Effects of dietary protein supplementation on rumen digesta kinetics and voluntary intake of rice straw in dairy cows

稲わらへのタンパク質飼料の補給が乳牛のルーメン内容物動態および自由摂取量に及ぼす影響

北海道大学　大学院農学院

生物資源科学専攻　博士後期課程

KYAW SAN WIN

2015

Effects of dietary protein supplementation on rumen digesta kinetics and voluntary intake of rice straw in dairy cows

稲わらへのタンパク質飼料の補給が乳牛のルーメン内容物動態および自由摂取量に及ぼす影響

By

KYAW SAN WIN

THESIS SUBMITTED FOR THE DEGREE OF Ph. D.

TO

DIVISION OF BIORESOURCES AND PRODUCTS SCIENCE

GRADUATE SCHOOL OF AGRICULTURE

HOKKAIDO UNIVERSITY

2015

CONTENTS

ACKNOWLEDEMEMT

Professor Dr. Seiji KONDO, Laboratory of Animal Production System, Division of Bioresources and Products Science, Graduate School of Agriculture, Hokkaido University, and advisor of this thesis, gave much of his precious time to instruct and teach me throughout the course of this study. I would like to show my grateful appreciations for his continuous friendly treatment, instructions and encouragements throughout this study, and for other kind helps he offered to me during my stay in Japan.

I would like to express the grateful appreciations and special thanks to my supervisor Dr. Koichiro UEDA, Associate Professor of Animal Production System, for his invaluable guidance, instructions, suggestions in research ideas, his expert and technical support for the completion of this study and for the cordial revision of the manuscript.

I also show my deepest sense of gratitude to Dr. Yasuo Kobayashi, Professor, Laboratory of Animal Nutrition, for his valuable advice and constructive criticism as a reviewer of the manuscript of this thesis.

I wish to offer my sincere thanks to Dr. Tomohiro MITANI, assistant professor of Laboratory of Animal Production System and senior colleagues Dr. Shingio TADA, Dr. Aye Sandar Cho for their helpful advises, suggestions and discussions and practical contributions to the study. Thanks are also due to my doctoral course classmates Mr. S. UCHIYAMA, Mr. Min Bo and other junior members Mr. H. KONDO, Mr. S. SATO, Mr. T. KONDO, Ms. M. KATSUMATA, Ms. A. YAMAKAWA, Mr. K. ISOMURA, Ms. H. NODA, Mr. S. TAKAKO, Mr. R. YAMATANI, Mr. Y. SUZUKI, Mr. R. NISHIDA and Mr. T. KITAGAWA for their assistances to complete and finish the experimental

works on farm. I express my grateful gratitude to the staffs of the Second Division of Animal Science, Experimental Farm, Graduate School of Agriculture, Hokkaido University, for their assistances to complete the experiments on farm.

I really desire to thank to the staff of International Students' Center of Hokkaido University and all of the staff of Student Affairs Section of Graduate School of Agriculture for their assistance and kind helps related with academic life in Japan. I wish to thank Committee of Education, Ministry of Livestock, Fishery and Rural Development, Republic of the Union of Myanmar; and special gratitude to Ministry of Science, Sport and Culture (Monbukagakusho), Japan for scholarship support to accomplish my study in Japan.

I sincerely wish to thank to my professors, Dr. Tin Htwe, Dr. Aung Than, Dr. Ni Ni Maw, Dr.Tin Ngwe, Dr. Myint Thein, Dr. Nwe Nwe Htin, Dr. Khin Hnin Swe and Dr. Mar Mar Win (rector) of University of Veterinary Science, Myanmar for their encouragement and moral support.

I am deeply indebted to my parents, my elder sisters and my younger brother for their ever moral support throughout my study. Finally, I would like to thank to my beloved wife, Daw Sandar Aye and lovely son, Aung Ko Ko Thant for their whole tolerance, understanding, encouragement and moral support to my study in Japan.

ABBREVIATIONS AND SYMBOLS USED

ADF : Acid Detergent Fiber

ADL : Acid Detergent Lignin

BW : Body Weight

CP : Crude Protein

CMRT : Compartment Mean Retention Time of particle

DM : Dry Matter

DMI : Dry Matter Intake

FP : Fine Particles

k1 : particle size reduction rate in the rumen

k2 : passage rate of small particles from the rumen

LP : Large Particles

NDF : Neutral Detergent Fiber

NH_3-N : Ammonia Nitrogen

OM : Organic Matter

RS : Rice Straw

SBM : Soybean Meal

SP : Small Particles

VFA : Volatile Fatty Acid

TABLE CONTENTS

FIGURE CONTENTS

Figure Page

Chapter 1

Introduction

Uses of rice straw as ruminant feed

In the present world, increasing human population can make a strong competition between human beings and livestock production animals for grain food which can be consumed by both. For 2020, there will be a deficiency of grain for human consumption about 100 million tons in the world. The global meat and milk consumption is expected to increase from 233 to 300 million tons and 568 to 700 million tons in the period of 2000 to 2020, respectively (FAO, 2008a). Therefore, milk and meat production from animals based on forages or roughages is critically important for future global requirements for increasing human population.

About 11% of the world land surface is arable land; 23.5% is permanent pasture, 32.4% forest and woodlands and 34.3% other land (Crabbe and Lawsen, 1981). However, 20% of all cultivated areas, 30% of forests and 10% of grasslands currently undergoes degradation; a quarter of the world's population is sustained by production on degraded soil (FAO, 2008b). Livestock production stands approximately 30% of global arable land (FAO, 2008a). A field of livestock production that regards for the critical problem is the availability of feedstuff resources. Since the observation of alternative /additional food and feedstuff ingredient is of essential importance as the global requirement for grains was extremely large for the production and strong and stiff competition between human beings and livestock industries for existing of food and feed materials (McCalla, 2009). Moreover, gradual reducing of soil quality, lack of water and climatic changes also continues to affect productivity of crop and forage plants, threatening severely the animal productivity (Pearson and Langridge, 2008). The

world production of cottonseed, rapeseed, soybean, sunflower seed and total grains were 13, 34, 165, 13 and 1800 millions tons, respectively in 2009-10 (USDA, 2011).

For developing countries in the tropical and sub-tropical regions of the world, agriculture takes part in a significant section to improve the rural development and to supply foods for the growing human population. Thus, an arable land for crop production may be used more extensively for human food and consequently animal production will use on giving the by-products or co-products from the food made for human consumption. The demand for meat and milk at a high rate also increase for rapid growing economies in several developing countries of Asia. Hence, many countries in this area including Myanmar urgently need to increase their livestock production.

However, the tropical regions in the world, ruminant animals rely on year-round grazing with natural pastures or some are offered with cut grass and crop residues. An increase in the occurrence of dry weather is, moreover, projected for south Asia, east Asia and southeast Asia (Walsh, 2004). The frequency and intensity of rainfall in many parts has increased, causing an increase in the number and severity of floods; the number of rainy days has actually decreased along with the total amount of precipitation (Gruza and Rankova, 2004). Nowadays, many of these areas face seasonal dry periods for a long time in which the availability of pastures reduces and impact decrease of its nutrient quality such as the content of digestible energy and nitrogen.

Nearly 80% of the world's rice is grown by small-scale farmers in many developing countries including southeast Asia (Table 1) and it is common to use rice straw for animal feeding. Roughages used for dairy farms in developing countries are by-products or co-products and wastes from the agricultural sector. They originate from

rice straw, wheat straw, maize stalks, sorghum stalks, legume stems, leaves and cane tops. Due to the multiple cropping of rice, the countries have a surplus of roughages for ruminants. One kilogram of cereal crop residue per kilogram of grain produced is available for animal feed, even after leaving ample residue to prevent soil erosion and maintain soil quality (Anderson, 1978). Millions of tons of fibrous materials are available for animal feed in the world but only a small part of them is actually used. Ruminant animals are economically important to human beings as they can utilize efficiently a great number of roughages for the production of milk, meat, wool, and power for cultivating agricultural fields by consuming these large amounts of agricultural crop residues. Devendra and Thomas (2002) stated that rice straw is the principal crop residue fed to more than 90% of the ruminant livestock in this area. The calculated utilization of rice straw (RS) for animal feed in Asia, including China and Mongolia, was 30-40% of the total rice straw production (Devendra, 1997).

However, RS is abundant, inexpensive, and wide use for ruminants, it is typically considered that the main limiting factors in the utilization of RS as a feed are related to the low voluntary intake, poor digestibility, high fiber content and insufficiency of other nutrients to support animal production. Commonly, the maximum intake of rice straw by ruminants is about 1.0 to 1.2 kg per 100 kg live weight reported by Devendra (1997). The voluntary feed intake is an utmost important factor for roughage influencing the amount of energy or nutrients availability for ruminants when offered *ad libitum* (Dulphy and Demarquilly, 1983). Voluntary feed intake is more important than all other factors in determining the feeding value of roughage diets. Like all other feeds, the feeding value of RS depends mainly on; (i) voluntary intake,

Table 1 Rice production and obtained residues of the top 10 rice-producing countries in the world in 2003

Number	Country	Rice production (million tons)	Rice husk (million tons)[a]	Rice straw (million tons)[a]
1	China	166.00	38.18	74.70
2	India	133.51	30.71	60.08
3	Indonesia	51.85	11.93	23.33
4	Bangladesh	38.06	8.75	17.13
5	Vietnam	34.61	7.96	15.57
6	Thailand	27.00	6.21	12.15
7	Myanmar	21.90	5.04	9.86
8	Philippines	13.17	3.03	5.93
9	Brazil	10.22	2.35	4.60
10	Japan	9.86	2.27	4.44
Total		506.18	116.42	227.78

[a] Calculated data (Adapted from NARC newsletter, 2004)

(ii) digestibility and (iii) efficiency of nutrients utilization (Crampton *et al.,* 1957; Van Soest, 1982; Minson, 1985). To produce more milk and meat from ruminants fed rice straw, it could be recognized that the increase in voluntary intake of RS by dairy cows is the most critical factor for small-scale dairy farmers utilizing mainly RS in developing countries, especially in southeast Asia.

Nutrients constraints of rice straw for ruminant feeding

Theander and Aman (1984) reported that RS consists of a relatively large proportion of leaf (60%) than other cereal straws such as barley (35%), oat (43%) and wheat (20-41%). RS has a high content of cell walls, comprised of cellulose, hemicelluloses and lignin. To breakdown those components, cellulase, hemicellulase and ligninase enzymes are required (Schiere and Ibrahim, 1989). The microorganisms in the reticulorumen of ruminants produce cellulase and hemicellulase although these enzymes are not produced by animals' themselves. However, the chemical composition of RS can vary between varieties and growing seasons, with greater nitrogen and cellulose contents in early-season rice than others (Shen *et al.,* 1998). The straw fiber is very difficult to degrade which is partly an intrinsic characteristic. Rice straw's leaves contain less neutral detergent fiber (NDF), however, more ash and acid-insoluble ash than the stems, resulted in lower *in vitro* dry matter (DM) digestibility of the leaves competed to the stems (Vadiveloo, 2000). In goats, DM digestibility for RS leaf was 56.2% and for RS stem was 68.5% in the *in vivo* study by Phang and Vadiveloo (1992).

When compared to other cereal straws, rice straw has a higher content of silica (12-16 vs. 3-5% of DM) and a lower content of lignin (6-7 vs. 10-12% of DM). Silica, one of the rice cell wall components, will be present in wide proportions range from 5 to

15% depending on rice variety (Vadiveloo, 1992) and the availability of this mineral in the soil (Agbagla-Dohnani *et al.,* 2003). Silica decreases palatability and degradability of RS in the rumen due to its direct action inhibiting colonization by ruminal microorganisms (Bae *et al.,* 1997; Agbagla-Dohnani *et al.,* 2003). Van Soest (2006) also reviewed the role of silica on the quality of RS. RS stems are more digestible than leaves because their silica content is lower than the leaves (Jackson, 1977a, Shinoda *et al.,* 1984), therefore the paddy crop should be harvested as close to the ground as possible if the straw is to be fed to ruminants agriculture. Furthermore, similar in all other straws, lignin is the main cause of low digestibility (Garrett *et al.,* 1979). Lignin cannot be broken down in the rumen due to the absence of ligninase enzyme. However, it has important indirect effects on livestock production through effects on degradability and feed intake.

Some protective compounds in plant are lignin, cutin and silica which are called physical barriers and can alter cell wall degradation. Moreover, the most readily digestible plant tissues in forages are existed inside the plant. Silica and lignin are deposited in the cell wall of epidermis and mechanical tissues of RS respectively which are barriers of the invasion of ruminal microbes into the inner tissues, subsequently cell wall constituents were little digested (Kawamura *et al.,* 1973). The most aromatic organic polymer (lignin) takes a role in resistance compressing forces preventing against consumption by insects and mammals, and is tolerance to the rate and degree of microbial degradation (Iiyama *et al.,* 1990). If the plant material is highly resistant to particle size reduction in the rumen, voluntary feed intake will be reduced because large particles of digesta cannot pass through the reticulo-omasal orifice into the lower digestive tract. Physical properties such as dustiness, rigidity or fragility can also

influence on chewing activity that causes particle size reduction. Therefore, structural factors of the plant cell wall and plant tissues could be important in determining voluntary intake as well as the impact of access of rumen microorganisms (Gharpuray *et al.*, 1983; Wilson and Hatfield, 1997).

Besides cell walls, rumen microorganisms require other nutrients for growth and metabolism (Hoover, 1986). The CP content of RS is very low. The maintenance requirement of CP for cattle is 7% of DM (National Research Council, 1981); therefore, the RS only does not provide enough amounts of digestible protein for normal growth of cattle. The voluntary feed intake declines in forages containing less than 7% CP (NRC, 2000). The voluntary intake of RS is less than 2% of body weight. Ruminants fed RS as a sole diet cannot usually gain body weight and sometimes will lose their weight (Toyokawa, 1978; Moran *et al.*, 1983). Devendra (1997) concluded that the main regards of intake and degradability of RS depend mainly on their morphological characteristics, the physical and chemical nature of the cell walls. Finally, these factors of RS, thus, can influence on the chewing behavior of ruminant animals and the extent of fiber fragmentation that can differ in the reticulorumen. For these reasons, RS can be regarded as a poor quality livestock feed for ruminant production. However, from the results of DM intake, digestibility and rumen fermentation, Oh *et al.* (1971) suggested that RS is better than barley straw. Without any supplement of other required nutrient resources, RS feeding alone will lead to poor performance of the animals because RS cannot consist of enough sugar, amino acids and minerals for efficient microbial growth (Doyle *et al.*, 1986).

Methods for improving the utilization of rice straw

In ruminants' livestock, experimentally or practically processing methods of RS can be grouped into (i) treatment methods and (ii) supplements without treatment. In recent years, many studies have been conducted on the physical and chemical characterization and utilization of RS as a ruminant feed (Shen *et al.,* 1998, Abou-El-Enin *et al.,* 1999; Vadiveloo, 2000, 2003). Numerous strategies of physical, chemical and biological treatments including supplementation method with other feed ingredients in order to increase the consumption of RS by ruminants had been tested (Reddy, 1996; Karunanandra and Varga, 1996a, b; Shen *et al.,* 1999; Vu *et al.,* 1999; Liu and Orskov, 2000; Selim *et al.,* 2004). Physical treatments include chopping (coarse or fine), soaking, steaming under pressure and gamma radiation. Many of these treatments are not practically for use on small-scale farms, as they require machines or industrial processing (Xing, 1988). However, small machines such as chopper or grinder to cut RS can be feasible. Biological treatment such as fungi or their enzymes treatment (Jalc, 2002; Sarnklong *et al.,* 2010) is too difficult to apply in developing countries for the lack of technology to produce large quantity of fungi to meet the requirement for industrial livestock. Chemicals such as sodium hydroxide, calcium hydroxide, ammonia and urea (using in chemical treatment) to improve the nutritive values and utilization of RS could be alkali, acidic or oxidative agents (Itoh *et al.,* 1979; Liu *et al.,* 1988; Hoek *et al.,* 1988). These alkali agents are absorbed into the cell walls and break down the ester bonds between lignin and hemicellulose and cellulose chemically, and cause the structural fiber swollen or weaken physically (Chenost and Kayouli, 1997; Lam *et al.,* 2001). However, livestock farmers can still refuse unfamiliar technology for unexpected risk, economical costs and labour availability for these new methods in some

developing countries. Therefore, one of possible and easy methods to improve the feeding value of RS without treatment may be a mean of suitable supplementation strategy to fulfill deficiency of essential nutrients (Doyle and Pearce, 1985; Xing, 1988).

As RS has very low nitrogen content and is difficult to degrade, it is clear that supplementation of RS with a protein source and available energy source can improve the performance and the ruminants' production. It is primarily important to supply the rumen microorganisms with the essential nutrients needed for self-multiplication and degradation of the cell walls of straw, leading to favorable conditions for maintenance of good cellulolysis (Chenost and Kayouli, 1997). For many years, supplementation to poor quality roughages is performed by feeding energy or nitrogen sources concentrates, special minerals, proteins or green forages. Small quantities of supplements such as minerals or proteins improve ruminal fermentation, subjecting to increased intake and digestibility (Schiere and De Wit, 1993 a, b). Therefore, various supplements may be used such as protein and energy concentrates, molasses, multi-nutrient blocks, green leaves, crop residues and locally available co-products. Utilization of non-conventional supplement is commonly used when straw is not treated but only supplemented with urea (Van der Hoek *et al.*, 1989) or urea molasses blocks (Leng *et al.*, 1991), easily digestible fiber source such as sugar beet pulp (Masuda *et al.*, 1999) and bean husk (Tin Ngwe, 2003). Supplement with high protein alfalfa hay increased the consumption of barley straw (Haddad, 2000).

There is little evidence of positive associative effect on forage intake (Henning *et al.*, 1980) and digestibility (Pordomingo *et al.*, 1991; Lardy *et al.*, 2004) when low levels of grains are supplemented. However, recent many studies indicate that forage intake and digestibility could be reduced by supplementary energy sources such as corn,

barley, or other cereal grains as a result of negative associative effects (Kartchner, 1980; Chase and Hibberd, 1987; Lusby and Wagner, 1987; Carey *et al.*, 1993). Supplementation of RS based diets with protein source concentrates has been reported to overcome the nutritional constraints, and thus increased voluntary intake, digestibility and improved animal performance by heifers and sheep (Church and Santos, 1981; Warly *et el.*, 1992a). In contrary, voluntary intake of RS was increased a little (Liu *et al.*, 1988) or not increased (Devendra, 1978) in sheep by soybean meal supplementation.

In case of high-producing dairy cows, the supplements can be major ingredient of the ration when poor quality roughages are given to these animals. The common oilseed by-products such as soybean meal, peanut meal, cottonseed meal, sesame meal and sunflower meal are used in dairy cattle ration for smallholder farmers in developing countries. In feeding field trials, a diet of RS supplemented with soybean meal elevated both rumen degradability and intake (Warly *et al.*, 1992) and supplementation can improve milk production as illustrated by supplements with cottonseed meal (Wanapat *et al.*, 1996) and with a urea-molasses-multi-nutrient block (Vu *et al.*, 1999; Wanapat *et al.*, 1999; Akter *et al.*, 2004). Supplements with a high content of protein meals can promote digestibility of RS (Wiedmeier *et al.*, 1983; Tin Ngwe, 1990) and increase intake of poor quality prairie hay (McCollum and Glayean, 1985; Guthrie and Wagner, 1988; Stoke *et al.*, 1988). This is possibly resulted from improved microbial growth in the rumen and positive associative effects on physical factors such as rumen digesta kinetics of RS between supplements and poor quality roughages. However, there is again no enough data on the effects of protein meal supplements on ruminal size reduction of large particles (LP) and ruminal passage of small particles (SP) of RS in dairy cows, which are relating to digestibility and intake of RS.

Relationship between rumination and supplements

Many researchers have investigated that rumination and mastication were also associated with feed intake control and limitations (Schalk and Amadon, 1928; Balch, 1971; Pearce and Moir, 1964; Weston and Hogan, 1967). Voluntary intake of poor quality roughages was related with the resistance of LP breakdown by chewing activity both during eating and ruminating (McLeod *et al.,* 1990) and decreased by the rumen fill of dry matter (Bines, 1971; Weston and Kennedy, 1984). In previous researches, the increase in voluntary intake and digestibility of RS by SBM supplementation was associated with the decrease in rumination activity in sheep (Warly *et al.,* 1992a, b). However, they also showed that the increased levels of SBM did not further stimulate to intake and rumination processes. Additionally, physical and chemical properties of feeds can affect the chewing activity of the ruminants (Mertens, 1997). Therefore, ingestive mastication contributes to the removal of cuticle, crushing or disrupting of plant tissues and reduction in size (Pond *et al.,* 1984). However, ruminating seems to be more effective than ingestive masticating with respect to comminution of feed particles (Ulyatt *et al.,* 1986). Weakened structure of LP during rumen fermentation contributed to the ease of breakdown during rumination (Murphy and Kennedy, 1993). Facilitation of ruminal fiber fermentation also affected the specific fragility of forage species (Suzuki *et al.,* 2000).

Furthermore, it is also clear that rumination is considerably influenced by changes of dietary chemical composition. The mode of chewing during rumination is an important role in the reduction of particle size of ingested feed; subsequently this could facilitate degradation by microbes in the rumen. Ruminating time was markedly decreased when urea was added to an oat straw diet in cows (Campling *et al.,* 1962),

SBM (Warly *et al.,* 1992) and wheat bran supplemented to a RS diet in sheep (Harumoto and Kato, 1979). This decrease of ruminating time in cows and sheep will be possibly related to the increased of fragility of straw and the improved size reduction of rumen digesta by activated microbial fermentation in these reports. Thus, efficiency of chewing for LP breakdown to SP during ruminating can vary due to ruminal fermentation activity. Furthermore, supplementing low quality straw-based diets with protein sources increases ruminal ammonia nitrogen concentration to improve the slow rate of straw fiber fermentation in the rumen (Fike *et al.,* 1995; Promkot *et al.,* 2007; Aye Sandar Cho *et al.,* 2012).

On the basis of above observations, it can be hypothesized that the breakdown efficiency for LP by chewing activity during ruminating could be greater for the protein supplemented RS than that for RS because supplements accelerated microbial activity in the rumen.

Moreover, the breakdown of LP would be variable among forages due to differences in cell wall fragility (McLeod *et al.,* 1990), stages of maturity and species (Ueda *et al.,* 1997) during both eating and ruminating. However, there is no enough information on the factors affecting the efficiency of ruminating chew for the LP breakdown of RS fed to dairy cows. Welch (1982) observed that large animals seem to be more efficient ruminators than small animals within one species. As rumination plays a primary role in particle size reduction of roughages, amount of LP fiber in the rumen are of importance in regulating roughage intake and productivity of ruminants. Therefore, cattle are able to ruminate more and hence eat a large amount of RS relative to body weight than sheep. However, there is little information on the contribution of ruminating process of RS in dairy cows.

The breakdown of size of rumen particles is completed by chewing during eating and ruminating, microbial fermentation and rumen motility (Reid *et al.* 1977). The particle size distribution in the DM rumen digesta for sheep fed wheat straw appears to differ from that in those offered RS (Doyle *et al.*, 1987). With wheat straw about 70% of DM rumen digesta is in particles of less than 1 mm in size while with RS only 50% of DM in this size. It will be necessary to consider that ingestive chewing is more effective for wheat straw than for RS. Therefore, ruminating chew is more required for RS than for wheat straw to reduce ruminal LP into SP that can pass through the rumen. However, the breakdown of LP into SP by ruminating chew in dairy cows fed RS supplemented with dietary protein source is very scarcely investigated or there is no information.

The effect of physical distension of the gut or 'rumen fill' can limit voluntary intake of ruminants. Thus, the food intake is primarily reduced by rumen capacity in ruminant animals. The roughage intake will be inhibited by slow digestion of dietary constituents or slow passage rate of undigested residues. When poor, medium and good quality hays were offered to sheep, the contents of DM in the digestive tract were not altered in the observation of Blaxter *et al.* (1961). They also suggested that the amount of feed consumption by sheep was determined by the capacity of their digestive tract. Hence, forage intake was regulated by ruminal fill, particulate passage and digestion rate (Balch and Campling. 1962; Ellis, 1978; Forbes. 1986).

The voluntary intake of poor quality roughages in ruminants is controlled by physical factors, particularly the size reduction rate of rumen particles and its passage rate from the reticulo-omasal orifice (Campling, 1969). Conrad (1966) observed that as RS was very poorly fermented, it had slow rate of disappearance in the rumen and slow

rate of passage from the rumen, resulting in decreased feed intake. Thus, the slow rates of degradation, disappearance in sheep (Jelan and Kabul, 1987) and passage of RS from the rumen in sheep (Liu *et al.,* 1988) are barriers of increasing voluntary intake of RS. The particle size reduction is the main limiting step to control these processes. However, there is no evidence on the interrelationship among the particle size reduction rate and passage rate and voluntary intake of RS in dairy cow.

Objectives of the current study

Since there is a certain associative effect between feeds, the digestibility of a mixture of feeds is the sum of digestibility of the individual ingredients in ruminants. Thus, one dietary feed ingredient can influence ruminal digestion of the other ingredient. While the positive associative effects for ruminal fiber fermentation between protein supplements and RS in chemical digestion are well published, there is no quantitative information in physical digestion of RS in dairy cows.

The hypothesis tested in this study is whether the size reduction rate and ruminal passage rate of RS particles can be increased when a dietary protein supplement is added to RS based diets in dairy cows. Another hypothesis in this study is whether rumination efficiency for breakdown of LP to SP of rumen digesta can be increased when protein supplement is added to RS due to ruminal fermentation.

For these objectives, following two experiments were done and discussed.

In experiment 1,

i) To clarify variation in ruminal fermentation parameters after once RS feeding per day when a protein source concentrate is supplemented to RS (Experiment 1).

ii) To know the effects of changes in diegsta weights and size distributions of RS

particles in the rumen (Experiment 1).

In experiment 2,

iii) To enumerate the rate of particle size reduction and ruminal passage rate of RS particles (Experiment 2).

iv) To know the mechanism of the breakdown of LP to SP of rumen digesta during ruminating, rumination efficiency for LP breakdown and rumen fills (Experiment 1 and 2).

v) To verify the mechanism of the rates of ruminal size reduction and passage of RS particles and voluntary intake of RS in dairy cows (Experiment 1 and 2).

Chapter 2

Effects of amount of soybean meal supplementation on particle size distribution and digesta weight in the rumen of dairy cows fed rice straw (Experiment 1)

2.1 Introduction

The proportion of rumen digesta particles larger than 1.18 mm in size reduces with time after feed offering. This is due to particle size reduction through microbial action and through a physical masticating by ruminating (Sekine *et al.,* 1992). Additionally, the fiber fermentation is facilitated when feed particle size is reduced because of the increase in the surface area of particles for ruminal microbes' attachments and attack (Gerson *et al.,* 1988). The size reduction of small particles less than the critical particle size (1.18 mm) is required for escaping particles from the rumen (Poppi *et al.*, 1980, 1981).

Differences in the resistance of particle size reduction of roughage during ruminating chew due to the accelerated microbial activity appear to cause the differences in particles sizes existing in the rumen that can affect ruminal fill weights. Casler *et al.* (1996) reported that fragility of roughage is related to the rate of particle size reduction during mastication. Thus, the characteristics of the particle size distribution of digesta are important for researching the kinetics of digesta particles in the rumen (Mertens and Ely, 1979; Mertens *et al.,* 1984).

The pattern and size reduction of large particles of RS in the rumen could be related to the rumen fill, rates of fermentation of small particles as well as barriers against passage of digesta particles from the rumen. However, rumen bacteria can

slowly ferment RS fiber under ammonia-insufficient condition and will retard the rate of particulate passage from the rumen. Bacterial activity in the rumen of dairy cows is often concealed or hindered by the availability of ammonia nitrogen. Supplementing poor quality straw based diets with protein sources elevates ruminal ammonia nitrogen concentration (Fike *et al.,* 1995) and increases fermentation of RS fiber (Aye Sandar Cho *et al.,* 2012). Therefore, protein content is basically considered as the main nutrient constraint in ruminants for increasing fiber digestion and intake of RS. Warly *et al.* (1994) suggested that increasing of protein supplementation is more effective for promoting particle size reduction of rumen digesta and hence reduces rumination in sheep fed RS. Nevertheless, information regarding the effects of protein supplement on changes of digesta weights and size distribution of RS in the rumen of dairy cows are lacking.

The objective of this chapter was to quantify the effects of soybean meal supplement (SBM) on chewing activity, changes in weights of rumen digesta and distribution of RS particle sizes with time after RS feeding by dairy cows fed once daily. The soybean meal supplementation level can be hypothesized to provide the different changes in weights of rumen digesta due to the improving microbial activity and the increasing fragility of RS.

2.2 Materials and methods

2.2.1 Animal care and management

This experiment was conducted at the Experimental Farm in the Field Science Center of Hokkaido University, Sapporo, Japan. The methods of feeding management and surgery for the ruminal cannulation of cows in this study were approved by the

Animal Care and Welfare Committee of Hokkaido University.

2.2.2 Experimental design and treatments

Six rumen-cannulated non-lactating Holstein cows, with a mean body weight 718 kg were used in a 3 x 3 Latin square design. They were housed in the stall barn throughout the experimental periods and two cows per treatment within one period were allocated to one of the following three experimental treatments: a) rice straw alone (SBM0), 2) rice straw+1.5 kg SBM (SBM1.5) per day and 3) rice straw+3.0 kg SBM (SBM3.0) per day. RS was chopped into about 1-2 cm in length. The daily allowance of RS was set at 7 kg fresh matter (FM) basis and were given to cows after feeding SBM at 0800 h once a day. Drinking water and mineral block salt were free accessed. The length for each period was lasted 22 days in which the first 9 days were for adaptation period and the remaining 13 days were for measuring period.

2.2.3 Data collection and sample analysis

Weight of feed residue was measured before feeding. Samples of feed offered and feed residue were sampled during the measuring period. The RS actual intake was determined by the difference between feed offered and feed residue during measuring period.

On the first day of measuring period, approximately 100 ml of rumen fluid was collected eight times with three hour interval. Rumen fluid samples were taken by a syringe through a catheter inserted into the rumen which was fixed on the rumen cannula. After taking the rumen fluid, pH of the fluid was measured immediately by using a digital pH meter (Horiba, B-212., Japan). A 1-mL of subsample of rumen fluid was mixed with 0.1-mL 25% meta-phosphoric acid and the resultant sample were centrifuged at 28,000 ×g for 10 minutes at 4˚C. The supernatant was used for the

analysis of VFA with gas chromatography (GC-20, Shimadzu, Kyoto, Japan) using a column (ULBON HR-20M; Shinwa Chemical Industries, Kyoto, Japan) with column temperature of 150 °C, injection temperature of 150 °C and carrier gas. Another 0.1-mL subsample was mixed with 20% NaCl and then analyzed for ammonia-N as described by Wetherburn (1967).

The digesta weight in the reticulo-rumen was measured by evacuating manually at 3 h, 6 h, 12 h, 18 h and 24 h after the beginning of RS offering on day 11, 14, 17, 20, and 22 during the collection period. These digesta samplings were scheduled at intervals no shorter than 2 days to avoid the possibility of an effect of the rumen evacuation on the subsequent rumen sampling. The total rumen digesta of each cow was manually mixed and a subsample was taken. The subsample was dried at 60 °C for 48h and ground through a 1-mm screen. The weight distribution of the different particle sizes of the rumen digesta was determined with another undried subsample by the wet sieving method with sieves of 1.18- and 0.15-mm aperture according to the technique of Ichinohe *et al.* (1994). The DM weights of ruminal large particles (LP: >1.18mm), small particles (SP; <1.18mm, >0.15mm) and fine particles (FP; <0.15mm) at each sampling time were measured. Ruminal disappearance rates of total digesta, LP, SP and NDF were calculated by exponential functional equation.

$$Y=Ce^{-kt}$$

where, Y=the weight of digesta particles or fraction in the rumen at time t (h), C=the initial weight, k=fractional disappearance rate (%/h).

Samples of offered feed, rumen digesta and sieved rumen particles were dried at 60 °C for 48h and ground to pass through a 1-mm screen for subsequent chemical analysis. Feed and rumen digesta samples were analyzed for DM, organic matter (OM),

crude protein (CP), NDF, acid detergent fiber (ADF) and acid detergent lignin (ADL). DM, CP and crude ash were determined according to the AOAC (1990). NDF, ADF and ADL for feed samples and NDF for rumen digesta were measured according to the methods described by Goering and Van Sorest (1975).

Eating time and ruminating time were recorded with time elapsed video tape–recorder on each digesta sampling day 21 and day 22. The amount of LP breakdown was calculated as the difference of DM weight LP between each digesta sampling intervals. Rumination efficiency was calculated by dividing LP breakdown by ruminating time.

2.2.4 Calculation of data and statistical analysis

All data were subjected to GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Unless otherwise stated, a significant effect was declared at $P<0.05$ for experiment.

2.3 Results

The chemical compositions of RS and SBM are shown in Table 2. Any residues of experimental diets were not observed in the current experiment. DM intake of RS by dairy cows was not reduced by increased level of SBM supplement, as shown in Table 3.

Mean values of pH, and NH_3-N are presented in Table 4. The mean pH values of rumen fluid at the different time points and its postprandial changes did not differ among treatments (Figure 1). The mean concentrations of NH_3-N were significantly increased with increasing levels of SBM. The increase in concentrations of NH_3-N with SBM supplement level were also significantly different at every time sample collection

Table 2 Chemical composition of rice straw and soybean meal (Experiment 1)

	Rice straw	Soybean meal
OM, % of DM	89.3	93.8
CP, % of DM	5.0	49.7
NDF, % of DM	72.6	13.9
ADF, % of DM	46.8	8.8
ADL, % of DM	5.3	0.5

DM: dry matter, OM: organic matter; CP: crude protein; NDF, ADF: neutral and acid detergent fiber; ADL: acid detergent lignin.

Table 3 Dry matter intake and neutral detergent fiber intake of dairy cows fed rice straw supplemented with 0, 1.5 and 3.0 kg of soybean meal (Experiment 1)

	SBM0	SBM1.5	SBM3.0	*P*-value
Rice straw DMI, kg/day	6.4	6.4	6.4	NS
Total DMI, kg/day	6.4 [c]	7.7 [b]	9.1 [a]	<0.01
Total NDFI, kg/day	4.6 [c]	4.8 [b]	5.0 [a]	<0.01

DMI: dry matter intake, NDFI: neutral detergent fiber intake, SBM0: 0 kg SBM supplementation per day, SBM1.5: 1.5 kg SBM supplementation per day, SBM3.0: 3.0 kg SBM supplementation per day.

[abc]Mean value followed by the same letter in the same row do not differ significantly

NS: non significance

after the beginning of the RS feeding in the current experiment (Figure 2). The peak concentration of NH_3-N in the rumen of dairy cows fed RS supplemented with both 1.5 and 3.0 kg of SBM reached at 6 h after feeding.

Mean of total VFA concentration and molar proportion of VFA are displayed in Table 4. The post feeding change of total VFA and molar proportion of VFA are shown form Figure 3 to Figure 9. The mean concentrations of total VFA were significantly increased with increasing levels of SBM. However, concentrations of total VFA in SBM3.0 were significantly larger than those in SBM0 and SBM1.5 at 6 h, 9 h and 12 h sample collection after the beginning of the RS feeding. Molar proportion of acetic acid (C2) was reduced and butyric acid (C4) was significantly increased by increasing levels of SBM supplementation but molar proportion of propionic acid (C3) did not change. Molar proportions of iso-butyric acid (iC4), iso-valeric acid (iC5) and valeric acid or pentanoic acid (C5) were significantly increased by increasing levels of SBM supplementation.

Daily time spent for eating, ruminating and chewing of the cows are expressed in Table 4. The daily RS allowance was eaten within 3 h for all treatments. Ruminating chew was not recorded within 3 h after the beginning of RS given. The daily time spent for eating in SBM1.5 was significantly shorter than in SBM0. Eating time spent per kg DM and NDF intake for SBM1.5 was significantly less than SBM0, while the values of SBM0 and SBM3.0 were not differed. The daily time spent for ruminating in SBM0 was significantly longer than in SBM1.5 and SBM3.0. When expressed as per kg DM and NDF intake, ruminating time for SBM0 treatment was also longer than those of SBM1.5 and SBM3.0 treatments. However, the daily total chewing activity differed significantly among treatments being longest for SBM0, middle for SBM3.0, shortest

Table 4 Rumen fluid components in the rumen of dairy cows fed rice straw supplemented with 0, 1.5 and 3.0 kg of soybean meal (Experiment1)

	SBM0	SBM1.5	SBM3.0	*P*-value
pH	7.0	7.0	6.9	NS
NH3-N, mg/dL	0.8 [c]	5.6 [b]	11.3 [a]	0.01
Total VFA, mmol/L	5.1 [b]	5.9 [a]	6.3 [a]	0.01
C2, mmol/100mmol	74.6 [a]	72.4 [b]	69.6 [c]	0.01
C3, mmol/100mmol	17.9	17.4	18.0	NS
iC4, mmol/100mmol	1.5 [c]	1.8 [b]	2.0 [a]	0.01
C4, mmol/100mmol	4.4 [c]	5.4 [b]	6.8 [a]	0.01
iC5, mmol/100mmol	1.4 [c]	2.2 [b]	2.6 [a]	0.01
C5, mmol/100mmol	0.2 [c]	0.8 [b]	1.0 [a]	0.01

For abbreviations see footnotes in Table 3, NH_3N: ammonia nitrogen, VFA: volatile fatty acids, C2: acetic acid, C3: propionic acid, iC4: iso-butyric acid, C4: butyric acid, iC5: iso-valeric acid, C5: valeric acid.

[abc]Mean value followed by the same letter in the same row do not differ significantly NS: non significance

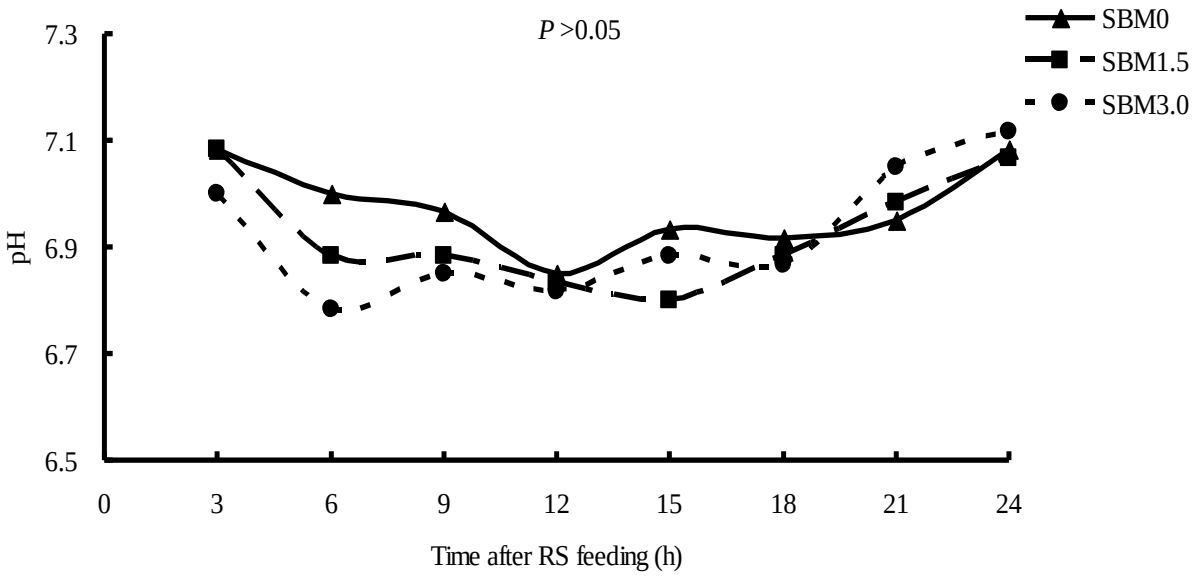

Figure 1 Changes of pH in the rumen fluid after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

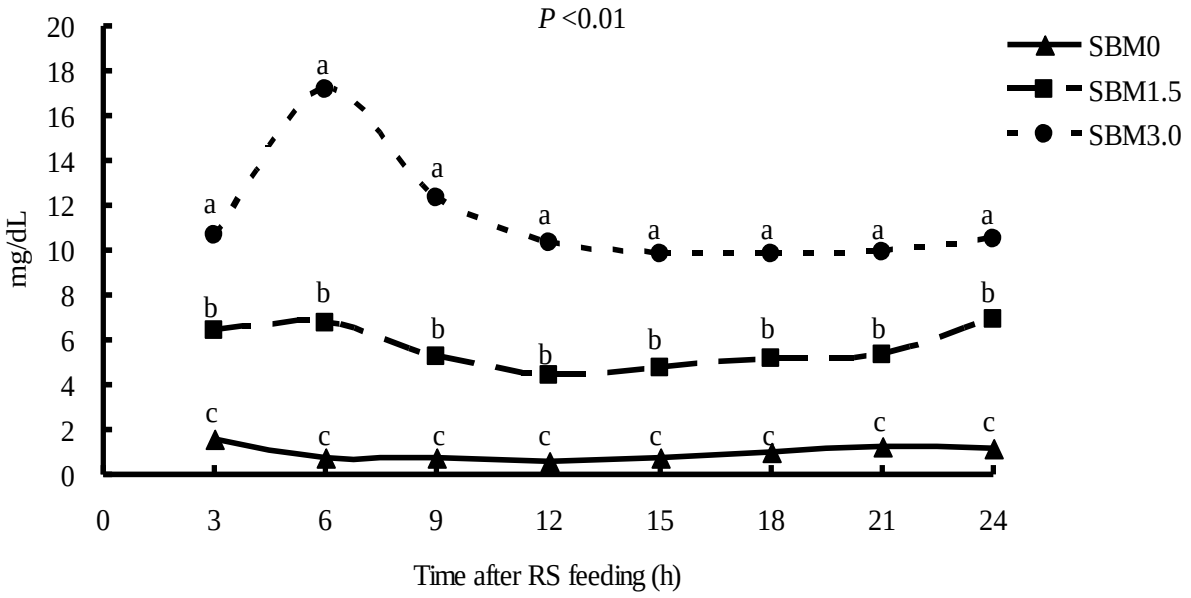

Figure 2 Changes in concentration of NH_3-N in the rumen fluid after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

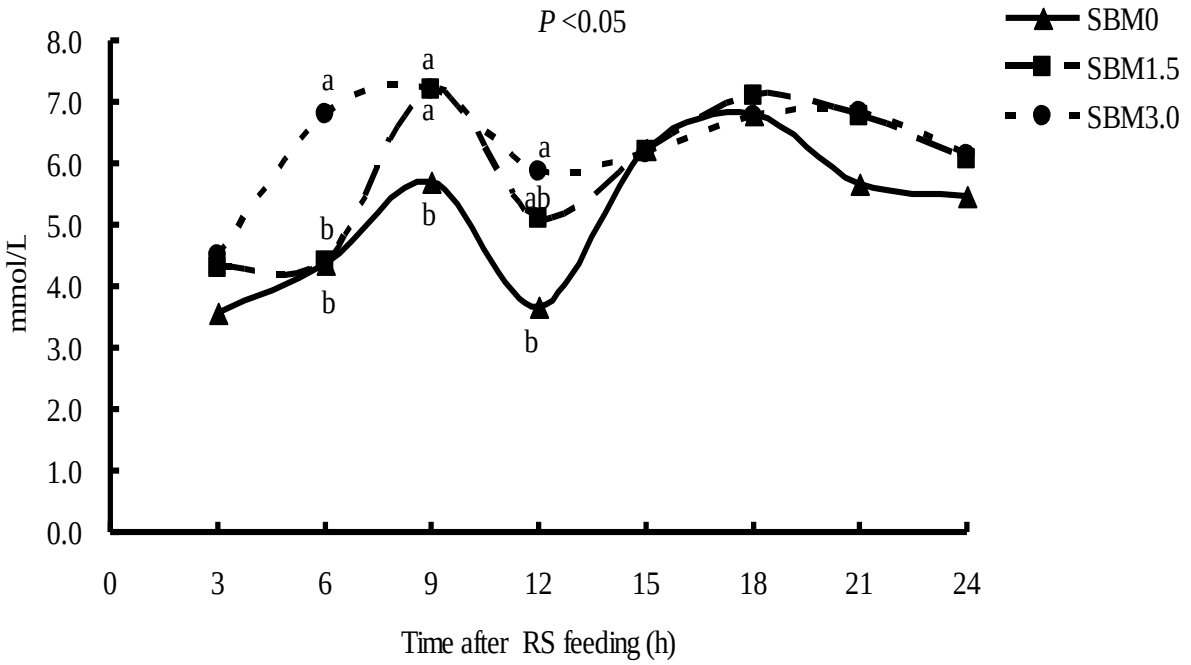

Figure 3 Changes in concentration of total VFA in the rumen fluid after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

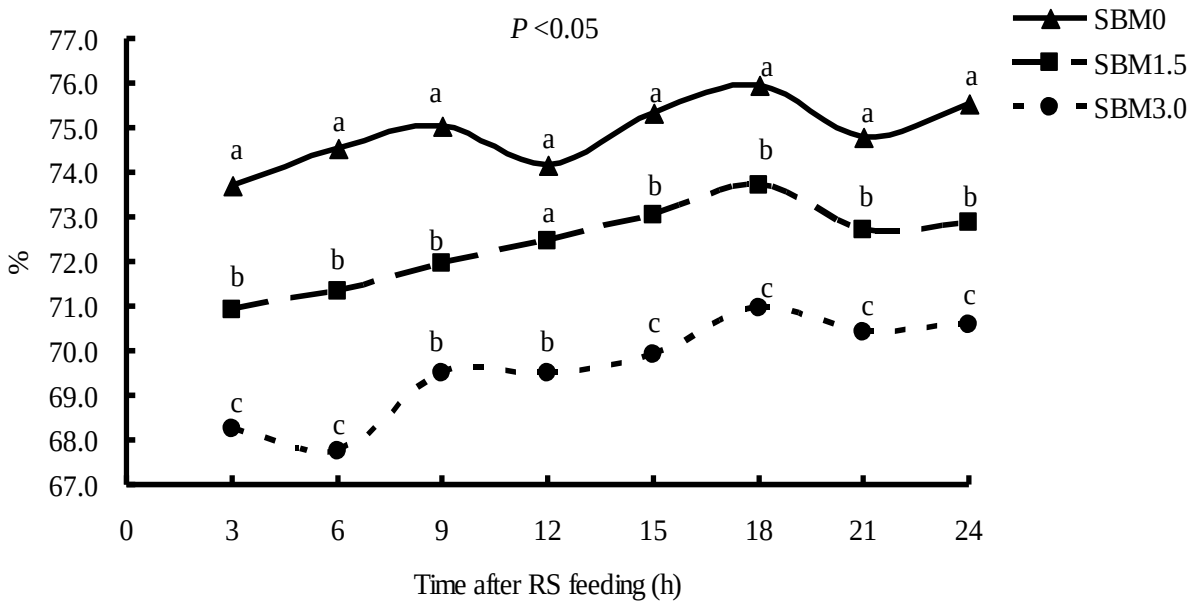

Figure 4 Changes in percentage of acetic acid in the rumen fluid after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

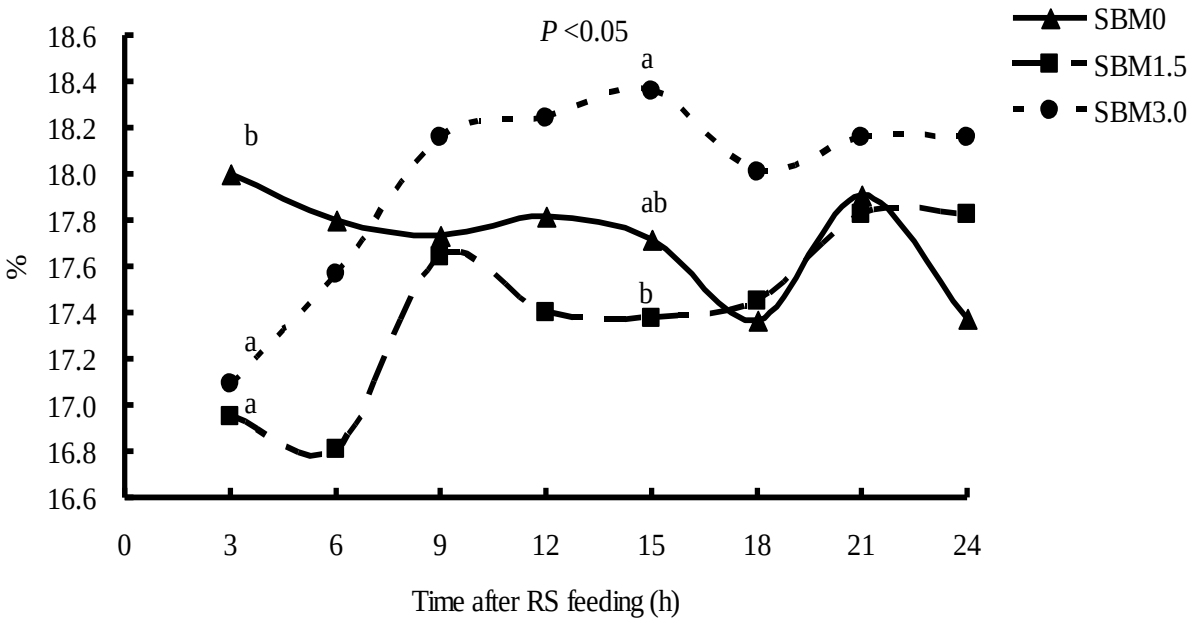

Figure 5 Changes in percentage of propionic acid in the rumen fluid after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

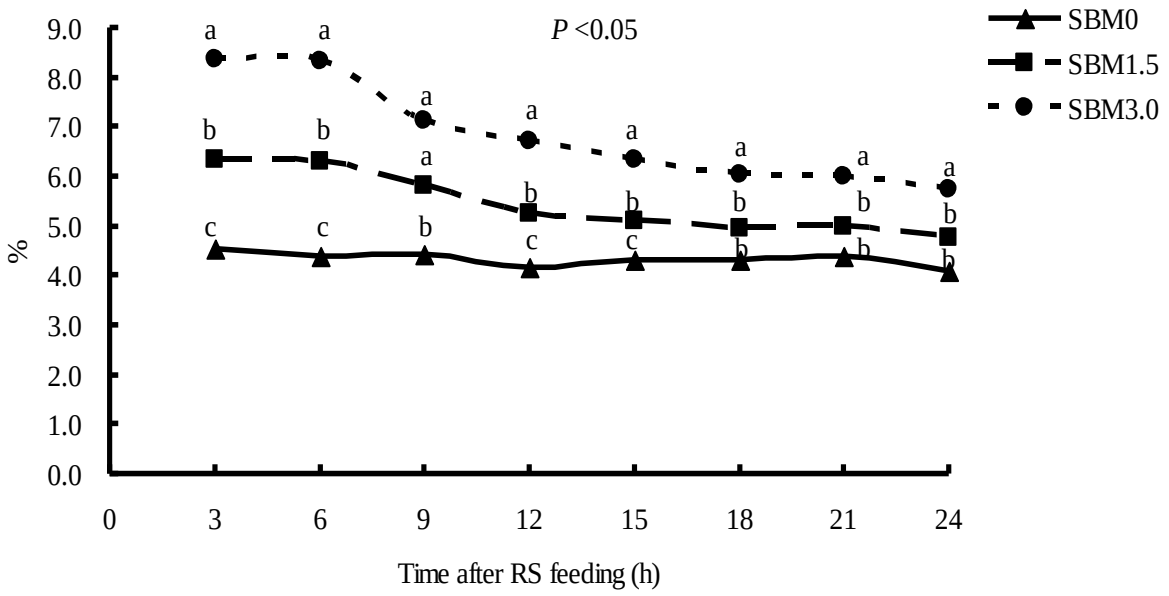

Figure 6 Changes in percentage of butyric acid in the rumen fluid after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

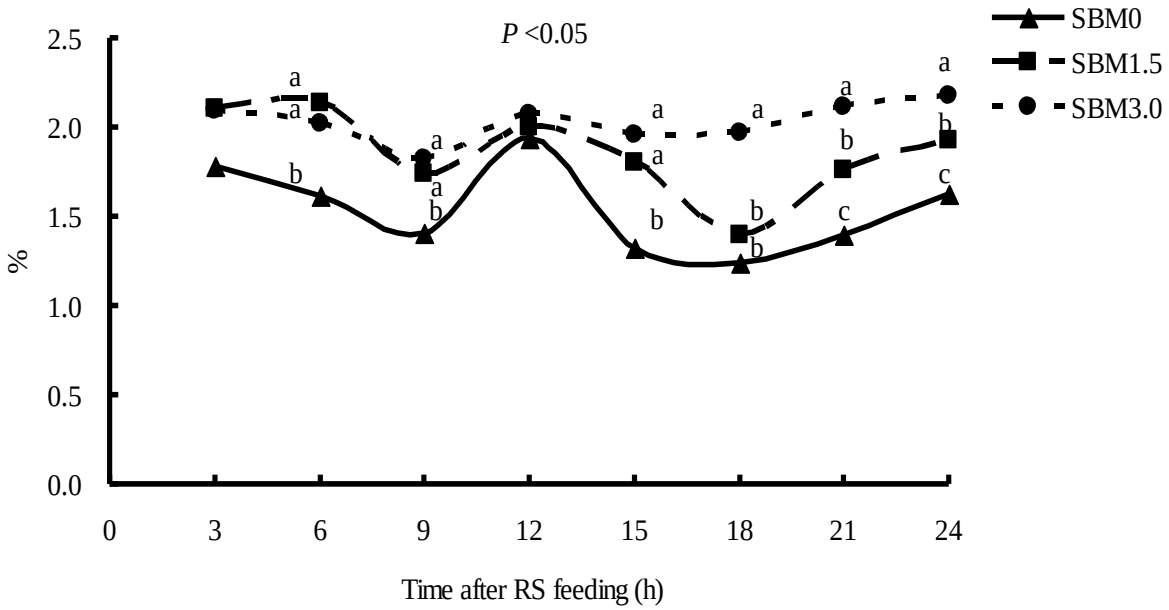

Figure 7 Changes in percentage of iso-butyric acid in the rumen fluid after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

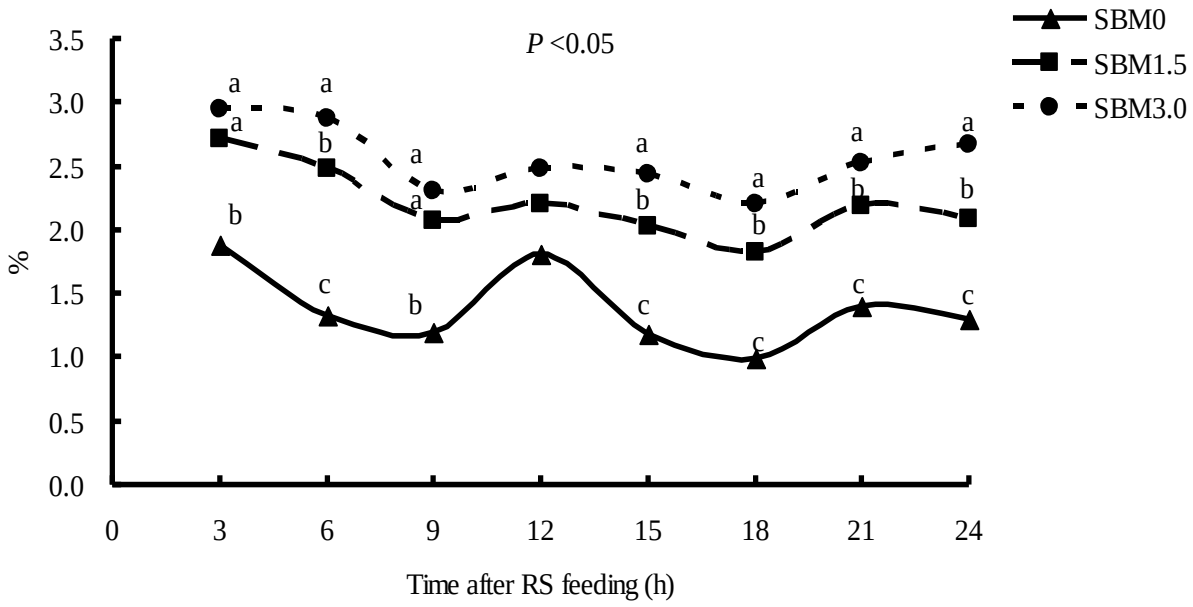

Figure 8 Changes in percentage of iso-valeric acid in the rumen fluid after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

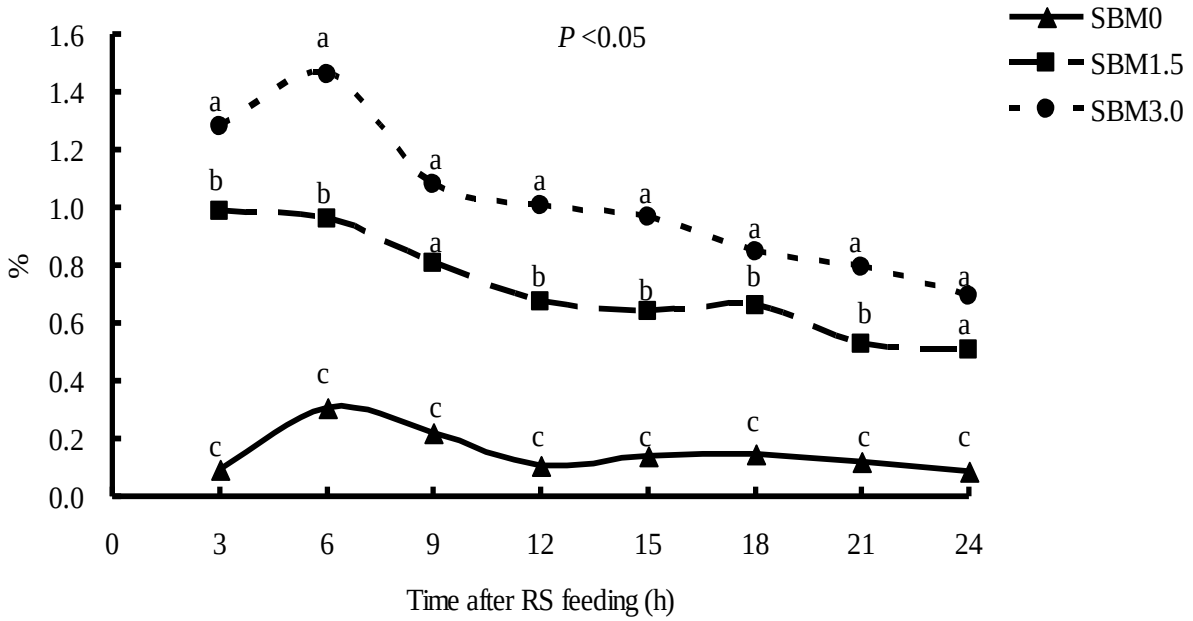

Figure 9 Changes in percentage of valeric acid in the rumen fluid after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

Table 5 Chewing time of dairy cows fed rice straw supplemented with 0, 1.5 and 3.0 kg of soybean meal (Experiment 1)

	SBM0	SBM1.5	SBM3.0	*P*-value
Eating time, min/day	120.1 [a]	92.1 [b]	109.6 [ab]	<0.05
min/kgDMI	18.9 [a]	14.5 [b]	17.2 [ab]	<0.05
min/kgNDFI	25.9 [a]	19.5 [b]	23.1 [ab]	<0.05
Ruminating time, min/day	377.6 [a]	333.4 [b]	353.8 [b]	<0.01
min/kgDMI	59.3 [a]	52.3 [b]	55.5 [b]	<0.01
min/kgNDFI	81.3 [a]	71.8 [b]	76.2 [b]	<0.01
Chewing time, min/day	498.2 [a]	425.5 [c]	463.4 [b]	<0.01
min/kgDMI	78.2 [a]	66.8 [c]	72.7 [b]	<0.01
min/kgNDFI	107.3 [a]	91.7 [c]	99.8 [b]	<0.01

For abbreviations see footnotes in Table 3.

[abc]Mean value followed by the same letter in the same row do not differ significantly

Table 6 Cumulative rumination time of dairy cows fed rice straw supplemented with 0, 1.5 and 3.0 kg of soybean meal after rice straw feeding (Experiment 1)

	SBM0	SBM1.5	SBM3.0	*P*-value
Time after RS feeding (min)				
0 h to 6 h	43.7	45.5	57.7	NS
0 h to 12 h	106.3	129.3	122.0	NS
0 h to 18 h	291.3 [a]	262.2 [b]	269.5 [b]	<0.05
0 h to 24 h	377.6 [a]	333.4 [b]	353.8 [b]	<0.01

For abbreviations see footnotes in Table 3.

[abc]Mean value followed by the same letter in the same row do not differ significantly

NS: non significance

for SBM1.5. This is the same when as a expressed as per kg DM and NDF intake.

Table 5 showed the cumulative rumination times of dairy cows. There was no significant effect for the cumulative ruminating time between 0 to 6 h and 0 to 12 h after RS feeding among treatments. However, significant effect was observed between 0 to 18 h and between 0 to 24 h after RS feeding among treatments in the current experiment. In these cumulative ruminating times of SBM0 were longer intervals than those of SBM1.5 and SBM3.0.

Fresh, dry and NDF weights of rumen digesta are displayed in Figures 10 (a, b, c). The fresh, dry and NDF digesta weights in the rumens of dairy cows significantly differed in all sampling times among treatments. Cows fed SBM3.0 had the smallest weights of FM, DM and NDF, SBM1.5 had the middle and SBM0 had the largest although the difference was not significant between SBM1.5 and SBM3.0 at 6 h after feeding.

The particle size distribution of total rumen digesta at each collected time is shown in Figures 11 (a, b, c). The proportion of LP decreased from 3 h to 24 h feeding for all measurements; 54.8% to 52.0% for SBM0, 68.8% to 55.6% for SBM1.5 and 61.5% to 53.4% for SBM3.0. The proportion of SP reduced from 3 h to 24 h after RS feeding; 20.5% to 18.3% for SBM0, 18.9% to 14.4% for SBM1.5 and 19.7% to 14.9% for SBM3.0. In contrary to the decreasing pattern of ruminal LP and SP proportion, the proportion of FP increased from 3 h to 24 h after feeding; 24.7% to 29.7% for SBM0, 12.3% to 30.1% for SBM1.5 and 18.8% to 29.4% for SBM3.0. The LP proportion for SBM3.0 tended to be higher than for SBM1.5 and SBM0. However, the SP proportion for SBM0 tended to be higher than proportion for SBM1.5 and SBM3.0. The significant differences observed at 12 h and 18 h after the beginning of RS feeding. The FP

proportion for SBM0 tended to be greater up to 12 h after RS feeding than for SBM1.5 and SBM3.0 and then lower.

The dry weights of LP, SP and FP are presented in Figures 12 (a, b, c). LP weight in the rumen reduced from 3 h to 24 h after feeding; 9.7 to 6.4 kg for SBM0, 10.6 to 6.1 kg for SBM1.5 and 8.6 to 4.8 kg for SBM3.0. The weights of SP also decreased from 3 h to 24 h after feeding; 3.6 to 2.2 kg for SBM0, 2.9 to 1.5 kg for SBM1.5 and 2.8 to 1.3 kg for SBM3.0. The weights of FP also gradually increased from 3 h to 12 h after feeding; 4.5 to 6.0 kg for SBM0, 1.8 to 4.8 kg for SBM1.5 and 2.6 to 3.6 kg for SBM3.0 and then FP weights gradually decreased from 12 h to 24 h in all treatments. This effect was seen significant weight reductions of LP (from 12 h to 18 h), SP (from 3 h to 24 h) and FP (from 6 h to 12 h) for cows supplemented with SBM compared to RS only cows.

Ruminal disappearance rates of total digesta, LP, SP and NDF of RS fiber are shown Table 6. All Disappearance rates were numerically greater for SBM1.5 and SBM3.0 than SBM0 cows although non-significant.

Large particles breakdown during ruminating and rumination efficiency for SBM supplemented cows tended to be greater than for SBM0 cows, but no further increase was observed by increasing levels of SBM (Table 7). Between 6 h to 12 h and between 12 h to 18 h, rumination efficiency of LP breakdown significantly differed between SBM supplemented and SBM0 cows, respectively.

Table 8 and 9 show mean proportions of hemicellulose, cellulose and lignin in the ruminal LP and SP of in the rumen digesta of SBM0, SBM1.5 and SBM3.0. Mean proportions of hemicellulose, cellulose and lignin in the ruminal LP and SP were not differed among treatments. The proportions of hemicellulose and cellulose in ruminal

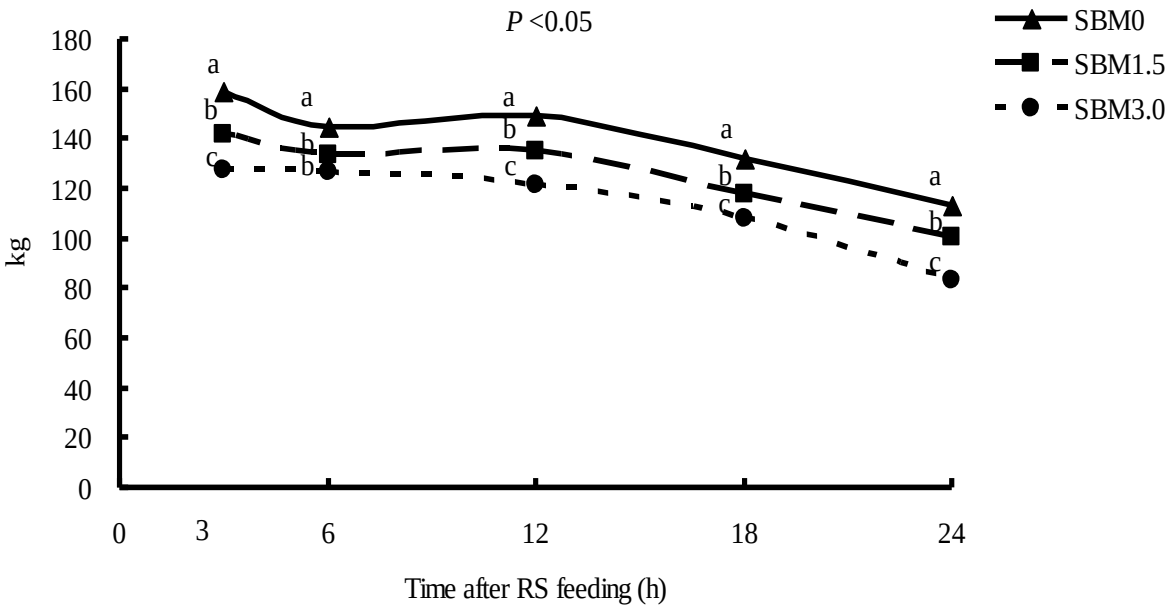

Figure 10a Changes in weights of fresh rumen digesta after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

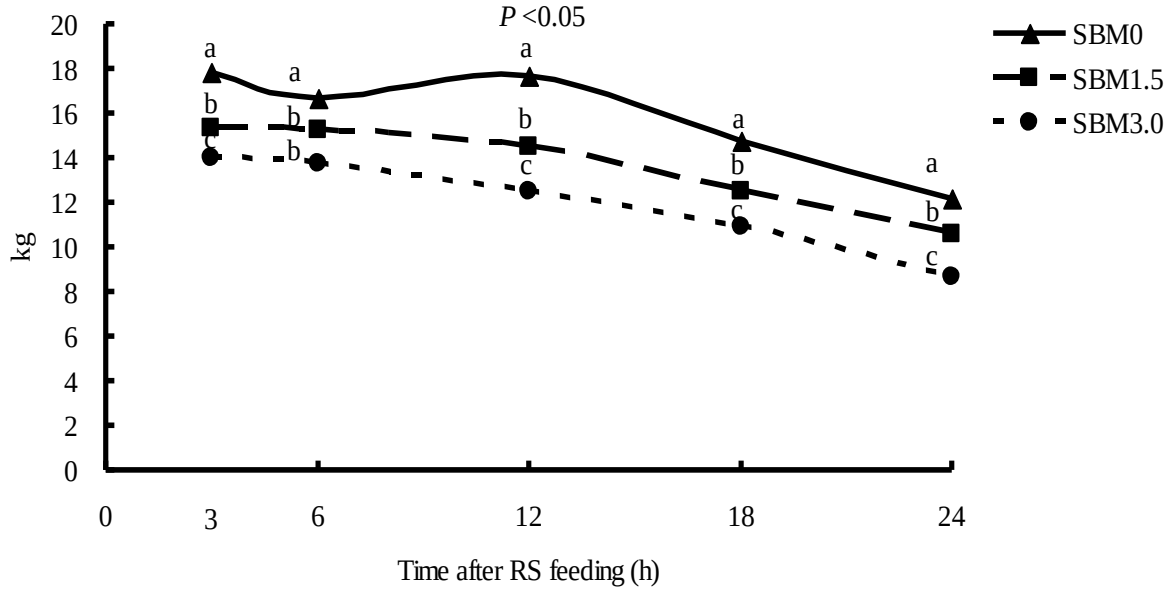

Figure 10b Changes in weights of dry rumen digesta after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

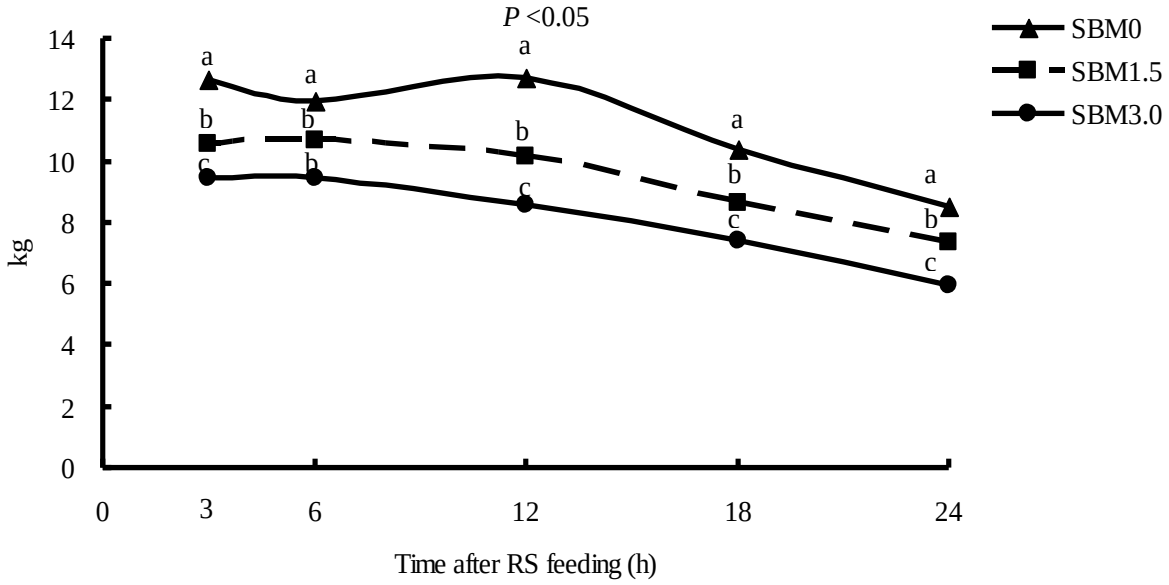

Figure 10c Changes in NDF weights of rumen digesta after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

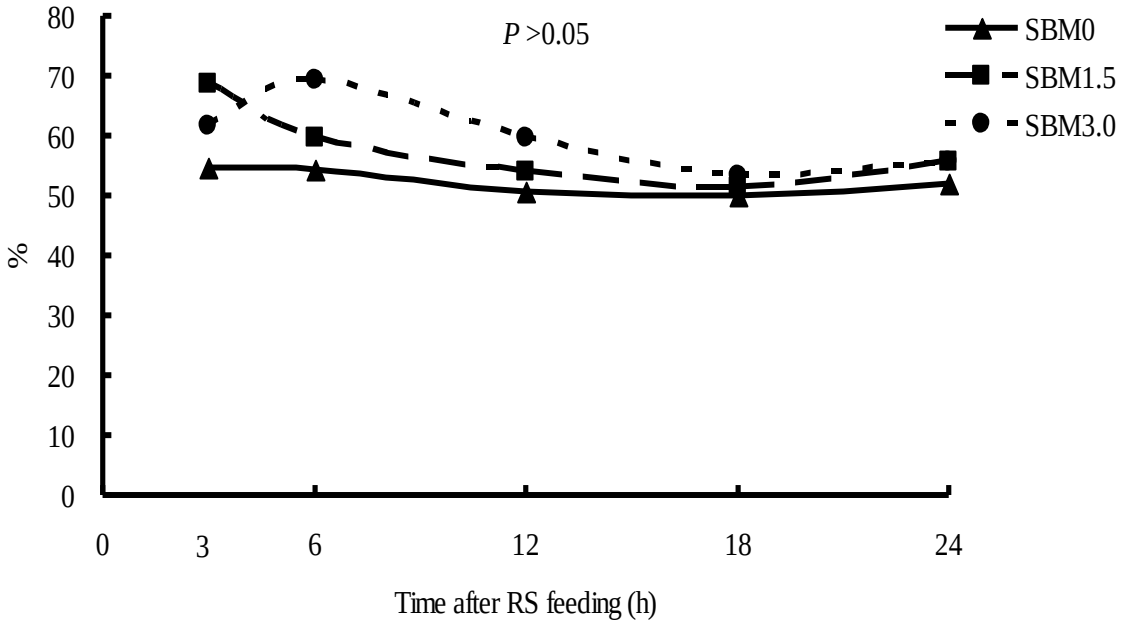

Figure 11a Changes in proportions of large particles in the rumen digesta after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

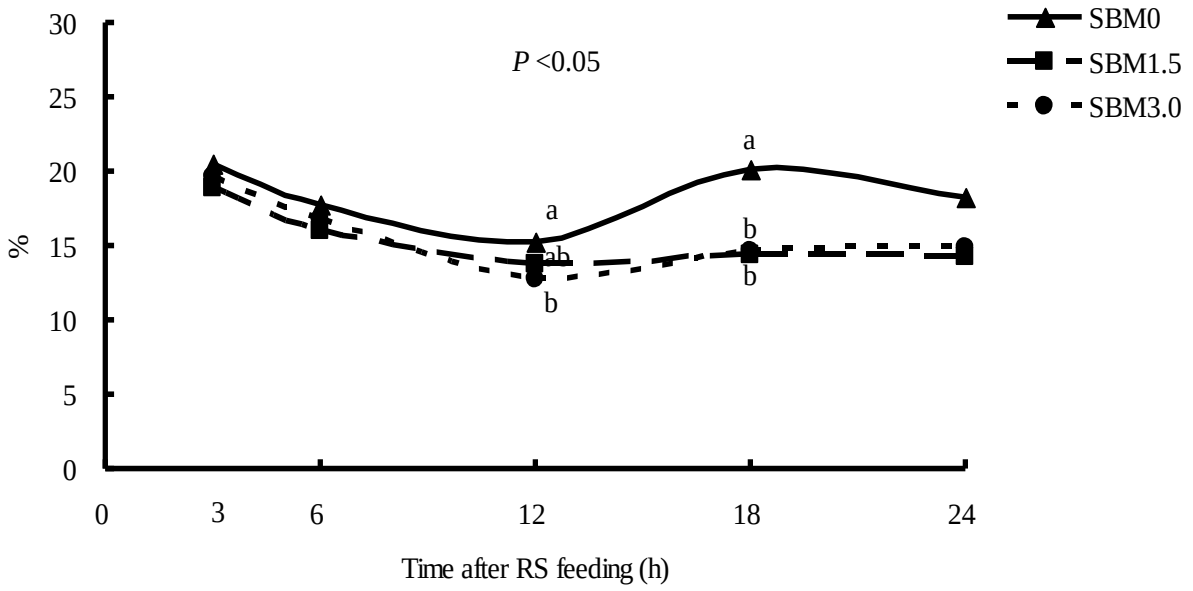

Figure 11b Changes in proportions of small particles in the rumen digesta after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

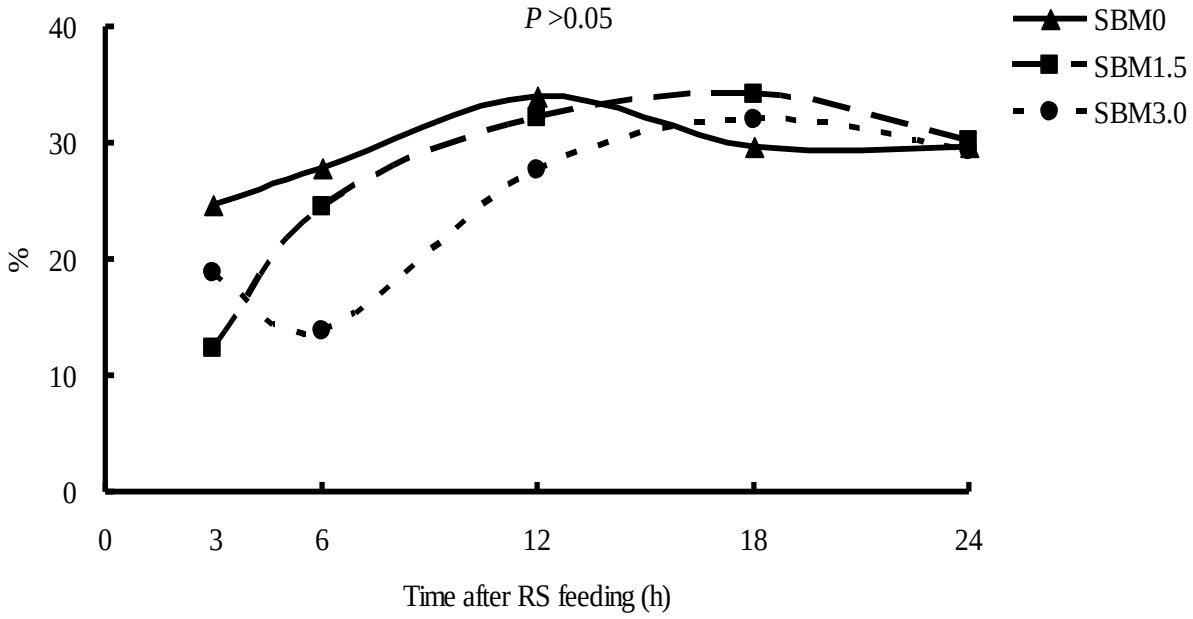

Figure 11c Changes in proportions of fine particles in the rumen digesta after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

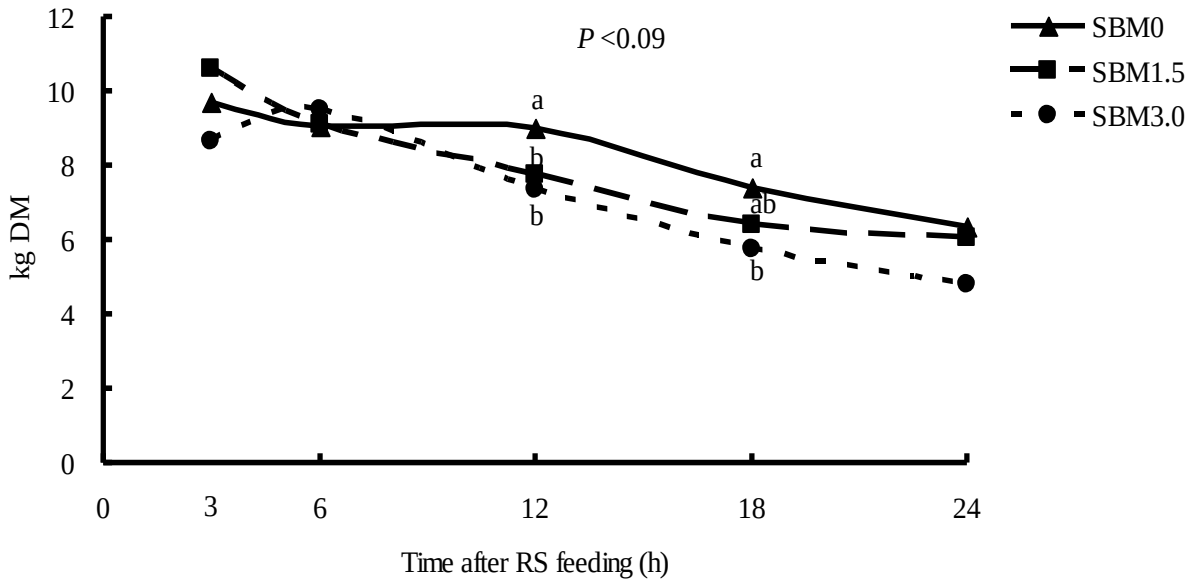

Figure 12a Changes in weights of large particles in the rumen digesta after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

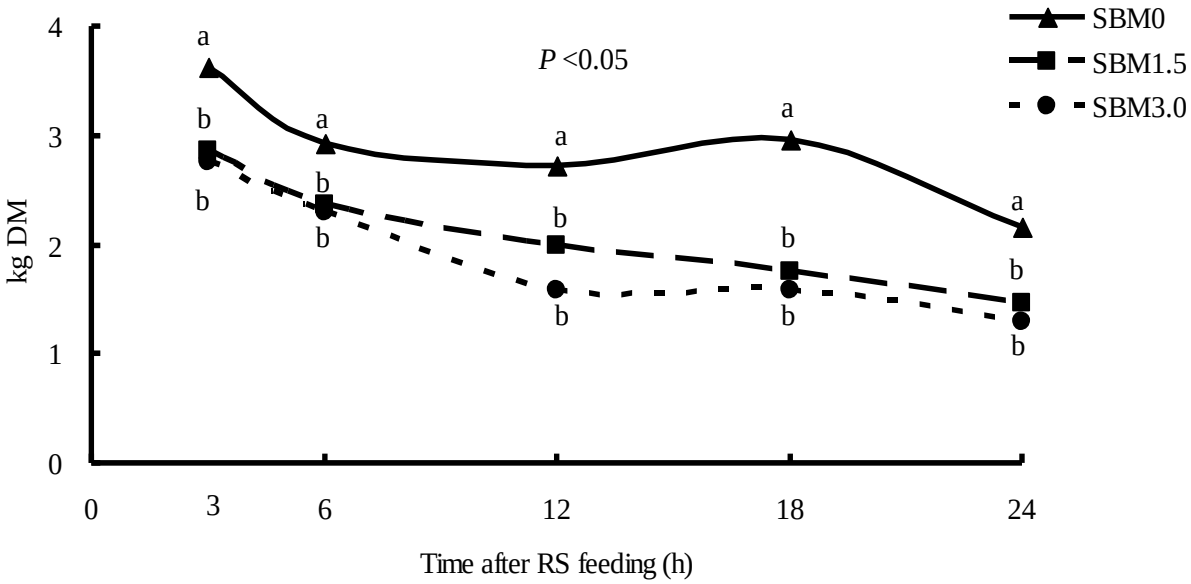

Figure 12b Changes in weights of small particles in the rumen digesta after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

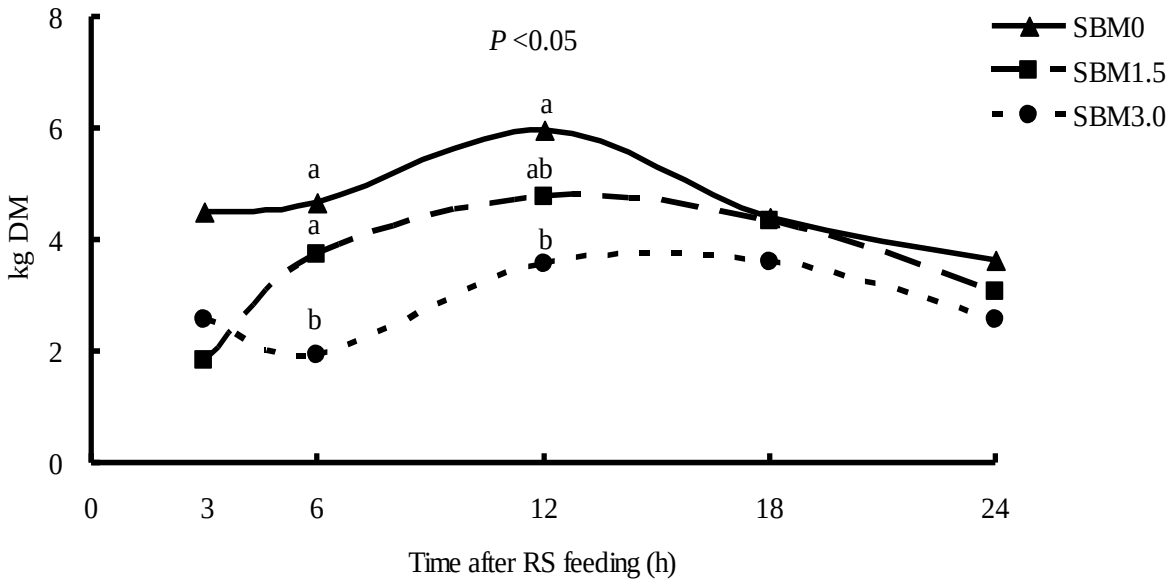

Figure 12c Changes in weights of fine particles in the rumen digesta after rice straw feeding in dairy cows supplemented with 0, 1.5 and 3.0 kg of soybean meal

Table 7 Ruminal disappearance rates of total digesta, large particles and small particles of rice straw in the rumen of dairy cows fed rice straw supplemented with 0, 1.5 and 3.0 kg of soybean meal (Experiment 1)

	SBM0	SBM1.5	SBM3.0	*P*-value
		%/h		
Total digesta	1.55	1.83	2.26	NS
LP	2.00	2.91	3.12	NS
SP	2.35	3.20	3.35	NS
NDF	1.62	1.87	2.25	NS

For abbreviations see footnotes in Table 3.

LP: large particles (>1.18mm), SP: small particles (<1.18 but >0.15mm), FP: fine particles (<0.15mm).

NS: non significance

Table 8 Large particles breakdown and rumination efficiency of dairy cows fed rice straw supplemented with 0, 1.5 and 3.0 kg of soybean meal

	SBM0	SBM1.5	SBM3.0	*P*-value
LP breakdown (kg/rumination interval)				
Time after RS feeding				
3 h to 6 h	0.21	0.61	-0.15	NS
6 h to 12 h	0.00 [b]	0.62 [a]	0.75 [a]	0.05
12 h to 18 h	0.42	1.15	1.23	NS
18 h to 24 h	0.66	1.21	1.63	NS
Rumination efficiency for LP breakdown (g/rumination, min)				
3 h to 6 h	8.72	22.01	-5.00	NS
6 h to 12 h	-0.07 [b]	4.68 [a]	6.35 [a]	0.01
12 h to 18 h	1.33 [b]	4.38 [a]	4.70 [a]	0.05
18 h to 24 h	1.75	3.70	4.61	NS

For abbreviations see footnotes in Table 3.

[abc]Mean value followed by the same letter in the same row do not differ significantly

NS: non significance

Table 9 Proportion of hemicellulose, cellulose and lignin in the ruminal large particles of dairy cows fed rice straw supplemented with 0, 1.5 and 3.0 kg of soybean meal (Experiment 1)

	SBM0	SBM1.5	SBM3.0	*P*-value
Hemicellulose proportion in ruminal LP after RS feeding (% of NDF)				
3 h	36.25	36.21	35.69	NS
6 h	35.97	36.08	36.38	NS
12 h	36.51	36.08	34.98	NS
18 h	36.32	35.21	34.68	NS
24 h	36.22	35.44	36.21	NS
Cellulose proportion in ruminal LP after RS feeding (% of NDF)				
3 h	53.12	53.01	53.50	NS
6 h	52.58	51.90	53.12	NS
12 h	51.07	52.37	53.86	NS
18 h	50.50	52.28	51.92	NS
24 h	50.80	52.67	51.43	NS
ADL proportion in ruminal LP after RS feeding (% of NDF)				
3 h	10.63	10.78	10.81	NS
6 h	11.45	12.02	10.49	NS
12 h	12.42	11.55	11.17	NS
18 h	13.18	12.51	13.40	NS
24 h	13.41	11.89	11.93	NS

For abbreviations see footnotes in Table 3.

Hemicellulose: NDF - ADF, Cellulose: ADF – ADL.

NS: non significance

Table 10 Proportion of hemicellulose, cellulose and lignin in the ruminal small particles of dairy cows fed rice straw supplemented with 0, 1.5 and 3.0 kg of soybean meal (Experiment 1)

	SBM0	SBM1.5	SBM3.0	*P*-value
Hemicellulose proportion in ruminal SP after RS feeding (% of NDF)				
3 h	36.70	35.73	35.41	NS
6 h	34.65	34.04	34.42	NS
12 h	35.50	35.18	35.06	NS
18 h	39.16	36.79	35.88	NS
24 h	36.80	37.01	36.89	NS
Cellulose proportion in ruminal SP after RS feeding (% of NDF)				
3 h	50.22	49.70	53.16	NS
6 h	52.25	52.59	52.73	NS
12 h	53.28	51.95	51.71	NS
18 h	47.64	49.21	50.58	NS
24 h	49.50	48.86	49.82	NS
ADL proportion in ruminal SP after RS feeding (% of NDF)				
3 h	13.08	14.57	11.43	0.05
6 h	13.10	13.37	12.85	NS
12 h	11.68	12.87	12.77	NS
18 h	13.20	14.00	13.54	NS
24 h	13.69	14.13	13.29	NS

For abbreviations see footnotes in Table 3.

Hemicellulose: NDF - ADF, Cellulose: ADF – ADL.

NS: non significance

LP and SP for SBM1.5 and SBM3.0 were slightly smaller and larger than for SBM0 but not significantly differ. The proportions of lignin in ruminal LP and SP was not differed among treatments.

2.4 Discussion

2.4.1 Fermentation parameters in the rumen

Mean NH_3-N concentrations in the rumen fluid significantly increased from 0.8 to 5.6 and 11.3 mg/dL in dairy cows fed SBM0, SBM1.5 and SBM3.0, respectively. This result agreed with that of several researchers (Rooke *et al.*, 1986; Krysl *et al.*, 1989; Stoke *et al.*, 1988). An ammonia concentration of 5 to 8 mg/ dL in the rumen fluid is sufficient to support maximum rates of microbial growth *in vitro* research (Satter and Slyter, 1974). Therefore, the increasing ruminal NH_3-N concentration could increase fiber fermentation of RS by improving microbial activity for dairy cows in the current experiment. Concentrations of ruminal NH_3-N in the present experiment were relatively lower than those in sheep reported by Warly *et al.* (1992a). This difference might probably be due to the different utilizing efficiency by dairy cows and sheep.

The time at which highest concentration of ruminal NH_3-N for SBM1.5 and SBM3.0 cows in the current study was differed from the results of Stoke *et al.* (1988) and Warly *et al.* (1992). This difference could possibly be due to the use of different basal roughage and amount of SBM. Moreover, Satter and Roffler (1981) reported that NH_3-N concentration normally reached a peak level at about 1-2 hr after feeding and then decreased gradually. However, SBM3.0 cows had about 10mg/dL NH_3N at all sampling times after feeding. It can maintain microbial activity throughout the day that can correlate with improving fiber digestion.

The total VFA concentration was largely increased by SBM supplementation, showing higher values in the order of SBM3.0 >SBM1.5>SBM0. This might associate with the reduced rumen NDF weights by increasing SBM supplement being SBM3.0 <SBM1.5 < SBM0. The greater VFA concentration in the presence of supplementary SBM indicates that the larger quantities of carbohydrate are fermented. In such case, CP degradation of SBM should be considered. SBM is highly degradable in the rumen as observed by Okubo *et al.* (1986) and Iriki and Abe (1987). Thus, when RS was supplemented with SBM with appropriate rate of CP degradation, RS fiber could be digested rapidly.

In SBM supplemented cows compared with SBM0 cows, molar proportion of acetate was reduced; however, molar butyrate and some iso-acids were increased. SBM supplement significantly increased concentrations of iC4, iC5 and C5 in SBM supplemented cows. The addition of isoacids and valeric acid to cultural medium of rumen microbes improved cellulose digestion (Bentley *et al.*, 1955; MacLeod and Murray, 1956; Gylswyk, 1970). These acids are required for growth of cellulolytic bacteria to increase fiber digestion (Bryant, 1973; Bryant and Robinson, 1962; Dehority *et al.*, 1967). However, when isoacids were supplemented to a low protein diets, fiber digestion was not improved in a number of studies (Cline *et al.*, 1966; Helmsely and Moir, 1963; Hungate and Dyer, 1956). The effect of SBM supplementation on the ruminal pH did not differ among treatments. At all sampling times, ruminal pH in three treatments was not less than 6.8 in this experiment. If pH reduced from 6.8 to 6.0, it can cause a fair depression in fiber digestion (Mould *et al.*, 1984).

2.4.2 Rumination

The daily time spent for ruminating per kg DM was significantly decreased by SBM supplementation regardless of amount of SBM. The current results were similar to those of Freer *et al.* (1962) and Warly *et al.* (1992) who found that the time spent for rumination per unit straw intake decreased by urea and SBM supplementation. However, the increasing level of SBM from 1.5 to 3.0 kg per day (0.2 to 0.4% of BW) did not additionally decrease the daily time spent for rumination. The present result was similar to the results observed by Warly *et al.* (1992a, 1994). The cumulative ruminating time in the current study was significantly reduced by SBM supplementation only at 18 h and 24 h after the beginning of RS feeding. Rumination time per kg DM that indicates the process by ruminants in comminuting ingested diet was considerably decreased by SBM supplement (Fujihara, 1981). Also when expressed per kg NDF intake, ruminating minutes was also obviously less with SBM-supplemented cows as compared to SBM0 cows in the current study. Furthermore, LP breakdown of RS during ruminating and rumination efficiency were increased by SBM supplementation but the significant effects occurred for 6 h and 12 h rumination intervals after RS feeding. This improvement of LP breakdown efficiency for protein-supplemented cows suggests that the alteration of RS anatomical structure; it could be softened or fragile by improved microbial fermentation by SBM.

2.4.3 Rumen digesta weights and size distribution

In the current experiment, fresh, dry and NDF weights of rumen digesta were significantly differed by SBM supplementation. Moreover, the pattern of particle size distribution and amount of various size particles was changed by SBM supplementation.

Dry weights of LP in the rumen were significantly reduced at 12 and 18 h after feeding by SBM supplementation. Dry weights of SP in the rumen were also decreased at all sampling times after feeding by SBM supplementation. The amount of large particles breakdown can be variable due to differences in cell wall fragility (McLeod et al., 1990) or the weakness of particles due to ruminal fermentation (Murphy and Kennedy, 1993). These differences among treatments could be interconnected with the rate of size reduction of LP and with the fiber fermentation rate for each size of ruminal particles of RS fiber due to accelerated microbial activity. As a result, disappearance rates of total digesta, LP and NDF of RS for SBM supplemented cows were greater than those for RS only cows in the current experiment.

In the current study, the LP weights at 12, 18 and 24 h after RS feeding: 1.2, 1.0 and 0.3 kg for SBM1.5 and 1.6, 1.7 and 1.4 kg for SBM3.0 were more reduced compared to SBM0. This indicates the rapid breakdown of LP rice straw by SBM supplement. The weights of SP in the rumens for both SBM supplemented cows reduced largely compared to SBM0 cows. The reduction in the weight of LP over 24 h after RS feeding was 34.0, 42.4 and 44.0% for SBM0, SBM1.5 and SBM3.0, respectively. Warly (1994) reported that the reduction in the proportion of LP over 24 h rice straw feeding was 23.0, 28.3 and 43.2% for low, medium and high crude protein (LCP, MCP, HCP) levels of supplementation by combining barley and SBM in sheep. This disagreement could be due to the different supplements used or the different rumination efficiency between cows and sheep. Welch (1982) reported that mature cow could ruminate 40 g/kg metabolic weight per and the sheep only ruminate 15 g/kg in the given period. The reduced proportion of LP in the rumen with time after feeding may be due to particle size reduction by microbial and masticating actions reported by Sekine *et*

al. (1992). However, the greater reduction of LP weights in dairy cows fed RS 1.5 SBM and 3.0SBM could be probably caused by the stronger attack by rumen microbes to fiber components and by increased rumination efficiency with this amount of SBM1.5. The microbial activity in the rumen was increased by 1.5 kg SBM supplementation in the current study. Because the rapid reduction of LP weights between SBM1.5 and SBM3.0 did not differ at 12 h and 18 h after feeding resulted in rumination efficiency for LP breakdown was also not differed between these two treatments by increased microbial activity.

Therefore, the diurnal change in the proportion and weight of LP and SP of RS in total rumen digesta might be caused by multi factors. For examples, there are different rigidity or fragility of RS, size reduction of LP by microbial activity and rumination chew (McLeod and Smith, 1989), through ruminal digestion (Tin Ngwe, 1990; Murphy and Nicoletti, 1984) and ruminal passage rate of RS particles (Warly, 1994). Also the decreasing in the weight of SP over 24 h after the beginning of RS given was 40.3%, 48.7% and 52.9% for SBM0, SBM1.5 and SBM3.0. The decreased in SP weight was related to higher ruminal disappearance and passage rates of small particles of RS for SBM1.5 and SBM3.0 than for SBM0 cows. The reduction of total weights of fresh, dry and NDF in the rumen of dairy cows was the largest for SBM3.0, middle for SBM1.5 and the least for SBM0. This indicates the accelerated ruminal passage rate of SP which could be caused by the increased LP breakdown by SBM supplementation.

2.4.4 Conclusion

It can be concluded that the weights of rumen digesta in dairy cows fed a RS based diet were decreased by increasing amount of SBM protein supplement. The post

feeding weights reduction of LP and SP in the rumen of supplemented cows were accelerated by SBM supplementation. However, ruminal dry LP and SP weights did not differ between SBM1.5 and SBM3.0. This acceleration can be related to the improvement of ruminal NH_3-N concentration and resultant increased microbial activity. The accelerated microbial activity might weaken the RS fiber structure in the rumen. This increased LP breakdown efficiency during rumination resulting fast clearance of LP in the rumen of dairy cows fed RS supplemented with SBM.

Further research must be required to verify the possible causes of reducing rumen digesta weights and changes of particles size distribution such as particle size reduction rate, ruminal passage rate and fragility of RS fiber according to the present amount of SBM supplement in dairy cows.

2.5 Summary

Mean rumen ammonia nitrogen concentrations increased as increasing level of SBM. The SBM1.5 and SBM3.0 cows showed the increased NH_3-N at all sampling times after SBM feeding and this improved microbial growth and activity. Total volatile fatty acids, butyrate and valerate concentrations in the rumen fluid were also significantly increased but acetate decreased by SBM supplementation. The total fresh, dry and NDF weights of rumen digesta for all treatments decreased post feeding. However, SBM3.0 had the lowest fresh, dry and NDF weights among treatments in all sampling times post feeding. Weights of ruminal LP also reduced for all treatments after offering. From 12 to 24 h post feeding, weights of ruminal LP for SBM0 were larger than for SBM1.5 and SBM3.0. On the other side, weights of ruminal SP for three treatments decreased after RS given. Ruminal SP weights of SBM0 were larger than for

SBM1.5 and SBM3.0 at 3, 6, 12, 18 and 24 h after post RS given. However, ruminal SP weights did not differ significantly between SBM1.5 and SBM3.0. Daily time spent in ruminating reduced by SBM supplement, however, did not additionally reduce by increasing SBM levels. During ruminating chew, LP breakdown and rumination efficiency for LP breakdown for SBM1.5 and SBM3.0 were significantly larger for SBM0 between 6 h to 12 h after post feeding. Therefore, dietary SBM supplement caused the early and rapid breakdown of LP rice straw regardless the amount of supplement.

Chapter 3

Increase of voluntary intake of rice straw in dairy cows when supplemented with soybean meal as affected by the rates of size reduction, passage and fermentation of ruminal particles (Experiment 2)

3.1 Introduction

In chapter 2, it was observed that weights of rumen digesta were reduced to a large extent by SBM supplementation, and the weights of LP and SP decreased rapidly in the rumen. It was probably due to enhancing cellulolysis and accelerating the rates of breakdown of LP rice straw in the reticulorumen which was caused by the increased NH_3-Nconcentration and fragility of RS with SBM supplementation.

Campling (1969) has suggested that voluntary intake of poor quality roughages such as RS by ruminants is controlled by physical factors, particularly the rate of breakdown of rumen digesta and its passage through the reticulo-omasal orifice. Furthermore, the rate of passage of digesta from the rumen depends on its rate of breakdown of particles in the rumen by microbial activity and by chewing activity. Therefore, to understand its mechanisms quantitatively, further study is required on increasing voluntary intake and changes of the rates of particle size reduction and ruminal passage of RS in dairy cows by dietary protein source supplementation. Although some studies have been conducted in this aspect; protein supplements increased the particle size reduction of RS, however, they are all in sheep. There is no quantitative information in dairy cows.

The hypothesis tested in this study is whether ruminal size reduction rate and passage rate of the RS fiber can be increased due to the improvement of ruminal

fermentation when protein supplement is added to RS.

The objectives of this study were to quantify particle size reduction rate and ruminal passage rate of RS, fermentation rate in the rumen when SBM was supplemented to RS fed dairy cows. To specify for the mechanism of the change of voluntary intake of RS, relationship between size reduction rate and ruminal passage rate of RS, this experiment was conducted under two levels of RS allowance (limited and *ad libitum*).

3.2 Materials and methods

3.2.1 Cows and treatments

Animal care and managements were the same as described in experiment 1. Six rumen-cannulated, non-lactating Holstein cows (660± 42.9 kg BW) were allocated to one of two dietary treatments in double cross-over design because of two feeding levels. Each experimental period consisted of 9 d adaptation period and 5 d measurement period. The two dietary treatments were RS only and RS plus 1.5 kg SBM per day. RS was chopped at a theoretical length of 10 mm using a chopper machine. Two equal portions of the experimental diets were offered at 0730 h and 1930 h. SBM was given to cows before RS feeding. The daily RS feed allowance was restricted at 6 kg (FM basis) in the first cross over design. During the second crossover design, RS was fed *ad libitum*. Cow was freely allowed to fresh water and trace mineral salt blocks. To measure the daily fecal excretion of the cows, 50 g of an external marker was fed every day immediately before each feeding of the treatment diets throughout the experiment. The maker for measuring fecal output was prepared from beet pulp pellets labeled with La using the immersion method, as described by Mader *et al.* (1984).

3.2.2 Data collection and sample analysis

The weight of feed refused was measured before every morning feeding. Samples of feed offered and refused were collected during the measurement period. The RS intake was determined by the difference between the feed offered and refused.

Eating time and ruminating time per day were measured by recording eating and ruminating activity with a time elapsed video tape recorder on the third and fourth day of each measurement period. The breakdown of LP during ruminating was calculated by ruminal disappearance rate of LP multiplied by the DM weight of LP in rumen digesta. To calculate rumination efficiency for LP breakdown, the LP breakdown was divided by rumination time (grams per minute of rumination).

On the first day of each collection period, Co-EDTA (5 g/250 mL water) was administered into the rumen at 0700 h to measure the ruminal liquid passage rate. Approximately 100 mL of rumen fluid was then collected eight times in a day with 3 h intervals. The measuring procedures of ruminal pH, NH_3-N and total VFA were the same described for experiment 1.

RS markers for measuring digesta kinetics labelled with Yb, Dy, Er, Nd, Gd, Sm, Ho, Pr and Ce were prepared by the method of Mader *et al.* (1984). The markers (100 g DM) were dosed into the rumen through a cannula at 2000 h on day 9 for Yb, at 0800 h on day 10 for Dy, at 1400 h on day 11 and day 12 for Er and Nd, at 0800 h and 1400 h on day 13 for Gd and Sm, at 0200 h, 0800 h and 1100 h on day 14 for Ho, Pr and Ce, respectively.

The rumen digesta was measured by manually emptying at 1400 h on the fifth day of the measurement period. The total rumen digesta of each cow was weighed and manually mixed and a subsample was taken. Several fecal collections were done every 3

h for 60 h, every 6 h for 60 to 96 h and every 8 h for 96 to 120 h after the marker dosing. The subsample was dried at 60°C for 48 h and ground through a 1-mm screen. The weight distribution of the different particle sizes of the rumen digesta was determined with another wet subsample by the wet sieving method with sieves of 1.18- and 0.15-mm aperture

Samples of offered feed, feces, rumen digesta, and sieved rumen digesta were dried at 60°C for 48 h and ground to pass through a 1-mm screen for subsequent chemical and marker analyses. Feed and feces samples were analyzed for DM, crude ash, CP, ash-free NDF, ash-free ADF. Ash-free ADL content was analyzed only for feed samples. Determination of FM, DM, OM, NDF and ADF content was also conducted for the rumen digesta, LP and SP samples. DM, CP, crude ash NDF, ADF, and ADL were determined by the same methods described in Chapter 1. Concentrations of rare earth elements (Yb and La) in the feces and La-labeled beat pulp pellets, that of (Yb, Dy, Er, Nd, Gd, Sm, Ho, Pr and Ce) in the total rumen digesta, sieved LP and SP particles as well as that of Co in the dried rumen fluid, were digested with 2:1 nitric and perchloric acid and determined using an inductively coupled plasma spectrometer (Eran DRC; Perkin-Elmer, United States).

Dried and ground samples of RS were subjected to measurements of *in situ* ruminal disappearance of DM and NDF. Triplicate 1.0-g samples were incubated in the rumens of dairy cows at 0, 6, 12, 24, 48 and 96 h. These samples were oven-dried at 103°C for 24 h, and weighed. Residual DM was expressed as a proportion of the initial amount of residue. Samples of residues were analyzed for disappearance of DM and NDF at each time point were calculated.

3.2.3 Calculation of data and statistical analysis

The nutrient digestibility in the total digestive tract was calculated from the nutrient intake and fecal excretion. Fecal DM excretion (kg DM/day) was calculated by the following equation: fecal DM (mg of La dosed per day)/ (mg of La per kg of fecal DM).

Ruminal liquid passage rate was calculated by exponential functional equation.

$$Y=Ce^{-kt}$$

where, Y=the concentration of marker in the compartment at time t(h), C=the initial concentration of marker, k= fractional outflow rate of the ruminal liquid (%/h).

Marker concentration of Ce, Pr, Ho, Sm, Gd, Nd, Er, Dy and Yb in rumen digesta were plotted in a graph against the time elapsed after administration, which are 3, 6, 12, 24, 36, 48, 72, 96 and 120 h, respectively. Marker concentrations in FP were calculated by subtracting the sum of LP and SP from the total digesta. The exponential decrease of the markers in total digesta, LP, SP and FP were fitted to the same model as for the analysis of rumen fluid passage rate. Then, the disappearance rates of total digesta, LP, SP and FP were calculated.

The rate of ruminal passage of RS was analyzed using the two-compartment, gamma age-dependent and independent model (G3G1 model) described by Pond and Ellis (1988). The mean outflow rates from the age-dependent compartment (k1) and the rate constant of outflow from the age-independent compartment (k2) were estimated by fitting a marker excretion curve to the model using the NLIN procedure of SAS (SAS Inst. Inc., Cary, NC). The rates of k1 and k2 can be regarded as the rate of size reduction of large particles into small particles and the rate of passage of small particles from the rumen, respectively. The compartmental (ruminal) mean retention time (CMRT) was

also calculated as 3/k1+1/k2.

All data were subjected to the statistical analysis using the GLM procedure of SAS (SAS 2004). Unless otherwise stated, the significant effect was declared at $P < 0.05$.

3.3 Results

The chemical composition of RS and SBM are shown in Table 11. The overall chemical components of RS and SBM were slight different between the experiment 1 and experiment 2 in this study. The contents of CP and ADL of both RS and SBM in experiment 2 were relatively greater than those in experiment 1.

Dry matter intake (DMI) and total tract apparent nutrient digestibility are presented in Table 12. In restricted feeding, DMI of RS was not reduced by SBM supplement. The NDF and ADF digestibility did not differ between treatments, whereas DM, OM and CP digestibility was increased in supplemented cows compared to control cows. In *ad libitum* feeding, a significant increase in DMI of RS occurred. Digestibilities of all nutrients except ADF were increased by SBM supplementation. The NDF and ADF digestibility of RS were 5.3% and 3.8% unit larger for supplemented cows than for RS cows in *ad libitum* feeding.

Results for pH, NH_3-N, VFA and ruminal liquid outflow rate are shown in Table 13. The concentration of NH_3-N in the rumen of supplemented cow was significantly greater than that in RS cows for both feeding levels. The mean ruminal NH_3-N concentrations of RS and RS+SBM were nearly about 0.2 and 4.3 mg/dL for both feeding levels. The total VFA concentration and ruminal pH were significantly affected by the SBM supplementation for both feeding levels. The mean total VFA

concentration in SBM supplemented cows was significantly larger than that in RS cow for both feeding levels. The total VFA concentration for supplemented cows in *ad libitum* feeding was 2.68 mmol/L units higher than that in restricted feeding.

Molar proportion of acetic acid (C2) was significantly reduced by SBM supplement but that of propionic acid (C3) and butyric acid (C4) did not changed in restricted feeding. However, molar proportion of C2 was reduced, that of C3 and C4 were increased in *ad libitum* feeding by supplementation of SBM. Molar proportions of iC4, iC5and C5 were significantly increased by SBM supplementation in *ad libitum* feeding. C5 was not detected in restricted feeding. The ruminal liquid outflow rate was not changed by SBM supplementation for both feeding levels.

Table 14 shows the results of *in situ* disappearance of DM and NDF for 0, 6, 12, 24, 48 and 96 h incubation in the rumen of cows. *In situ* DM disappearance of RS was greater for supplemented cows at 12 h and 24 h for restricted feeding and at 12 h , 24 h and 48 h for *ad libitum* feeding than in RS cows but not significant at 6 h and 96 h incubation. *In situ* NDF disappearance of RS was also higher for SBM supplemented cows at 12 h and 24 h for restricted feeding and at 24 h and 48 h for *ad libitum* feeding than in RS cows but not significant at 6 h and 96 h. *In situ* disappearances of DM and NDF after 48 h incubation were 10.6% units and 13.8% units greater for SBM supplemented cows than for RS cows in *ad libitum* feeding but not significant at this time in restricted feeding.

The values of daily spent for eating time, ruminating time and chewing time of RS cows and SBM supplemented cows for restricted and *ad libitum* feeding are shown in Table 15. The eating time per day, per kg NDF intake was not affected but that per kg DM intake was reduced by SBM supplementation for both RS feeding levels. The

Table 11 Chemical composition of rice straw and soybean meal (Experiment 2)

	Rice straw	Soybean meal
OM, % of DM	87.9	93.1
CP, % of DM	5.2	52.1
NDF, % of DM	74.1	13.2
ADF, % of DM	45.2	7.9
ADL, % of DM	7.1	2.1

For abbreviations see footnotes in Table 2.

Table 12 Dry matter intake and digestibility of dairy cows fed rice straw only or supplemented with soybean meal (Experiment 2)

	RS	RS+SBM	SEM	*P*-value
Experiment 1				
Rice straw DMI, kg/day	5.4	5.4	—	
Total DMI, kg/day	5.4	6.7	—	
Total tract digestibility (%)				
DM	34.0	45.5	1.51	0.01
OM	39.6	50.8	1.52	0.01
CP	-6.0	63.7	1.68	0.01
NDF	44.8	46.8	1.82	NS
ADF	46.4	46.7	1.83	NS
Experiment 2				
Rice straw DMI, kg/day	7.8	10.1	0.57	0.01
Total DMI, kg/day	7.8	11.4	0.65	0.01
Total tract digestibility (%)				
DM	37.7	48.6	1.54	0.01
OM	41.3	52.1	1.42	0.01
CP	7.9	60.0	2.97	0.01
NDF	47.5	52.8	1.36	0.05
ADF	45.7	49.5	1.31	0.07

For abbreviations see footnotes in Table 3.

RS: rice straw only, RS+SBM: rice straw + 1.5 kg SBM per day (FM basis).

NS: non significance

Table 13 Rumen fluid characteristics in the rumen of dairy cows fed rice straw only or supplemented with soybean meal (Experiment 2)

	RS	RS+SBM	SEM	*P*-value
Experiment 1				
pH	6.83	6.75	0.05	0.01
NH_3-N, mg/dL	0.17	4.66	0.24	0.01
Total VFA, mmol/L	5.90	6.38	0.36	0.01
C2, mmol/100mmol	75.83	73.11	0.49	0.01
C3, mmol/100mmol	18.50	18.41	0.24	NS
iC4, mmol/100mmol	0.09	1.14	0.05	0.01
C4, mmol/100mmol	5.54	5.80	0.36	NS
iC5, mmol/100mmol	0.04	1.54	0.07	0.01
C5, mmol/100mmol	0.00	0.00	—	—
Outflow rate, %/h	8.46	10.48	0.96	NS
Experiment 2				
pH	6.82	6.63	0.04	0.01
NH_3-N, mg/dL	0.28	4.02	0.43	0.01
Total VFA, mmol/L	6.88	9.56	0.24	0.01
C2, mmol/100mmol	76.14	72.74	0.30	0.01
C3, mmol/100mmol	18.44	18.70	0.20	0.05
iC4, mmol/100mmol	0.12	0.85	0.07	0.01
C4, mmol/100mmol	5.25	6.52	0.13	0.01
iC5, mmol/100mmol	0.04	1.14	0.06	0.01
C5, mmol/100mmol	0.00	0.05	0.02	0.05
Outflow rate, %/h	9.96	8.42	0.96	NS

For abbreviations see footnotes in Table 4 and Table 12.

NS: non significance

Table 14 *In situ* dry matter and neutral detergent fiber disappearances in the rumen of dairy cows fed rice straw only or supplemented with soybean meal (Experiment 2)

	RS	RS+SBM	SEM	*P*-value
DM disappearance				
Experiment 1	——— % ———			
0h	27.6	27.6	—	—
6h	29.2	29.9	0.55	NS
12h	31.8	33.2	0.73	NS
24h	38.9	44.8	0.79	0.01
48h	58.1	59.9	1.68	NS
96h	70.3	70.3	0.37	NS
Experiment 2				
0h	27.6	27.6	—	—
6h	30.5	31.4	0.41	NS
12h	32.4	34.0	0.28	0.01
24h	36.2	40.3	0.91	0.01
48h	45.5	56.1	1.71	0.01
96h	68.9	69.3	0.73	NS
NDF disappearance				
Experiment 1	——— % ———			
0h	10.3	10.3	—	—
6h	11.1	11.1	0.57	NS
12h	11.8	14.8	1.04	0.05
24h	21.6	29.4	1.06	0.01
48h	43.8	43.6	1.17	NS
96h	59.0	58.7	0.62	NS
Experiment 2				
0h	10.3	10.3	—	—
6h	8.5	8.7	0.61	NS
12h	9.7	11.3	1.07	NS
24h	12.0	19.4	1.50	0.01
48h	25.7	39.5	1.99	0.01
96h	57.6	58.1	0.61	NS

For abbreviations see footnotes in Table 2 and Table 12.

NS: non significance

Table 15 Chewing time of dairy cows fed rice straw only or supplemented with soybean meal (Experiment 2)

	RS	RS+SBM	SEM	*P*-value
Experiment 1				
Eating time, min/day	88.6	85.2	7.09	NS
min/kgDMI	16.2	12.7	1.22	0.05
min/kgNDFI	22.5	20.7	1.76	NS
Ruminating time, min/day	415.5	381.1	21.83	NS
min/kgDMI	76.6	55.9	3.50	0.05
min/kgNDFI	105.5	92.6	5.42	NS
Chewing time, min/day	508.6	462.2	25.16	NS
min/kgDMI	93.5	68.0	3.93	0.01
min/kgNDFI	128.9	112.3	6.26	NS
Experiment 2				
Eating time, min/day	239.0	273.3	15.54	NS
min/kgDMI	31.2	24.4	1.94	0.05
min/kgNDFI	41.1	35.8	3.46	NS
Ruminating time, min/day	516.6	585.1	27.61	NS
min/kgDMI	62.3	51.7	2.59	0.01
min/kgNDFI	89.0	75.8	3.53	0.05
Chewing time, min/day	755.6	858.3	32.87	0.01
min/kgDMI	98.4	76.1	3.55	0.01
min/kgNDFI	130.1	111.6	4.91	0.01

For abbreviations see footnotes in Table 3 and Table 12.

NS: non significance

ruminating time per day and per kg NDF intake did not differ between treatments for restricted feeding. However, for *ad libitum* feeding, ruminating time per day did not alter, while ruminating time per kg DM and NDF intake, was significantly reduced by supplementary SBM. In *ad libitum* feeding, the total chewing time per day, per kg DM intake and per kg NDF intake was also affected by SBM supplementation but significant difference was observed only expressed in per kg DM intake in restricted feeding.

The results for weight and particle size distribution of rumen digesta are shown in Table 16. The supplementation of SBM did not affect fresh rumen digesta weight for both feeding levels. No significant difference in rumen digesta DM and OM weights were found but rumen digesta NDF and ADF weights were significantly reduced by supplement for restricted feeding. However, opposite changes in these parameters were clearly seen in *ad libitum* feeding. Significant differences in rumen digesta DM and OM were observed but NDF and ADF weights did not differ in *ad libitum* feeding. The proportion of particle size retained on LP, SP and FP were not affected by SBM supplementation.

Ruminal digesta kinetics of RS for supplemented cows and control cows are shown in Table 17. The rates of large particle size reduction (k1) of RS for supplemented cows were significantly greater compared to RS cows for both feeding levels. The tendency of greater ruminal passage rate of small particles (k2) of RS was observed in SBM supplemented cows than in RS cows. The compartment mean retention time (CMRT) of RS particles in the rumen was not significantly (P=0.08) affected by SBM for restricted feeding but significantly reduced by SBM for *ad libitum* feeding.

Disappearance rates of total digesta, LP, SP, FP and FP+SP are presented by in Table 18. Ruminal disappearance rates of total digesta, LP and SP were significantly increased by SBM supplementation for restricted feeding although those of FP and SP+FP were not affected. For *ad libitum* feeding, ruminal disappearance rates of total digesta, LP, SP and SP +FP were increased by SBM supplementation. Values for LP breakdown and rumination efficiency for LP breakdown are shown in Table 19. In restricted feeding, LP breakdown during ruminating and rumination efficiency was not affected by SBM supplementation. However, *in ad libitum* feeding the LP breakdown and the rumination efficiency for LP breakdown were increased markedly in SBM supplemented cows compared to RS cows.

Table 16 Rumen digesta weights and particle size distribution in the rumen of dairy cows fed rice straw only or supplemented with soybean meal (Experiment 2)

	RS	RS+SBM	SEM	*P*-value
Experiment 1				
Digesta weight, kg				
FM	103.2	99.8	3.09	NS
DM	11.1	10.6	0.32	NS
OM	9.2	8.7	0.25	NS
NDF	7.8	7.2	0.17	0.05
ADF	5.1	4.7	0.11	0.05
Particle size distribution, %				
LP	52.1	54.0	3.96	NS
SP	35.5	34.2	4.13	NS
FP	12.3	11.8	1.31	NS
Experiment 2				
Digesta weight, kg				
FM	147.2	158.7	12.39	NS
DM	16.8	18.7	1.54	0.05
OM	14.1	15.7	1.31	0.05
NDF	12.0	12.8	1.08	NS
ADF	8.5	8.8	0.71	NS
Particle size distribution, %				
LP	50.9	55.9	2.74	NS
SP	30.3	26.8	2.31	NS
FP	18.8	17.3	4.59	NS

For abbreviations see footnotes in Table 2, 7 and 12, FM: fresh matter.

NS: non significance

Table 17 Rumen digesta kinetics of rice straw particles of dairy cows fed rice straw only or supplemented with soybean meal(Experiment 2)

	RS	RS+SBM	SEM	*P*-value
Experiment 1				
k1, %/h	4.46	5.47	0.41	0.05
k2, %/h	1.78	2.34	0.34	0.06
CMRT, h	104.94	78.61	10.56	0.08
Experiment 2				
k1, %/h	4.93	7.82	0.93	0.05
k2, %/h	1.72	1.94	0.17	0.07
CMRT, h	99.49	77.92	7.60	0.05

For abbreviations see footnotes in Table 12.

K1: particle size reduction rate in the rumen.

K2: ruminal passage rate of small particles in the rumen.

CMRT: compartment mean retention time.

Table 18 Ruminal disappearance rates of total rumen digesta, large particles, small and fine particles in the rumen of dairy cows fed rice straw only or supplemented with soybean meal (Experiment 2)

	RS	RS+SBM	SEM	*P*-value
Experiment 1	%/h			
Total digesta	2.31	2.32	0.13	NS
LP	3.74	4.56	0.31	0.05
SP	1.65	2.10	0.22	0.05
FP	2.44	3.42	0.46	NS
SP+FP	1.87	2.12	0.22	NS
Experiment 2	%/h			
Total digesta	1.89	2.21	0.16	0.05
LP	3.00	3.58	0.20	0.01
SP	1.77	2.15	0.29	0.05
FP	2.39	2.54	0.43	NS
SP+FP	1.59	1.93	0.20	0.05

For abbreviations see footnotes in Table 7. NS: non significance

Table 19 Large particles breakdown and rumination efficiency of dairy cows fed rice straw only or supplemented with soybean meal (Experiment 2)

	RS	RS+SBM	SEM	*P*-value
Experiment 1				
LP breakdown (kg/day)	3.37	3.72	0.26	NS
Rumination efficiency (g/min)	8.13	9.81	0.94	NS
Experiment 2				
LP breakdown (kg/day)	4.45	6.04	0.61	0.01
Rumination efficiency (g/min)	8.42	10.28	0.79	0.01

For abbreviations see footnotes in Table 7 and Table 12.

NS; non significance

3.4 Discussion

In the present study, OM, CP, NDF and ADL components of RS were relatively higher than those reported recently (Harumoto and Kato, 1979; Warly *et al*., 1992). This difference could be probably due to rice variety, type of soil, different fertilizer application and storage method. However, there was nearly same with the composition data analyzed by Liu *et al.* (1988). Therefore, the experimental RS is considered as a representative one distributed to the world.

3.4.1 Fiber fermentation in the rumen

For restricted feeding, *in situ* disappearances of DM and NDF of RS were greater in supplemented cows than in RS cows at 12 and 24 h after feeding in restricted feeding. Also, DM and NDF disappearance rates of RS fiber were greater in supplemented cows than in RS cows at 12, 24 and 48 h for *ad libitum* after feeding. Thus, SBM supplementation increased the rate of ruminal fiber fermentation of RS in the current study. This was similar to the report by Aye Sandar Cho *et al.* (2012) who observed that RS could be fermented rapidly when supplemented with dietary protein supplements *in vitro* study. This could be associated with the availability of NH_3-N for improvement of microbial activity in the rumen. It is well reported that supplementing low quality straw-based diets with protein sources increased ruminal NH_3-N concentration to improve the slow fiber fermentation of straw in the rumen (Fike *et al.*, 1995). For restricted and *ad libitum* feeding, in the current study, SBM supplementation increased NH_3-N concentrations in the rumen of cows at 4.66 and 4.02 mg/dL, respectively. Therefore, RS fiber fermentation and NH_3-N concentration in the rumen was positively related. However, these were lower than the level recommended by

Hume *et al.* (1970) reported that the maximum level of NH_3-N for rumen microbes was 13.3 mg/dL rumen fluid by *in vivo* study.

Total tract nutrients digestibilities of RS for supplemented cows were significantly greater than that for RS cows in *ad libitum* feeding. This agreed with the results of Wiedmeier *et al.*(1983) and Stoke *et al.*(1988), who suggested that increasing level of dietary CP could improve digestibility of DM, OM, CP and NDF of poor quality wheat straw and prairie hay. Increase for fiber digestibility in this study was contrasted with that in recent studies (Liu *et al.*, 1988; Warly *et al.*, 1992a). However, *in vivo* DM and OM digestibility of RS were increased from 3.0% to 7.0% points by SBM, groundnut meal and sesame meal supplementation (Tin Ngwe, 1990), which was relatively less than the values in the current study. In the current study, total tract DM, OM, ADF and NDF digestibility of RS for supplemented cows in *ad libitum* feeding were as much 3.0%, 1.3%, 2.8% and 6.0% units increased for supplemented cows in restricted feeding. This small increase in digestibility of RS between two levels of RS feeding could probably relate to low NH_3-N in the rumen. Perdok *et al.* (1988) revealed that the maximum intake and digestibility of RS by cattle was attained about 10-20 mg/dL NH_3-N level.

The total VFA concentration was significantly increased by supplementing of SBM. However, the total VFA concentration slightly increased (RS: 5.90 vs. RS+SBM: 6.38 mmol/L) for restricted feeding and largely increased (RS: 6.88 vs. RS+SBM: 9.56 mmol/L) for *ad libitum* feeding. It could be recognized that the slow rate of CP degradation of SBM would be suitable for RS fiber fermentation by the ruminal microbes. This increase of VFA production was supported by decrease of ruminal pH (Table 13).

Improved RS fiber fermentation in the rumen for the current experiment did partly relate to the increase in concentration of iC4, iC5 and C5 by SBM supplementation. Addition of isoacids can improve fiber digestion of alfalfa hay, orchard grass hay and corn silage *in vitro* cultures as suggested by Gorosito *et al.* (1985). However, a number of researches did not show increased fiber digestion when isoacids were added to a low protein diet (Cline *et al.,* 1966; Helmsley *et al.,* 1963).

3.4.2 Breakdown of large particles of RS and rumination efficiency

Daily time spent for eating of RS was unchanged by SBM supplementation for two levels of RS feeding in this experiment. However, the time require to eat 1 kg DM of RS was significantly reduced in supplemented cows compared to control cows. The current result indicated that the SBM supplemented cows spent a comparatively shorter time and rapidly breakdown RS particles through ingestive chewing than control cows. A major role of ingestive chewing is to form the bolus which cows can easily swallow by reducing the size of RS particles. This results supported that the findings of Harumoto and Kato (1979) and Warly *et al.* (1992) who described that eating rate of RS was increased in sheep when supplementary energy or protein sources were offered. However, when expressed as per kg NDF intake, eating time did not differ between treatments in *ad libitun* feeding. This indicated that eating time was not shorter than the increase of NDF intake. Therefore, time prolongation for ingestive chewing for breakdown RS particles was reduced by SBM supplementation.

In the experiment 2, the particle size distribution of the rumen digesta was measured at the time (1400h) in the final day of the measuring period when almost the entire morning diet had been eaten by cows. Thus, the particle size distribution of rumen

digesta strongly reflects the extent of particle size reduction via ingestive chewing. However, particle size distribution did not differ in the rumen between treatments for both levels of RS feeding. This result indicated that supplemented SBM in the RS-based diet was reduced in size to a same rate via ingestive chewing in spite of increase RS intake in *ad libitum* feeding.

Daily time spent for ruminating did not differ between SBM supplemented and RS cows for both levels of RS feeding in the present experiment. This finding support the previous results of Freer *et al.* (1962) and Warly *et al.* (1992) who reported that the time spent for rumination in cows and sheep fed oat straw and RS *ad libitum* was not influenced by urea and SBM supplementation. However, the time require to ruminate for breakdown of 1 kg DM large particles of RS was significantly decreased in SBM supplemented cows compared to control cows for restricted and *ad libitum* feeding (RS: 62.3 vs. RS+SBM: 51.7 min/kg DM). Therefore, the extent of particle size reduction via rumination chewing is a significant factor, because large particles that escape breakdown into small sizes by ingestive chewing must be broken down by rumination to become sufficiently small to pass the rumen. Therefore, the small extent of particle size reduction during ingestive chewing could increase the necessity of rumination chewing and prolong rumination time. In addition, when ruminating time expressed as per kg NDF intake, it was reduced due to improvement of microbial fermentation by SBM supplementation (RS: 89.0 vs. RS+SBM: 75.8 min/kg NDF) for *ad libitun* feeding. Results of ruminating time per kg DM and NDF of RS clearly indicated that large particles of RS were rapidly broken down by SBM supplementation.

However, ruminating chewing seems to be more efficient than ingesting chewing with regard to comminution of feed particles (Ulyatt *et al.* 1986). During

ruminating, the breakdown of LP of supplemented cows was significantly higher (1.59 kg/d) than RS cows. Rumination efficiency for LP breakdown of supplemented cows (1.86 g/min) was significantly larger than RS cows for *ad libitum* feeding but did not differ for restricted feeding in the current experiment. The larger dry matter intake was possible reason for the difference of rumination efficiency between supplemented and control cows. If amount of LP in regurgitated bolus during ruminating is directly proportional to ruminal contents, the increase of ruminal content could be related to the increase of rumination efficiency for LP breakdown. The current study can support the suggestion of Ueda *et al.* (1997) that the greater dry matter intake cause the higher rumination efficiency for LP breakdown.

Pond *et al.* (1984) reported that ingestive chewing contributes to removing of cuticle, crushing or crimping of plant tissues and reducing in particle size. Chewing activity increased physical damage, reduced particle size and promoted the surface area of the feed particles (Pan *et al.* 2003). Therefore, improved fibrolytic microbes with SBM supplementation easily invade and attach into the inner tissues of RS. The supplementary SBM could make RS fiber fragile and softened due to microbial fermentation in the current experiment.

3.4.3 Rumen digesta kinetics and rumen fill

In the present study, the mean particle size reduction rate (k1) for RS was clearly accelerated by SBM supplementation. The current data support those of Casler *et al.* (1996) that forage fragility is associated with the rate of particle size reduction when masticated; and chewing activity can reflect the physical and chemical properties of feeds (Mertens, 1997). The current result indicates that SBM supplementation alters

the rate of ruminal size reduction of RS, regardless of the levels of RS feeding; and besides the rate of k1 is 2.43 times greater in *ad libitum* feeding than in restricted feeding. The reduced rumination time expressed by per kg NDF intake and improved rumination efficiency for LP might be possibly associated with the presence of effect on the k1 of RS due to the supplementary SBM. The finding k1 in the present experiment confirmed the comments of the previous researchers (Murphy and Kennedy, 1993; Warly *et al.*, 1994) who reported that the weakened particles of forage by ruminal fermentation would facilitate and accelerate to breakdown of LP during ruminating. The positive response of associative effect could be obviously seen on ruminal size reduction rate of LP fiber of RS between SBM and RS.

The ruminal passage rate of RS small particles (k2) increased in restricted (P=0.06) and in *ad libitum* (P=0.07) feeding by SBM supplementation. Nevertheless, CMRT was clearly shortened in supplemented cows compared to RS cows for *ad libitum* feeding. The tendency for higher ruminal passage rate and shorter CMRT of RS particles was positively correlated to acceleration of ruminal size reduction and rumination efficiency for LP to SP in the current experiment. However, the MRT results of this experiment agreed with the results of Varga and Prigge (1982) who observed that no difference in mean retention time due to feed intake levels.

Ruminal passage rates of SP of RS for both RS feeding levels in the present experiment much differed from the results of Warly *et al.* (1994) who reported that 4.26 %/h for LCP, 4.0 %/h for MCP and 6 %/h for HCP. This different passage rate could probably due to be different treatments; the CP levels in RS diets were prepared by combination of barley and SBM in the study of Warly *et al.* (1994). This can possibly provide the higher rate of ruminal size reduction of RS than the present study.

However, the passage result in the current study was consistent with the findings of Liu (1988) who found that only SBM supplementation slightly increased the ruminal passage rate of RS particles. Poore *et al.* (1990) revealed that ruminal passage rates for hay and straw were unchanged when concentrate proportion increased from 30 to 60%, but passage rates for hay and straw retarded if concentrate proportion increased up to 90%. However, the amount of SBM supplement used in the present study was not too high.

It can be primarily assumed that if increased ruminal size reduction rate of RS particles with SBM supplementation cause a parallel response for ruminal passage rate of RS. However, the increase in ruminal passage rate of small particles of RS with SBM supplementation was not much large compared to k1. The small response in k2 with SBM supplementation could attribute to the specific gravity of SP. Wattiaux *et al.* (1991) suggested that a diet with high grain concentrate delayed the increasing of specific gravity of forage particles in associating with slow passage rate due to a higher gas production during fermentation.

Ruminal disappearance rates of total digetsa, LP and SP for supplemented cows were significantly faster than those for control cows in both restricted and *ad libitum* feeding. The present results were similar to Campling *et al.* (1962) who observed that infusion of urea increased the rate of disappearance and reduced the retention time of straw residues in the rumen.

Weights of rumen digesta DM and OM were significantly increased with elevated RS intake for *ad libitum* and were not differed for restricted feeding between treatments. However, weights of rumen digesta NDF and ADF significantly reduced between treatments in restricted and did not differ between treatments in *ad libitum*

feeding regardless of increasing NDF intake. Increased rumen digesta DM and OM for *ad libitum* feeing could possibly associate to ruminal passage rate of SP of RS. Difference k2 values between supplemented cows and RS cows were 0.80 %/h units for restricted and 0.22 %/h units for *ad libitum* feedings. Moreover, k2 for SBM supplemented cows (1.94 %/h) in *ad libitum* feeding was relatively slower than for SBM supplemented cows (2.34 %/h) in restricted feeding. Reduced rumen digesta NDF and ADF might probably relate to accelerated ruminal size reduction of LP of RS in the rumen. Difference in k1 values between supplemented and RS cows were 1.01 %/h units for restricted and 2.89 %/h units for *ad libitum* feeding. Additionally, k1 in supplemented cows (7.82 %/h) for *ad libitum* was relatively faster than in supplemented cows (5.47 %/h) for restricted feeding by SBM supplementation. Finally, ruminal disappearance rates of total digesta, LP, SP and SP+FP significantly increased due to accelerated k1 by SBM supplementation. Therefore, large amount of ruminal NDF fill can inhibit the intake of RS in *ad libitum* feeding. When high fiber forages were fed to sheep, the influences on rumen fill and intake might be great (Heaney *et al.*, 1963).

3.4.4 The relationship between voluntary feed intake and rates of ruminal size reduction and passage of RS

SBM supplementation significantly increased DMI of RS at *ad libitum* feeding in this current study. Such an improvement in intake could be possibly due to increase of size reduction and passage rates of RS fiber, resolving a ruminal nitrogen deficiency limiting microbial fiber digestion (Church and Santos, 1981; McCollum and Galyean, 1985) or changing host nutrient status (Kempton *et al.*, 1977). This result agreed to the results of Liu *et al.* (1988) and Warly *et al.* (1992a). Church and Santos (1981) reported

voluntary intake of wheat straw was also increased by SBM supplementation. However, this result was in disagreement with Devendra (1978) and Nguyen *et al.* (2008) reporting that the DMI of RS could not be increased by nitrogen source supplements.

The slow rates of particle size reduction, disappearance and passage of RS are intrinsic causes of reducing voluntary intake of RS. Feed intake of roughages is greatly related to the ruminal passage rate (Bawden, 1970; Coombe *et al.,* 1976; Poppi *et al.,* 1981a, b). Therefore, we presumed the supplementary SBM in cows fed RS could increase the passage rate of residual RS particles, hereby increasing intake of RS. However, increase in voluntary intake of RS by dairy cows was related to ruminal passage rate of small particles of RS in this study. This result was in agreement with Toyokawa (1978) who observed that the voluntary intake of RS was positively correlated to the passage rate of digesta from the rumen. The increased rate of particulate passage by cottonseed meal and SBM supplementation was a major factor correlated to the increased voluntary intake of poor prairie hay fed steers and beef cows (McCollum and Galyean, 1985; Stoke *et al.,* 1988). When feed intake increased, digestibility generally decreased (Van Soest, 1994), though this did not occur in the current experiment. Thus, the increase in voluntary intake of RS was positively correlated to the rate of ruminal size reduction of RS particles. Accelerated rate of ruminal size reduction of LP caused increasing rates of ruminal disappearance of total digesta, LP and SP of RS and hence increased in voluntary intake of RS in this study. Thus, the amount of SBM supplement at the level of 0.20% of BW was acceptable when considering fiber digestibility and voluntary intake of rice straw in the current experiment.

3.4.6 Conclusion

The present results clearly and quantitatively showed that the voluntary intake of RS by dairy cow was increased by soybean meal supplementation, and this was caused by improvements of rumen fiber fermentation, accelerated size reduction and passage of rumen digesta particles. The positive associative effect was distinctly existed in the rates of particle size reduction and ruminal passage of RS between dietary protein supplement and RS.

3.5 Summary

Mean ruminal NH_3-N and total VFA concentrations of RS+SBM cows were significantly greater than control cows for both restricted and *ad libitum* feedings. The breakdown of large particles of RS during ruminating and rumination efficiency significantly improved in RS+SBM cows compared to control cows for *ad libitum* feeding. *In situ* disappearance rate of rice straw DM was higher for RS+SBM than RS only from 12 to 24 h for restricted and from 12 to 48 h for *ad libitum* feeding. SBM supplementation increased the fiber fermentation of RS in the rumen. *In situ* disappearance rate of rice straw NDF was faster for RS+SBM than control cows from 12 to 24 h for restricted and from 24 to 48 h for *ad libitum* feeding. Ruminal disappearance rates of LP and SP for RS+SBM were faster than for RS only. The SBM supplementation accelerated the particle size reduction of RS and concomitant increase in ruminal passage rate of small particles of RS was relatively small. Voluntary intake of rice straw in cows fed SBM supplemented diet was 20% higher (10.1 vs. 7.8 kg DM/d, $P<0.01$) than that in cows fed rice straw only diet.

Chapter 4

General discussion and overall conclusion

General discussion

It is primarily important to know the voluntary feed intake, physical digestions and digestibility of rice straw in dairy cows at the whole animal scale in relation to animal production performance and individual differences by *in vivo* evaluation method. This study was carried out to inform the physical factors affecting the voluntary intake of rice straw by dairy cows. In chapter 4, the voluntary intake of RS will be discussed with reference rumen fermentation parameters, characteristics of rumen digesta, chewing activity and ruminal digesta kinetics as influenced by dietary protein supplement added to the RS diets.

By eight times collections of rumen fluid samples, ruminal pH did not differ significantly among treatments, especially did not drop less than 6.2 (Figure 1, Experiment 1). Mould and Orskov (1983) observed severe reduction in cellulolysis when pH dropped under 6.1. Mean ruminal NH_3-N concentrations significantly differed between the SBM supplemented and control diets (Figure 2, Experiment 1). However, the mean concentration of ruminal NH_3-N of SBM supplemented cows had 4.0 mg/dL (Table 13, Experiment 2) in *ad libitum* RS feeding, which is still lower than the normal level (10-20 mg/dL) reported by Perdok *et al.* (1988).

The rumen fills such as fresh, dry and NDF weights were significantly reduced by supplementary SBM (Figure 10a, b, c; Experiment 1). The higher SBM levels in RS diets would reduce the rumen fills in the present study. Proportions and weights of LP, SP and FP were also changed by SBM supplementation (Figures 11a, b, c and Figure 12a, b, c; Experiment 1). The reduction in weights of LP, SP and FP are caused by

improved microbial activity and chewing activity. SBM supplementation enhanced fast breakdown of large particles of rice straw.

Daily time spent for eating and ruminating were not affected by SBM supplement. When expressed per kg DM and NDF intake, eating and ruminating time was reduced by SBM supplement (Table 5, 6; Experiment 1 and Table 15 Experiment 2). The eating time per kg DM intake of RS was shorter for SBM supplemented cows than for control cows. The results in present study can support the findings of Warly *et al.* (1992) that the eating rate of RS (g/min) was increased by SBM supplementation in sheep. When animal fed quickly, the energy cost per unit DMI will be decreased, and vice versa (Osuji *et al.*, 1975). The breakdowns of LP during ruminating and rumination efficiency were significantly increased by SBM supplementation due to becoming fragile RS structure (Table 8, Experiment 1 and Table 19, Experiment 2). The method proposed by this study is possible to use the efficiency of ruminating chew for LP breakdown. Therefore, the chewing activity during ruminating is essential action for reduction of particles size of ingested feed and subsequently facilitates fiber degradation by microbes in the rumen. McLeod *et al.* (1990) concluded that the amount of large particle breakdown per unit chew could differ among forages due to variation in cell wall fragility or the weakened particles that might be readily broken down due to ruminal fermentation during ruminating (Murphy and Kennedy, 1993, Pearce and Moir, 1964). Hence, SBM supplemented cows needed the less rumination time for comminution of ingested RS to leave the rumen.

The slow rates of particle size reduction, disappearance and passage of RS are intrinsic causes of reducing voluntary intake of RS by dairy cows in the current study. Positive associative effects on the rate of gas production and organic matter digestibility

(OMD) were observed when RS was supplemented with protein supplements regardless their sources (Promkot *et al.,* 2007; Kiran *et al.,* 2007; Aye Sandar Cho *et al.*, 2012). However, their observation was based on *in vitro* ruminal incubation.

SBM supplementation significantly accelerated the size reduction rate of RS particle and concomitantly increased ruminal passage rate of small particle of RS. K1 values in experiment 2 confirmed the early and rapid breakdown of large particles of RS in experiment 1 by corresponding to the weight change of LP, SP and FP in the rumen (Table 17, Experiment 2 and Figure 12a, b, c; Experiment 1).

The mean retention times of RS particles were reduced by supplementary SBM. The small increase in ruminal passage rate of small particles of RS in the current study was possibly related to specific gravity. Particle size and specific gravity of ruminal digesta could be changed by sampling site; samples from the ventral sites in the rumen of cows were smaller and heavier than those from dorsal sites (Evan *et al.,* 1973). Moreover, the mechanical strength of wheat straw estimated by shear and tensile tests was reduced by incubating with cellulolytic rumen microbes (Fonty *et al*., 1999). The physical strength of rice straw was also largely decreased (Selim *et al.*, 2004) and the fragility of the inside and outside cell wall structures of barley straw increased by ammonia treatments (Goto *et al*., 1993). However, the physical strength and specific gravity of RS were not measured in the current study,

When RS diets are fed to dairy cows, protein concentrate supplements such as soybean meal, groundnut meal, sunflower seed meal, sesame meal and cottonseed meal are essential; especially for small dairy holders in Myanmar. The protein supplements can provide adequate nitrogen to the rumen microbes that are limited to growth and activity. SBM is highly and slowly degraded in the rumen (Okubo *et al*.,1986; Iriki and

Abe, 1987). However, Aye Sandar Cho *et al.* (2012) reported that the rate of gas production of SBM with single incubation was not significantly differed with groundnut and sesame meals. However, when SBM was mixed with RS, rates of gas production and OMD were greater than those for groundnut and sesame meals. They concluded that the CP degradation rate of other supplements could be too fast to match to the rice straw fermentation rate by rumen microbes than that of SBM. Based on the results of the current study, therefore, further study is needed by using other dietary protein sources such as groundnut and sesame meals in the relationship between voluntary intake and rumen digesta kinetics.

Moreover, the mechanism by which the protein concentrate supplement in the diet may change the physical strength and specific gravity of RS fiber. The connection in the specific gravity and the passage rate of RS particles is not clear in the present study. Future study in these aspects is also needed.

Overall conclusion

The results of this study indicated that supplemented dietary protein source increased ammonia nitrogen concentration and hence fiber fermentation in the rumen of dairy cows. Total digesta, LP and SP disappearances and the breakdown of LP during ruminating and rumination efficiency increased probably due to accelerated microbial fibrolytic activity that weakened RS fiber structure. As a result, the rate of size reduction of rumen digesta particles was accelerated and increased ruminal passage of digesta. Therefore, the weights of rumen digesta were reduced in supplemented cows. The voluntary intake of RS by dairy cow was increased by the fast digesta clearance when cows were fed RS with soybean meal supplement. Increased voluntary intake and

digestibility can elevate the nutrients intake and production performance of dairy cows. Finally, this study clearly showed that the positive associative effect in physical digestion of dairy cows was present on rate of ruminal size reduction of LP rice straw between dietary protein concentrate supplement and RS.

Chapter 5

Summary

(1) The physical factors affecting the voluntary intake of RS by dairy cows were studied in relation to ruminal fermentation parameters, changes of rumen digesta weights, characteristics of digesta particle sizes and ruminal digesta kinetics, all of which could be affected by dietary protein supplementation.

(2) Mean concentrations of ruminal NH_3-N and VFA were significantly higher in SBM supplemented cows than that control cows.

(3) Daily time spent for eating and ruminating were not affected by SBM supplement. When expressed per kg DM and NDF intake, eating and ruminating time were reduced SBM supplement.

(4) The method proposed by this study is possible to use the efficiency of ruminating chew for large particles breakdown. The breakdowns of large particles during ruminating and rumination efficiency were significantly increased by SBM supplementation because improved ruminal fibrolytic bacteria activity that weakened RS fiber structure.

(5) The total fresh, dry and NDF weights and weights of LP, SP and FP were reduced by SBM supplementation. SBM supplement caused rapid breakdown of large particles of RS. However, weights of LP and SP did not differ significantly between SBM1.5 and SBM3.0.

(6) SBM supplementation significantly increased or accelerated the particle size reduction rate of RS and disappearance rates of total rumen digesta, LP, SP and FP+SP.

(7) The ruminal passage rate of small particles of RS was slightly increased by the SBM supplementation. However, the total mean retention time of RS particles residues was shortened by supplementary SBM.

(8) Dietary soybean meal supplementation increased the voluntary intake and fiber digestibility of RS.

This study clearly and quantitatively showed that voluntary intake of RS was limited by the particle size reduction in dairy cows and this was largely improved by protein source supplementation. The positive associative effect in physical digestion of dairy cows was obviously seen in rate of ruminal size reduction of LP rice straw between dietary protein source supplement and RS.

References

Abou- El-Enin, O.H., J.G. Fadel and D.J. Mackill. 1999. Differences in chemical composition and fiber digestion of rice straw with, without, anhydrous ammonia from 53 rice varieties. Anim. Feed Sci. Technol. 79: 129-136.

Agbagla-Dohnani, A., P. Noziere, B. Gaillard, M. Puard and M. Doreau. 2003. Effect of silica content on rice straw ruminal degradation. J. Anim. Sci. 140: 183-192.

Akter, Y., M.A. Akbar, M. Shahjalal and T.U. Ahmed. 2004. Effect of urea molasses multi-nutrient blocks supplementation of dairy cows fed rice straw and green grasses on milk yield, composition, live weight gain of cows and calves and feed intake. Pak. J. Biol. Sci. 7: 1523-1525.

AOAC.1990. Official Methods of Analysis of Association of Official Analytical Chemists, 15th edn. Washington, DC.

Anderson, D.C. 1978. Use of cereal residue in beef cattle production systems. J. Anim. Sci. 46: 849-861.

Aye Sandar Cho, K. Ueda and S. Kondo. 2012. Evaluation of associative effects on ruminal digestion kinetics between rice straw and protein supplements. Hokkaido Animal Science and Agriculture Society. 54: 47-52.

Bae, H.D., T.A. McAllister, E.G. Kokko, F.L. Leggett, L.J. Yanke, K.D. Jakober, J.K. Ha, H.T. Shin and K.J. Cheng. 1997. Effect of silica on the colonization of rice straw by ruminal bacteria. Anim. Feed Sci. Technol. 65: 165-181.

Balch, C.C. 1971. Factors affecting the utilization of food by dairy cows. 6. The rate of contraction of the reticulum. Brit. J. Nutr. 6: 366-375.

Balch, C.C. and R.C. Campling. 1962. Regulation of voluntary food intake in ruminants. Nutr. Abstr. Rev. 32: 669-686.

Barton, K.J. and C.A. Hibberd. 1984. Effects of protein supplementation on ruminal fermentation of low quality native grass hay in steers. Oklahoma Agric. Exp. Sta. MP-116.pp: 165-167.

Bawden, R.J. 1970. The effect of nematode parasitism on the rate of passage of food residues through the alimentary tract of sheep. Br. J. Nutr. 24: 291-296.

Bentley, O. G., R.R. Johnson, T.V. Hershberger, J.H. Cline and A.L. Moxon. 1955. Cellulolytic factor activity of certain short chain fatty acids for rumen microorganisms *in vitro*. J. Nutr. 57: 389-400.

Bines, J.A.1971. Metabolic and physical control of food intake in ruminants. Proceedings of the Nutrition Society. 30: 116-122.

Blaxter, K.L., F.W. Wainman and R.S. Wilson. 1961. The regulation of food intake by sheep. Anim. Prod. 3: 51-61.

Bryant, M.P. 1973. Nutritional requirements of the predominant rumen cellulolytic bacteria. Fed. Proc. 32: 1809-1813.

Bryant, M.P. and I.M. Robinson. 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. J. Bacteriol. 84: 605-614.

Campling, R.C. 1969. Physical regulation of voluntary intake. In: Physiology of Digestion and Metabolism in the Ruminants. Proceedings of the Third International Symposium Cambridge, England. Oriel Press, pp. 226-234.

Campling, R.C., M. Freer, and C.C. Balch. 1962. Factors affecting the voluntary intake of food by cow. 3. The effect of urea on the voluntary intake of oat straw. Br. J. Nutr. 16:115-124.

Carey, D.A., J.S. Caton and M. Biondini. 1993. Influence of energy source on forage intake, digestibility, in situ forage degradation, and ruminal fermentation in

beef steers fed medium quality brome hay. J. Anim. Sci. 71: 2260-2269.

Casler, M.D., D.K. Schneider and D.K. Comb. 1996. Development and application of a selection criterion for particle size breakdown of smooth brome grass leaves. Anim. Feed Sci. Technol. 61: 57-71.

Chase, C.C. Jr and C.A. Hibberd. 1987. Utilization of low quality native grass hay by beef cows fed increasing quantities of corn grain. J. Anim. Sci. 65: 557-566.

Chenost, M. and C. Kayouli. 1997. Roughage Utilization in Warm Climates. FAO Animal Production and Health Paper 135, Rome.

Church, D.C. and A. Santos. 1981. Effect of graded levels of soybean meal and non-protein nitrogen molasses supplement on consumption and digestibility of wheat straw. J. Anim. Sci. 53: 1609-1615.

Cline, T.R., U.S. Garrigus and E.E. Hatfield. 1966. Addition of branched- and straight-chain volatile fatty acids to purified lamb diets and effects on utilization of certain dietary components. J. Anim. Sci. 25: 734-739.

Coombe, J.B., D.A. Dinius and W.E. Wheeler. 1979. Effect of alkali treatment on intake and digestion of barley straw by beef steers. J. Anim. Sci. 49: 169-176.

Conrad, H.R. 1966. Symposium on factors influencing the voluntary intake of herbage by ruminants: Physiological and physical factors limiting feed intake. J. Anim. Sci. 25: 227-235.

Crabbe, D. and S. Lawsen. 1981. The World Food Book. Nichols Publishing Co. New York.

Crampton, E.W., E.E. Lister and L.E. Lloyd. 1957. Voluntary intake of forage as a measure of its feeding value. J. Anim. Sci. 16: 1056. (Abstr.)

Dehority, B.A., H.W. Scott and P. Kowaluk. 1967. Volatile fatty acid requirements of cellulolytic rumen bacteria. J. Bacteriol. 94: 537-543.

Devendra, C. and D. Thomas. 2002. Crop-animal interactions in mixed farming systems in Asia. Agriculture System. 71: 27-40.

Devendra, C. 1978. Studies on the utilization of rice straw by sheep. V. The effects of different protein sources on voluntary feed intake. Malay. Agric. J. 51: 280-290.

Devendra, C.1997. Crop residues for feeding animals in Asia: Technology development and adoption in crop/livestock systems. In: Crop Residuals in Sustainable Mixed Crop/Livestock Farming System (Renard, C. eds). pp. 241-261. CAB International; Wallingford, UK.

Doyle, P.T. 1987. Supplements other than forages. In: The Nutrition of Herbivores (Hacker, J.B. and J.H. Ternouth, eds). pp. 429-464. Academic press Australia.

Doyle, P.T. and G.R. Pearce. 1985. Processing method to improve nutritive value of rice straw. Proceedings of 3rd Asian-Australasian Association of Animal Production (AAAP). Congr. Seoul. Vol. 1, pp. 82-98.

Doyle, P.T., C. Devendra and G.R. Pearce. 1986. Rice straw as a feed for ruminants. IDP, Canberra, Australia.

Dulphy, J.P. and C. Demarquilly. 1983. Voluntary feed consumption as attribute of feeds. In: Feed Information and Animal Production (Robards, G.E. and R.G. Packham, eds). pp. 135-156. Commonwealth Agricultural Bureaux. Slough.

Ellis, W.C. 1978. Determinants of grazed forage intake and digestibility. J. Dairy Sci. 61: 1828-1840.

Evans, E.W., G.R. Pearce, J. Burnett and S. Pillinger. 1973. Changes in some physical characteristics of the digesta in the reticulo-rumen of cows fed once daily. Br. J. Nutr. 29: 357-376.

Fike, G.D., D.D. Simmis, R.C. Cochran, E.S. Vanzant, G.L. Kutil and R.T. Brandt Jr.

1995. Protein supplementation of ammonia treated wheat straw: effect on performance and forage utilization of beef cattle. J. Anim. Sci.73: 1595-1601.

Food and Agriculture Organization (FAO). 2008a. Livestock, a major threat to environment. Rome, Italy. Available from URL: http://web.archive.org/web/20080328062709//http%3A//www.fao/org/newsroom/en/news/2006/1000448/index.html

Food and Agriculture Organization (FAO). 2008b. Land degradation on the rise- One fourth of the world's population affected says new study. Available from URL: http://www.fao.org/newsroom/en/news/2008/1000874/index.html.

Fonty, G., M.Chavarot, J. Lepitit, J. Canistro and R. Favier. 1999. Mechanical resistance of wheat straw after incubation in cultures of ruminal cellulolytic microorganisms. Anim. Feed Sci. Technol. 80: 297-307.

Forbes, J.M. 1986. The Voluntary Food Intake of Farm Animals. Butterworth & Co. Ltd. London and Boston.

Freer, M., R.C. Campling and C.C. Balch. 1962. Factors affecting the voluntary intake of forage by cows. 4. The behavior and reticular motility of cows receiving diets of hay, oat straw and oat straw with urea. Br. J. Nutr. 16: 279-295.

Fujihara, T. 1981. Eating and rumination behaviour in sheep given silage made from the fibrous residue of ladino clover. J. Agric. Sci. Camb. 97: 485-488.

Garrett, W.N., H.G. Walker, G.O. Hohler and M.R. Hart. 1979. Response of ruminants to diets containing sodium hydroxide or ammonia treated rice straw. J. Anim. Sci. 48: 92-103.

Gerson, T., A.S.D. King, K.E. Kelly and W.J. Kelly. 1988. Influence of particle size and surface area on *in vitro* rate of gas production, lipolysis of triacyglycerol and

hydrogenation of linoleic acid by sheep rumen digesta or *Ruminococcus flavefaciens*. J. Agric. Sci. (Camb.). 110: 31-37.

Gharpuray, M.M., Y.H. Lee and L.T. Fan. 1983. Structural modification of lignocelluloses by pretreatments to enhance enzymatic hydrolysis. Biotechnol. Bioeng. 25: 157-172.

Goering, H.K. and P.J. Van Soest. 1975. Forage fiber analysis. agricultural research service. Agricultural Handbook No.379. US Department of Agriculture, Washiton.

Gorosito, A. R., J.B. Russell and P.J. Van Soest. 1985. Effect of carbon-4 and carbon-5 volatile fatty acids on digestion of plant cell wall *in vitro*. J. Dairy Sci. 68: 840-847.

Goto, M., Y. Yokoe, K. Takabe, S. Nishikawa and O. Morita. 1993. Effects of gaseous ammonia on chemical and structural features of cell walls in spring barley straw. Anim. Feed Sci. Technol. 40: 207-221.

Gruza, G. and E. Rankova. 2004. Detection of changes in climate state, climate variability and climate extremity. In: World Climate Change Conference, Moscow (Izrael, Y., G. Gruza, S. Semenov and I. Nazarov, eds). pp. 90–93. Moscow, Institute of Global Climate and Ecology.

Guthrie, M.J. and D.G. Wagner. 1988. Influence of protein or grain supplementation and increasing levels of soybean meal on intake, utilization and passage rate of prairie hay in beef steers and heifers. J. Anim. Sci. 66: 1529-1537.

van Gylswyk, N.O. 1970. The effect of supplementing a low protein hay on the cellulolytic bacteria in the rumen of sheep and on the digestibility of cellulose and hemicellulose. J. Agric. Sci. (Camb.). 74: 169-180.

Haddad, S.G. 2000. Associative effects of supplementing barley straw diets with alfalfa hay on rumen environment and nutrient intake and digestibility for ewes. Anim. Feed Sci. Technol. 87: 163-171.

Harumoto, T. and M. Kato. 1979. Effect of wheat bran supplement to rice straw diet on the ruminating behaviour in sheep. Bull. of the Fac. of Agric. Shimane University. 13: 21-26.

Heaney, D.P., W.J. Pigden, D.J. Minton and G.I. Pritchard. 1963. Effect of pelleting on energy intake of sheep from forages cut at three stages of maturity. J. Anim. Sci. 22: 752-757.

Helmsley, J.A. and R.J. Moir. 1963. The influence of higher volatile fatty acids on the intake of urea-supplemented low quality cereal hays by sheep. Aust. J. Agric. Res. 14: 509-517.

Henning P.A., Y.V. Linden, M.C. Matthorpe, W.K. Nauhaus and H.M. Swartz. 1980. Factors affecting the intake and digestion of roughage by sheep fed maize straw supplemented with maize grain. J. Anim. Sci. 94: 565-573.

Hiltner, P. and B. A. Dehority. 1983. Effects of soluble carbohydrates on digestion of cellulose by pure cultures of rumen bacteria. Appl. Environ. Microbiol. 46: 642-648.

Hoek, R.V.D., G.S. Muttetuwegama and J.B. Schiere. 1988. Overcoming the nutritional limitations of rice straw for ruminants. I. Urea ammonia treatment and supplementation with rice bran and glicirida for lactating Surti buffaloes. Asian-Aust. J. Anim. Sci. 1: 201-208.

Hooper, A. P and J.G. Welch. 1984. Change in functional specific gravity and particle size ten forages. J. Dairy Sci. 67 (Suppl. 1): 248. (Abstr.).

Hoover, W.H. 1986. Chemical factors involved in ruminal fiber digestion. 69: 2755-2766.

Hume, I.D., R.J. Moir and M. Somers. 1970. Synthesis of microbial protein in the rumen. I. Influence of the level of nitrogen intake. Aust. J. Agric. Res. 21: 283-296.

Hungate, R.E. and I.A. Dyer. 1956. Effect of valeric and isovaleric acids on straw utilization by steers. J. Anim. Sci. 15: 485-488.

Ichinohe. T., T. Tamura, K. Ueda, M. Okubo and Y. Asahida. 1994. The particle size distribution of ingested boli, rumen digesta and feces in sheep in fed orchardgrass hay harvested at different stages of maturity. Anim. Sci. Technol. J. 65: 701-708.

Iiyama, K., T.B.T. Lam and B.A. Stone. 1990. Phenolic acid bridges between polysaccharides and lignin in wheat internodes. Phytochem. 29: 733-737.

Iriki, T. and M. Abe. 1987. Rumen by-pass proteins in growing calves. Proc. Jpn. Soc. Anim. Nutr. Metab. 32: 149-171. (in Japanese)

Itoh, H., Y. Terashima and N. Tohroi. 1979. Evaluation of ammonia treatment for improving the utilization of fibrous materials in low quality roughages. J. Zootech. Sci. 50: 54-61.

Jackson, M.G. 1977a. Rice straw as livestock feed. World. Anim. Rev. 23: 25-31.

Jalc, D. 2002. Straw enrichment for fodder production by fungi. In: The Mycota XI Agricultural Applications (Kempken, F. eds). pp. 19-38. Springer-Verlag, Berlin, Heidelberg.

Jelan, Z.A. and M.A. Kabul. 1987. Degradation characteristics of some agro-industrial by-products. Proceedings of 4th Asian-Australasian Association of Animal

Production (AAAP). Congr. Hamilton. Vol. 2, pp. 291-295.

Kartchner, R.J. 1980. Effects of protein and energy supplementation of cows grazing native winter range forage on intake and digestibility. J. Anim. Sci. 51: 432-438.

Karunanadaa, K. and G.A. Varga. 1996a. Colonization of rice straw by white-rot fungi (*Cyathus stercoreus*): Effect on ruminal fermentation pattern, nitrogen metabolism, and fiber utilization during continuous culture. Anim. Feed Sci. Technol. 61:1-16.

Karunanadaa, K. and G.A. Varga. 1996b. Colonization of rice straw by white-rot fungi (*Cyathus stercoreus*): cell wall monosaccharides, phenolic acids, ruminal fermentation characteristics and digestibility of cell wall fiber components *in vitro*. Anim. Feed Sci. Technol. 63: 273-288.

Kawamura, O., T. Senshu, M.Horiguchi and T. Matsumoto. 1973. Histochemical studies on the rumen digestion of rice straw cell wall and on the chemical determination of its non nutritive residue. Tohoku J. Agric. Res. 24: 183-191.

Kempton, T. J., V.V. Nolan and R.A. Leng. 1977. Principles for the use of nonprotein nitrogen and by-pass protein in diets for ruminants. World Anim. Rev. 22: 18-24.

Kiran, D. and U. Krishnamoorthy. 2007. Rumen fermentation and microbial biomass synthesis indices of tropical feedstuffs determined by the *in vitro* gas production technique. Anim. Feed Sci. Technol. 134: 170-179.

Krysl, L.J., M.E. Branine, A.U. Cheema, M.A. Funk and M.L. Galyean. 1989. Influence of soybean meal and sorghum grain supplementation on intake, digesta kinetics, ruminal fermentation, site and extent of digestion and microbial

protein synthesis in beef steers grazing Blue Grama rangeland. J. Anim. Sci. 67: 3040-3051.

Lam, T.B., T.K. Kadoya and K. Iiyama. 2001. Bonding of hydroxycinnamic acids to lignin: ferulic and p-coumaric acids are predominantly linked at the benzyl position of lignin, not the b-position, in grass cell walls. Phytochem. 57: 987-992.

Lardy, G.P., D.N. Ulmer, V.L. Anderson and J.S. Caton. 2004. Effects of increasing level of supplementary barley on forage intake, digestibility, and ruminal fermentation in steers fed medium-quality grass hay. J. Anim. Sci. 82: 3662-3668.

Leng, R.A., T.R. Preston, R. Sansoucy and P.J.G. Kunju. 1991. Multinutrient blocks as a strategic supplement for ruminants. World Anim. Rev. 67: 11-20.

Liu, J.X., M. Okubo and Y. Asahida. 1988. Effects of soybean meal supplementation on fiber digestion in the rumen and voluntary intake of rice straw by sheep. J. Zootech. Sci. 59: 1034-1039.

Liu, J.X. and E.R. Orskov. 2000. Cellulase treatment of untreated and steam pre-treated rice straw-effect on *in vitro* fermentation characteristics. Anim. Feed Sci. Technol. 88: 189-200.

Lusby, K.S. and D.G. Wagner. 1987. Effects of Supplements on Feed Intake. In: Feed Intake by Cattle (Owen, F.N. eds). pp. 173-181. Oklahoma State University, Stillwater, OK.

MacLeod, R.A. and J.F. Murray. 1956. Some factors affecting cellulose digestion by rumen microorganisms *in vitro*. J. Nutr. 60: 245-259.

Mader. T.L., R.G. Teeter and G.W. Horn. 1984. Comparison of forage labeling

techniques for conducting passage rate studies. J. Anim. Sci: 58, 208-212.

Masuda, Y., S. Kondo, M. Shinjo and I. Goto. 1999. Effect of sugar-beet pulp supplementation on fiber degradation of grass hay in the rumen of goats. Asian-Aust. J. Anim. Sci. 12. 186-188.

McCalla, A.F. 2009. World food prices: Causes and consequences. Canad. J. Agricul. Econ. 57: 23-34.

McCollum, F.T. and M. L. Glayean. 1985. Influence of cottonseed meal supplementation on voluntary intake, rumen fermentation and rate of passage of prairie hay in beef steers. J. Anim. Sci. 60: 570-577.

McLeod, M.N, P.M. Kennedy and D.J. Minson. 1990. Resistance of leaf and stem fraction of tropical forage to chewing in cattle. Br. J. Nutr.63: 105-119.

McLeod, M.N. and B.R. Smith. 1989. Eating and ruminating behaviour in cattle given forages differing in fiber content. Anim. Prod. 48: 503-511.

Mertens, D.R. 1997. Creating a system for meeting the fiber requirements of dairy cows. J. Dairy Sci. 80: 1463-1481.

Mertens, D.R., T.L. Strawn and R.S. Cardoza. 1984. Modelling ruminal particle size reduction: its relationship to particle size description. In Techniques in particle size analysis of feed and digesta in ruminants (Kennedy, P.M. eds). pp. 134-141. Can. Soc. Anim. Sci. Edomonton.

Mertens, D.R. and L.O. Ely. 1979. A dynamic model of fiber digestion and passage in the ruminant for evaluating forage quality. J. Anim. Sci. 49: 1085-1095.

Milne, J. A., Alison Christie and A.J.F. Russel. 1979. The effects of nitrogen and energy supplementation on the voluntary intake and digestion of heather by sheep. J. Agric. Sci. Camb. 92: 635-643.

Minson, D.J. 1985. Fiber as a limit to tropical animal production. Proceedings of 3rd Asian-Australasian Association of Animal Production (AAAP). Congr. Seoul. Vol. 1, pp: 108-119.

Moran, J.B., K.B. Satoto and J.E. Dawson. 1983. The utilization of rice straw fed to Zebu cattle and swamp buffalo as influenced by alkali treatment and Leucaena supplementation. Aust. J. Agric. Res. 34: 73-84.

Mould, F.L., E.R. Orskov and S.A. Gauld. 1984. Associative effects of mixed feeds. I. Effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis *in vivo* and dry matter digestion of various roughages. Anim. Feed Sci. Technol. 10: 15-30.

Mould, F.L. and E.R. Orskov. 1983. Manipulation of rumen fluid pH and its influence on cellulolysis *in sacco*, dry matter degradation, and the rumen microflora of sheep offered either hay or concentrates. Anim. Feed Sci. Technol. 10:1-9.

Murphy, M.R. and P.M. Kennedy. 1993. Particle dynamics. In; Quantitative aspects of ruminant digestion and metabolism (Fobes J.M. and J. France, eds.). pp. 87-105. CAB International. Walligford, UK.

Murphy, M.R. and J.M. Nicoletti, 1984. Potential reduction of forage and rumen digesta particle size by microbial action. J. Dairy Sci. 67: 1221-1226.

NARC newsletter (rice special issue), May 2004.

Nguyen, V.T., D.G. St. Louis, A.I. Orr and B.J. Rude. 2008. Supplementing of maize or soybean hull to cattle fed rice straw: intake, apparent digestion, *in situ* disappearance and ruminal dynamics. Asian-Aust. J. Anim. Sci. 21: 807-817.

National Research Council (NRC). 1981. Nutrient requirements of dairy cattle, 5th edn. National Academy Press, Washington, DC.

National Research Council (NRC). 2000. Nutrient requirements of dairy cattle, 7th Rev edn. National Academy Press, Washington, DC.

Oh, J.H., W.C. Weir and W.M. Longhurst. 1971. Feed value for sheep of cornstalk, rice straw and barley straw as compared with alfalfa. J. Anim. Sci. 32: 343-347.

Okubo, M., M. Hanada, J. Sekine, Y. Miura and Y. Asahida. 1986. The rumen degradability of protein for various feedstuffs. J. Fac. Agr. Hokkaido Univ. 63: 49-53.

Osuji, P.O., J.G. Gordon and A.T.F. Webster. 1975. Energy exchanges associated with eating and rumination in sheep given grass diets of different physical forms. Brit. J. Nutr. 34: 59-71.

Pan, J., S. Koike, T. Suzuki, K. Ueda, Y. Kobayashi, K. Tanaka and M. Okubo. 2003. Effect of mastication on degradation of orchardgrass hay stem by rumen microbes: fibrolytic enzyme activities and microbial attachment. Anim. Feed Sci. Technol. 106: 69-79.

Papas, A.M., S.R. Ames, R.M. Cook, C.J. Sniffen, C.E. Polan and L. Chase. 1984. Production responses of dairy cows fed diets supplemented with ammonium salts of iso C-4 and C-5 acids. J. Dairy Sci. 67: 276-293.

Pearson, L. and J. Langridge. 2008. Climate change vulnerability assessment: Review of agricultural productivity. CSIRO Climate Adaptation Flagship Working paper No.1. Available from URL: http://www.csiro.au/resources/CAF-Working-Papers

Pearce, G.R. and R.J. Moir. 1964. Rumination in sheep. I. The influence of rumination and grinding upon the passage and digestion of food. Aust. J. Agric. Res. 15: 635-644.

Perdok, H.B., R.A. Leng, S.H. Bird, G. Habib and M. Van Houtert. 1988. Improving livestock production from straw-based diets. In: Increasing Small Ruminant Productivity in Semi-arid areas (Thomson, E.F. and F.S. Thomson, eds). pp. 81-91. ICARDA, Syria.

Phang, O.C. and J. Vadiveloo. 1992. Effects of varieties, botanical fractions and supplements of palm oil by-products on the feeding value of rice straw in goats. Small Rumin. Res. 6: 295-301.

Pond, K.R., W.C. Ellis and D.E. Akin. 1984. Ingestive mastication and fragmentation of forages. J. Anim. Sci. 58: 1567-1574.

Pond, K.R. and W.C. Ellis. 1988. Compartment models for estimating attributes of digesta flow in cattle. Br. J. Nutr. 60: 571-595.

Pordomingo, A.J., J.D. Wallace, A.S. Freeman and M.L. Galyean. 1991. Supplementary corn grain for steers grazing native rangeland during summer. J. Anim. Sci. 69: 1678-1687.

Poore, M.H., J.A. Moore and R.S. Swingle. 1990. Different passage rates and digestion of neutral detergent fiber from grain and forages in 30, 60 and 90 % concentrate diets fed to steers. J. Anim. Sci. 68: 2965-2972.

Poppi, E.C., B.W. Norton, D.J. Minson and R.E. Hendricksen. 1980. The validity of critical size theory for particles leaving the rumen. J. Agric. Sci. 94: 275-280.

Poppi, D.P., D.J. Minson and J.H. Ternouth. 1981. Studies of cattle and sheep eating leaf and stem fractions of grasses. I. The voluntary intake, digestibility and retention time in the reticulorumen. Aust. J. Agric. Res. 32: 99-108.

Promkot, C., M. Wanapat and P. Rowlison. 2007. Estimation of ruminal degradation and intestinal digestion of tropical protein resources using the *nylon bag* technique

and three-step *in vitro* procedure in dairy cattle on rice straw diets. Asian-Aust. J. Anim. Sci. 20: 1849-1857.

Reid, C.S.W., M.J. Ulyatt and J.A. Munro. 1977. The physical breakdown of feed during digestion in the rumen. Proc. N.Z. Soc. Anim. Prod. 37: 173-175.

Reddy, D.V. 1996. Evaluation of rice straw-poultry droppings based rations supplemented with graded levels of rice bran in fistulated buffaloes. Anim. Feed Sci. Technol. 58: 227-237.

Rooke, J.A., M.A. Overend and D.G. Amstrong. 1986. The effects upon rumen microbial protein synthesis of giving sheep diets of rolled barley and hay supplemented with increasing quantities of soybean meal. J. Agric. Sci. Camb. 106: 635-638.

Sarnklong, J., W.Cone, W. Pellikaan and W.H. Hendriks. 2010. Utilization or rice straw and different treatments to improve its feed value for ruminants: A review. Asian-Aust. J. Anim. Sci. 23: 680-692.

SAS. 1994. User's guide. 6th ed. SAS Inc. Carry. NC.

Satterl, L.D. and R.E. Roffler. 1981. Influence of nitrogen and carbohydrate inputs rumen fermentation. In: Recent developments in ruminants nutrition (Haresign, W. and D.J.A. Cole, eds). pp. 115-139. Butterworths.

Satter, L.D. and L.L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. Br. J. Nutr. 32: 199-208.

Schalk, A.F. and R.S. Amadon. 1928. Physiology of the ruminant stomach (bovine). Study of the dynamic factors. North Dakota Agric. Exp. Stn. Bull. 216: 1-64.

Schiere, J.B. and J.De Wit. 1993a. Feeding standards and tropical feeding system (A review). Anim. Feed Sci. Technol. 43: 121-134.

Schiere, J.B. and A.J. Nell. 1993b. Feeding of urea treated rice straw in the tropics. I. A review of its technical principles and economics. Anim. Feed Sci. Technol. 43: 135-147.

Schiere, J.B. and M.N.M. Ibrahim. 1989. Feeding of urea-ammonia treated rice straw: A compilation of miscellaneous reports produced by the Straw Utilization Project (Sri Lanka). Pudoc, Wageningen.

Sekine, J., Y. Imaki, Y. Kuninishi, R. Oura, H. Miyazaki, M. Okamoto and Y. Asahida. 1992. Effect of time after feeding on distribution of feed particles in the gastrointestinal tract of sheep given orchardgrass hay once a day. Asian-Aust. J. Anim. Sci. 5: 55-61.

Selim, A.S.M., J. Pan, T. Takano, T. Suzuki, S. Koike, Y. Kobayashi and K. Tanaka 2004. Effect of ammonia treatment on physical strength of rice straw, distribution of straw particles and particle-associated bacteria in sheep rumen. Anim. Feed Sci. Technol. 115: 117-128.

Shen, H.S., F. Sundstol, E.R. Eng and L.O. Eik. 1999. Studies on untreated and urea-treated rice straw from three cultivation seasons: 3. Histological investigations by light and scanning electron microscopy. Anim. Feed Sci. Technol. 80: 151-159.

Shen, H.S., D.B. Ni and F. Sundstol. 1998. Studies on untreated and urea-treated rice straw from three cultivation seasons: 1. Physical and chemical measurements in straw and straw fractions. Anim. Feed Sci. Technol. 73: 243-261.

Shinoda, M., S. Horii and A. Abe. 1984. Comparison of nutritive nature of rice straw parts, and effect of alkali treatment on them. Bull. Nat. Inst. Anim. Ind. 42: 21-27. (in Japanese with English summary)

Shriver, B.J., W.H. Hoover, J.P. Sargent, R.J. Crawford, Jr and W.V. Thayne. 1986. Fermentation of a high concentrate diet as affected by ruminal pH and digesta flow. J. Dairy Sci. 69: 413-419.

Stoke, S.R., A.L. Goetsch and K.M. Landis. 1988. Feed intake and digestion by beef cows fed prairie hay with different levels of soybean meal and receiving postruminal administration of antibiotics. J. Anim. Sci. 66: 1778-1789.

Suzuki, T., J. Pan, K. Izumi, Y. Ueda, K. Ueda, K. Tanaka and M. Okubo. 2000. Relationship between conmminution of feed particles during rumination and fiber fermentation in cattle. Anim. Sci. J. 71: J331-J337.

Terry, R.A., J.M.A. Tilley and G.E. Outen. 1969. Effect of pH on cellulose digestion under *in vitro* conditions. J. Sci. Food. Agric. 20: 317-320.

Theander, O. and P. Aman. 1984. Anatomical and chemical characteristics. In: Straw and Other Fibrous By-products as Feed. (Sundstol, F. and E. Own, eds). pp. 45-78. Developments in Animal Veterinary Sciences, 14. Elsevier, Amsterdam.

Tin Ngwe. 2003. Study on effects of chickpea (*Cicer Arietinum*) husk and lablab bean (*Dilichos Lablab*) husk, as sources of easily digestible fiber, on rice straw digestion and ruminal characteristics in sheep. Ph.D. Thesis, Graduate School of Agriculture, Hokkaido University, Japan.

Tin Ngwe. 1990. The effects of different sources of protein supplement on the utilization of rice straw based diet in castrated growing young calves. M.Agr. Thesis, Graduate School of Agriculture, Hokkaido University, Japan.

Toyokawa, K. 1978. Studies on the utilization of rice straw for ruminants, especially on the features of rumen fermentation, the decomposition and stagnancy of infermentable materials in the rumen and the methods of increasing voluntary

intake of rice straw. Bull. Fac. Agric. Hirosaki Univ. 30: 47-130. (in Japanese with English summary)

Tyrrel, H.F. and P.W. Moe. 1975. Effect of intake on digestive efficiency. J. Dairy Sci. 58: 1151-1157.

Ueda, K., T. Ichinohe, T. Tamura, M. Okubo and Y. Asahida. 1997. Efficiency of chewing for breakdown of large particles during eating and ruminating in sheep fed hays. Anim.Sci. Technol. J. 68: 917-925.

Ulyatt, M.J., D.W. Dellow, A. John, C.S.W. Reid and G.C. Waghorn.1986. Contribution of chewing during eating and rumination to the clearance of digesta from the ruminoreticulum. In: Control of Digestion and Metabolism in Ruminants (Milligan L.P., W.L. Grovum and A. Dobson eds). pp. 498-515. Englewood Cliffs. New Jersey.

USDA. 2011. Oilseeds: World markets and trade, foreign agricultural service. Available from URL: http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1290

Vadiveloo, J. 2003. The effect of agronomic improvement and urea treatment on the nutritional value of Malaysian rice straw varieties. Anim. Feed Sci. Technol. 108: 33-46.

Vadiveloo, J. 2000. Nutritional properties of the leaf and stem of rice straw. Anim. Feed Sci. Technol. 83: 57-65.

Vadiveloo, J. 1992. Varietal differences in the chemical composition and *in vitro* digestibility of rice straw. J. Agric. Sci. 119: 27-33.

Vander Hoek, R., G.S. Muttetuwegama and J.B. Schiere. 1988. Overcoming the

nutritional limitations of rice straw for ruminants: 1. Urea ammonia treatment and supplementation of straw for lactating Surti buffaloes. Asian-Aust. J. Anim. Sci.1: 201-208.

Van Soest, P.J. 2006. Review: rice straw, the role of silica and treatments to improve quality. Anim. Feed Sci. Technol. 130: 137-171.

Van Soest, P.J.1994. Nutritional ecology of the ruminant, 2nd edition. Cornell University Press, NY, USA.

Van Soest, P.J., J.B. Robertson and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583-3597.

Van Soest, P.J. 1982. Nutritional ecology of the ruminant. O and B Books Inc., Corvallis, OR.

Varga, G.A. and E.C. Prigge. 1982. Influence of forage species and level of intake on ruminal turnover rates. J. Anim. Sci. 55: 1498-1504.

Vu, D.D., L.X. Cuong, C.A, Dung and P.H. 1999. Use of urea-molasses-multinutrient block and urea-treated rice straw for improving dairy cattle productivity in Vietnam. Pre. Vet. Med. 38: 187-193

Walsh, K.J.E. 2004. Tropical cyclones and climate change: unresolved issues. Climate Res., 27: 77–84.

Wanapat, M., A. Petlum and O. Pimpa. 1999. Startegic supplementation with a high quality feed block on roughage intake, milk yield and composition and economic return in lactating dairy cows. Asian-Aust. J. Anim. Sci. 12: 901-903.

Wanapat, M., K. Sommart and K. Saardrak. 1996. Cottonseed meal supplementation of dairy cattle fed rice straw. Livestock Research for Rural Development. 8.

Available from URL:
http://fao.org/ag/AGa/AGAP-/FRG/FEEDback/1rrd/1rrd8/3/metha83.htm

Warly, L. 1994. Study on improving nutritive value of rice straw and physico-chemical aspects of its digestion in sheep. Ph. D. Thesis. The United Graduate School of Agriculture Sciences. Tottori University, Japan.

Warly, L., A. Fariani, O.P. Mawuenyegah, T. Matsui, T. Fujihara and T. Harumoto. 1994. Study on the utilization of rice straw by sheep. IV. The effect of soybean meal and barley supplementation on eating and rumination behaviour. Asian-Aust. J. Anim. Sci. 7: 273-277.

Warly, L., T. Matsui, T. Harumoto and T. Fujihara. 1992a. Study on the utilization of rice straw by sheep. I. The effect of soybean meal supplementation on the voluntary intake of rice straw and ruminal fermentation. Asian-Aust. J. Anim. Sci. 5: 687-693.

Warly, L., T. Matsui, T. Harumoto and T. Fujihara. 1992b. Study on the utilization of rice straw by sheep. II. The effect of soybean meal supplementation on the eating and rumination behaviour. Asian-Aust. J. Anim. Sci. 5: 695-698.

Wattiaux, M.A., D.R. Mertens and L.D. Satter. 1991. Effect of source and amount of fiber on kinetics of digestion and specific gravity of forage particles in the rumen. J. Dairy Sci. 74: 3872-3883.

Welch, J.G. and A.M. Smith. 1975. Milk fat depression and citrus pulp on rumination activity. J. Dairy Sci. 58: 678-681.

Welch, J.G.1982. Rumination, particle size and passage from the rumen. J. Anim. Sci. 54: 885-894.

Weston, R.H. and J.P. Hogan. 1967. The digestion of chopped and ground roughages by

sheep. I. The movement of digesta through the stomach. Aust. J. Agric. Res. 18: 789-801.

Weston, R.H. and P.M. Kennedy. 1984. Various aspects of reticulorumen digestive function in relation to diet and digesta particle size. In: Techniques in particle size analysis of feed and digesta in ruminants (Kennedy, P. M. eds). pp. 1-17. Can. Soc. Anim. Sci. Edomonton.

Wetherburn, M.W. 1967. Phenol-hypochlorite reaction for determination of ammonia. Analytical Chemistry. 36: 971-974.

Wiedmeier, R.D., J.R. Males and C.T. Gaskins. 1983. Effect of dietary crude protein on the dry matter digestibility of wheat straw diets in cattle. J. Anim. Sci. 57: 1568-1575.

Wilson, J.R. and R.D. Hatfield. 1997. Structural and chemical changes of cell wall types during stem development: consequences for fiber degradation by rumen microflora. Aust. J. Agric. Res. 48: 165-180.

Xing, T. 1988. Possibility and possible methods of improving the nutritive value of cereal straw without pre-treatment. Asian-Aust. J. Anim. Sci. 1:115-134.