing 35% brewers grain and 64% ground corn had higher NDF and ADF digestibility than pelleted complete diet containing 58 % ground corn, 17% alfalfa meal, 17% peanut hull and 7% soybean meal in lambs.

Grant *et al.* (1974) concluded that buffaloes have utilized feed containing high levels of cellulose. Moreover, rumen microbes of buffaloes have greater fibrolytic activity than those of cattle when fed a highly fibrous diet (Homma 1986; Kennedy *et al.* 1992; Wanapat *et al*. 2000). Therefore, the feeding of the TMR silage containing large amount of brewers grain to buffaloes is likely to have the advantage of fiber digestion compared to the conventional concentrate.

Intake, BW change and milk production in buffaloes

The BW changes in buffaloes were not different between CTL and T1, whereas the values increased with the amount of TMR silage feeding (Table 2.5). The increase of BW change is likely due to higher intake of the TMR silage.

Paul *et al.* (2002) reported the nutrient requirements for buffaloes, which mentioned that the daily requirements of DM, CP and TDN for riverine buffalo were 5.35 kg, 485 g and 3.16 kg for maintenance of 400 kg body BW, respectively. The corresponding data for producing 1.0 kg milk with 7.0% fat were 768 g, 101 g and 453 g, respectively (Paul *et al.* 2002). Accordingly, the daily requirement of DM, CP and TDN for buffaloes of 400 kg BW and 3.0 kg MY with 7.0% fat can be calculated to be 7.65 kg, 788 g and 4.52 kg, respectively. The daily intake of DM, CP and TDN of buffaloes in the present study was regarded as high as 135-167%, 97-151% and 115-153% of the requirements, respectively (Table 2.5). Because the basal diet was considered to contain the minimum protein and energy requirements, the nutrient intake of the buffalo in T2 was considered to be much higher than the maintenance and milk production. It is considered that buffaloes used in the present study were at the middle to late lactating periods, therefore, the energy intake in T2 exceeded the requirement of milk production performance of the buffaloes and the energy surplus might have been allocated to the body accumulation. Buffaloes at the early lactation period may improve the milk production performance by feeding with T2 diet.

The yields of milk fat and total solid tended to increase in T2 compared to C and T1 (P = 0.12). Staples *et al.* (1984) reported that the yields of milk fat increased by increasing the fiber contents in the diets fed to the cattle. Armentano and Pereira (1997) stated that significantly positive correlation (r = 0.4) was observed among the forage NDF and milk fat concentrations. In the present study, the NDFom intake in T2 was higher than that in CTL ($p<0.05$). Besides the improvement of MY, the increase of NDFom intake may have positive effect on the milk fat yield in the buffaloes.

The FCMY/DM intake and FCMY/TDN intake had no significant differences among the treatments (Table 2.7). This result suggested that the increase of milk production in T2 was as a result of an increased DM intake and TDN intake.

In conclusion, the supplementation of TMR silage with brewers grain significantly increased the DM intake of buffaloes without any negative effects. The CP intake, TDN intake and BW were increased with the amount of TMR silage. Moreover, the milk production (MY and FCMY) of buffaloes increased with the amount of TMR silage. The TMR silage mixed in the present study was designed an economically beneficial feed compared to the conventional concentrate in Nepal. The purchased cost of maize flour, mustard oil cake, rice bran, wheat bran, salt, and vitamin and mineral mixture were 15.6, 15.5, 20.0, 22.5, 9.0 and 81.0 Nepal Rupees (NRs/kg) on a DM basis, respectively, and the brewers grain mixed with TMR silage were sold at 1 NRs/kg on a wet basis at the local beer brewery. Since the dairy farming integrated with rice production is preferred by farmers in this region, rice straw is available free of charge. Thus the feed costs (NRs DM/heads/day) of CTL, T1 and T2 were calculated as 49.8, 22.4 and 43.8, respectively. Since the MY in CTL and T1 were similar, the feeding the TMR silage as much as the conventional concentrate has the advantage of reducing the feed cost.

Therefore, the more TMR silage feeding than minimum feeding level of conventional concentrate has the possibility to enhance milk production of the buffaloes in Nepal economically.

CHAPTER 3

Studies on supplementary desalted mother liquor on digestibility of nutrients, ruminal fermentation, and energy and nitrogen balance in Thai native cattle

3.1 Introduction

In Asian countries, the demand for livestock production is increasing due to the increasing human population, rapid economic development and improved living standards. To correct for the shortage of feed caused by the increased livestock numbers, several Asian countries are importing animal feedstuff from foreign countries. In the tropics, most ruminants are fed low-quality roughage, agricultural crop residues and industrial by-products, which basically contain low levels of fermentable carbohydrate and low levels of good-quality protein (Wanapat 1999). Although, in these areas, rice bran or especially soy bean meal are the main crude protein (CP) resources for the animal feeding (Wanapat 1999), the price of them is not still kindness for the livestock farmers. Since high feed cost is the main constraint against the increase in the animal production, the moderate ingredients such as palm kernel oil cake, cassava chip or cassava pulp would be preferred to utilize as the animal feeding (Tipu *et al*. 2014; Chanjula 2007). Even though these ingredients could be adequate for the animal feeding, attention should be paid to not only the price but also the chemical composition of the diets, such as dry matter (DM) contents, because they are related to their palatability.

On the other hand, recently, large amounts of agro-industry by-products from the processing of seasonings have been generated in Thailand. Desalted mother liquor (DML) is one of the nucleic acids related compounds [disodium inosine-5′-monophosphate (IMP), disodium guanosine-5′-monophosphate (GMP)] for food additives produced by a seasoning company in the country. In the process of obtaining DML, culture medium containing carbohydrates from tapioca and crude sugar, ammonium as nitrogen source and various minerals is used for fermentation. The by-product, DML, contains large amount of salt and small amount of nucleic acids which might be utilized in the feeding of animals as salt and non-protein nitrogen supplements. In support of this potential use of DML, Kanyinji *et al*. (2009) reported that the responses in terms of nutrient intake, digestibility, ruminal fermentation and nitrogen balance in goats that were fed high amounts of concentrate with supplementary inosine and urea were similar. Kimura *et al*. (2010) performed *in vitro* fermentation using roughage and concentrate as substrates, and compared the resulting gas production and the digestibility of supplementary guanosine and inosine. They found that fermentation using the roughage substrate resulted in higher gas production and higher digestibility of DM when guanosine and inosine were added than when urea was added, but with the concentrate substrate, the gas production and the digestibility of DM were lower under guanosine and inosine than under urea supplementation (Kimura *et al.* 2010).

Although DML has so far only been used in agricultural fertilizers for upgrading soil fertility, it has potential for use in animal additives because of its high nitrogen content related to nucleic acids. When large amounts of DML are fed to animals, however, attention should be paid to its high sodium chloride (NaCl) concentration, which is approximately 50% on a DM basis. Cardon (1953) mentioned that increasing the salt concentration in the rumen fluid may have a detrimental effect on rumen microorganisms even though salt may not have any direct toxic effect on the animal. According to the National Agriculture and Food Research Organization (NARO) (2010), an NaCl concentration up to 5.0% in the diet on a DM basis has no negative effect on beef cattle. Moreover, the National Research Council (1980) reported that the maximum tolerable level of salt fed to cattle was 9.0% on a DM basis.

The objective of this study was to determine an adequate level of supplementary DML utilizing the moderate ingredients that could be fed to Thai native cattle in terms of the digestibility of nutrients, nitrogen balance, blood metabolites and ruminal fermentation. In addition, a respiration trial was also conducted in order to obtain more precise information of the diets mixed the novel ingredients on energy metabolism.

3.2 Materials and Methods

The experiment was conducted at the Khon Kean Animal Nutrition Research and Development Center, Khon Kean, Thailand, from July to September 2013. The animals used in the experiment were managed according to the guidelines of the Kyoto University Ethics Committee as an experiment on vertebrate animal species that are expected to produce little or no discomfort, and the experimental procedures were consistent with the Ethical Principles for the Use of Animals for Scientific Purposes of the National Research Council of Thailand.

Feeding of animals and experimental design

DML was provided by a seasoning factory in Kamphaeng Phet, Thailand. The DM content of DML was 25.2%, and the CP and NaCl contents were 25.5% and 50.2% on a DM basis, respectively. DML contained 9.83% IMP and 7.94% GMP on a DM basis. The chemical compositions of the ingredients and the proportions of the ingredients in the present study are shown in Tables 3.1 and 3.2. The composition of the control (C) diet was 15.2% rice bran, 23.4% soy bean meal, 27.1% palm kernel cake, 14.7% cassava chip, 17.6% cassava pulp, 1.0% vitamin mixture and 1.0% commercial salt on a

DM basis. Three alternative diets with different levels of added DML were prepared as the treatments (T1: 1.0%, T2: 2.0%, T3: 3.0% NaCl concentrations with supplementary DML on a DM basis). All the diets were formulated using the National Research Council (NRC) (1996) guidelines to contain 16% CP and 68% total digestible nutrient (TDN). To balance the CP and TDN levels among the diets, the rice bran, soybean meal and cassava pulp contents were decreased, and the palm kernel cake and cassava chip contents were increased as the DML contents increased.

Table 3.1 Chemical composition of ingredients (% on a dry matter basis)

	Rice barn	Soybean meal	Palm kernel oil cake	Cassava chip	Cassava pulp	Rice straw
Dry matter†	86.3	86.4	92.3	86.1	15.0	88.8
Crude protein	14.0	45.5	14.1	2.0	2.5	3.6
Ether extract	12.0	0.4	8.0	0.1	0.5	1.6
Non-fibrous carbohydrate	36.9	28.0	1.9	89.0	54.0	9.3
Neutral detergent fiber	27.0	16.0	69.0	6.7	40.6	68.8
Acid detergent fiber	11.1	10.3	44.0	4.7	27.7	42.3
Crude ash	10.1	10.2	7.0	2.2	2.4	16.7

†As-fed basis

Table 3.2 Proportions of ingredients in the diets (% on a dry matter basis)

Ingredients	Diets			
	C	T1	T2	T3
Rice bran	15.2	18.0	9.8	5.0
Soy bean meal	23.4	18.8	16.8	14.0
Palm kernel cake	27.1	34.0	42.2	50.0
Cassava chip	14.7	12.7	17.0	20.4
Cassava pulp	17.6	13.8	9.8	4.5
Desalted mother liquor	0.0	1.7	3.4	5.1
Commercial salt	1.0	0.0	0.0	0.0
Vitamin and mineral mix	1.0	1.0	1.0	1.0

Four castrated male Thai native cattle (226.5 ± 14.2 kg) were used in a 4 × 4 Latin square design experiment. The animals were housed individually in metabolic crates. A 14-day preliminary adjustment period was followed by a 5-day period during which all feces and urine were collected to evaluate apparent digestibility and nitrogen balance. The animals were given the experimental diets and rice straw daily at levels equal to 1.2% and 0.8% of body weight (BW), respectively, on a DM basis. Water was accessible at all times. The feed intake was calculated as the difference between the amounts of feed supplied to and rejected by each animal during the 5-day collection periods. The BW of the animals was measured before and after each collection period.

During the sample collection period, all feces and urine were obtained and weighed daily after the morning feeding. These samples were respectively mixed for each animal receiving each treatment. Parts of the diets and feces were oven-dried at 60°C for 48 h and ground with a Willey mill to pass a 1-mm screen for chemical analyses. Urine was collected in plastic tanks containing 600-800 mL of 20% sulfuric acid to prevent the loss of ammonium nitrogen. The volume of urine was measured daily, and 500-mL representative samples were collected.

Ruminal fluid was sampled via orogastric tubing from each animal before the morning feeding and at 4 h after feeding ,at the stable and peak phase of ruminal fermentation, respectively (Ishida *et al*. 2012; Onodera 2001), on day 19 of each period. The ruminal fluid pH was measured immediately, and samples were separated from feed articles through four layers of gauze. Samples were frozen (−20°C) for later analysis of volatile fatty acid (VFA) and NH_3-N. Avoiding acute changes of blood metabolite concentrations after feeding (Okada 2011), blood samples were collected from the jugular vein into heparinized tubes before the morning feeding on the final day of each period.

Respiration trials and gas analyses

Respiration experiments were carried out on all animals after each treatment and period. During the sample collection period, a 5-day total feces and urine collection period was followed by a 2-day period (excluding the calibration time required for the analyzers) during which air was collected for a period of 24 h (from the 09.00 hours feeding to the next day's 09.00 hours feeding). Expiratory gas was collected using a ventilated hood system as described by Suzuki *et al*. (2008). The system consisted of a head box, flow cell (thermal flow cell FHW-N-S; Japan Flow Cell, Ltd., Japan), oxygen analyzer (Xentra 4100; Servomex, Ltd., Crowborough, UK), and carbon dioxide and methane analyzers (infra-red gas analyzer VIA300; Horiba, Kyoto, Japan). Gas analyzers were calibrated against certified gases (Kasidis, Ltd., Thailand) with known gas concentrations. The gas sample was analyzed at five positions (i.e., ambient air and four different head boxes) using a set of gas analyzers. In this automated system, the gas sampling point switches among the five positions at 90 seconds intervals. The first 60 seconds allow gas concentration stabilization before measurements; the final 30 seconds are for data acquisition. The system gas analyzers measured carbon dioxide and methane by a carbon dioxide analyzer (infrared system) and a methane analyzer (infrared system). The data collection system is recorded automatically for 24 h.

Chemical analyses

The dried samples of supplied feed and refused feed were analyzed for DM, CP, ether extract (EE), crude fiber and crude ash according to methods 934.01, 988.05, 920.39, 962.09 and 942.05, respectively, of the Association of Official Analytical Chemists (2000). The organic matter (OM) was calculated as weight loss through ashing. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents of the samples were determined according to Van Soest *et al*. (1991). The samples of feces were analyzed for DM, CP, OM, EE, NDF and ADF as stated above. Samples of urine were analyzed for urinary nitrogen using the Kjeldahl procedure described by the Association of Official Analytical Chemists (AOAC) (2000). The concentration of NaCl was determined by Mohr's titration method (Fischer & Peter 1968).

The VFA was measured qualitatively and quantitatively using a gas chromatograph (GC-14B; Shimazu, Tokyo, Japan) equipped with a thermal conductivity detector and a GC-2014 glass column (3.2 mm×2.1 m; TZ-3 Thermon-3000; Shinwa Chemical, Kyoto, Japan). The analytical conditions were as follows: column oven temperature, 140°C; injector temperature, 210°C; detector temperature, 250°C. The pH was measured using a handheld glass electrode pH meter. Ruminal NH_3-N was determined by the microdiffusion method (Conway 1962).

The concentrations of albumin (Alb), blood urea nitrogen (BUN), blood sugar, cholesterol, creatine, glutamate oxaloacetate (GOT), glutamate pyruvate transaminase (GPT), non-esterified fatty acid (NEFA) and blood protein were analyzed using an Olympus AU400 automatic analyzer (Olympus, Hamburg, Germany).

The gross energy (GE) contents of oven-dried feed, feces and urine were determined using an automatic isoperibol calorimeter (6400; Parr, Moline, IL, USA).

Calculations of feed values

The digestibility of each diet was calculated from the amount of nutrients in the feed consumed and the amount of nutrients that remained in the feces. The nitrogen balance was determined from the nitrogen intake and nitrogen losses into the feces and urine, the energy balance from the energy intake and energy loss in the feces, urine, methane and heat production (HP). TDNs were calculated using the following formula: TDN = 5.81 + 0.869 × digestible DM (Heaney & Pigden 1963). Non-fibrous carbohydrate (NFC) was calculated as follows: NFC = 100 – CP – NDF – crude EE – crude ash (NRC 2001). HP (kJ/day) was calculated using the equation (Brouwer 1965), $HP = 16.18 \times O_2 + 5.02 \times CO_2 - 2.17 \times CH_4 - 5.99 \times N$, where O_2, CO_2 and CH_4 represent volumes of oxygen (l/day) consumed, carbon dioxide (l/day) and methane produced (l/day), respectively, and N is the quantity of urinary nitrogen excreted (g/day).

Statistical analysis

Data on digestion, ruminal VFA, and methane emission were analyzed according to treatment using the MIXED procedure of SAS (1998). The statistical model was $Y_{ijkl} = \mu + P_i + T_j + A_k + e_{ijkl}$, where Y_{ijkl} is the observed value, μ is the overall mean, P_i is the fixed effect with the experimental period, T_j is the fixed effect with treatment, A_k is the random effect of animal and e_{ijkl} is the residuals. Differences amongst the least squares means were analyzed by the Tukey-Kramer method and were considered statistically significant at $P < 0.05$. Differences at $0.05 < P < 0.10$ were accepted as showing tendencies toward significance.

3.3 Results

Chemical composition

Table 3.3 shows the chemical composition and GE of the experimental diets. Similar CP and GE contents were observed among the diets. The NaCl content of the C diet was 1.1%, and the other diets that contained DML ranged from 1.1% to 3.4% depending on the level of DML supplementation. The NDF and ADF contents were highest in the T3 diet, followed by the T2, T1 and C diets; on the other hand, the NFC contents were ranked in the inverse order.

Table 3.3 Chemical composition and gross energy of experimental diets

	Concentrate				
Item	C	T1	T2	T3	Rice straw
Chemical composition (% on a DM basis)					
Dry matter†	58.6	59.6	62.9	73.5	83.7
Crude protein	16.6	16.5	16.4	16.5	2.3
Ether extract	7.2	7.5	7.6	8.5	1.2
Non-fibrous carbohydrate	35.7	35.9	34.0	31.0	6.5
Neutral detergent fiber	29.9	31.2	31.5	33.0	75.5
Acid detergent fiber	17.5	17.9	18.5	19.0	47.1
NaCl content (g/kg DM)	1.1	1.2	2.2	3.4	-
Gross energy (MJ/kg DM)	18.6	19.1	18.3	18.5	16.2

DM, dry matter. C, supplied 1.0% of commercial salt; T1, supplied 1.7% of desalted mother liquor (DML); T2, supplied 3.4% of DML; T3, supplied 5.1% of DML.

Nutrient intake, apparent digestibility and TDN

Table 3.4 shows the nutrient intake, apparent digestibility and TDN in each treatment. The DM and CP intakes did not differ significantly among the treatments.

The EE intake in T3 tended to be the highest and that in C was the lowest ($P = 0.095$). The NFC intakes in C and T1 were significantly higher than those in T2 and T3 ($P < 0.05$). A higher NDF intake was observed in T2 and T3 than in C and T1 ($P < 0.05$). The ADF intake in T3 was higher than those in C and T1 ($P < 0.05$).

No significant difference in the apparent digestibilities of DM, OM, CP, EE and NFC were observed among the treatments; however, the C treatment showed a nonsignificant trend of higher NDF and ADF digestibilities compared to the other treatments ($P = 0.092$ and $P = 0.096$, respectively). There was no significant difference in TDN among the treatments.

Table 3.4 Nutrient intake, apparent digestibility and TDN of experimental diet

	Treatment				
Item	C	T1	T2	T3	SEM
Intake (kg/day)					
Dry matter	3.80	3.84	3.91	4.00	0.07
Crude protein	0.394	0.393	0.386	0.419	0.007
Ether extract	0.179	0.188	0.197	0.202	0.004
Non-fibrous carbohydrate	0.90^{c}	0.87^{c}	0.82^{b}	0.73^{a}	0.01
Neutral detergent fiber	1.94^{a}	1.92^{a}	1.98^{b}	2.02^{b}	0.01
Acid detergent fiber	1.17^{a}	1.18^{ab}	1.21^{bc}	1.23^{c}	0.06
Apparent digestibility (%)					
Dry matter	64.2	64.3	63.5	63.7	1.1
Crude protein	65.1	66.1	65.2	66.6	1.2
Ether extract	88.5	90.1	89.1	90.4	0.5
Non-fibrous carbohydrate	82.3	87.9	84.6	84.0	1.6
Neutral detergent fiber	62.1	58.8	59.8	58.6	1.4
Acid detergent fiber	60.0	57.2	57.9	56.3	1.4
TDN (% on a dry matter basis)	68.9	68.7	67.6	67.2	1.0

Means in the same row with different superscripts differ significantly ($P < 0.05$). TDN, total digestible nutrient (5.81 + 0.869 * digestible dry matter); C, supplied 1.0% of commercial salt; T1, supplied 1.7% of desalted mother liquor (DML); T2, supplied 3.4% of DML; T3, supplied 5.1% of DML; SEM, standard error of means.

Ruminal fermentation

The ruminal fermentation characteristics at 4 h after feeding are presented in Table 3.5. The ruminal pH of the animals administered the C, T1, T2 and T3 treatments 4 h after feeding were 6.67, 6.82, 6.75 and 7.04, respectively, and the ruminal pH in animals fed the C diet had a lower tendency ($P = 0.079$). The total VFA concentrations in C and T2 were higher than those in T1 and T3 ($P < 0.05$). The molar proportions of propionic acid tended to be lower in the animals receiving the C and T2 treatments ($P = 0.091$).

Table 3.5 Effect of feeding the experimental diets on rumen fermentation at 4 h after feeding

Item	Treatment				SEM
	C	T1	T2	T3	
pH	6.67	6.82	6.75	7.04	2.12
NH_3-N (mg/dL)	12.8	12.8	13.8	13.4	1.2
Total VFA (mmol/l)	169.4^{b}	158.3^{a}	169.8^{b}	148.5^{a}	18.5
VFA composition (mol%)					
Acetic acid (C2)	68.9	65.1	68.0	63.3	2.4
Propionic acid (C3)	25.9	28.6	27.1	30.2	2.0
n-butyric acid	7.2	7.4	7.2	7.6	0.2
i-butyric acid	0.56	0.58	0.51	0.62	0.12
C2 : C3	3.3	3.6	3.4	3.8	0.4

Means in the same row with different superscripts differ significantly ($P < 0.05$). VFA, volatile fatty acid; C, supplied 1.0% of commercial salt; T1, supplied 1.7% of desalted mother liquor (DML); T2, supplied 3.4% of DML; T3, supplied 5.1% of DML; SEM, standard error of means.

Nitrogen balances

Table 3.6 shows the nitrogen balances of each treatment. While there was no significant difference in the nitrogen excretion into urine between the C and T2 treatments, that in T3 was the highest among the treatments and was higher according to the amount of DML ($P < 0.05$). The results for nitrogen retention were the inverse of those for urinary excretion ($P < 0.05$). Among the T1, T2 and T3 treatments, the lowest nitrogen retention was observed in T3 followed by T2 and T1 ($P < 0.05$).

Table 3.6 Nitrogen balances of animals fed experimental diets (g/day)

Item	Treatment				SEM
	C	T1	T2	T3	
Intake	63.2	63.4	62.7	62.0	1.5
Excretion					
Feces	23.1	22.3	23.5	23.0	0.9
Urine	18.2^{b}	15.8^{a}	18.8^{b}	19.9^{c}	1.3
Retention	21.9^{b}	25.3^{c}	20.5^{b}	18.1^{a}	1.8

Means in the same row with different superscripts differ significantly ($P < 0.05$). C, supplied 1.0% of commercial salt; T1, supplied 1.7% of desalted mother liquor (DML); T2, supplied 3.4% of DML; T3, supplied 5.1% of DML; SEM, standard error of means.

Blood metabolites

The blood metabolite concentrations of each treatment are shown in Table 3.7. The plasma total cholesterol concentrations of animals fed the T1, T2 and T3 were higher than those of animals fed the C treatment diet ($P < 0.05$). The other metabolite concentrations did not differ significantly among the treatments.

Table 3.7 Effect of feeding on blood metabolites

Item	Treatment				SEM
	C	T1	T2	T3	
Protein (g %)	7.1	7.2	7.3	7.2	0.1
Blood sugar (mg %)	76.0	77.0	76.3	72.5	2.0
NEFA (mmol/L)	0.23	0.19	0.19	0.17	0.04
BUN (mg %)	10.0	10.5	10.1	9.4	0.9
Albumin (g %)	3.0	3.7	3.8	3.7	0.1
Cholesterol (mg %)	167.0[a]	183.8[b]	178.3[b]	182.3[b]	2.9
GOT (U/L)	50.3	50.8	48.8	52.0	2.1
GPT (U/L)	18.5	22.3	24.0	27.8	2.6
Creatine (mg %)	2.10	2.20	2.10	2.20	0.04

Means in the same row with different superscripts differ significantly (P < 0.05). NEFA, non-esterified fatty acid; BUN, blood urea nitrogen; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; C, supplied 1.0% of commercial salt; T1, supplied 1.7% of desalted mother liquor (DML); T2, supplied 3.4% of DML; T3, supplied 5.1% of DML; SEM, standard error of means.

Energy balances

The energy balances, including the results of the respiration trial, are shown in Table 3.8. The metabolic energy (ME) intake on the basis of metabolic body weight in T1 and T3 were higher than in C (P < 0.05). The energy losses into methane and HP were significantly higher in C and T2 than T3 (P < 0.05). T3 and T1 had the highest energy retentions among the treatments, followed by T2 and then C (P < 0.05). Although the ratios of digestible energy (DE) to GE, ME to GE and ME to DE showed no significant differences, T1 and T3 showed a trend toward higher ratio of ME to GE, i.e. metabolizability, and higher ratio of ME to DE (P = 0.089). The ratio of energy loss into methane and HP to GE in T3 was lower than that in C and T2 (P < 0.05). The ratios of energy retention to GE in T1 and T3 were higher than in T2, and that in C was the lowest (P < 0.05).

Table 3.8 Energy balance of animal fed experimental diets

Item	Treatment				SEM
	C	T1	T2	T3	
Body weight	233.8	233.1	233.3	236.7	3.9
Intake (kJ/BWkg$^{0.75}$)					
GE	1114.6	1150.4	1139.1	1147.9	9.4
DE	387.5	400.2	418.4	407.6	13.2
ME	608.7[a]	660.3[b]	633.7[ab]	662.1[b]	26.1
Energy loss into (kJ/BWkg$^{0.75}$)					
Feces	387.5	400.2	418.4	407.6	13.2
Urine	23.1[b]	19.5[a]	21.5[ab]	21.7[ab]	1.3
Methane	84.4[b]	79.1[ab]	81.0[b]	74.9[a]	2.2
Heat production† (kJ/BWkg$^{0.75}$)	406.6[b]	390.0[ab]	396.2[b]	374.3[a]	4.5
Energy retention (kJ/BWkg$^{0.75}$)	215.5[a]	288.2[c]	255.2[b]	314.6[c]	27.27
DE/GE	0.671	0.677	0.664	0.672	0.011
ME/GE	0.558	0.591	0.573	0.587	0.020
ME/DE	0.831	0.872	0.864	0.874	0.016
Feces/GE (MJ/100MJ GE intake)	34.8	34.8	36.4	35.0	1.1
Urine/GE (MJ/100MJ GE intake)	1.9	1.7	1.9	1.9	0.8
Methane/GE (MJ/100MJ GE intake)	7.6[b]	6.9[ab]	7.1[b]	6.6[a]	0.2
Heat production†/GE (MJ/100MJ GE intake)	36.4[b]	33.9[ab]	34.7[b]	32.6[a]	0.4
Retention/GE (MJ/100MJ GE intake)	32.2[a]	41.8[c]	37.8[b]	44.6[c]	3.8

Means in the same row with different superscripts differ significantly (P < 0.05). †Calculated by HP (kJ/day) = 16.18 * O2 (l/day) + 5.02 * CO2 (l/day) – 2.17 * CH4 (l/day) – 5.99 * Urinary N (g/day) (Brouwer, 1965). BW, body weight; GE, gross energy; DE, digestible energy; ME, metabolic energy; C, supplied 1.0% of commercial salt; T1, supplied 1.7% of desalted mother liquor (DML); T2, supplied 3.4% of DML; T3, supplied 5.1% of DML; SEM, standard error of means.

3.4 Discussion

The DM contents of the C diet were the lowest, followed by the T1, T2 and T3 diets (Table 3.3). This was related to the inclusion rate of the cassava pulp, the DM content of which was 15.0% on a DM basis, and the proportions of the cassava pulp in C, T1, T2 and T3 diets were 17.6%, 13.8%, 9.8% and 4.5% on a DM basis, respectively (Tables 3.1 and 3.2). Lahr *et al*. (1983) reported that DM intake decreased with the increase of dietary water content. In the present study, the animals in all treatments completely ate the offered diets at 2% of body weight on a DM basis in a short time (Table 3.4). This implies that the palatability of all diets in the present study was similar, and the variety of DM contents in the diets had no negative effect on the DM intake. The NaCl contents in the C, T1, T2 and T3 treatments were 1.1%, 1.2%, 2.2% and 3.4% on a DM basis, respectively. None of the treatments had adverse effects on feed intake, although Masters *et al.* (2005) demonstrated that increasing the sodium in the diet significantly decreased feed intake in sheep.

Higher NDF and ADF intakes were observed as the amount of palm kernel cake increased in the diets in which the NDF and ADF contents were high (Table 3.4). The tendency of the NDF and ADF digestibilities to be lower in T3 might not have been affected by the inclusion of DML but was affected by the inclusion of palm kernel cake. O'Mara *et al*. (1999) noted that palm kernel cake that had poorly digested components, such as lignin, cellulose and the least digestible non-carbohydrate fraction, had a high ADF content. Costa *et al*. (2010), who assessed levels of up to 40% palm kernel cake in sheep concentrate, observed that the addition of around 30% palm kernel cake allowed for greater consumption of the fibrous fractions. In the present study, the T3 diet contained 50% palm kernel cake (Table 3.2), and its NDF content was the highest of all the diets (Table 3.3), which might have resulted in the lowest NDF digestibility. The trend of a higher molar percent of propionic acid in T1 and T3 compared to C may also have been related to the NDF and ADF digestibility (Table 3.6).

The normal range of the ruminal pH is between 5.5 and 7.0, and the outer limits for the ruminal pH lie between 4.5 and 7.5 (Dohority 2003). The ruminal pH in each treatment in the present study appeared to be within the normal range (between 6.7 and 7.0; Table 3.6). The ruminal pH in animals fed the C diet tended to be the lowest among the treatments ($P < 0.078$), and the total VFA concentration in C was higher than those in T1 and T3 ($P < 0.05$). This was likely due to the greater amounts of cassava chip and cassava pulp in the C diet; together these components accounted for 32.3% of the C diet on a DM basis (Table 3.2). Suksombat *et al*. (2007) have reported that both cassava chip and cassava pulp contain high NFC contents that are easily digested in the rumen. The NFC contents of cassava chip and cassava pulp in the present study were 89.0% and 54.0% on a DM basis, respectively (Table 3.1). Dohority (2003) and Maghsoud *et al*. (2008) reported that a high NFC content in a diet enhances ruminal fermentation, which in turn results in an increase in total VFA and a decrease in the ruminal pH. Several studies have demonstrated that addition of NaCl has no effect on the ruminal molar percent of normal butyric acid (Roger *et al*. 1979; Leibholz *et al*. 1980; Croom *et al*. 1982), which agrees with the present finding that there were no significant differences among the different treatments in the molar proportions of normal or iso-butyric acids in the rumen (Table 3.6).

Previous studies stated the relationship between water intake and urine volume in sheep and cattle (Kelly *et al*. 1955; Winchester & Morris 1956; Weeth & Lesperance

1965). The average amounts of water intake and urine in the present study were highest in animals fed the T3 diet (17.3 L/day and 10.7 kg/day), followed by those administered the T2 (11.0 L/day and 7.5 kg/day), C (16.7 L/day and 7.0 kg/day) and T1 (9.6 L/day and 4.7 kg/day) diets. Spek *et al*. (2012) reported that the increased water intake with increased dietary Na levels increased outflow of rumen microbial protein to the intestine, which in turn, led to an increase in intestinal absorption of derivatives of microbial nucleic acids, resulting in an increased urinary non-urea (non-protein) nitrogen. In the present study, the urinary excretion of nitrogen was the highest in animals given the T3 diet ($P < 0.05$), which might have been due to an increase in water intake due to the high DML-derived salt content of this diet (Table 3.5). Consequently, the highest content of nucleic acid (non-protein nitrogen) related compounds in DML in T3 might have been affected directly to an increase in intestinal absorption of derivatives of microbial nucleic acids.

The plasma total cholesterol concentrations of animals offered DML supplementation (the T1, T2 and T3 diets) were higher than those of animals offered commercial salt (C diet; $P < 0.05$; Table 3.7). This might have been caused by a higher EE intake due to the high EE content of the palm kernel cake (Tables 3.3 and 3.4). Solomon *et al*. (1992) reported increased serum cholesterol concentrations in lambs fed palm oil. Nestel *et al*. (1978) proposed that an increase in dietary fat stimulated intestinal cholesterol synthesis to meet the increased demand for the absorption and transport of fat in ruminants. In spite of the higher NaCl intake from the DML, which resulted in the T3 diet inducing the highest water consumption, the plasma creatine concentration was normal (2%; Kaneko *et al*. 2008), which indicated that the higher NaCL intake had no negative effect on renal function.

While no significant difference was observed in the GE and DE intakes on the basis of metabolic body size, the ME intake on the basis of metabolic body size was higher in the T1 and T3 diets compared to the C diet ($P < 0.05$; Table 3.8). This was likely due to the energy loss into urine and methane, i.e., to the lower methane emission in T3 and the lower energy excretion in urine in T1. The methane emissions from cattle on the basis of metabolic body size (g/kgBW$^{0.75}$) in the present study were highest in animals fed the C and lowest in those given the T3 diet ($P < 0.05$; C: 1.53; T1: 1.44; T2: 1.47; T3: 1.36). This was not likely due to the amount of NaCl concentrate but rather to the EE contents in the experimental feed. Generally, high-fat feed is considered to inhibit methane production by stimulating propionate production and inhibiting the protozoa activity, in addition to inhibiting cellulolytic bacteria and feed digestion in the rumen. The EE content in the present study was highest in the T3 feed, and the molar proportions of propionic acid tended to be higher in the T1 and T3 feeds (Table 3.6). These results were consistent with several previous reports. Sondakh *et al*. (2012) described that the coconut cake meal had high EE contents and medium-chain fatty acids, which can inhibit the production of methane, resulting in the increase of propionic acid production. In addition, Czerkawski *et al*. (1966) reported that fat-rich feeds such as coconut cake meal, brewers grain, and rice bran have unsaturated fatty acids, which are hydrogenated by rumen microbes, resulting in low hydrogen pressure, which is a pre-requisite for a reduction in methane production. In addition, the energy loss into urine in the present study was also highest in animals fed the C and lowest in those fed the T1 diet. The value of the combined energy loss into urine and methane was related to the lower ME intake on the basis of metabolic body size in animals fed the C diet compared to those fed the T1 or T3 diets. The higher HP in C and lower HP in T3 could be explained by the values of the oxygen consumptions and carbon dioxide and emissions. HP was calculated using the equation described by Brouwer (1965), and the amounts of daily oxygen consumptions (L/day), carbon dioxide (L/day) and methane (L/day) emissions were used in this equation. Although the amounts of daily methane emissions in C were the highest among the treatments, the amounts of oxygen consumptions and carbon dioxide emissions were also the highest among the treatments. Itoh (1977) stated that HP is affected to a large degree by the amounts of oxygen consumptions and carbon dioxide emissions. Consequently, the energy retention levels in T2 and T3 were higher than that in C.

The purchased cost of rice bran, soy bean meal, palm kernel oil cake, cassava chip, cassava pulp, salt, and vitamin and mineral mixture were 11.0, 15.8, 4.3, 1.6, 0.01, 6.7 and 33.5 Thai Bahts (TBs/kg) on a FM basis, respectively, and DML supplemented in the present study were not for sale so far. Thus the feed costs (TBs FM/head/day) of C, T1, T2 and T3 diets were calculated as 46.6, 44.9, 38.3 and 30.3, respectively. Since the feed cost of C and T1 diets were similar, T2 and T3 diets could reduce approximately 18% and 35% of the feed cost compared to C diet.

Hence the formulations of each feed in the present study were diversified to adjust the CP and TDN contents accurately; further studies will be needed to define the effects of DML supplementation clearly. The present results suggested that the animal feeds utilizing the moderate ingredients such as palm kernel oil cake, cassava chip or cassava pulp with DML supplementation at NaCl concentration 3% might be reduced the nitrogen balance for the animals, however, that at 1% or 2% can be replaced with commercial salt without adverse effects on the digestibility of nutrients, blood metabolites or ruminal fermentation in Thai native cattle. Therefore, DML could be a valuable alternative ingredient due to its low cost and high protein content, resulting in a reduction in the feed cost.

CHAPTER 4

General Discussion

In the previous chapters, several by-product feedstuffs in the tropical Asia were evaluated for animal feeds. Our findings provided in Chapter 2 indicated that supplying more TMR silage with brewers grain than minimum feeding level of conventional concentrate has a possibility to enhance milk production of the buffaloes in Nepal. The results obtained in Chapter 3 suggested that desalted mother liquor (DML) could be a valuable alternative ingredient due to its low cost and high protein with NaCl content, resulting in a reduction in the feed cost without nutrient deficiency.

4.1 Utilization of brewers grain for TMR silage

Several studies were reported on the use of brewers grain as TMR silage. Nishino *et al*. (2003) reported that wet brewers grain is a sustainable by-product for ensiling and could be expected to improve stability of TMR silage against aerobic deterioration when ensiled with various feeds as TMR. Wang and Nishino (2008b) reported that DMI was higher in brewers grain treated TMR silage than soybean card residue treated TMR silage when fed after opening for aerobic exposure. In addition, Wang *et al*. (2011) evaluated the effect of ensiling on fermentation quality and aerobic stability of a total mixed ration (TMR) containing wet brewers' grains and corn straw, and stated that after stored for 28 days TMR silage containing 40% of brewers grain showed great fermentation quality and aerobic stability. This is consistent with the present study in Chapter 2 showing that TMR silage can have low pH, high lactic acid content and low ammonia nitrogen content. These results could be attributed to water-soluble carbohydrates or some non-water-soluble hemicelluloses in the other ingredients being used by inoculated lactic acid bacteria, which resulted in lactic acid fermentation (Winters et al. 1998).

4.2 Nutrient utilization in buffaloes

We attempted to evaluate the net energy for lactation and conversion efficiency of production energy for milk energy in the buffaloes using the values in the present study in Chapter 2 (Table 4.1), as following the equitation described by Paul *et al*. (2002).

$TDNI_m$ (kg/day) = 35.3 × MBW (kg $BW^{0.75}$)

$TDNI_p$ (kg/day) = TDNI - $TDNI_m$

MEI_p (Mcal/day) = $TDNI_p$ × 3.62

NE_l (Mcal/day) = (0.0913 × milk fat (%) + 0.3678) × milk yield (kg/day)

k_l (%) = NE_l / MEI_p × 100

$TDNI_m$ is a part of total digestible nutrient intake utilized for maintenance, $TDNI_p$ is a part of total digestible nutrient intake utilized for production, MEI_p is a part of metabolic energy intake utilized for production, NE_l is net energy for lactation, k_l is a conversion efficiency of production energy for milk energy.

Table 4.1 Nutrient intake, net energy for lactation and conversion efficiency of production energy for milk energy in buffaloes

		Treatment			
		CTL	T1	T2	SEM
TDNI	$kgBW^{0.75}$	57.2^{a}	60.3^{a}	75.4^{b}	3.1
$TDMI_{m}$	kg/day	3.2	3.1	3.2	0.1
$TDMI_{p}$	kg/day	2.0^{a}	2.2^{a}	3.7^{b}	0.3
MEI_{p}	Mcal/day	7.2^{a}	8.0^{a}	13.3^{b}	1.2
NE_{l}	Mcal/day	2.2^{a}	2.2^{a}	2.8^{b}	0.1
k_{l}	%	30.9^{b}	26.9^{ab}	20.8^{a}	2.7

Means within the same row with different superscripts significantly differ (*P*<0.05). CTL, fed concentrate at 0.6% of BW; k_l, a conversion efficiency of production energy for milk energy; MEI_p, a part of metabolic energy intake utilized for production; NE_l, net energy for lactation; T1, fed TMR at 0.6% of BW; T2, fed TMR at 1.2% of BW; TDNI, total digestible nutrient intake; $TDNI_m$, a part of total digestible nutrient intake utilized for maintenance; $TDNI_p$, a part of total digestible nutrient intake utilized for production.

The $TDNI_p$ was increased by increasing of feed intake (Table 4.1 and Table 2.3). The MY and FCMY also increased statistically in T2 (Table 2.6). This suggested that the milk production increment in T2 may be a result of increased $TDNI_p$. However, the conversion efficiency of production energy for milk energy was lower in T2. Bovera *et al.* (2007) reported that the conversion efficiency of NE_l was high when the energy supply was close to the requirement in lactating buffaloes. Because the BW increased in T2 in the present study, CPI was considered to be a contributing factor to increased body reserves. It is considered that the energy intake in T2 exceeded above the requirement of milk production performance of the buffaloes and the energy surplus might have been allocated to the body accumulation, as described in Chapter 2. There were contradictory observations for nutrient use between cattle and buffalo in literature.

Katiyar and Bisth (1988a,b) observed higher ($P < 0.01$) digestibility of fibrous fractions for buffaloes than mature cattle. Similarly, Robles *et al*. (1971) observed higher ($P < 0.01$) digestibility of crude protein and crude fiber in buffalo than in cattle. The digestibility of NDF and ADF in TMR silage was higher than that in conventional concentrate (Table 2.4). Grant *et al*. (1974a,b) and Ichikawa and Homma (1986) reported that buffaloes used feed nutrients better ($P < 0.05$) than cattle when fed with poor quality rations containing a high level of cellulose. The TMR silage in the present study included 29.3% brewers grain which contains high levels of cellulose (Mussatto *et al.* 2006). Buffaloes in the present study might utilize the high levels of cellulose in brewers grain. Wora-anu *et al*. (2000) found that ruminal cellulolytic, proteolytic and amylolytic bacteria of swamp buffaloes were significantly higher than those found in cattle fed similar diets. In addition, Lapitan *et al*. (2004) stated that the digestibility of dry matter, organic matter and nitrogen free extract tended to be high in crossbred water buffalo than in crossbred cattle, and crossbred water buffalo had higher average daily gain than crossbred cattle. These results suggested that, in the present study, buffaloes may have a tendency to use extra protein intake for body reserves rather than for milk production.

4.3 Seasoning by-products for animal feed additives

Unusual by-product resources are regarded as the most non-conventional resources of energy and protein that used in ruminant diets. Limited information is currently available and they are immensely in nutrient composition. DML used in Chapter 3 is one of the nucleic acids related compounds [disodium inosine-5′-monophosphate (IMP), disodium guanosine-5′-monophosphate (GMP)] for food additives produced by a seasoning company. This DML is a novel seasoning by-product from IMP and GMP production processes, however, several studies were conducted using similar seasoning by-product, the concentrated demineralized solution (CDS), which is from monosodium glutamate (MSG) production processes. CDS contained 42% moisture, more than 20% crude protein, 2.6% glutamic acid, other amino acids, and various vitamins. Yoshimura *et al*. (1994) reported that the digestible crude protein and TDN in CDS for goats were 14.5% and 31.9% on a fresh matter, respectively. The apparent digestibility of crude protein, crude fat and soluble nitrogen-free extract for goats were 72%, 56% and 47%, respectively. Yoshimura and Kawakita (1995) showed that no differences in the feed intake between cows given feed containing 5% CDS and those given no CDS. This was coincident with our results in Chapter 3 that the DM intake did not differ significantly among the treatment (Table 3.4). Kanyinji *et al*. (2009) reported that the responses in terms of nutrient intake, DM intake in goats that were fed high amounts of concentrate with supplementary inosine and urea were similar. Yoshimura (2001) investigated the feeding value of CDS for fattening cattle by giving them feed containing soybean meal, urea or CDS for five months. The results indicated that cattle fed CDS showed a higher daily weight gain, consumption and feed conversion rate than cows fed the urea and soybean cake. The fattening study using DML has not conducted yet, however, if the chemical composition of DML including the amount of amino and/or nucleic acid contents is similar to those of CDS, DML has the potential to utilize the fattening period. Yoshimura *et al*. (1995) concluded that these finding suggested that nitrogen components of CDS added to the feed of cow within a range of 5% are effectively used.

In the present study, adding 5.1% of DML was increased nitrogen excretion into urine, resulted in decreased the nitrogen retention (Table 3.6).

4.4 Conclusion

In the present thesis, we evaluated the nutrient values and availabilities of agro-industry by-products in tropical Asia. In Tarai, Nepal, at the time we had studied, feeding brewers grain was generally limited on near the factories. However, within the last 5 years, brewers grain has become recognized by livestock farmers as premier feedstuffs for lactating cows and buffaloes of high merit, and livestock farmers have to pay for getting brewers grain. In that area, brewers grain is no more residues but already feedstuffs for the animals. In Thailand, the challenge and research for improved efficiency of use of DML is ongoing so far. If the price of by-products is kept lower than that of conventional feedstuffs such as maize, rice bran and soybean meal, then it may also lead to cheaper feed and possibly cheaper animal products in tropical Asia.

REFERENCES

REFERENCES

Abrams SM, Shenk JS. Westerhaus MO. 1983. Capability of near infrared reflectance spectroscopy (NIR) to determine silage quality. *Journal of Dairy Science* **66**, 183.

Aibibula Y, Okine A, Hanada M, Murata S, Okamoto M, Goto M. 2007. Effect of replacing rolled corn with potato pulp silage in grass silage-based diets on nitrogen utilization by steers. *Asian Australasian journal of animal sciences* **20**, 1215-1221.

Alexiev AI. 1998. Breeding and management of river buffalo in Europe, Egypt and Iraq. In: Antonio B (eds), *Buffalo production and research*, Chapter 3, pp. 59-76 FAO Press, Rome.

Ammerman CB, Arrington LR, Loggins PE, McCall JT, Davis GK. 1963. Tomato By-Products as Feedstuffs, Nutritive Value of Dried Tomato Pulp for Ruminants. *Journal of Agricultural and Food Chemistry* **11**, 347-349.

Armentano L, Pereira M. 1997. Measuring the effectiveness of fiber by animal response trials. *Journal of Dairy Science* **80**, 1416-1425.

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

Bampidis VA, Robinson PH. 2006. Citrus by-products as ruminant feeds: A review. *Animal Feed Science and Technology* **128**, 175-217.

Barnett AJG. 1951. The colorimetric determination of lactic acid in silage. *Journal of Biochemical* **49**, 527-529.

Batajoo KK, Shaver RD. 1998. *In situ* dry matter, crude protein, and starch degradabilities of selected grains and by-product feeds. *Animal Feed Science and Technology* **71**, 165-176.

Bath DL. 1981. Feed by-products and their utilization by ruminants. In: Upgrading Residues and By-Products for Animals, Huber, J.T. (edn). *CRC press*, pp. 1-16

Bovera F, Cutrignelli MI, Calabro S, Piccolo G, Tudisco R, D'Urso S, Infascelli F. 2007. Use of two different dietary energy and protein contents to define nutritive requirements of lactating buffalo cows. *Journal of Animal Physiology and Animal Nutrition* **91**, 181–186.

Brouwer E. 1965. Report of sub-committee on constants and factors. In: Proceedings of the Third EAAP Symposium, *Energy Metabolism*, Vol. 11, pp. 441-443. Academic Press, London, England.

Cai Y, Benno Y, Ogawa M, Kumai S. 1999. Effect of applying lactic acid bacteria isolated from forage crops on fermentation characteristics and aerobic deterioration of silage. *Journal of Dairy Science* **82**, 520–526.

Cao Y, Takahashi T, Horiguchi K. 2009. Effects of addition of food by-products on the fermentation quality of a total mixed ration with whole crop rice and its digestibility, preference, and rumen fermentation in sheep. *Animal Feed Science and Technology* **151**, 1-11.

Cardon BP. 1953. Influence of a high salt intake on cellulose digestion. *Journal of Animal Science* **12**, 536-540.

Carpemtero MC, Holding AJ, McDonald P. 1969. Fermentation studies on lucerne. *Journal of the Science of Food and Agriculture* **20**, 677-681.

Cavani C, Bianconi L, Monggardi D. 1990. Soybean hulls and cereal distillers in dairy sheep feeding. In: 9th National congress of SIPAOC, pp. 609-610. Grado.

Central intelligence agency (CIA). 2015. The world factbook.
Available from URL:
https://www.cia.gov/library/publications/resources/the-world-factbook/geos/th.html

Chanjula P, Ngampongsai W, Wanapat M. 2007. Effects of replacing ground corn with cassava chip in concentrate on feed intake, nutrient utilization, rumen fermentation characteristics and microbial populations in goats. *Asian-Australasian Journal of Animal Science* **20**, 1557-1566.

Chumpawadee S, Pimpa O. 2009. Effect of fodder tree as fiber sources in total mixed ration on feed intake, nutrient digestibility, chewing behavior and ruminal fermentation in beef cattle. *Journal of animal veterinary advances* **8**, 1279-1284.

Conway EJ. 1962. *Microdiffusion Analysis and Volumetric Error*, 5th edn. *Crosby Lockwood*, London.

Coppock CE, Bath DL, Harris BJr. 1981. From feeding to feeding system. *Journal of dairy science* **64**, 1230-1249.

Costa DA, Ferreira GDG, Araújo CV, Colodo JCN, Moreira GR, Figueiredo MRP. 2010. Intake and digestibility of diets with levels of palm kernel cake in sheep. *Revista Brasileira de Saude e Producao Animal* **11**, 783-792.

Croom Jr WJ, Harvey RW, Froetschel M, Linnerud A. 1982. High levels of sodium chloride in beef cattle diets. *Canadian Journal of Animal Science* **62**, 217-227.

Czerkawski JW, Blaxter KL, Wainman FW. 1966. The metabolism of oleic, linoleic, and linolenic acids by sheep related to there on methane production. *British Journal of Nutrition* **20**, 349-362.

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

Devendra C. 2008. Improvement of animal agriculture in small farms and landless systems in Asia. *Proceedings of the 16th Asian Australasian Association of Animal Production Societies Animal Science Congress*, pp. 19. Hanoi, Vietnam.

Dohority BA. 2003. *Rumen Microbiology*, Nottingham University Press, Nottingham.

Ensminger ME, Olentine CG. 1978. Feeds and Nutrition. Ensminger Publishing Company, (1st edn) pp. 1417.

Eruden B, Nishida T, Matsuyama H, Hosoda K, Shioya S. 2005. Effect of addition of various levels of green tea grounds silage at the feed intake and milk production in lactating dairy cows. *Nihon Chikusan Gakkaiho* **76**, 295–301. *(In Japanese)*

Fischer RB, Peter DG. 1968. *Basic theory and quantitative chemical analysis*. 3rd edn. pp. 375-396. Saunders PA.

Food and Agriculture Organization of the United Nations (FAO*).* 2012. *Livestock Information [homepage on the internet]. Sector Analysis and Policy Branch, FAO, Rome; [cited 10 March 2014]. Available from URL:* http://faostat.fao.org/site/339/default.aspx

Food and Agriculture Organization of the United Nations (FAO*).* 2013. FAOSTAT. Available from URL: http://faostat3.fao.org/home/index.html

Gasa J, Castrillo C, Baucells MD, Guada JA. 1989. By-products from the canning industry as feedstuff for ruminants: digestibility and its prediction from chemical composition and laboratory bioassays. *Animal Feed Science and Technology* **25**, 67-77.

Gill RS. 1998. Management systems for river and swamp buffaloes. In: Proceeding of Symposium series 1 of *the 8th World Conference Animal Production*. In June 28-July 4, 1998. pp. 256-262. Seoul, Korea.

Grant RJ, Van Soest PJ, McDowell RE. 1974a. Influence of rumen fluid source and fermentation time on in vitro true dry matter digestibility. *Journal of Dairy Science* **57**, 1021–1205.

Grant RJ, Van Soest PJ, McDowell RE, Perez CB. 1974b. Intake, digestibility and metabolic loss of Napier grass by cattle and buffaloes when fed wilted, chopped and whole. *Journal of Animal Science* **39**, 423–434.

Grasser LA, Fadel JG, Garnet I, DePeters EJ. 1995. Quantity and economic importance of nine selected by-products used in California dairy rations. *Journal of Dairy Science* **78**, 962-971.

Ham GA, Stock RA, Klopfenstein TJ, Larson EM, Shain DH, Huffman RP. 1994. Wet corn distillers byproducts compared with dried corn distiller grains with soluble as a source of protein and energy for ruminants. *Journal of animal science* **72**, 3246-3257.

Hayashi Y, Devkota NR, Kumagai H. 2007. Effects of field pea (*Pisum sativum* L.) hay feeding on DM intake and milk production of Murrah buffaloes (*Bubalus bubalis*) fed rice straw *ad libitum*. *Animal Science Journal* **78**, 151-158.

Heaney DP, Pigden WJ. 1963. Interrelationships and conversion factors between expressions of the digestible energy value of forages. *Journal of Animal Science* **22**, 956-960.

Homma H. 1986. Cellulase activities of bacteria in liquid and solid phases of the rumen digesta of buffaloes and cattle. *Japanese Journal of Zootechnical Science* **57**, 336-341.

Huuskonen A, Khalili H, Joki-Tokola E. 2008. Inclusion of barley fiber and barley protein in a total mixed ration for growing dairy bulls. *Acta agriculturae scandinavica, Section A* **58**, 37-44.

Ichikawa T, Homma H. 1986. Comparative studies on feeding buffalo and cattle. 1. DM intake and nutrients digestibility. *Japan Journal of Tropical Agriculture* **30**, 251–256.

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

Ipharraguerre IR, Clark JH. 2003. Soyhulls as alternative feed for lactating cows. *Journal of dairy science* **86**, 1052-1073.

Itoh M. 1977. Evaluation of error in the measurement of nitrogen and energy lost in urine, CH4-production, O2-consumption, CO2-production, RQ and heat production with cattle. *Nihon Chikusan Gakkaiho* **48**, 347-353. (In Japanese)

Johnson COLE, Huber JT, King KJ. 1987. Storage and utilization of brewers wet grains in diets for lactating dairy cows. *Journal Dairy Science* **70**, 98-107.

Kaneko JJ, Harvey JW, Bruss ML. 2008. *Clinical Biochemistry of Domestic Animals*. 6th edn. *Academic Press,* Burlington, MA.

Kanyinji F, Kumagai H, Maeda T, Kaneshima S, Yokoi D. 2009. Effects of supplementary inosine on nutrient digestibility, ruminal fermentation and nitrogen balance in goats fed high amount of concentrate. *Animal Feed Science and Technology* **152**, 12-20.

Katiyar RC, Bisth GS. 1988a. Nutrient utilization in Murrah buffalo and Hariana Cattle: a comparative study with oat-hay-based rations. *Proceedings of the Second World Buffalo Congress*, Vol. 2, pp. 189–193. New Delhi, India.

Katiyar RC, Bisth GS. 1988b. Nutrient utilization in Murrah buffalo and Hariana Cattle: a comparative study with lucerne-hay-based rations. *Proceedings of the Second World Buffalo Congress*, Vol. 2, pp. 194–198. New Delhi, India.

Kayouli C, Lee S. 1999. Silage from by-products for smallholders. In: ‘t Mannetje L (ed.), *FAO Plant Production and Protection Paper*, Vol. 161, pp. 85-95. FAO, Rome, Italy.

Kelly CF, Bond TE, Ittner NR. 1955. Water cooling for livestock in hot climates. *Agricultural Engineering* **35**, 173.

Kennedy PM, Boniface AN, Liang ZJ, Muller D, Murray RM. 1992. Intake and digestion in swamp buffaloes and cattle. 2. The comparative response to urea supplements in animals fed tropical grasses. *Journal of Agricultural Science (Cambridge)* **119**, 243–254.

Kimura S, Oishi K, Hirooka H, Yokoi D, Kumagai H. 2010. Effects of supplementary nucleic acids on *in vitro* ruminal fermentation of roughage and concentrate as substrate. *Nihon Chikusan Gakkaiho* **81**, 457-466. (In Japanese)

Kondo M, Kita K, Yokota H. 2007. Ensiled or oven-dried green tea by-product as protein feedstuffs: Effects of tannin on nutritive value in goats. *Asian Australasian journal of animal sciences* **20**, 880-886.

Kyuma T, Saitoh O, Satoh T, Ieki T. 2009. Ensiling characteristics of brewer's grain and feeding performance for fattening Japanese Black steers. *Bulletin of the Shinshu University Alpine Field Center* **7**, 47-53.

Lahr DA, Otterby DE, Johnson DG, Linn JG, Lundquist RG. 1983. Effects of moisture content of complete diets on feed intake and milk production by cows. *Journal of Dairy Science* **66**, 1981–1900.

Lapitan RM, Del-Barrio AN, Katsube O, Tokuda T, Orden EA, Robles AY, Fujihara T, Cruz LC, Kanai Y. 2004. Comparison of feed intake, digestibility and fattening performance of Brahman grade cattle (*Bos indicus*) and crossbred water buffalo (*Bubalus bubalis*). *Animal Science Journal* **75**, 549-555.

Leibholz J, Kellaway RC, Hargreave GT. 1980. Effects of sodium chloride and sodium bicarbonate in the diet on the performance of calves. *Animal Feed Science and Technology* **5**, 309-314.

Macedo CAB, Mizubuti IY, Pereira ES, Ribeiro ELA, Ramos BMO, Mori RM, Pinto AP, Moreira FB, Bonin MN. 2007. Apparent digestibility and nitrogen use of diets with different levels of fresh orange pulp. *Archivos de zootecnia* **56**, 907-917.

Maghsoud B, Akbar T, Hossein J, Ali MG. 2008. Evaluation of some by-products using *in situ* and *in vitro* gas production techniques. *American Journal of Animal and Veterinary Sciences* **3**, 7–12.

Masters DG, Rintoul AJ, Dynes RA, Pearce KL, Norman HC. 2005. Feed intake and production in sheep fed diets high in sodium and potassium. *Australian Journal of Agricultural Research* **56**, 427–434.

Matsuyama H, Horiguchi K, Takahashi T, Kayaba T, Ishida M, Nishida T, Hosoda K, Park WY. 2004. Effects of ruminal dosing of mechanical stimulating brush and feeding of brewer's grains silage on methane emission of Holstein steer fed high concentrate diet. *Animal Science Journal (Japan)* **75**, 395-404.

McCarthy FD, Norton SA, McClure WH. 1990. Utilization of an ensiled wet brewers' grain-corn mixture by growing lambs. *Animal Feed Science and Technology* **28**, 29-38.

McDonald P, Whittenbury R. 1973. The Ensilage Process. In Butler GW, Bailey RW (eds), *Chemistry and Biochemistry of Herbage*. Vol. 3, pp. 33. Academic Press, New York.

Mirzaei AA, Maheri NS. 2008. Nutritive value of some agro-industrial by-products for ruminants-A review. *World journal of Zoology* **3**, 40-46.

Moya D, Mazzenga A, Holtshausen L, Cozzi G, Gonzalez LA, Calsamiglia S, Gibb DG, McAllister TA, Beauchemin KA, Schwartzkopf-Genswein K. 2011. Feeding behavior and ruminal acidosis in beef cattle offered a total mixed ration or dietary components separately. *Journal of Animal science* **89**, 520-530.

Murdock FR, Hodgson AS, Riley Jr. RE. 1981. Nutritive value of wet brewers grains for lactating dairy cows. *Journal of Dairy Science* **64**, 1826-1832.

Mussatto SI, Drangone G, Roberto IC. 2006. Brewer's spent grain: generation, characteristics and potential applications. *Journal of Cereal Science* **43**, 1-14.

National Agriculture and Food Research Organization (NARO). 2010. *Japanese Feeding Standard of beef cattle (2009). Japan Livestock Industry Association,* Tokyo. *(In Japanese)*

National Research Council (NRC). 2001. *Nutrient requirement of dairy cattle,* 7th edn. National Academy Press. Washington, DC.

National Research Council (NRC). 1996. *Nutrient Requirement of Beef Cattle,* 7th edn.

National Academy Press, Washington, DC.

National Research Council (NRC). 1980. *Mineral Tolerance of Domestic Animals*. National Academy Press, Washington, DC.

Nestel PJ, Poyser A, Hood RL, Mills SC, Willis MR, Cook LJ, Scott TW. 1978. The effect of dietary fat supplements on cholesterol metabolism in ruminants. *The Journal of Lipid Research* **19**, 899–908.

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

Okada K. 2011. Veterinary systems of production animals. In: Japanese College of Veterinary Internal Medicine (eds), *Veterinary Internal Medicine*, Large Animals 2nd edn, pp. 310-317. Buneido Shuppan Ltd., Tokyo. (In Japanese)

O'Mara FP, Mulligan FJ, Cronin EJ, Rath M, Caffrey PJ. 1999. The nutritive value of palm kernel meal measured in vivo and using rumen fluid and enzymatic techniques. *Livestock Production Science* **60**, 305-316.

Onodera R. 2001. *Dobutsu Eiyo Shikenho (Methods for Animal Nutrition Experiments)* ed. by Ishibashi. pp. 407-416. Yokendo. Tokyo. (In Japanese)

Patro BN and Bhat PN. 1979. Inheritance of production traits in buffaloes. *Indian Journal of Animal Science* **49**, 10-14.

Paul SS, Mandal AB, Pathak NN. 2002. Feeding standards for lactating riverine buffalo in tropical conditions. *Journal of Dairy Research* **69,** 173-180.

Pirmohammadi R, Golgasemgarebagh A, Azari AM. 2007a. Effects of ensiling and drying of white grape pomace on chemical composition, degradability and digestibility for ruminants. *Journal of animal and veterinary advance* **6**, 1079-1082.

Pirmohammadi R, Teimouri yansari A, Afshar hamidi B, Manafiazar G. 2007b. Effect of different fibrous and nonfiber carbohydrate levels on nutrients digestibility of total mixed ration using *in vivo* in buffalo. *Italian Journal of Animal Science* **6**, 476-479.

Prasad J, Tyagi AK, Neeraj. 1999. *Principles and Practices of Animal Nutrition. Kalyani Publishers*, Ludhiana.

Robles AY, Ordoveza AL, Aranas TJ. 1971. The feeding value of Napier grass (*Pennisetum purpureum Schumach*) for cattle and carabaos. *Philippine Journal of Animal Science* **8**, 123–130.

Rodrigues MA. Guedes CM, Rodrigues AL, Cone JW, Van Gelder AH, Ferreira LMM, Santos AS, Sequeira CA. 2008. Evaluation of the nutritive value of apple pulp mixed with different amounts of wheat straw. *Livestock research for rural development* **20**, 1654-1659.

Rogers JA, Davis CL, Clark JH. 1982. Alteration of rumen fermentation, milk fat synthesis, and nutrient utilization with mineral salts in dairy cows. *Journal of dairy science* **65**, 577-586.

Solomon MB, Lynch GP, Lough DS. 1992. Influence of dietary palm oil supplementation on serum lipid metabolites, carcass characteristics and lipid composition of carcass tissues of growing ram and ewe lambs. *Journal of Animal Science* **70**, 2746-2751.

Sondakh EHB, Yusiati IM, Hartadi H, Suryanto E. 2012. The Effect of Methanogenic Inhibitor Feed on Propionic Acid and Lamb Meat Chemical Quality. *Journal of* *the Indonesian Tropical Animal Agriculture* **37**, 183-188.

Spek JW, Bannink A, Gort G, Hendriks WH, Dijkstra J. 2012. Effect of sodium chloride intake on urine volume, urinary urea excretion, and milk urea concentration in lactating dairy cattle. *Journal of dairy science* **95**, 7288-7298.

Staples CR, Davis CL, McCoy GC, Clark JH. 1984. Feeding value of wet corn gluten feed for lactating dairy cows. *Journal of Dairy Science* **67**, 1214-1220.

Statistical Analysis System (SAS). 1998. *SAS/STAT User's Guide Version 6*. SAS Institute, Cary, NC.

Suksombat W, Lounglawan P, Noosen P. 2007. Energy and protein evaluation of five feedstuffs used in diet in which cassava pulp as main energy source for lactating dairy cows. *Suranaree Journal of science and technology* **14**, 99-107.

Sunvold GD, Hussein HS, Fahey GC, Merchen NR, Reinhart GA. 1995. *In vitro* fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using fecal inoculum from cats, dogs, horses, humans, and pigs and ruminal fluid from cattle. *Journal of animal science* **73**, 3639-3648.

Suzuki T, Phaophaisal I, Pholsen P, Narmsilee R, Indramanee S, Nitipot P, Chaokaur A, Sommart K, Khotprom N, Panichpol V, Nishida T. 2008 *In vivo* nutritive value of Pangola grass (*Digitaria eriantha*) hay by a novel indirect calorimeter with a ventilated hood in Thailand. *JARQ* **42**, 123-129.

Thu VN, Udén P. 2000. Effect of work and urea-molasses cake supplementation on live weight and milk yield of Murrah buffalo cows. *Asian Australasian journal of animal science* **13**, 1329-1336.

Tipu MA, Ahmad F, Khalique A, Haque MN, Mirza RH, Tayyab U. 2014. Replacement of cotton seed cake with palm kernel cake in growing Nili-ravi buffalo male calves. *Journal of animal and plant sciences* **24**, 24-27.

Tyrrell TF, Reid JT. 1965. Prediction of the energy value of cow's milk. *Journal of Dairy Science* **48,** 1215-1223.

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

Wanapat M, Ngarmasang A, Kokhuntot S, Wachirapakom C, Rowlinson P. 2000. A comparative study on the ruminal microbial population of cattle and swamp buffalo raised under traditional village conditions in the northeast of Thailand. *Asian–Australasian Journal of Animal Sciences* **13**, 918–921.

Wanapat M. 1999. *Feeding of ruminants in the tropics based on local fed resources*. pp. 236. Academic Press, Khon Kean, Thailand.

Wang F, Nishino N. 2008a. Ensiling of soybean curd residue and wet brewers 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.

Wagenhoffer Z. 2007. Achievements of research in the field of beef cattle. In: Animal Production and Animal Science Worldwide WAAP Book of the year 2006, pp. 127-134. Academic Press, Wagenigen.

Weeth HJ, Lesperance AL. 1965. Renal function of cattle under various water and salt loads. *Journal of Animal Science* **24**, 441–447.

West JW, Ely LO, Martin SA. 1994. Wet brewers grains for lactating dairy cows during hot, humid weather. *Journal of Dairy Science* **77**, 196-204.

Westendorf ML. 2000. Food waste to animal feed: an introduction. In: Westendorf ML (edn), *Food Waste to Animal Feed*, pp. 3-15. Iowa State University Press, Ames, IA.

Winchester CF, Morris MJ. 1956. Water intake rates of cattle. *Journal of Animal Science* **15**, 722–740.

Winters AL, Merry RJ, Müller M, Davies DR, Pahlow G, Müller M. 1998. Degradation of fructans by epiphytic and inoculant bacteria during ensiling of grass. *Journal of applied microbiology* **84**, 304–312.

Wora-anu S, Wanapat M, Wachirapakorn C, Nontaso N. 2000. Effect of roughage to concentrate ratio on ruminal ecology and voluntary feed intake in cattle and swamp buffaloes fed on urea-treated rice straw. *Asian–Australasian Journal of Animal Sciences* **13**, 236-236

Xu C, Cai Y, Zhang J, Ogawa M. 2007. Fermentation quality and nutritive value of a total mixed ration silage containing coffee grounds at ten or twenty percent of dry matter. *Journal of Animal Science* **85**, 1024–1029.

Yoshimura (2001) Environmental conservation in the fermentation industry In: *International Science and Technology Co-operation Towards Sustainable Development,* OECD **35**. pp. 335-342.

Yoshimura M, Kawakita T, Yoshizumi T. 1994. L-glutamic acid mother liquor fermented from cane molasses as feed additive. *Nippon Nogeikagaku Kaishi* **68**, 1463-1473.

Yoshimura M, Kawakita T. 1995. Practical use of L-glutamic acid mother liquor as a feed additive. *Nippon Nogeikagaku Kaishi* **69**, 347-356.

Yoshimura M, Wanchai C, Sommaneewan C, Vearasilp T. 1995. L-glutamic acid mother liquor fermented from hydrolyzed cassava starch (glucose solution) as a feed additive. *Nippon Nogeikagaku Kaishi* **69**, 337-345.

Zalikarenab L, Pirmohammadi R, Teimuriyansari A. 2007. Chemical composition and digestibility of dried white and red grape pomace for ruminants. *Journal of Animal and Veterinary Advances* **6**, 1107-1111.