Chapter 4

Effects of fermented TMR on digestibility, ruminal fermentation, nitrogen utilization, blood characteristics, and ruminal methane production in sheep

4.1. Introduction

There is a large and growing body of evidence that most of the climatic warming observed over the last 50 years is attributable to human activity (IPCC, 2001). This increase in the Earth's average temperature is considered one of the most important global environmental issues. Methane is an important greenhouse gas, second only to carbon dioxide in its contribution to global warming. Per molecule, methane is approximately 30 times more potent as a greenhouse gas than carbon dioxide, but it has a relatively short atmospheric lifetime of approximately 10 years compared to more than 200 years for carbon dioxide (Moss, 1993). Reducing greenhouse gas emissions remains a top priority for stemming global warming (Mathison et al., 1998; Moss et al., 2000). The world population of ruminants is a major source of methane, contributing approximately 15% of the total atmospheric methane flux (Sahoo et al., 2000). Methane production occurs during microbial fermentation of feed within the rumen and represents a loss in productive energy for the animal (Beauchemin and McGinn, 2005) amounting to up to nearly 12% of total energy intake (Johnson and Ward, 1996; Giger-Reverdin and Sauvant, 2000; Johnson et al., 2000). Diet composition or type and the level of feed intake can have a major effect on methane production (Johnson and Johnson, 1995; Moss et al., 2000;

Benchaar et al., 2001). The forage-to-feed concentrate ratio (F/C) of the ration impacts rumen fermentation and hence the acetate-to-propionate ratio (A/P) declines with the F/C ratio (Moss et al., 2000). Thus, high-concentrate diets should reduce methane production (Fahey and Berger, 1988). Johnson and Johnson (1995) reported a methane energy loss of 6–7% gross energy intake when forages were fed at the maintenance plane of nutrition; this decreased to 2–3% when high-grain concentrates were offered at near ad libitum intake levels. A similar effect was reported for grass silage supplemented with barley (Moss et al., 1995). A diet that is high in grain or supplemented with soluble carbohydrates shifts the fermentation pattern in the rumen and results in a more hostile environment for methanogenic bacteria, which increases the passage rate of food, reduces ruminal pH and certain populations of protozoa, and inhibits or eliminates ruminal ciliates and methanogenic bacteria (Van Soest, 1982). A similar report showed that a low rumen pH regulates methane production (Lana et al., 1998). In addition, over a final pH range of 6.5 to 5.3, methane production is highly correlated with A/P (Russell, 1998).

Many studies have investigated how to inhibit methane production by ruminants to help address global climate change (e.g., Moss et al., 2000). Direct inhibition of methanogenesis by halogenated methane analogues and related compounds has been widely demonstrated *in vitro* (Van Nevel and Demeyer, 1995; McCrabb et al., 1997; Kung et al., 1998) but further *in vivo* testing is essential. Ionophoric antibiotics such as monensin depress methane production *in vitro* (Van Nevel and Demeyer, 1992); however, long-term *in vivo* trials have shown that this inhibition does not persist (Runpler et al., 1986). Malate, which is converted to propionate via fumarate, also stimulates propionate production and inhibits methanogenesis *in vitro* (Martin and Streeter, 1995), but actually increases methane production under rumen-simulating conditions, although this was

largely explained by the stimulation of fiber digestion, and the methane produced per unit dry matter did in fact drop (Carro et al., 1999). Bacteria that carry out reductive acetogenesis have been isolated from the rumen (Morvan et al., 1994), but they are few in number, and attempts to increase acetogenesis have not been successful, largely because reductive acetogens under rumen-like conditions are unable to compete with methanogenie archaea (Demeyer et al., 1996; Nollet et al., 1997, 1998). A methane-oxidizing bacterium was isolated from the gut of young pigs; it decreased methane accumulation when added to rumen fluid *in vitro* (Valdes et al., 1997); however, the validity of this approach *in vivo* has yet to be tested. Infusing the rumen of sheep with pure saponin (Lu and Jorgensen, 1987) or feeding them saponin-containing plants (Teferedegne et al., 1999) decreases protozoa populations; however, even if a practical on-farm method to remove protozoa from the rumen can be found, the effects of defaunation on methane emissions cannot be considered in isolation (Moss et al., 2000), as rumen ciliate protozoa play an active role in ruminal fiber breakdown (Coleman, 1986) and thus defaunation may adversely impact fiber digestion (Jouany and Ushida, 1999). Thus, defaunation to decrease methane production would have to be balanced against the effects on fiber and protein metabolism. Horiguchi and Takahashi (2001) reported that supplementing feed with rapeseed oil reduced methane emissions by sheep. Similarly, including fat in the diet markedly decreases methane production, but the effect depends on the fat source used (Machmuller et al., 1998); moreover, the effects of fat on methane production are not limited to those mediated via protozoa, i.e., lipids inhibit methanogenesis even in the absence of rumen protozoa (Dohme et al., 1999), possibly due to the toxicity of long-chain fatty acids (Henderson, 1973). In addition, fat may significantly inhibit fiber breakdown in the rumen (Machmuller and Kreuzer, 1997), the

severity of which also depends on the fat used (Machmuller et al., 1998). Both *Saccharomyces cerevisiae* yeast and *Aspergillus oryzae* fungi have been shown to both decrease (Frumholtz et al., 1989; Mutsvangwa et al., 1992) and increase (Martin and Nisbet, 1990) methane production.

Treating straw with urea and/or a mixture of urea and calcium hydroxide followed by storage can reduce methane production per kg digested OM and increase energy balance (Sahoo et al., 2000). Sunflower oil, ionophores, and possibly some yeast products may decrease methane energy loss in cattle, but oil supplementation impairs fiber digestibility (McGinn et al., 2004). Hindrichsen et al. (2005) showed that low enteric methanogenesis associated with high fiber excretion does not inevitably lead to compensatory increases in methane emission during slurry storage. Jordan et al. (2006) showed that although coconut oil-treated diets decrease methane, they also decrease digestibility, resulting in an extended finishing time with implications for lifetime methane emissions. (Nkrumah et al., 2006) found that residual feed intake (RFI) was correlated with daily methane production and energy lost as methane, and that methane production was 28% and 24% lower in low-RFI animals than high- and medium-RFI animals, respectively. Essential oils appear to have no effect on methane emissions, whereas fumaric acid may improve ruminal fermentation but does not lead to a measurable reduction in methane emissions (Beauchemin and McGinn, 2006). However, some lauric acid oils reduce methanogenesis and increase VFA production, especially in culture with ground corn (Yabuuchi et al., 2006). In general, research on methanogenesis has clearly revealed that methane production in the rumen is a rather sensitive process that can easily be inhibited by several additives (Van Nevel and Demeyer, 1996). Still, no single additive or intervention that is unequivocally effective has yet been identified, and

the shortcomings of each possible inhibitor are wide ranging, from having only a temporary effect to being toxic to the animal, leaked into the environment (a problem only if the inhibitor is not biodegradable), having low digestibility (lipid supplementation), or not being practical (defaunation; Van Nevel and Demeyer, 1996). Therefore, the ideal methane inhibitor must be extremely specific with persistent and long-lasting action, harmless to the animal, and must not leave behind residues in edible products (Van Nevel and Demeyer, 1996).

As already mentioned, the inhibition of methane production is normally accompanied by an increase in propionate production (Wolin, 1975). Furthermore, propionate production uses hydrogen and lactic acid (Moss et al., 2000). To date, the effect of fermented TMR on methane production in the rumen has not been investigated *in vivo*, although it has been found that a high LAC diet leads to low methane production *in vitro* (Cao et. al., in press, a).

The objectives of this experiment were to evaluate the effects of TMR with a high LAC on the digestibility, fermentation characteristics, ruminal methane production and energy loss, and blood characteristics of sheep.

4.2. Materials and Methods

The animal experiment was conducted with permission from the Committee of Animal Experimentation under the institutional guidelines for animal experiments at the Faculty of Agriculture, Yamagata University.

4.2.1. Preparation of WCR silage and TMR

WCR (Haenuki) was cultivated using conventional methods in a paddy field on an experimental farm at Yamagata University, Japan, harvested at the full-ripe stage,

prepared into a mini roll bale silage (50 kg), and stored outdoors (9–32°C) for 240 days of fermentation. After fermentation, the characteristics of the WCR silage were as follows: DM, 45.6%; pH 4.53; LAC, 1.41%; acetic acid, 1.40%; NH_3-N/TN, 6.81%; Flieg's score, 36.8; and V-score, 89.6.

The silage was cut to a length of 2 cm. As shown in Table 4.1, TMR was prepared using compound feed (Kitanihon-kumiai Feed, Yamagata, Japan); WCR silage; dried beet pulp (Zennou, Tokyo); a vitamin–mineral supplement (Snow Brand Seed, Iwate, Japan; vitamin A, 5,000,000 IU/kg; vitamin D3, 1,000,000 IU/kg; vitamin E, 2 g/kg; vitamin K3, 0.2 g/kg; vitamin B1, 0.5 g/kg; vitamin B2, 1 g/kg; vitamin B6, 0.1 g/kg; vitamin B12, 0.001 g/kg; nicotinic acid, 6 g/kg; choline chloride, 2 g/kg; calcium pantothenate-D, 10 g/kg; Mn, 0.16 g/kg; Zn, 0.7 g/kg; Fe, 0.55 g/kg; Cu, 0.14 g/kg; I, 0.33 g/kg; Co, 0.04 g/kg; methionine, 1 g/kg; lidocaine hydrochloride, 0.5 g/kg); and RB (Yamagata University Farm, Yamagata, Japan). Experimental treatments included controls (i.e., no lactic bacteria added and not fermented) or fermented TMR silage (FTMR; lactic bacteria added and fermented) with the addition of *Lactobacillus plantarum Chikuso-1* (Snow Brand Seed, Sapporo, Japan) at a rate of 5 mg/kg (i.e., 5 ppm) of fresh TMR. The TMR ingredients and proportions are listed in Table 4.2. CP and TDN content for the two TMR were calculated according to Standard Tables of Feed Composition in Japan (2001) (National Agricultural Research Organization, 2001). The proportions of WCR silage, feed concentrate, dried beet pulp, and vitamin–mineral supplement were fixed at 30%, 25%, 13.5%, and 1.5% of TMR DM, respectively, and the remaining 30% of TMR (DM) and the RB were mixed. The FTMR silage was adjusted with water to 55% moisture, and 100 kg were ensiled in two drum can silos of 200 L volume (Minikon-silo; KD-Service, Tokyo, Japan). To produce fresh TMR, WCR silage

was separated from the other ingredients, ensiled in two drum can silos alone, and the feed concentrate, beet pulp, vitamin–mineral supplement, and RB were mixed and ensiled in a separate drum can. The samples were stored outdoors (9–32°C) for 60 days, and the same proportions were fed to animals.

4.2.2. Feeding of animals and experimental design

Four Suffolk sheep (49.5 ± 3.2 kg) were used in a 2 (treatments) × 2 (periods) cross-over design experiment. The sheep were individually housed in metabolic cages and fed TMR at 2% body weight (BW) on a DM basis once daily at 09:00 h. The metabolizable energy maintenance level was set according to *Nutrient Requirements of Sheep* (NRC, 1985). Water was accessible at all times. A 7-day preliminary adjustment period was followed by a 5-day period during which all feces and urine were collected.

Blood samples were also collected by venipuncture of the jugular vein before the morning feeding and at 2 and 4 h after the morning feeding on day 12 of each period, and stored in vacutainer tubes (VENOject II; TERUMO, Tokyo, Japan). Hematocrit values were determined in duplicate using micro-hematocrit tubes and centrifuging in a 12,000 rpm centrifuge (Hemato Crit Kokusan Co., Okusan, Tokyo, Japan) for 5 min (Dacie and Lewis, 1975) and plasma was prepared by centrifugation (12,000 rpm, 20 min, 4°C) and stored at -20°C until later analysis of glucose and urea-N.

Ruminal fluid was sampled immediately before the morning feeding and at 2 and 4 h after feeding on day 13 of each period. Ruminal fluid pH was measured immediately, and samples were separated from feed articles through two layers of gauze. Samples were frozen (-20°C) for later analysis of VFA and NH_3-N.

4.2.3. Methane emission from expiratory gas

A 5-day feces and urine collection period was followed by a 2-day period (excluding

the calibration time required for analyzers) during which time air was collected for a period of 24 h (from 09:00 h feeding to next day 09:00 feeding). Expiratory gas was collected using a head hood-type respiration chamber (85 cm wide × 45 cm deep × 90 cm tall; Nishida et al., 2007), dehydration device, gas pump (85–95 L/min; JP-80 Vacuum pump; Tokyo Deodorant, Tokyo, Japan), gas flow meter (N6 LPG; Aichi Tokei Denki, Nagoya, Japan), sampling bag (60 L), and vinyl hose (I.D., 20 mm). The chamber had a hinged door through which feed and water were provided, and a fan was installed to circulate air throughout the respiration chamber. Each end was fitted with additional individual chambers for dehydration, connected to the device by a hose. The dehydration device, which was connected to a gas pump by a hose, filtered moisture from the chamber air, and the gas pump moved the air from the chamber into a gas flow meter (via a hose) and at the same time helped provide fresh air into the chamber. The gas flow meter measured the total volume of air leaving the chamber for 24 h and was connected to a sampling bag by a hose fitted with a control valve so that the collected air sample was less than 60 L for 24 h. Four sampling bags of expiratory gas were collected and analyzed for methane content.

4.2.4. Chemical analysis

The WCR TMR silages and feces were dried in a forced draft oven at 60°C for 48 h and ground into a 2-mm powder with a sample mill (Foss Tecator; Akutalstuku, Tokyo, Japan). The DM, CP, EE, and ash were analyzed according to methods 934.01, 976.05, 920.39, and 942.05, respectively, of the Association of Official Analytical Chemists (AOAC, 1990). The organic matter (OM) was calculated as weight loss upon ashing. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest et al. (1991). Heat-stable amylase and sodium sulfite were used in the NDF

procedure, and the results were expressed without residual ash. Urinary N was determined using the Kjeldahl procedure described by the AOAC (1990). Gross energy (GE) was determined using an automatic bomb calorimeter (OSK 150, Ogawa Sampling, Tokyo, Japan).

The fermentation products of the silages were determined using cold-water extracts. Wet silage (50 g) was homogenized with 200 mL sterilized distilled water and stored at 4°C overnight (Cai et al., 1999). The pH was measured using a glass electrode pH meter (Horiba D-21; Horiba, Kyoto, Japan). Lactic acid and NH_3-N were analyzed according to Takahashi et al. (2005). The VFA was steam-distilled and measured qualitatively and quantitatively using gas chromatography (G5000-A; Hitachi, Tokyo, Japan) equipped with a thermal conductivity detector and a glass column (Unisole F-200, 3.2 mm × 2.1 m). The analytical conditions were as follows: column oven temperature, 140°C; injector temperature, 210°C; detector temperature, 250°C. To assess silage quality, we calculated Flieg's scores from the lactic acid and VFA concentrations and V-scores from the NH_3-N/total N and VFA concentrations (Takahashi et al., 2005). The pH, VFA, and NH_3-N concentrations in ruminal fluid samples were measured using the same methods as for the TMR silage filtrates.

Serum glucose and urea-N were determined using the glucose C II Test-wako kit (Wako Pure Chemical Industries, Osaka, Japan) and urea-N B Test-wako (Wako Pure). Cholesterol, glutamic oxaloacetic transaminase (GOT), and glutamic pyruvic transaminase (GPT) were determined using the cholesterol E Test-wako (Wako Pure), and transaminase C II Test-wako kits (Wako Pure).

Gas samples were analyzed (Horiguchi and Takahashi, 2001) for methane by gas chromatography (G-5000A; Hitachi, Tokyo, Japan). The analytical conditions were as

follows: column oven temperature, 80°C; injector temperature, 100°C; detector temperature, 110°C.

4.2.5. Calculations and statistical analysis

The energy lost as methane was calculated as the total methane produced in liters per day at standard temperature and pressure (STP) × 9.45 kcal/L (Brouwer, 1965).

Chemical composition, pH, organic acids, and NH_3-N/TN were analyzed using a one-way ANOVA. Digestion and methane emission data were analyzed as a 2 × 2 Latin square using the General Liner Model procedure of SAS (1995), with diet, period, and animal included as factors. Tukey's test (SAS, 1995) was used to identify differences ($P < 0.05$) between means.

4.3. Results

4.3.1. Chemical composition

The chemical composition and GE content of TMR are listed in Table 4.3. DM, OM, nitrogen-free extract (NFE), and NFC were lower but EE, CF, crude ash (CA), and GE were higher in FTMR ($P < 0.05$) than in controls. There were no significant differences in CP, ADF, and NDF between the two TMR silages.

4.3.2. pH and organic acid content

FTMR silage had lower pH and higher LAC, acetic acid, and NH_3-N/TN compared to controls (Table 4.4). As indicated by the high Flieg's scores and V-scores, the silage was good quality.

4.3.3. Nutrient digestibility and nitrogen retention

Nutrient digestibility, nutrient content, and nitrogen retention in the two TMR silages are shown in Table 4.5. The apparent digestibility of FTMR was higher than

control TMR, and there were remarkable differences in CP, EE, CF, NDF, and GE. DM, OM, and ADF also tended to be higher in FTMR ($P = 0.0518$, $P = 0.0650$ and $P = 0.0741$, respectively). TDN, digestible crude protein (DCP), and digestible energy (DE) were higher in FTMR. There was no significant difference in nitrogen retention, nitrogen intake, or allantoin levels between the two TMR silages, although fecal and urinary excretion of nitrogen were significantly lower and higher for FTMR, respectively. There was no significant difference in DM intake per metabolic body weight between the two TMR silages.

4.3.4. Ruminal fermentation

The ruminal pH of the control and FTMR groups were 6.64–7.28 and 6.40–7.20, respectively; the difference was non-significant before and 4 h after feeding (Table 4.6) but significantly higher in FTMR 2 h after feeding. Total VFA did not differ between the two silages before feeding, but was significantly higher in FTMR 2 and 4 h after feeding. The molar proportions of acetic acid in FTMR were not affected by dietary TMR, but tended to increase before and 4 h after feeding ($P = 0.0761$ and $P = 0.0714$, respectively). Propionic acid was significantly higher in FTMR 2 h after feeding, while isobutyric acid was not affected by dietary TMR. Although butyric acid did not differ between the two silages before feeding, it was significantly lower in FTMR 2 and 4 h after feeding. Isovaleric acid, valeric acid, and the A/P ratio were not affected by dietary TMR. NH_3-N concentration did not differ between the two silages before feeding, but was higher in FTMR 2 and 4 h after feeding.

4.3.5. Energy intake and methane emission

There was no significant difference in daily GE intake between the two TMR silages (Table 4.7). Compared to controls, FTMR significantly decreased daily methane

emission per sheep by 25.1%, per DM (L/kg) intake by 24.7%, per digestible DM by 28.4%, and per metabolic BW by 25.5%. FTMR also significantly decreased the daily energy lost as methane per sheep by 25.1%, per kg metabolic BW by 25.4%, and per percent GE intake by 25.9%.

4.3.6. Blood characteristics

The blood data are shown in Table 4.8. Hematocrit, Glucose, urea-U, GOT, and GPT were 30.44–36.00%, 57.04–66.40 mg/dL, 13.06–16.43 mg/dL, 45.31–53.78, and 4.79–6.09, respectively; none was affected by dietary TMR. Although glucose was significantly lower in FTMR before feeding, there was no significant difference between the two TMR 2 and 4 h after feeding.

4.4. Discussion

The main losses in low-DM silages are associated with the fermentation process and effluent loss (Hameleers et al., 1999). Hameleers et al. (1999) reported DM losses of 0.16–0.06 for crops with a DM range of 200–350 g/kg, and that losses caused by the fermentation process varied from 0.10 to 0.05 at a DM range of 200–450 g/kg. In the present experiment, DM content was lower in FTMR than in controls, because both moisture (55%) and OM were lower in the FTMR. In addition, FTMR had a higher LAC, and lactic acid was extracted out of the sample and mixed with EE, resulting in a higher EE content (9.4%) than in controls (7.4%). During fermentation, even a small amount of water-soluble carbohydrate degraded by lactic bacteria could decrease NFE and NFC levels (Cai and Ohmomo, 1995; Cai et al., 2001, 2003). However, Shioya (2008) reported no significant differences in NFC between fresh and fermented TMR silage. In the present experiment, FTMR had a lower DM and NFC but higher GE than controls. This

could be attributable to the higher EE, CF, and NDF contents in FTMR.

To prepare high-quality TMR silage, we added LAB (Cai, 2001; Cai et al., 2003). As indicated by the low pH, high LAC, and high Flieg's scores and V-scores, the TMR silage was of good quality. This is consistent with a previous study (Shioya, 2008) that showed that TMR silage has a low pH, high LAC, and low butyric acid levels. In the present study, the WCR silage had a small amount of organic acids (i.e., lactic acid, acetic acid, propionic acid, and butyric acid) and NH_3-N. Therefore, the FTMR had a significantly higher LAC than controls.

Shioya (2008) reported that the DM intake for FTMR silage was higher than for fresh TMR (i.e., not fermented), and that ruminants preferred FTMR silage, which was also more digestible, although there were no significant differences in TDN or GE loss among treatments. In the present experiment, FTMR silage had more EE and GE with CF and NDF but less NFC than controls; at the same time, there were significant increases in the apparent digestibility of CP, EE, CF, NDF, and GE and an increasing (but non-significant) trend in the apparent digestibility of DM and OM ($P = 0.0518$ and $P = 0.0650$, respectively). This is consistent with a recent report that the digestibility of NDF significantly increased as NDF increased and NFC decreased in TMR made with cassava (Kanjanapruthipong and Buatong, 2004). In addition, FTMR had more nutrients, TDN, DCP, and DE, and resulted in less fecal excretion of nitrogen and allantoin but higher urinary excretion of nitrogen. There was no significant difference in DM intake between the two TMR silages, in contrast to the findings of Shioya (2008).

Erfle et al. (1982) found that ruminal pH varied from greater than 7 to less than 5 as VFA production decreased from 80 to 50 mmoles/day. Ruminal pH has been implicated in the development of bloating (Jones and Lyttleton, 1972), and there is an inverse

relationship between ruminal pH and gas production (Waghorn, 1991). In addition, pH values below 6 increase the stability of foam, which interferes with the normal eructation of gases and therefore, contributes to the frothy bloat (Gutierrez et al., 1963; McArthur and Miltimore, 1969). Furthermore, gas in the rumen forms bubbles that rise through the rumen contents and coalesce to form a dorsal gas pocket in the rumen (Clarke and Reid, 1974). Consequently, a low ruminal pH should increase bloat susceptibility by increasing the production and entrapment of gas in the rumen (Bretschneider et al., 2007). Indeed, Bretschneider et al. (2001) reported that corn silage fed before grazing at levels of intake ranging from 0.5 to 1 kg of DM/100 kg BW decreased the incidence of pasture bloat in cattle. In the present experiment, ruminal pH was always within the normal range for sheep (Russell and Hino, 1985). Although ruminal pH was significantly lower for FTMR 2 h after feeding, there were no significant differences between the two silages before feeding or 4 h after feeding. Total VFA increased by 5.55–8.84 mmol/dL and 5.92–12.72 mmol/dL in controls and FTMR, respectively, from before feeding to 2 h after feeding, but slightly decreased 4 h after feeding. FTMR had higher total VFA 2 and 4 h after feeding, and the consumption of fermentable carbohydrates led to a marked postprandial decrease in ruminal pH (Nocek, 1997; Chaucheyras-Durand et al., 2008). FTMR had more fermentable matter due to its high nutritive digestibility, which led to more VFA and a lower pH. Similar to total VFA, the molar concentrations of acetic acid and propionic acid in both controls and FTMR also increased from before feeding to 2 and 4 h after feeding, although acetic acid levels were slightly (non-significantly) higher in FTMR before (P = 0.0761) and 4 h after (P = 0.0714) feeding than in controls, and propionic acid was slightly higher in FTMR 2 h after feeding. This might have happened because, after feeding, the NFC, feed concentrate, or effective fiber may have been

degraded by bacteria into propionic acid, thereby decreasing ruminal pH and the A/P ratio; at the same time, lactic acid either from the FMTR or via bacterial fermentation in the rumen may have used hydrogen from the fermentation reaction to further increase propionic acid (Lana et al., 1998; Russell, 1998; Moss et al., 2000). Similar to previous studies (Lana et al., 1998; Russell, 1998), ruminal pH in the present experiment declined in sheep fed FTMR from 7.2 before feeding to 6.33 at 2 h after feeding and 6.40 at 4 h after feeding, while the A/P ratio declined as much as 12.2% before feeding and 14.6% 4 h after feeding, likely because the high CF, NDF, and fiber contents all produce acetic acid (Russell, 1998). Kim et al. (2006) found that feeding wormwood (*Artemisia montana*) silage instead of rice straw increased DM, OM, CP, EE, and NDF, as well as ruminal propionic and butyric acid. In contrast, the present study showed that feeding FTMR silage instead of non-fermented TMR also increased ruminal propionic acid but decreased ruminal butyric acid 2 and 4 h after feeding. Similar to total VFA, the NH_3-N concentration in controls and FTMR increased 2 h after feeding, and tended to decline between 2 and 4 h after feeding. NH_3-N production was higher 2 and 4 h after feeding in FTMR than in controls, possibly due to its higher CP digestibility. In addition, Kanjanapruthipong and Buatong (2004) reported that acetic acid, propoinic acid, the A/P ratio, and fiber digestibility all significantly increase with an increasing content of non-forage NDF from cassava residues.

There are two known mechanisms for the conversion of lactic acid or pyruvic acid to propionic acid (Leng, 1970). When lactate acid is secondarily fermented in the rumen by lactate-utilizing bacteria such as *Megasphaera elsdenii, Selenomonas ruminantium*, and *Veillonella parvula*, propionate is generally produced as a major product (Dawson et al., 1997; Russell and Wallace, 1997) and this can reduce methanogenesis because electrons

are used during propionate formation. If hydrogen is then used to convert lactic acid to propionic acid in the rumen (Moss et al., 2000), the hydrogen will decrease, which in turn will inhibit the conversion of hydrogen and CO_2 to methane. Russell (1998) reported that over a pH range of 6.5 to 5.3, methane production is highly directly correlated with the A/P ratio. In the present experiment, the ruminal pH of sheep fed FTMR declined from 7.20 before feeding to 6.33 at 2 h after feeding and 6.40 at 4 h after feeding, while the A/P ratio also decreased by up to 14.6%, indicating that the higher LAC of the FTMR diet may have led to the production of propionic acid and, accordingly, lowered methane production.

Many studies have reported lower plasma glucose concentrations in animals fed restrictively fermented silage (Smith et al., 1993; Miettinen and Huhtanen, 1997; Heikkilä et al., 1998) or in those that exhibited increased ruminal butyrate (Huhtanen et al., 1993; Miettinen and Huhtanen, 1996). For animals fed silage-based diets, a close relationship exists between mean rumen molar propionate proportions and mean plasma glucose concentrations (Shingfield et al., 2002). However, despite the possibility that diets based on restrictively fermented silages are probably more limited in glucose supply than extensively fermented silages, it appears that ruminants can adjust to a lower supply by more efficiently extracting glucose from arterial blood supplies (Miettinen and Huhtanen, 1997). In the present experiment, all blood characteristics tested fell into the normal range for sheep (Kaneko, 1989). There were no differences between sheep fed different silages, except for glucose, which was lower in FTMR-fed sheep than in controls before feeding. It is not clear why this trend was observed, but it could be linked to the higher LAC induced by FTMR feed, and the corresponding lower concentrations of butyric acid but higher concentrations of propionic acid 2 and 4 h after feeding.

4.5. Summary

The effects of fermented TMR silage on the nutritive value, nitrogen retention, ruminal fermentation, methane production, and plasma parameters of feed were evaluated. Four Suffolk sheep (49.5 ± 3.2 kg) were used in a 2 (treatments) × 2 (periods) cross-over design experiment. Experimental treatments included control TMR (i.e., not fermented) or fermented TMR silage (FTMR). Each contained compound feed, WCR silage, dried beet pulp, a vitamin–mineral supplement, and RB. WCR silage (Haenuki) was cultivated using conventional methods in a paddy field, harvested at the full-ripe stage, prepared into a mini roll bale silage (50 kg), stored outdoors (9–32°C) for 240 days of fermentation, and cut to a length of 2 cm. FTMR was adjusted with water to 55% moisture, and then ensiled in drum can silos for fermentation. To prevent control TMR from fermenting, WCR silage was kept apart from the other ingredients and ensiled alone in drum can silos. The other ingredients were mixed and ensiled in separate drum cans. A digestion trial was conducted to investigate apparent digestibility, nitrogen retention, ruminal fermentation characteristics, and plasma parameters over a 5-day test period. For 2 days of the test period, a head hood-type respiration chamber was used to measure methane production. FTMR had significantly lower ($P < 0.05$) DM, OM, NFE, and NFC but significantly higher LAC, EE, CF, ash, and GE than controls. Apparent digestibility of CP, EE, CF, NDF, and GE was higher for FTMR, which also had higher TDN, DCP, and DE levels. There were no significant differences in ruminal pH between TMR types before feeding or 4 h after feeding, although pH was significantly higher in FTMR 2 h after feeding. Total VFA and NH_3-N was higher and butyric acid was lower for FTMR 2 and 4 h after feeding, while propionic acid was higher only 2 h after feeding. FTMR

significantly decreased daily methane emissions per sheep by 25.1% and daily methane energy lost as a percent of GE intake by 25.9%. FTMR had lower glucose before feeding, while all plasma parameters remained within the normal range for sheep. These results show that fermented TMR silage with WCR and food by-products had higher digestibility and resulted in lower methane production than non-fermented TMR, and was also a safe feed for ruminants.

Table 4.1. Chemical composition and gross energy of whole crop rice silage (WCRS), feed concentrate, vitamin–mineral supplement (VMS), beet pulp, and rice bran used in the total mixed ration (TMR)

	WCRS	Concentrate[1]	VMS[2]	Beet pulp	Rice bran
DM[3] (%)	45.6	89.9	91.0	90.7	90.3
Organic matter (OM; %DM)	87.9	91.6	88.6	94.9	87.6
Crude protein (CP; %DM)	10.2	20.6	21.7	8.4	16.8
Ether extract (EE; %DM)	2.6	3.2	3.1	0.7	24.2
Nitrogen-free extract (%DM)	44.0	54.2	49.6	68.5	38.1
Crude fiber (%DM)	31.1	13.6	14.2	17.3	8.4
NFC[4] (%DM)	16.1	30.5	23.2	33.7	17.4
Crude ash (CA; %DM)	12.1	8.4	11.4	5.1	12.4
Acid detergent fiber (%DM)	34.3	16.8	15.7	25.6	10.3
Neutral detergent fiber (%DM)	59.0	37.3	40.6	52.1	29.1
Gross energy (MJ/kg DM)	18.1	18.9	18.4	18.5	22.5

[1]Formula feed ("Koushi Ikusei Special Mash" made by Zenno, TDN: 70.0%, CP: 12.0% in fresh matter).

[2]Commercial vitamin–mineral supplement product (Snow brand seed, Iwate, Japan).

[3]Dry matter.

[4]Nonfibrous carbohydrate (100 – CP – EE – NDF – CA).

Table 4.2. Ingredient proportions and nutrient composition of TMR[1]

	Treatment	
	Control	FTMR[2]
Ingredient		
WCRS[3] (% DM[4])	30	30
Concentrate[5] (% DM)	25	25
Vitamin-mineral supplement[6] (% DM)	1.5	1.5
Beet pulp (% DM)	14	14
RB[7] (% DM)	30	30
Nutrient composition		
CP[8] (% DM)	12.8	12.8
TDN[9] (% DM)	74.3	74.3

[1]Total mixed ration.

[2]Fermented total mixed ration.

[3]Whole crop rice silage.

[4]Dry matter.

[5]Formula feed ("Koushi Ikusei Special Mash" made by Zenno, TDN: 70.0%, CP: 12.0% in fresh matter).

[6]Commercial vitamin–mineral supplement product (Snow brand seed, Iwate, Japan).

[7]Rice bran.

[8]Crude protein.

[9]Total digestible nutrients.

Table 4.3. Chemical composition and gross energy of TMR[1]

	Treatment		SEM[3]	*P* value
	Control	FTMR[2]		
DM[4] (%DM)	68.8[b]	44.5[a]	0.0411	<0.0001
Organic matter (%DM)	90.8[b]	89.8[a]	0.0146	0.0006
Crude protein (CP; %DM)	14.6	15.0	0.1325	0.2139
Ether extract (EE; %DM)	7.4[b]	9.4[a]	0.0168	0.0001
Nitrogen-free extract (%DM)	50.7[b]	46.1[a]	0.2577	0.0076
Crude fiber (%DM)	18.2[b]	19.2[a]	0.1274	0.0310
NFC[5] (%DM)	26.4[b]	19.3[a]	0.5515	0.0177
Crude ash (CA; %DM)	9.2[b]	10.2[a]	0.0146	0.0006
Acid detergent fiber (%DM)	21.0	21.7	0.7246	0.5592
Neutral detergent fiber (NDF; %DM)	42.5	46.1	0.5227	0.0656
Gross energy (MJ/kg DM)	19.5[b]	19.8[a]	0.0004	0.0006

[1]Total mixed ration.

[2]Fermented total mixed ration.

[3]Standard error of the mean.

[4]Dry matter.

[5]Nonfibrous carbohydrate (100 – CP – EE – NDF – CA).

[a,b]Means within rows with different letters differ at $P < 0.05$.

Table 4.4. Fermentative characteristics of TMR[1]

	Treatment		SEM[3]	*P* value
	Control	FTMR[2]		
Dry matter (%)	68.8[b]	44.5[a]	0.04	<0.0001
pH	5.24[b]	3.97[a]	0.01	0.0188
Lactic acid (% DM[4])	0.55[b]	7.34[a]	0.15	<0.0001
Acetic acid (% DM)	0.46[b]	0.99[a]	0.10	0.0090
Propionic acid (% DM)	0.01	0.01	0.00	0.0502
Butyric acid (% DM)	0.03	0.03	0.00	0.4297
NH_3-N/TN[5] (%)	2.63[b]	5.43[a]	0.17	<0.0001
Flieg's score	-	100	-	-
V-score	-	96.2	-	-

[1]Total mixed ration.

[2]Fermented total mixed ration.

[3]Standard error of the mean.

[4]Dry matter.

[5]Total nitrogen.

[a,b]Means within rows with different letters differ at $P < 0.05$.

Table 4.5. Nutrient digestibility, nutrient content, and nitrogen retention in the TMR[1] fed to wethers

	Treatment		SEM[3]	*P* value
	Control	FTMR[2]		
DM intake (g/kg $BW^{0.75}$/day)	53.14	52.66	0.51	0.5486
Apparent digestibility				
Dry matter (%)	65.6	68.9	0.94	0.0518
Organic matter (%)	71.1	73.8	0.84	0.0650
Crude protein (%)	64.8[b]	70.4[a]	0.75	0.0023
Ether extract (%)	78.9[b]	84.0[a]	1.14	0.0282
Nitrogen-free extract (%)	79.9	80.4	0.84	0.7432
Crude fiber (%)	48.2[b]	55.7[a]	1.54	0.0146
Nonfibrous carbohydrate (%)	92.5	91.3	0.86	0.4268
Acid detergent fiber (%)	49.1	54.9	1.86	0.0741
Neutral detergent fiber (%)	58.6[b]	65.5[a]	1.10	0.0043
Gross energy (%)	70.9[b]	74.2[a]	0.83	0.0334
Nutrient content				
Total digestible nutrients (% DM[4])	71.8[b]	75.5[a]	0.70	0.0174
Digestible crude protein (% DM)	9.4[b]	10.5[a]	0.10	0.0004
Digestible energy (MJ/kg DM)	13.8[b]	14.6[a]	0.01	0.0133
Nitrogen retention				
Nitrogen intake (g/day)	23.34	23.98	0.81	0.6105
Fecal excretion of nitrogen (g/day)	8.21[b]	7.08[a]	0.20	0.0081
Urinary excretion of nitrogen (g/day)	9.76[b]	11.81[a]	0.45	0.0185
Nitrogen retention (g/day)	5.38	5.09	0.47	0.6823
Allantoin (g/day)	1.69	1.61	0.17	0.7527

[1]Total mixed ration.

[2]Fermented total mixed ration.

[3]Standard error of the mean.

[4]Dry matter.

[a,b]Means within rows with different letters differ at $P < 0.05$.

Table 4.6. pH, volatile fatty acid (VFA), and ammonia-N of rumen fluid of sheep fed TMR[1]

	Hours after feeding	Treatment		SEM[3]	*P* value
		Control	FTMR[2]		
pH	0	7.28	7.20	0.09	0.5760
	2	6.71[a]	6.33[b]	0.06	0.0039
	4	6.64	6.40	0.11	0.1751
Total VFA (mmol/dL)	0	5.55	5.92	0.32	0.4675
	2	8.84[a]	12.72[b]	0.79	0.0134
	4	8.74[a]	11.62[b]	0.74	0.0386
Acetic acid (A) (mol%)	0	47.87	50.68	0.83	0.0761
	2	53.17	54.61	2.46	0.6944
	4	51.63	55.09	1.10	0.0714
Propionic acid (P) (mol%)	0	33.79	31.64	1.98	0.5045
	2	37.05[a]	39.24[b]	0.54	0.0296
	4	38.02	38.33	1.21	0.8621
Isobutyric acid (mol%)	0	3.46	2.77	1.05	0.6548
	2	0.43	0.41	0.07	0.9035
	4	0.43	0.49	0.10	0.7111
Butyric acid (mol%)	0	14.40	14.42	0.94	0.9863
	2	13.89[a]	8.66[b]	0.73	0.0042
	4	15.12[a]	9.75[b]	1.24	0.0255
Isovaleric acid (mol%)	0	5.49	5.11	0.37	0.5237
	2	1.31	2.89	0.70	0.1626
	4	0.94	2.15	0.49	0.1511
Valeric acid (mol%)	0	1.38	1.37	0.10	0.9324
	2	1.15	1.61	0.35	0.4248
	4	1.05	1.43	0.12	0.0755
A/P[4]	0	1.43	1.64	0.11	0.2363
	2	1.47	1.40	0.12	0.6828
	4	1.36	1.44	0.05	0.3028
NH_3-N (mg/dL)	0	5.64	5.09	0.42	0.4000
	2	8.77[a]	18.33[b]	1.58	0.0055
	4	5.93[a]	16.20[b]	1.68	0.0071

[1]Total mixed ration.
[2]Fermented total mixed ration.
[3]Standard error of the mean.
[4]Acetic acid/propionic acid ratio.
[a,b]Means within rows with different letters differ at $P < 0.05$.

Table 4.7. Energy intake, methane emission, and heat production of sheep fed TMR[1]

	Treatment		SEM[3]	*P* value
	Control	FTMR[2]		
Gross energy (GE) intake				
(kJ/day)	19559.2	19810.4	672.3	0.8058
(kJ/kg $BW^{0.75}$/day)	1038.1	1045.3	1.0	0.6433
Methane emission				
(L/day)	39.84^{b}	29.84^{a}	0.82	0.0001
(L/kg DMI[4]/day)	39.87^{b}	30.03^{a}	1.29	0.0017
(L/kg DDM[5]/day)	60.87^{b}	43.61^{a}	2.10	0.0012
(L/kg $BW^{0.75}$/day)	2.12^{b}	1.58^{a}	0.1	0.0007
Methane energy				
(kJ/day)	1576.4^{b}	1180.8^{a}	32.6	0.0001
(kJ/kg $BW^{0.75}$/day)	83.8^{b}	62.5^{a}	2.3	0.0007
% of GE in take	8.08^{b}	5.99^{a}	0.26	0.0013

[1]Total mixed ration.

[2]Fermented total mixed ration.

[3]Standard error of the mean.

[4]Dry matter intake.

[5]Digestible dry matter.

[a,b]Means within rows with different letters differ at $P < 0.05$.

Table 4.8. Hematocrit, glucose, urea-N, cholesterol, GOT[1], and GPT[2] in blood plasma of sheep fed fermented or unfermented TMR[3]

	Hours after feeding	Treatment		SEM[5]	*P* value
		Control	FTMR[4]		
Hematocrit (%)	0	33.63	35.13	1.29	0.4674
	2	34.50	36.00	1.21	0.4330
	4	30.44	32.81	1.63	0.3992
Glucose (mg/dL)	0	63.87[b]	57.04[a]	1.27	0.0092
	2	60.21	57.30	1.64	0.3537
	4	65.77	66.40	2.26	0.8798
Urea nitrogen (mg/dL)	0	13.06	14.20	1.20	0.5283
	2	16.26	14.95	1.54	0.5708
	4	16.43	14.59	0.67	0.1035
GOT (IU/L)	0	53.78	47.66	5.3	0.4462
	2	48.24	45.51	3.91	0.6447
	4	45.31	47.54	3.11	0.6482
GPT (IU/L)	0	6.09	4.94	0.57	0.2231
	2	5.05	4.82	0.44	0.7229
	4	4.91	4.79	0.39	0.8326

[1]Glutamic oxaloacetic transaminase.

[2]Glutamic pyruvic transaminase.

[3]Total mixed ration.

[4]Fermented total mixed ration.

[5]Standard error of the mean.

[a,b]Means within rows with different letters differ at $P < 0.05$.

Chapter 5

In vitro examination of the mechanism of methane suppression by whole crop rice total mixed ration silage

5.1. Introduction

Many studies have described the modification of methane production by manipulating feed rations without using additives. For example, Blaxter and Clapperton (1965) showed that methane production (kJ per 100 kJ in feed) decreased simply by feeding more. At a maintenance-level feeding rate, methane production increases with increasing apparent digestibility, but the opposite is true when feeding at levels three times maintenance (Van Nevel and Demeyer, 1996). Structural carbohydrates such as cellulose and hemicellulose yield more methane per unit fermented substrate (Holter and Young, 1992), while using more feed concentrate lowers pH in the rumen and thus inhibits methane bacteria (Demeyer and Henderickx, 1967). Decreased methanogenesis is accompanied by higher molar proportions of propionate, in agreement with interspecies hydrogen transfer reactions (Van Nevel et al., 1974). Taking into account this relationship, any intervention at the dietary level (ration composition and feeding) should shift VFA proportions in the rumen in favor of propionate and thus result in lower methane production per unit of feed fermented (Van Nevel and Demeyer, 1996). Such a decreased acetate-to-propionate (A/P) ratio can be induced by several interventions, such as increasing the proportion of easily fermentable carbohydrates by decreasing roughage amounts in the diet (Oshio et al., 1987; Cecava et al., 1990); physically grinding or pelleting roughage or heat-treating grain (Zinn, 1987; Moore et al., 1992); lessening the

frequency of feeding, which tends to increase molar proportions of propionate (Sutton et al., 1986); or feeding more hay concentrate-based diets (Jentsch and Hoffmann, 1994). It has long been recognized that adding cereal grains to ruminant diets helps decrease methane and increase propionate production, although the cause of this fermentation shift is not clear (Van Kessel and Russell, 1996). Some starch-fermenting ruminal bacteria produce propionate, but starch feeding can also decrease ruminal pH, leading to a marked shift in ruminal bacteria; moreover, methanogenesis is pH-dependent, and no methane may be detected at pH values less than 6.0 (Van Kessel and Russell, 1996). Recent reports (Demirel and Scherer, 2008) have suggested that the roles of acetotrophic and hydrogenotrophic methanogens are of paramount importance in the anaerobic conversion process, no matter what type of substrate is being digested, but that the optimum pH range for acetotrophic methanogens is between 6.6 and 7.3, and acetotrophic methanogens are strongly inhibited below a pH of 6.2.

The results described in chapter 2 and 4 showed that *in vitro* methane production decreases when rich-lactic acid whole crop rice (WCR) total mixed ration (TMR) silage is incubated, and that sheep fed fermented TMR (FTMR) silage at maintenance level exhibit lower methane production regardless of the greater apparent digestibility of the FTMR. However, the causes of these fermentation shifts are unclear. Therefore, the objectives of the following experiments were to examine the effects of pH and LAC on the A/P ratio and methane production *in vitro*.

5.2. Materials and Methods

5.2.1. Incubation trial 1

This experiment evaluated the effects of adding lactic acid or starch on *in vitro* dry

digestibility and methane production in WCR TMR silage.

5.2.1.1. Donor wethers

Two adult wethers (average initial body weight, 78.5 kg) fitted with rumen cannulae were used as donors of ruminal fluid. The animals were fed basal diets of 50% reed canary grass (*Phalaris arundinacea* L.) hay and 50% commercial feed concentrate (Koushi-Ikusei-Special; Kitanihon-Kumiai-Feed, Miyagi, Japan) at a maintenance energy level (20 g DM/kg body weight daily) and had free access to clean drinking water. They were fed once daily at 09:00 h and were cared for according to the animal care and use guidelines of the Faculty of Agriculture, Yamagata University.

5.2.1.2. In vitro incubations

TMR silages prepared as described in Chapter 1 (TC:RB:WGTW = 10%:10%:10%) were used as substrates for *in vitro* cultures. TMR silages were ground to pass through a 0.5-mm filter.

Rumen fluid was collected through rumen cannulae 2 h after feeding and diverted into plastic bottles. The fluids were filtered through four layers of cheesecloth and then combined on an equal volume basis. The compound filtrate was bubbled with CO_2 to keep it anaerobic, and stored at 39°C in a water bath. The compound filtrate was used to inoculate the fermentors.

The buffer was CO_2-bubbled McDougal's artificial saliva (pH 6.8; McDougal, 1948). The experiment treatments consisted of controls (no additions) and silage with either lactic acid, starch, or lactic acid + starch added.

The compound filtrate was mixed with McDougal's artificial saliva at a ratio of 1:4 (v/v). Then 50 ml diluted rumen fluid were transferred to 128-ml serum bottles containing 0.5 g ground TMR (control), 5% lactic acid, 5% starch, or 5% lactic acid and starch (all

percentages of FM), and flushed with O_2-free CO_2. The tubes were capped with a butyl rubber stopper and sealed with an aluminum cap. Incubation was done in triplicate at 39°C for 6 h in a water bath with a reciprocal shaker (100 strokes/min).

5.2.1.3. In vitro methane production

To terminate the fermentation by bacteria at the end of incubation, 25 μL of formaldehyde solution (35%) were injected into serum bottles, which were immediately sealed with rubber stoppers and then cooled at room temperature. Gas samples were collected by air syringe from the serum bottles and injected into a gas chromatograph (GC323; GL Sciences, Tokyo, Japan) equipped with a thermal conductivity detector and stainless steel column (WG-100 SUS 1.8 m ×6.35 mm OD (outer diameter)), and methane production from each serum bottle was measured. The analytical conditions were as follows: column oven temperature, 50°C; injector temperature, 50°C; detector temperature, 50°C.

5.2.1.4. In vitro DM digestibility and VFA production

Separate sub-samples of the supernatant were taken to determine VFA concentration. The bottles were rinsed with warm water to remove solid residues. The residues were then oven-dried at 60°C and stored for further analysis. A total of 2 g dried residue was oven-dried at 135°C and stored for later analysis of DM digestibility (Horii et al., 1971).

To measure total VFA, ruminal fluid was steam-distilled and titrated using sodium hydroxide (Hamada, 1971). The dried VFA salt was separated and quantified using gas chromatography (G-5000A; Hitachi, Tokyo, Japan) equipped with a thermal conductivity detector and a glass column (Unisole F-200, 3.2 mm × 2.1 m). The analytical conditions were as follows: column oven temperature, 140°C; injector temperature, 210°C; detector temperature, 250°C.

5.2.2. Incubation trial 2

This experiment evaluated the effects of ruminal pH (adjusted using phosphoric acid buffer) and fermented TMR silage with a high LAC on *in vitro* DM digestibility, ruminal fermentation, and methane emission.

5.2.2.1. Donor wethers

The two wethers described in 5.2.1.1 were used.

5.2.2.2. In vitro incubations

Non-fermented WCR TMR (control) or fermented WCR TMR silage (FTMR), prepared as described in Chapter 4, were used as substrates for *in vitro* cultures. The TMR silages were ground to pass through a 0.5-mm filter.

The rumen fluid was collected and treated as described in 5.2.1.2. In this experiment, McDougal's artificial saliva was replaced with phosphate buffers with pH values adjusted to 5, 6, and 7, respectively. To prepare phosphate buffer solution, 0.947% Na_2HPO_4 (A) and 0.906% KH_2PO_4 (B) were first prepared. The pH 5 buffer (1000 ml) consisted of A (10 ml) and B (990 ml); pH 6 buffer (1000 ml) consisted of A (120 ml) and B (880 ml); and pH 7 buffer (1000 ml) consisted of A (611 ml) and B (389 ml). The three phosphate buffers were stored at 4°C until use.

The compound filtrate of rumen fluid was mixed with the three buffers at a ratio of 1:4 (v/v), respectively. Serum bottles (128 ml) with 0.5 g ground control TMR or 0.5 g ground FTMR were flushed with O_2-free CO_2, respectively, 50 ml diluted rumen fluid were added, and then the tubes were immediately capped with a butyl rubber stopper and sealed with an aluminum cap. Incubation was done in sixplicate at 39°C for 6 h in a water bath with a reciprocal shaker (100 strokes/min).

5.2.2.3. In vitro methane production and ruminal pH

To terminate the fermentation by bacteria at the end of incubation, 25 μL of formaldehyde solution (35%) were injected into the serum bottles, which were immediately sealed with rubber stoppers and then cooled at room temperature until methane measurements were taken. The final pH of the three serum bottles of gas was measured using a glass electrode pH meter (Horiba D-21; Horiba, Kyoto, Japan). Gas samples were collected by air syringe from the serum bottles and injected into a gas chromatograph (GC323; GL Sciences, Tokyo, Japan) equipped with a thermal conductivity detector and stainless steel column (WG-100 SUS 1.8 m ×6.35 mm OD (outer diameter)). The analytical conditions were as follows: column oven temperature, 50°C; injector temperature, 50°C; detector temperature, 50°C.

5.2.2.4. In vitro DM digestibility

Separate sub-samples of the supernatant were taken to determine VFA concentration. The bottles were rinsed with warm water to remove solid residues. The residues were then oven-dried at 60°C and stored for further analysis. A total of 2 g dried residue was oven-dried at 135°C and stored for later analysis of DM digestibility (Horii et al., 1971).

5.2.3. Incubation trial 3

This experiment evaluated the effects of the pH of rumen fluid taken either before or after feeding, and the effects of high-LAC FTMR silage, on *in vitro* DM digestibility, ruminal fermentation, and methane emission.

5.2.3.1. Donor wethers

The two wethers described in 5.2.1.1 were used.

5.2.3.2. In vitro incubations

Non-fermented WCR TMR (control) or fermented WCR TMR silage (FTMR), prepared as described in Chapter 4, was used as the substrate for *in vitro* cultures. The TMR silages were ground to pass through a 0.5-mm filter.

To obtain rumen fluid with different pH values, samples were collected through the rumen cannulae before feeding and 2 and 4 h after feeding, diverted into plastic bottles, and filtered through four layers of cheesecloth. The two filtrates were then combined on an equal volume basis. The three resulting compound filtrates were bubbled with CO_2 to keep them anaerobic and stored at 39°C in a water bath. The three compound filtrates were used to inoculate the fermentors. The buffer was CO_2-bubbled McDougal's artificial saliva (pH 6.8; McDougal, 1948).

The three compound filtrates were mixed with McDougal's artificial saliva at a ratio of 4:1 (v/v), respectively. The pH values of the three diluted rumen fluids were 7.08 (before feeding), 5.86 (2 h after feeding), and 6.56 (4 h after feeding). Then 50 ml of diluted rumen fluid were transferred to 128-ml serum bottles containing 0.5 g ground control or FTMR, and flushed with O_2-free CO_2. The tubes were capped with a butyl rubber stopper and sealed with an aluminum cap. Incubation was done in sixplicate for every treatment at 39°C for 6 h in a water bath with a reciprocal shaker (100 strokes/min).

5.2.3.3. In vitro methane production and ruminal pH

To terminate fermentation by bacteria at the end of incubation, 25 μL of formaldehyde solution (35%) were injected into the serum bottles, which were immediately sealed with rubber stoppers and then cooled at room temperature until methane measurements were taken. The final pH values of the three samples were measured using a glass electrode pH meter (Horiba D-21; Horiba, Kyoto, Japan). Gas samples were collected by air syringe from the serum bottles and injected into a gas

chromatograph (GC323; GL Sciences, Tokyo, Japan) equipped with a thermal conductivity detector and stainless steel column (WG-100 SUS 1.8 m ×6.35 mm OD (outer diameter)). The analytical conditions were as follows: column oven temperature, 50°C; injector temperature, 50°C; detector temperature, 50°C.

5.2.3.4. In vitro DM digestibility and VFA production

Separate sub-samples of the supernatant were taken to determine VFA concentration. The bottles were rinsed with warm water to remove solid residues. The residues were then oven-dried at 60°C and stored for further analysis. A total of 2 g dried residue was oven-dried at 135°C and stored for later analysis of DM digestibility (Horii et al., 1971).

To measure total VFA, ruminal fluids were steam-distilled and titrated using sodium hydroxide (Hamada, 1971). Dried VFA salt was separated and quantified using gas chromatography (G-5000A; Hitachi, Tokyo, Japan) equipped with a thermal conductivity detector and a glass column (Unisole F-200, 3.2 mm × 2.1 m). The analytical conditions were as follows: column oven temperature, 140°C; injector temperature, 210°C; detector temperature, 250°C.

5.2.4. Statistical analyses

Analyses were conducted using the general linear model procedure (SAS institute, Cary, NC, USA). The *in vitro* DM digestibility, ruminal methane, VFA concentrations, and A/P ratio in trial 1 were subjected to a one-way ANOVA, while *in vitro* DM digestibility and ruminal pH in trial 2 were subjected to a two-way ANOVA, and *in vitro* DM digestibility, ruminal pH, methane production, VFA concentrations, and A/P ratio were subjected to a two-way ANOVA. Tukey's test was used to identify differences ($P < 0.05$) between means.

5.3. Results

5.3.1. Incubation trial 1

The addition of lactic acid did not affect *in vitro* DM digestibility of TMR (Table 5.1), methane production per digestible DM (L/kg DDM), total VFA, acetic acid, propionic acid, or A/P ratio, while the addition of starch significantly increased ($P < 0.05$) the *in vitro* DM digestibility of TMR and VFA content, and decreased methane production (L/kg DDM) and molar concentrations of valeric acid. Similarly, the addition of lactic acid + starch also increased DM digestibility and VFA content, and decreased methane production (L/kg DDM).

However, because neither the addition of starch or lactic acid + starch affected either propionic acid or the A/P ratio, these results do not illustrate the effects of high-LAC TMR on DM digestibility, A/P, or methane production. Therefore, the following experiment was conducted.

5.3.2. Incubation trial 2

Methane was not detected in all buffer treatments, and therefore VFA concentration could not be determined.

Buffer pH significantly affected the *in vitro* DM digestibility and final ruminal pH (Table 5.2). Diet did not affect *in vitro* DM digestibility, but had a significant affect on final ruminal pH. There were no interactions between buffer and diet. When buffer pH was 7, the DM digestibility of FTMR was higher than controls. Final ruminal pH was higher for FTMR than controls for all buffer treatments.

However, because methane was not detected in any buffer treatment, these results do not illustrate the effects of high-LAC TMR on DM digestibility, A/P ratio, or methane

production. Therefore, the following experiment was conducted.

5.3.3. Incubation trial 3

As shown in Table 5.3, the initial pH of the mixed ruminal fluid significantly affected the *in vitro* DM digestibility, methane production (L) per kg DM, methane production (L) per kg DDM, final pH, total VFA, and propionic acid concentration. The diet also remarkably affected the *in vitro* DM digestibility, methane production (L) per kg DM, methane production (L) per kg DDM, total VFA, acetic acid, propionic acid, valeric acid, and the A/P ratio, and there was a significant interaction effect between pH and diet on methane production (L) per kg DM.

When initial pH was 7.08, the final pH for controls and FTMR were 6.47 and 6.54, respectively; total VFA concentration was higher for FTMR, but DM digestibility, methane production, and A/P ratio did not differ significantly between the two silages, although FTMR increased DM digestibility by 4.2% and decreased methane production and the A/P ratio by 5.2% and 6.7%, respectively. When initial pH was 6.56, the final pH for controls and FTMR were 6.34 and 6.38; FTMR increased (but not significantly) DM digestibility by 9.6%, significantly decreased methane production (L) per kg DM and per kg DDM, and slightly decreased (but not significantly) the A/P ratio by 2.3%. When initial pH was 5.86, DM digestibility and methane production were lower but total VFA and propionic acid were higher compared to the other buffers. Although there were no significant differences in DM digestibility and methane production (L) per kg DM between controls and FTMR, the FTMR increased DM digestibility by 14.2% and decreased methane production (L) per kg DM by 11.5%, decreased methane production (L) per kg DDM, decreased acetic acid and A/P ratio, and increased propionic and valeric acids, compared to controls.

5.4. Discussion

Lactic acid is an important intermediate of carbohydrate fermentation in the rumen of grain-fed animals, and in animals offered silage it may make up as much as 15% of consumed DM (Gill et al., 1986). The biochemical pathways for the conversion of lactate to acetate, propionate, and butyrate are well documented (Baldwin et al., 1962), but the factors that determine the relative amounts of lactate that are metabolized by the individual pathways are not well understood (Gill et al., 1986). Both bacterial species and rumen pH may influence the relative amount of individual VFA produced (Van Kessel and Russell, 1996; Lana et al., 1998; Russell, 1998). Furthermore, ruminal lactic acid may be absorbed (Waldo and Schultz, 1956) and could thus contribute to glucose metabolism (Gill et al., 1986). The efficient utilization of digestion end products can be significantly influenced by the nature of the VFA produced and absorbed in relation to the relative abundance of gluconeogenic (propionate) and non-gluconeogenic (acetate and butyrate) precursors (MacRae and Lobley, 1982). Therefore, depending on its metabolic fate in the rumen, lactic acid arising from grass silage may have a significant effect on the overall efficiency of energy utilization in silage diets (Gill et al., 1986). Acetate is the major end-product of lactate metabolism, but a diet high in carbohydrate or lactate or both increases propionate production (Baldwin et al., 1962). However, propionate is the principal end product of lactate metabolism in ruminants fed grass silage (Chamberlain et al., 1983). Furthermore, when high starch diets are fed to ruminants, *Streptococcus bovis* often predominates in the rumen and produces lactate as the major fermentation product (Asanuma and Hino, 2002). When lactate is secondarily fermented in the rumen by lactate-utilizing bacteria such as *Megasphaera elsdenii, Selenomonas ruminantium,* and

Veillonella parvula, propionate is generally a major product (Dawson et al., 1997; Russell and Wallace, 1997), and this may reduce methanogenesis as electrons are used for propionate formation (Asanuma and Hino, 2002).

In incubation trial 1, although lactic acid, starch, or lactic acid + starch were directly infused into rumen fluid and incubated together with TMR, neither acetic acid nor propionic acid or butyric acid differed among the four treatments. This result is not consistent with previous studies (Baldwin et al., 1962; Dawson et al., 1997; Russell and Wallace, 1997; Asanuma and Hino, 2002). Compared to controls, incubation with lactic acid did not affect DM digestibility, methane production, or total VFA concentration. However, adding either starch or lactic acid + starch increased DM digestibility and total VFA concentration. These results are consistent with previous studies (Cameron et al., 1991; Knowlton et al., 1998; Reynolds et al., 2001; Bechmant and Weiss, 2005) showing that starch increases DM digestibility and total VFA production but decreases methane production and that when ruminants eat high-starch diets their rumen has lower acetate but higher propionate concentrations (Silveira et al., 2007). Therefore, to investigate the effect of pH and a high-LAC diet on the A/P ratio and methane production, incubation trial 2 was conducted.

In incubation trial 2, control TMR and high-LAC FTMR were incubated in rumen fluid with three different pH values, respectively, but methane was not detected in any treatment. Phosphate may have inhibited methane production.

Therefore, incubation trial 3 used rumen fluids collected before feeding and 2 and 4 h after feeding to obtain samples with high, low, and intermediate pH, respectively. Although the amount of protozoa or bacteria may differ among rumen fluids collected at different times, any such difference should not affect the comparison between control

TMR and FTMR. Compared to controls, FTMR silage increased DM digestibility in all treatments, consistent with previous reports (Kanjanapruthipong and Buatong, 2004; Shioya, 2008). When hay or cracked corn was incubated with rumen fluid obtained from ruminants fed 90% feed concentrate, pH decreased to 6.5–5.3 (Russell, 1998), the A/P ratio decreased (Lana et al., 1998; Russell, 1998), and methane production was highly correlated with A/P but dependent on pH and substrate. In the present experiment, when the initial pH was 7.08, the final pH for controls and FTMR were 6.47 and 6.54, respectively; FTMR did not significantly increase propionic acid or decrease either the A/P ratio or methane production. When the initial pH was 6.56, the final pH for controls and FTMR was 6.34 and 6.38, respectively, similar to previous reports (Lana et al., 1998; Russell, 1998), and although FTMR decreased methane production, it did not decrease the A/P ratio. When initial pH was 5.86, FTMR significantly decreased methane production (L) per kg DDM, acetic acid concentration, and A/P ratio, and increased the concentrations of propionic acid and valeric acid. This was again likely due to the production of propionate by lactate-utilizing bacteria, which reduce methanogenesis by using electrons (Dawson et al., 1997; Russell and Wallace, 1997).

5.5. Summary

Adding lactic acid did not affect the *in vitro* DM digestibility, methane production, and total VFA concentration, while adding either starch or lactic acid + starch increased *in vitro* DM digestibility and total VFA. Phosphate might inhibit methane production. The FTMR silage increased the *in vitro* DM digestibility in all treatments and decreased the A/P ratio and methane production, which, however, was also affected by ruminal pH.

Table 5.1. Measurements of dry matter digestibility, methane production, and VFA concentration *in vitro* after 6 h of incubation of TMR[1] silage

	Treatment				SEM[2]	*P* value
	Control	Lactic acid	Starch	Lactic acid + Starch		
DM[3] digestibility (%)	38.0[b]	36.5[b]	41.3[a]	43.2[a]	0.40	<0.0001
Methane production (L/kg DDM[5])	15.1[a]	15.2[a]	14.1[b]	13.1[c]	0.18	0.0003
Total VFA (mmol/dL)	5.4[b]	5.3[b]	5.9[a]	5.8[a]	0.04	<0.0001
Acetic acid (mol%)	44.2	45.3	45.8	45.4	0.81	0.705
Propionic acid (mol%)	36.9	36.3	37.6	37.4	0.47	0.4475
Isobutyric acid (mol%)	0.6	0.2	0.2	0.1	0.13	0.2833
Butyric acid (mol%)	12.4	11.7	11.5	11.8	0.75	0.8903
Isovaleric acid (mol%)	2.4	3.0	1.9	2.1	0.47	0.466
Valeric acid (mol%)	3.44[a]	3.41[ab]	3.07[b]	3.16[ab]	0.08	0.0257
A/P[4]	1.2	1.25	1.22	1.22	0.04	0.8989

[1]Total mixed ration

[2]Standard error of the mean

[3]Dry matter

[4]Acetic acid/propionic acid ratio

[a,b]Means within rows with different letters differ at $P < 0.05$

Table 5.2. Measurements of dry matter digestibility and ruminal pH *in vitro* after 6 h of incubation of TMR[1] silages

	Buffer pH:5		Buffer pH:6		Buffer pH:7		SEM[3]	Effects		
	Control	FTMR[2]	Control	FTMR	Control	FTMR		Buffer	TMR	Buffer×TMR
DM[3] digestibility (%)	33.94	35.44	30.12	32.08	40.75[a]	44.25[b]	1.36	0.0002	0.1648	0.8644
pH	4.97[a]	5.06[b]	5.13[a]	5.22[b]	5.62[a]	5.69[b]	0.02	<0.0001	<0.0001	0.8715

[1]Total mixed ration

[2]Fermented total mixed ration

[3]Standard error of means

[4]Dry matter

[a,b]Means within rows with different letters differ at $P < 0.05$

Table 5.3. Measurements of dry matter digestibility, methane production, and VFA concentration *in vitro* after 6 h of incubation of TMR[1] silages

	pH:7.08		pH:5.86		pH:6.56		SEM[3]	Effects		
	Control	FTMR[2]	Control	FTMR	Control	FTMR		pH	TMR	pH×TMR
DM[4] digestibility (%)	50.82	52.93	36.77	41.98	46.26	50.70	1.62	<0.0001	0.0169	0.6575
Methane production (L/kg DM)	8.75	8.63	4.07	3.6	7.59[a]	6.53[b]	0.14	<0.0001	0.009	0.031
Methane production (L/kg DDM[5])	17.23	16.34	11.15[a]	8.58[b]	16.48[a]	12.90[b]	0.49	<0.0001	0.0001	0.0721
pH	6.47	6.54	5.66	5.62	6.34	6.38	0.05	<0.0001	0.6323	0.5521
Total VFA (mmol/dL)	6.60[a]	7.06[b]	8.53	8.42	7.47	7.69	0.06	<0.0001	0.0046	0.0036
Acetic acid (A) (mol%)	44.39	42.63	46.35[b]	42.98[a]	44.63	45.10	0.51	0.1057	0.0104	0.0307
Propionic acid (P) (mol%)	32.93	34.01	35.43[b]	36.98[a]	34.91	35.99	0.33	0.0001	0.0019	0.7889
Butyric acid (mol%)	18.54	17.48	15.40[b]	16.93[a]	17.24	15.94	0.63	0.1026	0.6901	0.2048
Isovaleric acid (mol%)	2.25	3.19	0.79	0.71	0.69	0.70	0.42	0.0130	0.5898	0.6934
Valeric acid (mol%)	1.66	2.36	1.94[b]	2.41[a]	2.40	2.27	0.15	0.2584	0.0435	0.1200
A/P[6]	1.35	1.26	1.31[b]	1.16[a]	1.28	1.25	0.03	0.1444	0.0046	0.2024

*Ruminal fluid was obtained from two ruminally fistulated sheep before the morning feeding and 2 and 4 h after feeding.

[1]Total mixed ration

[2]Fermented total mixed ration

[3]Standard error of means

[4]Digestible dry matter

[5]Digestible dry matter

[6]Acetic acid/propionic acid ratio

[a,b]Means within rows with different letters differ at $P < 0.05$

Chapter 6

General discussion

An obvious way to improve Japan's low feed self-sufficiency rate is by producing rather than importing feed for domestically raised animals (Ogino et al., 2008). Therefore, utilizing WCR and food by-products to prepare TMR silage would help address this matter. In addition, it would also return into use currently abandoned paddy fields and decrease waste and pollution, as most food by-products are presently burned, dumped into landfills, or released into the local environment (Kondo et al., 2004a; Xu et al., 2007a; Xu et al., 2007b; Xu et al., 2008). Furthermore, TMR silage may alleviate energy costs associated with drying and transporting wet byproducts and facilitate preservation in silos regardless of the composition of byproducts (Imai, 2000). Feeding TMR to ruminants also stabilizes rumen function and helps prevent self-selection by the animals (Coppock et al., 1981). The use of TMR silage also reduces labor costs, as measuring and mixing feed rations is not necessary. Moreover, unpalatable byproducts may be incorporated into rations, because the fermentation process during ensiling often improves odor and flavor. In addition, TMR silage appears to resist aerobic deterioration after a sufficient ensiling period (Wang and Nishino, 2008b).

The present series of studies evaluated the effects of food by-products on the fermentation characteristics of WCR TMR silage, and the nutrient digestibility, nitrogen retention, ruminal fermentation characteristics, methane production, and animal plasma parameters related to various silages including high-LAC FTMR silage.

6.1. Fermentation quality

WCR is usually insufficient in sugars and lactic acid bacteria, and thus WCR silage typically has a low LAC, complicating its long-term storage, which largely depends on lactic acid fermentation (Cai et al., 2003). WCR silage with either *Chikuso-1* or FJLB added has better fermentation characteristics (Cai et al., 2003; Hiraoka et al., 2003; Takahashi et al., 2005), especially when the two types of LAB are used in combination. In addition, adding NFC-rich food by-products may improve NFC-deficient WCR.

The first experiment, described in Chapter 2, clearly demonstrated that both LAB and the ration formula of food by-products significantly influenced the fermentation quality of WCR TMR silage. Although all silages were high quality, with low pH, high LAC, low NH_3-N/TN, and high Flieg's and V-scores, adding LAB decreased pH and increased LAC, consistent with previous reports (Cai et al., 2003; Hiraoka et al., 2006b). Of the various byproduct ration formulas investigated, the addition of 30% TC increased LAC the most, likely because TC has more NFC than WGTW, and RB has a large amount of oxidation lipid, which prevents lactic acid fermentation (Yokota and Ohshima, 1997). Moisture content may also have a major effect on fermentation quality, and Xu et al. (2004b) found that adjusting the moisture level to 65% led to the best-quality TMR silage. Similarly, Seki et al. (2000) reported that a suitable proportion of food byproduct for quality TMR is 15% per DM of TMR. However, to take full advantage of the benefits of food by-products, the studies presented herein prepared TMR silages with 30% by-products per DM of TMR; the content of CP and TDN met the requirements for the fattening prophase beef cattle (Japanese Feeding Standard for Beef Cattle; Agriculture, Forestry, and Fisheries Research Council Secretariat, MAFF, 2000).

Experiment 2, described in Chapter 2, assessed the effects of preparing WCR TMR

with molasses and LAB on LAC, ruminal fermentation, and methane production. Molasses is a fermentable carbohydrate (Maiga and Schingoethe, 1997) that has been used successfully with grass silage (Archibald et al., 1960; Alli et al., 1984; Wuisman et al., 2006; Wang and Nishino, 2008b). WCR TMR silage with molasses and LAB increased LAC and reduced NH_3-N concentration, consistent with previous reports (Alli et al., 1984; Cai, 2001; Cai et al., 2003).

6.2. Nutrient digestibility

In experiment 1 and 2, described in Chapter 2, TMR silage with LAB or LAB and molasses increased *in vitro* DM digestibility, likely because the high levels of LAB shortened the activity period of other microorganisms in the initial stage of fermentation, thereby decreasing DM loss (Cai and Ohmomo, 1995; Cai et al., 1999; Cai, 2001; Shioya and Cai, 2004; Hiraoka et al., 2005, 2006a,b). These results are in agreement with previous studies showing improved CF and ADF digestibilities (Takahashi et al., 2005); improved apparent digestibilities of DM, OM, CF, and NFE of alfalfa (Cao et al., 2002); and increased *in situ* DM disappearance in crop silage, stover silage, and WCR silage with the addition of LAB (Yamamoto et al., 2004).

Ration formula also had an effect. Similar to the first experiment assessing ration vs. fermentation quality, TMR silages with 30% RB led to the best results, increasing DM digestibility after 6 h incubation. This was likely due to the high CP and EE content of RB and because it is a high-energy feed (Warren and Farrell, 1990; Forster et al., 1993; Yokota and Ohshima, 1997; Amissah et al., 2003; Enishi and Kawashima, 2003). However, in experiment 2, described in Chapter 3, TMR silages with 30% TC increased nutrient digestibility *in vivo*, while in an *in situ* incubation experiment, TMR silage with

RB exhibited more DM, CP, and NDF degradation than TMR with 30% TC. These results show that TMR silages with 30% RB had higher *in vitro* DM digestibility but lower *in vivo* nutrient digestibility than TMR with 30% TC (Cao et al., in press, b).

6.3. Nitrogen retention

In experiment 2, described in Chapter 3, although nitrogen intake for TMR silages with TC and WGTW was higher than for RB or control silages, based on urinary excretion, nitrogen retention did not differ among the silages (Cao et al., in press, b). In experiment 1, described in Chapter 4, animals that ate FTMR excreted less fecal nitrogen, but more urinary nitrogen, and therefore FTMR silage also did not affect nitrogen retention, consistent with some previous reports (Takahashi et al., 2005; Horiguchi and Takahashi, 2007). However, Cao et al. (2002) reported that alfalfa TMR silage without LAB increased nitrogen retention.

6.4. Ruminal fermentation characteristics

In vitro incubation experiments in Chapter 2 showed that although TMR silage with LAB did not affect the concentration of total VFA in the rumen, it decreased acetic acid and increased propionic acid. This is similar to previous reports showing a tendency for increased propionic acid in cows 1 or 2 h after feeding on alfalfa silage with LAB (Cao et al., 2002); increased propionic acid but decreased A/P ratio in sheep fed WCR silage with LAB (Takahashi et al., 2005); and increased propionic acid but unaffected total VFA 2 h after feeding on green soybean stover silage with LAB (Horiguchi and Takahashi, 2007). Increases in propionic acid concentrations in rumen fluid might be attributable to high LACs in silage, because when pH is appropriate, rumen microflora convert lactic acid to propionate (Baldwin et al., 1962; Leng, 1970; Van Kessel and Russell, 1996; Lana et al., 1998; Russell, 1998; Moss et al., 2000; Takahashi et al., 2005). However, Weinberg et al.

(2003b) found that silage with LAB increased total VFA concentrations in rumen fluid compared to silage without LAB, while Weinberg et al. (2004) found that all silages (with and without LAB) had a consistent effect on total VFA content compared to diets without silage, and that LAB passed from silage samples to rumen fluid *in vitro* and persisted.

WCR TMR silage with M-LAB increased acetic acid and decreased butyric acid, but despite a high LAC, did not affect either propionic acid concentrations or the A/P ratio. Wing et al. (1998) reported similar results with a diet that included 6% molasses. However, Waldo and Schultz (1960) reported that a diet with molasses reduced acetic acid and increased butyric acid. Why M-LAB in the present study increased acetic acid and decreased butyric acid is not clear.

In experiment 1, described in Chapter 4, FTMR decreased ruminal pH 2 h after feeding but remained within the normal range (Russell and Hino, 1985). The content of total VFA increased for both controls and FTMR from before feeding to 2 h after feeding, but then slightly decreased 4 h after feeding. FTMR increased total VFA concentrations 2 and 4 h after feeding. The consumption of readily fermentable carbohydrates generally leads to a marked postprandial decrease in ruminal pH (Nocek, 1997; Chaucheyras-Durand et al., 2008), and the FTMR likely had more fermentable matter due to its high nutritive digestibility, resulting in a greater VFA and lower pH. Similar to total VFA, the molar concentrations of acetic and propionic acid also increased for both controls and FTMR from before feeding to 2 and 4 h after feeding; however, both acids tended to increase more for FTMR than for controls before feeding and 4 h after feeding. This may have occurred because after feeding, the concentrate, NFC, and/or effective fiber from the feed may have been degraded by bacteria, producing propionic acid and lowering ruminal pH, which in turn, after reaching 5.3 (Russell, 1998) or 5.7 (Lana et al.,

1998), decreased the A/P ratio (Lana et al., 1998; Russell, 1998). At the same time, lactic acid either from the FMTR or from bacterial fermentation may have converted hydrogen to propionic acid (Moss et al., 2000). In addition, FTMR with a high fiber content (e.g., CF, NDF) produces more acetic acid than a low-fiber diet (Russell, 1998), which could explain why FTMR had a tendency to increase acetic acid compared to controls. Feeding wormwood (*Artemisia montana*) silage instead of rice straw may increase ruminal propionic and butyric acid (Kim et al., 2006). In contrast, the present study showed that feeding FTMR silage instead of non-fermented TMR increased ruminal propionic acid but decreased ruminal butyric acid 2 and 4 h after feeding. The reason for this is not clear. Finally, similar to total VFA content, NH_3-N concentration for both controls and FTMR increased 2 h after feeding and tended to decline from 2 to 4 h after feeding; however, FTMR led to more NH_3-N production than did control feed 2 and 4 h after feeding, possibly due to its higher CP digestibility.

6.5. Ruminal methane production

Both methane and propionic acid production in the rumen use hydrogen and thus can be considered competitive pathways for hydrogen use (Leng, 1970; Moss et al., 2000). Lactic acid converts hydrogen into propionic acid and thus higher LACs lead to lower hydrogen levels (Baldwin et al., 1962; Gill et al., 1986). In addition, secondary fermentation of lactate in the rumen by lactate-utilizing bacteria produces propionate (Dawson et al., 1997; Russell and Wallace, 1997), which may reduce methanogenesis as electrons are used for propionate formation. Ruminal methane production may also contribute to hydrogen concentration (Demirel and Scherer, 2008). Therefore, in experiment 1, 2 and 3, described in Chapters 2, 4, and 5, respectively, the high-LAC TMR

decreased ruminal methane production because hydrogen was used by lactic acid to produce propionate. However, at a low pH (6.5–5.3), methane production is highly correlated with the A/P ratio, i.e., when pH decreased the A/P, methane production is inhibited. The results of experiment 3, described in Chapter 5, are in agreement: at an initial pH of 5.86, more propionate was produced, and both the A/P ratio and ruminal methane production decreased.

6.6. Plasma parameters

In experiment 2, described in Chapter 3, WCR TMR silage with food by-products did not affect the plasma concentrations of hematocrit and glucose. For silages with TC or WGTW, this was likely due to their high CP content, which increases urea-N concentrations as it is digested. Although silage with RB had CP levels similar to those in controls, its CP was highly degraded during the primary stages of fermentation. Several studies have reported lower plasma glucose concentrations in cows fed restrictively fermented silage compared to high lactate silage (Smith et al., 1993; Miettinen and Huhtanen, 1997; Heikkilä et al., 1998). For animals fed silage-based diets, a close relationship exists between rumen molar propionate proportions and plasma glucose concentrations (Shingfield et al., 2002). In experiment 1, described in Chapter 4, there were no differences in plasma parameters between controls and FTMR, except for glucose, which was lower in sheep fed FTMR. Why this occurred in the present study is not clear, and previous studies are inconsistent. For example, Shingfield et al. (2002) found that ruminants fed silage had lower plasma glucose concentrations than animals fed hay-based diets, while several other reports (Smith et al., 1993; Miettinen and Huhtanen, 1997; Heikkilä et al., 1998) showed that ruminants fed high-lactate silage had higher

plasma glucose concentrations. Nonetheless, all of the plasma parameters in the present studies were within the normal range (Kaneko, 1989).

Chapter 7

General summary and conclusion

It is increasingly important for countries to develop an efficient way to produce and utilize domestic feed, to improve the feed self-sufficiency rate for domestically raised animals and reduce negative environmental impacts. Therefore, the aims of the present studies were to investigate how (1) adding food by-products and lactic acid bacteria affects the fermentation quality of WCR TMR silage and its *in vitro* digestion characteristics, ruminal fermentation, and methane emissions; (2) adding food by-products affects the *in situ* degradation characteristics and *in vivo* nutritive value and ruminal fermentation of WCR TMR silage; (3) fermented TMR affects nutritive value, ruminal fermentation, and methane emissions; and (4) WCR TMR silage suppresses methane emission *in vitro*.

1. Effects of adding food by-products and lactic acid bacteria on the fermentation quality of WCR TMR silage, and its *in vitro* digestion characteristics, ruminal fermentation, and methane emissions

Two laboratory experiments were performed to examine how the ration formula of food by-products (i.e., TC, RB, and WGTW) with or without LAB and with or without molasses plus LAB (M-LAB) would affect the fermentation characteristics, ruminal fermentation, and methane emissions of WCR TMR silage. In experiment 1, TMR silages were prepared using the following food by-products at 30% TMR of DM: TC, RB, and WGTW at a ration formula of 10, 20 and 30%, respectively. Each silage also contained

WCR, feed concentrate, beet pulp, and a vitamin–mineral supplement at 30, 25, 13.5, and 1.5% TMR of DM, respectively. Ten samples were prepared with and ten samples were prepared without LAB (*Chikuso-1*, 5 ppm FM, and FJLB, 2% FM). In experiment 2, TMR silages were prepared with TC, WCR silage, feed concentrate, beet pulp, and a vitamin–mineral supplement at 30, 30, 25, 13.5, and 1.5% TMR. One additional sample was prepared with and one sample without M-LAB (M, 4%; *Chikuso-1*, 5 ppm). After 60 days of fermentation, all experimental treatments in experiment 1 were high quality, with low pH values (< 3.8) and high LAC (> 2.72% FM). The LAC for 30% TC TMR silage was the highest among treatments. The 30% RB TMR silage also had the highest *in vitro* DM digestibility and lowest methane production per DDM. All silages supplemented with LAB had a significantly lower pH, higher LAC, higher *in vitro* DM digestibility, and lower methane production per DDM than control silages.

In experiment 2, there were no notable differences in chemical composition between silages with and without M-LAB. Both silages had low pH and NH_3-N content and high LAC and V-scores, indicating good quality. LAC was significantly higher in silage with M-LAB than without it. Compared to TMR silage without M-LAB, TMR silage with M-LAB had 5.6% higher and 2.0% lower *in vitro* DM digestibility and methane production, respectively. Total VFA and the molar concentrations of propionic, isovaleric, and valeric acid did not differ between the two silages, while acetic and butyric acid were significantly higher and lower, respectively, for M-LAB silage. The results demonstrate that high-quality WCR TMR silage can be prepared from food by-products such as TC, RB, and WGTW. All silages prepared with 30% TC had high LAC. All silages prepared with 30% RB had high DM digestibility and low methane production. Adding LAB always promoted lactic fermentation and hindered methane production, and led to high

DDM. Adding M-LAB further increased LAC and augmented fermentation, and although the high LAC decreased ruminal methane production and increased propionic acid, the changes were not significant. Further studies are required to determine how high-LAC WCR TMR silage depresses methane production in ruminants *in vivo*, using feeding experiments.

2. Effects of adding food by-products on the fermentation quality of WCR TMR silage, and its *in situ* degradability and *in vivo* digestibility, preference, and ruminal fermentation

Four sheep were used in a 4×4 Latin square design experiment to study the fermentation quality, digestibility, and preference of WCR TMR silage with food by-products. Experimental treatments included control silage (no food by-products) and silages prepared with either TC, RB, or WGTW, all at 30% TMR of DM. Silages ensiled for 60 days were high quality, with low pH (< 4.06) and NH_3–N content and high LAC. TC silage had significantly more lactic acid than controls. TC and RB treatments led to more CP, EE, and GE, while WGTW treatment increased CP and ADF. Preference was also affected by food by-products. The mean relative intake of control, TC, RB, and WGTW silages were 0.485, 0.671, 0.397, and 0.447, respectively. Animals preferred silages in the following order: with TC, control, with RB, with WGTW. There were no differences in ruminal pH or total VFA, but ruminal NH_3-N was highest in TC silage (P = 0.0191) 2 h after feeding. The molar proportion of acetic acid in ruminal fluid was highest for WGTW silage (P = 0.0004) 4 h after feeding. Propionic and isobutyric acid were higher for RB silage (P = 0.0277 and P = 0.0368, respectively) than for WGTW silage

before feeding, and isovaleric and valeric acid were higher for RB silage ($P = 0.0183$ and $P = 0.0113$, respectively) than for either WGTW or control silage before feeding. Among the three food by-products, TC was the most digestible. RB silage had faster *in situ* degradation than control, TC, and WGTW silage. These findings suggest that food by-products can be used to make high-quality WCR TMR silage, and that silage with TC is more digestible, and is preferred by ruminants.

3. Effects of FTMR silage on *in vivo* nutritive value, nitrogen retention, digestibility, ruminal fermentation, methane emissions, and plasma parameters

Four Suffolk sheep (49.5 ± 3.2 kg) were used in a 2 (treatments) × 2 (periods) cross-over design experiment. Experimental treatments included either control TMR (not fermented) or FTMR silage. Both TMR contained WCR silage, compound feed, dried beet pulp, RB, and a vitamin–mineral supplement. WCR (Haenuki) silage was cultivated using conventional methods in a paddy field, harvested at the full-ripe stage, prepared into a mini roll bale (50 kg), stored outdoors (9–32°C) for 240 days of fermentation, and cut to a length of 2 cm. FTMR silage was adjusted with water to 55% moisture, and ensiled in drum can silos for fermentation. To prevent the fermentation of control TMR, the WCR silage was kept apart from the other ingredients and ensiled in a separate drum can silo. The other ingredients were mixed and ensiled in another drum can. A digestion trial was conducted to investigate apparent digestibility, nitrogen retention, ruminal fermentation characteristics, and plasma parameters over a 5-day test period. For 2 days of the test period, a head hood-type respiration chamber was used to measure methane

production. FTMR silage had a significantly lower DM, OM, NFE, and NFC but higher EE, CF, ash content, GE, LAC, TDN, DCP, DE, and apparent digestibility of CP, EE, CF, NDF, and GE than control TMR. Ruminal pH did not significantly differ between the TMR before feeding or 4 h after feeding but was higher for FTMR 2 h after feeding. FTMR had more total VFA and NH_3-N but less butyric acid 2 and 4 h after feeding, and less propionic acid 2 h after feeding than controls. FTMR significantly decreased daily methane emissions per sheep and daily methane energy lost as a percent of GE intake by 25.1% and 25.9%, respectively. FTMR had significantly less glucose before feeding than controls; however, all plasma parameters were within the normal range. These results show that FTMR WCR silage with food by-products reduces the energy lost to methane production, has high digestibility, and is safe for ruminants.

4. *In vitro* examination of how WCR TMR silage suppresses methane emissions

Incubating silage with lactic acid did not affect *in vitro* DM digestibility, methane production, or total VFA concentration. However, adding either starch or lactic acid + starch increased *in vitro* DM digestibility and total VFA concentration. Phosphate might inhibit methane production. TMR silage increased *in vitro* DM digestibility and decreased the A/P ratio and methane production in all treatments. However, ruminal pH may also affect methane production.

In conclusion, these results from a series of experiments suggest that WCR FTMR prepared with food by-products is useful as a feed for a ruminant animal, and that WCR FTMR is a high quality feed with both high lactic acid content and high nutritive value for

a ruminant animal, and that the high-LAC TMR can efficiently decrease ruminal methane production because hydrogen was used by lactic acid to produce propionate.

要約

食品残渣などの未利用資源を家畜用飼料として有効利用することが地球環境保全の立場からも注目されている。また，日本において，荒廃が進んでいる水田の利用を図るためと飼料自給率向上のために飼料イネサイレージ（WCRS）を家畜（牛）用飼料として利用する技術が急速に普及している。さらには，各種食品残渣由来の粗飼料と濃厚飼料を混合して乳酸発酵させて利用する発酵 TMR の調製と給与技術が開発されているが，実用レベルでの技術までには達していないと判断される。そこで，本研究では飼料イネと食品残渣等を活用した新しいタイプの発酵 TMR の調製と発酵品質の向上，および発酵 TMR を反芻家畜に給与した場合の第一胃内で消化特性，特に，消化率の改善とメタンガス生成の抑制に関する知見を得ることを目的とした。そのために，（1）食品残渣と乳酸菌の利用が飼料イネ TMR の発酵品質改善並びに *in vitro* による乾物消化率およびメタンと揮発性脂肪酸生成に及ぼす影響，（2）食品残渣を利用した飼料イネ発酵 TMR の *in situ* による乾物消失率および *in vivo* による飼料消化率と嗜好性，第一胃内容液および血液性状に及ぼす影響，（3）ヒツジにおける飼料イネ発酵 TMR の給与が第一胃内容液性状および呼気からのメタン放出量に及ぼす影響，（4）飼料イネ発酵 TMR の *in vitro* 法によるメタン生成抑制するメカニズムを検討した。

1．食品残渣と乳酸菌の利用が飼料イネ TMR サイレージの発酵品質改善並びに

in vitro による乾物消化率およびメタンと揮発性脂肪酸生成に及ぼす影響

本研究では食品残渣と乳酸菌を利用して調製した飼料イネ TMR サイレージが，発酵品質，*in vitro* 培養での乾物消化率，メタン生成および揮発性脂肪酸濃度に及ぼす影響について検討した。試験 1 は，トウフ粕，米ヌカおよび緑茶殻を乾物当たり 10，20 および 30%の割合で組み合わせを変えて，配合飼料（25%），ビートパルプ（13.5%）および WCRS（30%）に混合し，乳酸菌（畜草 1 号 5ppm（FM））および付着乳酸菌事前培養液 2%（FM））の添加，無添加による計 20 処理区の飼料イネ発酵 TMR をパウチ法により調製し，60 日間室温で貯蔵した後開封した。試験 2 は，トウフ粕，配合飼料，ビートパルプおよび WCRS をそれぞれ乾物当たり 30, 25, 13.5 および 30%の割合で混合し，乳酸菌（畜草 1 号 5ppm（FM））と糖蜜（4%（FM））の添加および無添加の 2 処理とした。試験 1 において，飼料イネ発酵 TMR の pH は，全ての処理区で 3.8 以下，乳酸含量は 2.72%（FM）以上の良質のサイレージに調製出来た。また，トウフ粕を 30%配合すると乳酸含量は，高くなった。米ヌカを 30%配合すると乾物消失率は高く，単位可消化乾物量当たりのメタン生成量は低くなった。さらに，乳酸菌を添加すると，pH は低く（$P<0.05$），乳酸含量と *in vitro* による乾物消化率は高く（$P<0.05$），単位可消化乾物量当たりのメタン生成量が低くなった（$P<0.05$）。試験 2 において，化学成分は処理区の間に有意差が認められなかった。全ての TMR の pH および揮発性塩基態窒素含量が低く，乳酸含量が高い良質なサイレージを調製出来，乳酸菌と糖蜜を添加すると乳酸含量は更に高くなった（$P<0.05$）。乳酸菌と糖蜜の添加は *in vitro* 乾物消化率を 5.6%高くし，メタン放出量を 2.0%減少させ

た。また，揮発性脂肪酸総量，プロピオン酸および吉草酸濃度は処理区に差がなかったが，乳酸菌と糖蜜無添加区に比べて添加区は，酢酸が高くなり（$P<0.05$），酪酸が低くなった（$P<0.05$）。以上のことから，食品残渣を利用した良質な飼料イネ発酵TMRを調製出来た。トウフ粕を30%配合すると乳酸含量は，高くなり，米ヌカを 30%配合すると乾物消化率は高く，単位可消化乾物量当たりのメタン生成量は低くなった。また，乳酸菌を添加すると，pHは低く，乳酸含量と乾物消化率は高く，単位可消化乾物量当たりのメタン生成量が低くなった。

2．食品残渣を利用した飼料イネ発酵 TMR の *in situ* による乾物消失率ならびに *in vivo* による成分消化率と嗜好性，第一胃内容液および血液性状に及ぼす影響

ヒツジを 4 頭用いた，食品残渣を利用した飼料イネ発酵 TMR の成分消化率，嗜好性および第一胃内容液性状，ならびに牛における *in situ* 消化特性について検討した。発酵 TMR の調製に用いた飼料および試験処理は飼料イネ，市販配合飼料およびビートパルプに食品残渣であるトウフ粕，米ぬかおよび緑茶殻をそれぞれ乾物あたり 30%配合した 3 つ種類の試験区，および食品残渣を利用しなかった対照区を設定し，ヒツジ4頭を用いて 4 × 4 のラテン方格法による消化試験を実施した。飼料イネ発酵 TMR の pH は，全ての処理区で 4.06 以下，乳酸含量は 2.4%（FM）以上の良質のサイレージに調製出来た。トウフ粕を利用したサイレージは乳酸含量が高く（$P<0.05$），ヒツジに給与すると，成分消化率が高くなる傾向を示し（$P<0.05$），嗜好性も良くなった。しかし，米ヌカおよび緑茶殻を利用したサイレージは対照区に比べて CP と EE の消化率が高くなったが，嗜好

性が低下した。第一胃内容液の pH および VFA 総量は，処理区の間に有意差が認められなかった。食品残渣単味の *in vivo* 消化率は，トウフ粕が最も高かった。牛における *in situ* 培養試験により，DM，CP および中性デタージェント繊維の消失率は，投入から 20 時間まで，米ヌカを利用した発酵 TMR が高かったが，その後，トウフ粕を利用した発酵 TMR が高くなった。

３．ヒツジにおける飼料イネ発酵 TMR の給与が第一胃内容液性状および呼気からのメタン放出量に及ぼす影響

本研究では，飼料イネ発酵 TMR の給与が第一胃内容液性状および呼気からのメタン放出量に及ぼす影響を検討した。試験処理は米ヌカ，市販配合飼料およびビートパルプを WCRS に配合して 60 日間貯蔵した発酵 TMR を試験区とし，米ヌカ，市販配合飼料およびビートパルプをヒツジに給与する直前に，WCRS に配合したフレッシュ TMR を対照区とし，ヒツジを 4 頭用いた反転法により消化試験と呼吸試験を実施した。給与飼料の乳酸含量は，発酵 TMR7.34%（DM）とフレッシュ TMR0.55%（DM）より著しく高かった（$P < 0.05$）。一般成分の中で，DM，有機物，可溶無窒素物および非構造性炭水化物は発酵 TMR がフレッシュ TMR より低かったが（$P < 0.05$），EE，CF，Ash および GE は発酵 TMR がフレッシュ TMR より高かった（$P < 0.05$）。消化試験により，フレッシュ TMR に比べて，発酵 TMR の CP，EE，CF，NDF および GE の消化率，可消化養分総量，可消化粗タンパク質および DE が増加した（$P < 0.05$）。また，呼気試験により，発酵 TMR はフレッシュ TMR より一日当たりメタン放出量が 25.1%減少し

（$P < 0.05$），メタンからのエネルギー損失量を25.9%減少した（$P < 0.05$）。

４．飼料イネ発酵 TMR の *in vitro* 法によるメタン生成抑制するメカニズムの検討

本研究では，飼料イネ発酵 TMR を *in vitro* 法により第一胃内容液で培養してメタン生成の抑制に関するメカニズムを検討した。試験 1 において，乳酸添加による乳酸含量の違いが飼料イネ発酵 TMR の *in vitro* メタン生成に及ぼす影響を検討した。乳酸だけの添加では可消化乾物当たりのメタン生成量，VFA およびプロピオン酸濃度に影響しなかったが，デンプンおよびデンプン＋乳酸を添加すると，DM 消化率および VFA 総量が増加し，可消化乾物当たりメタン生成量が減少した。しかし，デンプンおよびデンプン＋乳酸の添加はプロピオン酸濃度に影響しなかった。そのために，試験 2 において，リン酸バッファーを利用した pH の違いが *in vitro* メタン生成に及ぼす影響を検討した。その結果，6 時間培養したすべての処理でメタンを検出出来なかった。試験 3 において，飼料の給与前，給与後 2，4 時間に採取したルーメン液の pH（7.08，5.86 および 6.56）の違いが発酵 TMR の *in vitro* メタン生成に及ぼす影響を検討した。培養開始時点の pH が 5.86 および 6.56 とも，発酵 TMR はフレッシュ TMR よりメタン生成量が低くなったが（$P < 0.05$），培養開始時点 pH が 5.86 の時，6 時間培養後 pH が 5.6 であり，発酵 TMR からプロピオン酸生成を増加させ，酢酸：プロピオン酸比が減少した。これらの結果から，第一胃内で，発酵 TMR に含んでいる乳酸からプロピオン酸を生成するとき水素を取り込み，メタン生成を抑制するには

5.6程度の低いpHが必要だと推測された。

以上の結果から，食品残渣を利用した飼料イネ発酵TMRは反芻家畜用飼料として有益であり，乳酸発酵を促進させて消化率と栄養価を向上させることが分かった。また，地球温室効果ガスである第一胃からのメタンガスの生成を抑制する効果があり，そのメカニズムの一端を明らかに出来た。

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References

Agriculture, Forestry and Fisheries Research Council Secretariat, MAFF, 2000, Japanese Feeding Standard for beef Cattle (2000). Japan Livestock Industry Association, Tokyo, Japan, pp. 29-41.

Aguilera, J.f., Bustos, M., Molina, E., 1992. teh degradability of legume seed meals in the rumen: effect of heat treatment. Anim. Feed Sci. Technol. 36, 101-112.

Alli, I., Fairbairn, R., Noroozi, E., Baker, B.E., 1984. The effects of molasses on the fermentation of chopped whole-plant leucaena. J. Sci. Food Agric. 35, 285-289.

Amissah, J.G.N., Ellis, W.O., Oduro, I., Manful, J.T., 2003. Nutrient composition of bran from new rice varieties under study in Ghana. Anim. Feed Sci. Technol. 14, 21-24.

AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Araba, A., Byers, F.M., Guessous, F., 2002. Patterns of rumen fermentation in bulls fed barley/molasses diets. Anim. Feed Sci. Technol. 97, 53-64.

Archibald, J.C., Kuzinesk, J.W., Russell, S., 1960. Grass silage quantity as affected by crop composition and by additives. J. Dairy Sci. 43, 1648-1655.

Arosemena, A., Depeters, E.J., fadel, J.G., 1995. Extent of variability in nutrient composition within selected by-procuct feedstuffs. Anim. Feed Sci. Technol. 54, 103-120.

Asanuma, N., Hino, T., 2002. Regulation of fermentation in a ruminal bacterium, streptococcus bovis, with special reference to rumen acidosis. Anim. Sci. J. 73, 323-325.

Baldwin, R.L., Wood, W.A., Emery, R.S., 1962. Conversion of lactate-C^{14} to propionate

by the rumen microflora. J. Bacteriol. 83, 907-913.

Barker, S.B., Summerson, W.H., 1941. The colorimetric determination of lactic acid in biological material. J. Biol. Chem. 138, 535-554.

Batajoo, K.K., Shaver, R.D., 1994. Impact of nonfiber carbohydrate on intake, digestion, and milk production by dairy cows. J. Dairy Sci. 77, 1580-1588.

Bauchop, T., Mountfort, D.O., 1981. Cellulose fermentation by a rumen anaerobic fungus in both the absence & the presence of rumen methanogens. Appl. Environ. Microbiol. 42, 1103-1110.

Beauchemin, K.A., McGinn, S.M., 2005. Methane emissions from feedlot cattle fed barley or corn diets. J. Anim. Sci. 83, 653-661.

Beauchemin, K.A., McGinn, S.M., 2006. Methane emissions from beef cattle: Effects of fumaric acid essential oil, and canola oil. J. Anim. Sci. 84, 1489-1496.

Bechmant, J.L., Weiss, W.P., 2005. Nutrient digestibility of diets with different fiber to starch ratios when fed to lactating dairy cows. J. Dairy Sci. 88, 1015-1023.

Belyea, R.L., Steevens, B.J., Restrepo, R.J., Clubb, A.P., 1989. Variation in composition of by-product feeds. J. Dairy Sci. 72, 2339-2345.

Benchaar, C., Pomar, C., Chiquette, J., 2001. Evaluation of dietary strategies to reduce methane production in ruminants: A modeling approach. Can, J. Anim. Sci. 81, 563-574.

Blaxter, K.L., Clapperton, J.L., 1965. Prediction of the amoout of methane produced by ruminants. Br. J. Nutr. 19, 511-522.

Bretschneider, G., Peralta, M., Santini, F.J., Fay, J.P., Faverin, C., 2007. Influence of corn silage supplementation before alfalfa grazing on ruminal environment in relation to the occurrence of frothy bloat in cattle. Anim. Feed Sci. Technol. 136, 23-37.

Bretschneider, G., Santini, F.J., fay, J.P., faverin, C., 2001. Effects of maize silage supplementation before lucerne grazing on the occurrence of bloat in cattle. NZ J. Agric. Res. 44, 241-251.

Broudiscou, L., Van Nevel, C.J., Demeyer, D.I., 1990. Incorporation of soya oil hydrolysate in the diet of defaunated or refaunated sheep: effect on rumen fermentation *in vitro*. Arch. Tierernahr. 40, 329-337.

Brouwer, E., 1965. Report of sub-commitee on constants and factors. In: Proceeding of the Third EAAP Symposium on Energy Metabolism. Pulication no. 11, Academic Press, London, pp. 441-443.

Cai, Y., 2001. The role of lactic acid bacteria in the preparation of high fermentation quality. Jpn. J. Grassl. Sci. 47, 527-533.

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

Cai, Y., Fujita, Y., Murai, M., Ogawa, M., Yoshida, N., Kitamura, R., Miura, T., 2003. Application of lactic acid bacteria (*Lactobacillus plantarum Chikuso*-1) for silage preparation of forage paddy rice. Jpn. J. Grassl. Sci. 49, 477-485.

Cai, Y., Masuda, N., Fujita, Y., Kawamoto, H., Ando, S., 2001. Development of a new method for preparation and conservation of tea grounds silage. Anim. Sci. J. 72, 536-541.

Cai, Y., Ohmomo, S., 1995. Effect of lactic acid bacteria and cellulase on the changes in microflora during fermentation, gas production and dry matter loss in silage. (Jpn.) Anim. Sci. Technol. 66, 941-948.

Caipintero, G.M., Holding, A.J., McDonald, P., 1969. Fermentation studies on lucerne. J.

Sci. Food Agric. 20, 677-681.

Cameron, M.R., Klusmeyer, T.H., Lynch, G.L., Clark, J.H., 1991. Effects of urea and starch on rumen fermentation, nutrient passage to the duodenum, and performance of cows. J. Dairy Sci. 74, 1321-1336.

Cao, L.M., Goto, M., Karita, S., Yamamoto, Y., Mizutani, M., Deguchi, Y., 2002. Effect of fermented juice of epiphytic lactic acid bacteria on the fermentation quality of alfalfa (*Medicago sativa* L.) silage and its energy and nitrogen utilization by dry cows. Jpn. J. Grassl. Sci. 48, 227-235.

Cao, Y., Takahashi, T., Horiguchi, K., in press a. Effect of food by-products and lactic acid bacteria on fermentation quality and *in vitro* dry matter digestibility, ruminal methane and volatile fatty acid production in total mixed ration silage with whole-crop rice silage. Jpn. J. Grassl. Sci.

Cao, Y., Takahashi, T., Horiguchi, K., in press b. Effects of addition of food by-procucts on the fermentation quality of a total mixed ration with whole crop rice and its digestibility, preference, and rumen fermentation in sheep. Anim. Feed Sci. Technol. (2008), doi: 10.1016/J.anifeedsci.2008.10.010.

Carro, M.D., Lopez, S., Valdes, C., Ovejero, F.J., 1999. Effect of D, L-malate on mixed ruminal microorganism fermentation using the rumen simulation technique (RUSITEC). Anim. Feed Sci. Technol. 79, 279-288.

Cecava, M.J., Merchen, N.R., Rerger, L.L., Nelson, D.R., 1990. Effect of energy level and feeding frequency on site of digestion and postruminal nutrient flows in steers. J. Dairy Sci. 73, 2470-2479.

Chamberlain, D.G., Thomas, P.C., Anderson, F.J., 1983. Volatile fatty acid proportions and lactic acid metabolism in the rumen in sheep and cattle receiving silage diets. J.

Agric. Sci. Camb. 101, 47-58.

Chaucheyras-Durand, F., Walker, N.D., Bach, A., 2008. Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. Anim. Feed Sci. Technol. 145, 5-26.

Chen, M.C., Ammerman, C.B., Henry, P.R., Palmer, A.Z., Long, S.K., 1981. Citrus condensed molasses solubes as an energy source for ruminants. J. Anim. Sci. 53, 253-259.

Clapperton, J.L., 1974. The effect of trichloroacetamide chloroform & linseed oil given into the rumen of sheep on some of the end products of rumen digestion. Brit. J. Nutr. 32, 155-161.

Clarke, R.T.J., Reid, C.S.W., 1974. Foamy bloat of cattle. A review. J. Dairy Sci. 57, 753-785.

Coleman, G.S., 1986. The distribution of carboxymethyl celulase between fractions taken from the rumens of sheep containing no protozoa or one of five different protozoal population. J. Agric. Sci. Camb. 106, 121-127.

Conway, E.J., 1962. Microdiffusion Analysis and Volume Tric Error, 5th Edn. Crosby Lockwood, London, pp. 322.

Coppock, C.E., Bath, D.L., Harris, B., 1981. From feeding to feeding systems. J. Dairy Sci. 64, 1230-1249.

Cunningham, K.D., Cecava, M.J., Johnson, T.R., Ludden, P.A., 1996. Influence of source and amount of dietary protein on milk yield by cows in early lactation. J. Dairy Sci. 79, 620-630.

Czerkawski, J.W., Christie, W.W., Breckenridge, G., Hunter, M.L., 1995. Changes in rumen metabolism of sheep giver increasing amounts of linseed oil in their diet. Brit.

J. Nutr. 34, 25-44.

Dacie, I.N., Lewis, S.M., 1975. Practical Hematology, fifth ed. Churchill Livingtone, London, pp. 237-241.

Dawson, K.A., Rasmussen, M.A., Allison, M.J., 1997. Digestive disorgers and nutritional toxicity. In: Hobson, P.N., Stewart, C.S., (Eds), The Rumen Microbial Ecosystem, 2nd edn. Blackie Academic and Professional, London. pp. 633-660.

De Campos, R.M.L., Hierro, E., Ordóñez, J.A., Bertol, T.M., Terra, N.N., De la Hoz, L., 2007. Fatty acid and volatile compounds from salami manufactured with yerba mate (Ilex paraguariensis) extract and pork back fat and meat from pigs fed on diets with partial replacement of maize with rice bran. Food Chem. 103, 1159-1167.

Demeyer, D., Henderickx, H.K., 1967. Methane production *in vitro* by mixed rumen bacteria. Biochem. J. 105, 271-277.

Demeyer, D.I., De Graeve, K., 1991. Differences in stoinchiometry between rumen and hindgut fermentation. J. Anim. Physiol Anim. Nutr. 22, 50-61.

Demeyer, D.I., Fiedler, D., De Graeve, K.G., 1996. Attempted induction of reductive acetogenesis into the rumen fermentation *in vitro*. Reprod. Nutri. Dev. 36, 233-240.

Demirel, B., Scherer, P., 2008. The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. Rev. Environ. Sci. Biotechnol. 7, 173-190.

Dohme, F., Machmuller, A., Estermann, B.L., Ptister, P., Wasserfallen, A., Kreuzer, M., 1999. The role of the rumen ciliate protozoa for methane suppression caused by coconut oil. Lett. Appl. Microbiol. 29, 187-193.

Dong, Y., Bae, H.D., McAllister, T.A., Mathison, G.W., Cheng, K.J., 1997. lipid-induced depression of methane production & digestibility in the artificial rumen system

(RUSTIEC). Can, J. Anim. Sci. 77, 269-278.

Enishi, O., Kawashima, T., 2003. Analysis of *in situ* ruminal digestive characteristics and nutritive value of wash-free rice by-product. Jpn. J. Grassl. Sci. 49, 471-476.

Enishi, O., Kuroiwa, R., Saeki, M., Kawasima, T., 2005. Evaluation of protein characteristics of underutiliaed by-products as feedstuff for ruminant. Jpn. J. Grassl. Sci 51, 281-288.

Enishi, O., Shijimaya, K., 1998. The fermentation patterns of rice whole crop silage. Jpn. J. Grassl. Sci. 44, 179-181.

Erfle, D.J., Boila, R.J., Teather, R.M., Mahadevan, S., Sauer, F.D., 1982. Effect of pH on fermentation characteristics and protein degradation by rumen microorganisms *in vitro*. J. Dairy Sci. 65, 1457-1464.

Erfle, J.D., Sauer, F.D., Mahadevan, S., 1986. Energy metabolism in rumen microbes. in: Milligan, L.P., Grovum, W.L., Dobson, A. (Eds.), Control of Digestion and Metabolism in Ruminants. Prentice Hall, Englewood Cliffs, New Jersy, pp. 81-99.

Eruden, B., Nishida, T., Matsuyama, H., Hosoda, K., Shioya, S., 2005. Effect of the addition of various levels of green tea grounds silage at on the feed intake and milk production in lactation dairy cows. Anim. Sci. J. 76, 295-301.

Eruden, B., Nishida, T., Matsuyama, H., Hosoda, K., Shioya, S., Nonaka, K., Yamada, A., 2007. Effect of main roughages on the palatability by lactation dairy cows of total mixed rations with green tea waste silage. Jpn. J. Grassl. Sci. 52, 232-236.

Fadel, J.G., 1999. Quantitative analses of selected plant by-product feedstuffs, a global perspective. Anim. Feed Sci. Technol. 79, 255-268.

Fadel, J.G., Depeters, E.J., Arosemena, A., 2000. Compsition and digestibility of beet pulp with and without molass and dried using three methods. Anim. Feed Sci.

Technol. 85, 121-129.

Fahey, G.C., Berger, L.L., 1988. Carbohydrate nutrition in ruminants. In: Church, D.C., (Ed.), The ruminant animal: Digestive Physiology and Nutrition. Prentice Hall, Englewood Cliffs, New Jersey. pp. 269-297.

Finlay, B.J., Esteban, A.G., Clarke, K.J., Williams, A.G., Embley, T.M., Hirt, R.R., 1994. Some rumen ciliates have endosymbiotic methanogens. FEMS microbiol. Lett. 117, 157-162.

Fonty, G., Williams, A.G., Bonnemoy, F., Morvan, B., Wither, S.E., Gouet, P., 1997. Effect of Methanobrevibacter sp MF1 inoculation on glycoside hydrolase and polysaccharide depolymerase activities, wheat straw degradation and volatile fatty acid condentrations in the rumen of gnotobiotically-reared lambs. Anaerob 3, 383-389.

Forster, L.A., Jr., Goetsch, A.L., Galloway, D.L., Sr., Johnson, Z.B., 1993. Feed intake, digestibility, and live weight gain by cattle consuming forage supplemented with rice bran and (or) corn. J. Anim. Sci. 71, 3105-3114.

Frumholtz, P.P., Newbold, C.J., Wallace, R.J., 1989. Influence of Aspergillus oryzae fermentation extract on the fermentation of a basal ration in the rumen simulation technique (Rusitec). J. Agric. Sci. Camb. 113, 169-172.

Furumoto, F., 2002. Making and conservation of tatal mixed ration use of by-products. Jpn. J. Grassl. Sci. 48, 64-68.

Garcia-Lopez, P.M., Kung, L.J., Odom, J.M.I., 1996. *In vitro* inhibition of microbial methane production by 9, 10-anthraquinone. J. Anim. Sci. 74, 2276-2284.

Gatel, F., 1994. Protei quality of legume seeds for non-ruminant animals: a literaturea review. Anim. Feed Sci. Technol. 45, 317-345.

Giger-Reverdin, S., Sauvant, D., 2000. Methane Production in Sheep in Relation to Concentrate Feed Composition from Bibliographic Data. In: Ledin, I., Morand-Fehr, P. (Eds), 8th Seminar of the Sub-Network on Nutrition of the FAO-CIHEAM Inter-Regional Cooperative Research and Development Network on Sheep and Goats. INRA, Cahiers-Options-Mediterraneennes, Grignon, France, pp. 43-46.

Gill, M., Siddons, R.C., Beever, D.E., 1986. Metabolism of lactic acid isomers in the rumen of silage-fed sheep. Brit. J. Nutr. 55, 399-407.

Goga, T., Saito, T., Yoshida, M., Ishizaki, S., Muroi, S., Seki, M., Shimizu, K., Kato, Y., Uchida, T., Sunaga, S., Kimura, Y., Kawashima, K., Takenaka, A., Enishi, O., Terada, F., 2001. Effects of NDF and fat rich by-products on performance of early lactating cows. Anim. Sci. J. 72, 351-358.

Granzin, B.C., Dryden, G.M., 2005. Monensin supplementation of lactating cows fed tropical grasses and cane molasses or grain. Anim. Feed Sci. Technol. 120, 1-16.

Gutierrez, J., Essig, H.W., Williams, P.P., Davis, R.E., 1963. Properties of a slime isolated from the rumen fluid of cattle bloating on clover pasture. J. Anim. Sci. 22, 506-509.

Hamada, T., 1971. Physicochemical analytical method for nutritional experiments. In: Morimoto, H. (Ed.), Animal Nutrition Testing Method, 1st edn. Yokendo, Tokyo, Japan, pp. 428-431.

Hameleers, A., Leach, K.A., Offer, N.W., Roberts, D.J., 1999. The effects of incorporating sugar beet pulp with forage maize at ensiling on silage fermentation and effluent output using drum silos. Grass Forage Sci. 54, 322-335.

Hatano, R., shinano, T., Taigen, Z., Okubo, m., Zuowei, L., 2002. Nitrogen budgets and environmental capacity in farm systems in a large-scale karst region, southern China. Nutrient Cycling in agroecosystems 63, 139-149.

Hatch, E.F., Beeson, W.M., 1972. Effect of different levels of cane molasses on N and energy utilization in urea ration for steers. J. Anim. Sci. 35, 854-860.

Heikkilä, T., Toivonen, V., Huhtanen, P., 1998. Effects of and interactions between the extent of silage fermentation and protein supplementation in lactating dairy cows. Agric. Food Sci. Finl. 7, 329-343.

Henderson, C., 1973. The effects of fatty acids on pure cultures of rumen bacteria. J. Agric. Sci. Camb. 81, 107-112.

Hindrichsen, I.K., Wettstein, H.-R., Machmuller, A., Jörg, B., Kreuzer, M., 2005. Effect of the carbohydrate composition of feed concentratates on methane emission from dairy cows and their slurry. Environ. Monit. Assess. 107, 329-350.

Hiraoka, H., Yamamoto, Y., Oide, I., Tanaka, Y., Inui, K., Urakawa, S., 2005. Effect of fermented TMR based whole crop silage prepared in lap silo on resistance to aerobic deterioration. Jpn. J. Grassl. Sci. 52 (Special), 124-125.

Hiraoka, H., Yamamoto, Y., Urakawa, S., Mizutani, M., Yamada, H., Inui, K., Karita, S., Goto, M., 2003. Effect of fermented juice of epiphytic lactic acid bacteria on fermentation quality and its β-carotene and phytin phosphorus contents of whole crop silage of rice plant (*Oryza sativa*. L). Jpn. J. Grassl. Sci. 49, 460-464.

Hiraoka, H., Yamamoto, Y., Urakawa, S., Yamada, H., Karita, S., Goto, M., 2006a. Effect of fermented juice of epiphytic lactic acid bacteria on fermentation quality of rice whole crop silage in the undesirable harvesting condition. Jpn. J. Grassl. Sci. 2, 89-94.

Hiraoka, H., Yamamoto, Y., Urakawa, S., Yamada, H., Karita, S., Goto, M., 2006b. Effective fractions and micro biota of fermented juice of epiphytic lactic acid bacteria (FJLB) of rice plant (*Oryza sativa* L.). Jpn. J. Grassl. Sci. 51, 379-384.

Holter, J.B., Young, A.J., 1992. Methane production in dry and lactating holstein cows. J. Dairy Sci. 75, 2165-2175.

Horiguchi, K., Sato, H., Takahashi, T., 2002. Effect of safflower oil supplementation on methane emission from expiratory gas, digestibility and characteristics of rumen liquor in sheep fed with high concentrate diets. Jpn. J. Sheep Sci. 39, 17-24.

Horiguchi, K., Takahashi, T., 2001. Control of methane emission from expiratory gas by supplement of rapeseed oil in sheep fed with different ratios of roughage and concentrate. In: Takahashi, J., Young, B.A., (Eds.), Greenhouse Gases and Animal Agriculture. Dairy Japan, Tokyo, Japan, pp. 359-362.

Horiguchi, K., Takahashi, T., 2007. Fermentation quality and nutritive value of green soybean stover silage. Grassl. Sci. 53, 27-31.

Horii, S., Kurata, Y., Hayashi, Y., Tanabe, S., 1971. Physicochemical analytical method for nutritional experiments. In: Morimoto, H. (Ed.), Animal Nutrition Testing Method, 1st edn. Yokendo, Tokyo, Japan, pp. 280-298.

Huhtanen, P., Miettinen, H., Ylinen, M., 1993. Effect of increasing ruminal butyrate on milk yield and blood constituents in dairy cows fed a grass silage-based diet. J. Dairy Sci. 76, 1114-1124.

Ide, T., 2002. Feeding techniques of tofu cake for total mixed rations of dairy cows. Jpn. J. Grassl. Sci. 48, 73-77.

Ikwuegbu, O.A., Sutton, J.D., 1982. The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. Brit. J. Nutr. 48, 365-375.

Imai, A., 2000. Silage making and utilization of high moisture by-products. Jpn. J. Grassl. Sci. 47, 307-310.

Imai, A., 2002. utilization of tofu cake on the fattening cattle. jpn. J. Grassl. Sci. 48,

78-82.

IPCC, 1994. in: Houghton, J.H., Meria Filho, L.G., Bruce, J., Lee, H., Callander, B.A., Haites, H., Harris, N., Maskel, K. (Eds), Climate change 1994. Cambridge University Press, New York, 1994, pp. 25-27.

IPCC, 2001. Climate change 2001: Synthesis report. Contribution of Working Groups Ⅰ, Ⅱ and Ⅲ to the Third Assessment Report of the IPCC. Intergovernmental Panel Climate Change. Wason, RB., Core Writing team, (Eds.), Cambridge Univ. Press, Cambridge, UK, and New York, USA.

Ishibashi, T., 2007. Present and problems of feeds. Anim. Sci. J. 78, 1-13.

Ishida, S., 2002. Development and feeding effect of mixed silage ration of highmoisture by-products. Jpn. J. Grassl. Sci. 48, 69-72.

Islam, M.R., Ishida, M., Ando, S., Nishida, T., Yoshida, N., Arakawa, M., 2004. Effect of variety and stage of maturity on nutritive value of whole crop rice silage for ruminants: *In situ* dry matter and nitrogen degradability and estimation of metabolizable energy and metabolizable protein. Asian-Aust. J. Anim. Sci. 17, 1541-1552.

Islam, M.R., Nishida, T., Ando, S., Ishida, M., Yoshida, N., Arakawa, M., 2001. Effect of variety, stages of maturity and botanical fractions on phosphorous concentration and *in situ* degradability characteristics of phosphorous of whole crop rice silage. Anim. Feed Sci. Technol. 92, 237-248.

Japan Grassland Agriculture and Forage Seed Association, 2001. Guide Book for Quality Evaluation of Forage. Japanese Society of Grassland Science, Tokyo, Japan, pp. 40-42, 91-92.

Jentsch, W., Hoffmann, L., 1994. Influence of the energy metabolizability (Q) on ennerqy

maintenance requirement and utilization of the metabolizable energy for deposition in ruminants. 1. energy and nutrients digestibility. Arch. Anim. Nutr. 46, 61-76.

Johnson, D.E., Johnson, K.A., Ward, G.M., Branine, M.E., 2000. Ruminants and other animals, Chapter 8. In: Khalil, M.A.K. (Ed.). Atmospheric Methane: Its Role in the Global Environment. Springer-Verlag, Berlin Heidelberg, Germany, pp. 112-133.

Johnson, D.E., Ward, G.M., 1996. Estimate of animal methane emissons. Environ. Monit. Assess. 42, 133-141.

Johnson, K.A., Johnson, D.E., 1995. Methane emission from cattle. J. Anim. Sci. 73, 2483-2492.

Jones, W.T., Lyttleton, J.W., 1972. Bloat in cattle XXXVI. Further studies on the foaming properties of soluble leaf proteins. NZ J. Agric. Res. 15, 267-277.

Jordan, E., Lovett, D.K., Monahan, F.J., Callan, J., Flynn, B., O'Mara, F.P., 2006. Effect of refined coconut oil or copra meal on methane output and on intake and performance of beef heifers. J. Anim. Sci. 84, 162-170.

Jouany, J.P., Ushida, K., 1999. The role of protozoa in feed digestion. Asian-Aust. J. Anim. Sci. 12, 113-128.

Kagawa, Y., 2005. Standard tables of food composition in Japan fifth revised and enlarged edition. Kagawa nutrition university publishing division, Tokyo, p62-63, 228-229.

Kajikawa, H., 1996. Utilization and characteristics of by-product feed in our country. Animal husbandry 50, 63-69.

Kaneko, J.J., 1989. Normal blood analyte values in large animals. In: Kaneko, J.J. (Ed.), Clinical Biochemistry of Domestic Animals 4th Ed. Academic Press, Orlando, Florida, USA, pp. 884-888.

Kanjanapruthipong, J., Buatong, N., 2004. Effects of replacing nonfiber carbohydrates with nonforage detergent fiber from Cassava residues on performance of dairy cows in the tropics. Asian-Aust. J. Anim. Sci. 17, 967-972.

Kano, M., Horiguchi, K., Takahashi, T., 2000a. Effect of slow release fertilizer application on recovery rate of nitrogen, mineral contert, fermented quality and nutritional yield of rice plant (*Oryza sativa* L.). Jpn. J. Grassl. Sci. 46, 188-191.

Kano, M., Takahashi, T., Kayaba, T., 2000b. Effect of applied fermented daily cow waste on slurry nitrogen utilization, mineral content, fermented uality and nutritional yield of rice plant (*Oryaz sativa* L.). Jpn. J. Grassl. Sci. 45, 379-387.

Kellogg, D.W., Owen, F.G., 1969. Relation of ration sucrose level and grain content to lactation performance and rumen fermentation. J. Dairy Sci. 52, 657-662.

Kim, S.C., Adesogan, A.T., Kim, J.H., Ko, Y.D., 2006. Influence of replacing rice straw with wormwood (*Artemisia montana*) silage on feed intake, digestibility and ruminal fermentation characteristics of sheep. Anim. Feed Sci. Technol. 128, 1-13.

Knowlton, K.F., Glenn, B.P., Erdman, R.A., 1998. Performance, ruminal fermentation, and site of starch digestion in early lactation cows fed corn grain harvested and processed differently. J. Dairy Sci. 81, 1972-1984.

Kobayashi, R., Sato, K., Hattori, I., Konishi, T., 2007. Optimum planting density and fertilizer application rate of forage rice under ratoon croping in the certral kyushu region. Jpn. J. Grassl. Sci. 53, 208-214.

Kondo, M., Kita, K., Yokota, H., 2004a. Feeding value to goats of whole-crop oat ensiled with green tea waste. Anim. Feed Sci. Technol. 113, 71-81.

Kondo, M., Nakano, M., Kaneko, A., Agata, H., Kita, K., Yokota, H., 2004b. Ensiled green tea waste as partial replacement for soybean meal and alfalfa hay in lactating

cows. Asian-Aust. J. Anim. Sci. 17, 960-966.

Kowalcyzck, J., Ørskov, E.R., Robinson, J.J., Stewart, C.S., 1977. Effect of fat supplementation on voluntary food intake and rumen metabolism in sheep. Brit. J. Nutr. 37, 251-257.

Krizsan, S.J., Randby, Å.T., 2007. The effect of fermentation quality on the voluntary intake of grass silage by growing cattle fed silage as the sole feed. J. Anim. Sci. 85, 984-996.

Kung, L.J., Hession, A.O., Bracht, J.P., 1998. Inhibition of sulfate reduction to sulfide by 9, 10-anthraquinone *in vitro* ruminal fermentations. J. Dairy Sci. 81, 2251-2256.

Lana, R.P., Russell, J.B., Van Amburgh, M.E., 1998. The role of pH in regulating methane and ammonia production. J. Anim. Sci. 76, 2190-2196.

Lanigan, G.W., 1961. Studies on ensilage. I. A comparative laboratory study of molasses and sodium metabisulphite as aids to the conservation of lucerne. Aust. J. Agric. Res. 12, 1023-1038.

Leng, R.A., 1970. Formation and production of volatile fatty acids in the rumen. In: Phillipson, A.T. (Ed.), Physiology of Digestion and Metabolism in the Ruminant, Oriel Press, Newcastle upon Tyne, England, pp. 406-421.

Lopez, S., McIntosh, F.M., Wallace, R.J., Newbold, C.J., 1999a. Effect of adding acetogenic bacteria on methane production by mixed rumen microorganisms. Anim. Feed Sci. Technol. 78, 1-9.

Lopez, S., Valdes, C., Newbold, C.J., Wallace, R.J., 1999b. Influence of sodium fumarate on rumen fermentation *in vitro*. Brit. J. Nutr. 81, 59-64.

Lu, C.D., Jorgensen, N.A., 1987. Alfafa saponines affect site and extent of nutrient digestion in ruminants. J. Nutr. 117, 319-327.

Machmuller, A., Kreuzer, M., 1997. Methane suppression by coconut oil & associated effects on nutrient & energy balance in sheep. Pro. Soc. Nutri. Physiol. 6, 65-72.

Machmuller, A., Ossowski, D.A., Wanner, M., Kreuzer, M., 1998. Potertial of various fatty feeds to reduce methane release from rumen fermentation *in vitro* (Rusitec). Anim. Feed Sci. Technol. 77, 117-130.

MacRae, J.C., Lobley, G.E., 1982. Some factors which influence thermal energy losses during the metabolism of ruminants. Livestock Prod. Sci. 9, 447-456.

Maiga, H.A., Schingoethe, D.J., 1997. Optimaizing the utilization of animal fat and ruminal bypass proteins in the diets of lactating dairy cows. J. Dairy Sci. 80, 343-352.

Maltz, E., Devir, S., Kroll, O., Zur, B., Spahr, S.L., Shanks, R.D., 1992. Comaprative responses of lactating cows to total mixed rations or computerized individual condentrates feeding. J. Dairy Sci. 75, 1588-1603.

Martin, S.A., 1998. Manipulation of ruminal fermentation with organic acids: review. J. Anim. Sci. 76, 3123-3132.

Martin, S.A., Nisbet, D.J., 1990. Effects of Aspergillus oryzae fermentation extract on fermentation of amino acids, bermudagrass and starch by mixed ruminal micro-organisms *in vitro*. J. Anim. Sci. 68, 2142-2149.

Martin, S.A., Nisbet, D.J., 1992. Effects of direct fed microbials on rumen microbial fermentation. J. Dairy Sci. 75, 1736-1744.

Martin, S.A., Streeter, M.N., 1995. Effect of malate on *in vitro* mixed ruminal microorganism fermentation. J. Anim. Sci. 73, 2141-2145.

Mathison, G.W., Okine, E.K., McAllister, T.A., Dong, Y., Galbraith, J., Dmytruk, O.I.N., 1998. Reducing methane emissions from ruminant animals. J. Appl. Animal. Res. 14,

1-28.

Matsuyama, H., Shioya, S., Ishida, M., Nishida, T., Hosoda, K., Bayaru, E., Ando, S., Islam, M.R., Yoshida, N., 2005. Feed characteristics of rice plant (*Oryza sativea* L. *cv., Hamasari, Yumetoiro, Hokuriku*-184) as whole crop silage. Jpn. J. Grassl. Sci. 51, 289-295.

Matsuyama, H., Shioya, S., Nishida, T., Hosoda, K., Bayaru, E., Yoshida, N., Ishida, M., 2006. Nutritive value of rice plants (*Oryza sativa* L. cv. Kusayutaka, Hamasari, Kusahonami) as whole crop silage. Jpn. J. Grassl. Sci. 51, 385-389.

McArthur, J.M., Miltimore, J.E., 1969. Bloat investigations. The pH of rumen contents and its role in legume bloat. Can, J. Anim. Sci. 49, 56-67.

McCrabb, G.J., Berger, K.T., Magner, T., May, C., Hunter, R.A., 1997. Ihhibiting methane production in Brahman cattle by dietary supplementation with a novel compound % the effects on growth. Aust. J. Agric. Res. 48, 323-329.

McDonald, P., Purves, D., 1956. Effects of the addition of molasses on the composition and digestibility of field silages. J. Sci. Food Agric. 7, 189-196.

McDougal, E.I., 1948. Studied on ruminant saliva. 1. The composition and output of sheep's saliva. Biochem. J. 43, 99-109.

McGinn, S.M., Beauchemin, K.A., Goates, T., Colombatto, D., 2004. Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. J. Anim. Sci. 82, 3346-3356.

McPherson, J.G., Shanmugam, G., Moiola, R.J., 1998. Fan-deltas and braid deltas - Varieties of coarse-grained deltas - Reply. Geol. Soc. Am Bull. 100, 1309.

McSweeney, C.B., Palmer, B., McNeill, D.M., Krause, D.O., 2001. Microbial interactions with tannins: nutritional consequences for ruminants. Anim. Feed Sci.

Technol. 91, 83-93.

Miettinen, H., Huhtanen, P., 1996. Effects of the ratio of ruminal propionate to butyrate on milk yield and blood metabolites in dairy cows. J. Dairy Sci. 79, 851-861.

Miettinen, H., Huhtanen, P., 1997. Effects of silage fermentation and post-ruminal casein supplementation in lactating dairy cows: 2-Enerby metabolites and plasma amino acids. J. Sci. Food Agric. 74, 459-468.

Migeotte, M.J., 1948. Spectroscopic eviderce of methane in the earth's atmosphere. Phys. Rev. 73, 519-520.

Miller, T.L., Wolin, M.J., 1973. Formation of hydrogen and formate by Ruminococcus albus. J. Bacteriol. 116, 836-846.

Miron, J., Zuckerman, E., Adin, G., Solomon, R., Shoshani, E., Nikbachat, M., Yosef, E., Zenou, A., Weinberg, Z.G., Chen, Y., Halachmi, I., Ben-Ghedalia, D., 2007. Comparison of two forage sorghum varieties with corn and the effect of feeding their silages on eating behavior and lactation performance of dairy cows. Anim. Feed Sci. Technol. 139, 23-39.

Moore, J.A., Poore, M.H., Eck, T.P., Swingle, R.S., Huber, J.T., Arana, M.J., 1992. Sorghum grain processing and buffer addition for early lactation cows. J. Dairy Sci. 75, 3465-3472.

Morimoto, H., 1969. Animal Nutrition Science (1st Edn). Yokendo, Tokyo, pp. 39-40.

Morvan, B., Doré, J., Rieu-Lesme, F., Foucat, L., Fonty, G., Gouet, P., 1994. Establishment of hydrogenutilizing bacteria in the rumen of the newborn lamb. FEMS microbiol. Lett. 117, 249-256.

Moss, A.R., 1993. Methane: Global Warming and Production by Animals. Chalcombe, Canterbury, UK. pp.105.

Moss, A.R., Givens, D.I., Garnsworthy, P.C., 1995. The effect of supplementing grass silage with barley on digestibility, in sacco degradability, rumen fermentation and methane production in sheep at two levels of intake. Anim. Feed Sci. Technol. 55, 9-33.

Moss, A.R., Jouany, J.-P., Newbold, J., 2000. Methane production by ruminants: its contribution to global warming. Ann. Zootech. 49, 231-253.

Mutsvangwa, T., Edward, I.E., Topps, J.H., Paterson, G.F.M., 1992. The effect of dietary inclusion of yeast culture (Yea-Sacc) on patterns of rumen fermentation, food intake and growth of intensively fed bulls. Anim. Prod. 55, 35-40.

National Agricultural Research Organization, NARO, 2001, Standard Tables of Feed Composition in Japan (2001). Japan Livestock Industry Association, Tokyo, Japan, pp. 220-221.

Nishida, T., Eruden, B., Hosoda, K., Matsuyama, H., Xu, C., Shioya, S., 2007. Digestibility, methane production and chewing activity of steers fed whole-crop round bale corn silage preserved at three maturities. Anim. Feed Sci. Technol. 135, 42-51.

Nishino, N., Hattori, I., Kishida, Y., 2007. Alcoholic fermentation and its prevention by Lactobacillus buchneri in whole crop rice silage. Lett. Appl. Microbiol. 44, 538-543.

Nishino, N., Waga, H., Yoshida, M., Shiota, H., 2004. Microbial counts, fermentation products, and aerobic stability of whole crop corn and a total mixed ration ensiled with and without inoculation of *Lactobacillus casei* or *Lactobacillus buchneri*. J. dairy Sci. 87, 2563-2570.

Niwa, Y., 2001. Silage making and utilization of high moisture by-products (part 1) 4. making silage from tofu-cake and utilization. Jpn. J. Grassl. Sci. 47, 23-326.

Nkrumah, J.D., Okine, E.K., Mathison, G.W., Schmid, K., Li, C., Basarab, J.A., Perice, M.A., Wang, Z., Moore, S.S., 2006. Relationships of feedlot feed efficiency, perfomance, and feeding behavior with metabolic rate, methane production, and energy partitionting in beef cattle. J. Anim. Sci. 84, 145-153.

Nocek, J.E., 1997. Bovine acidosis: implications on laminitis. J. Dairy Sci. 80, 1005-1028.

Nollet, L., Demeyer, D., Verstraete, W., 1997. Effects of 2-bromoethanesulfonic acid and Peptostreptococccus products ATCC 35244 addition on stimulation of reductive acetogenesis in the ruminal ecosystem by selective inhibition of methanogenesis. Appl. Environ. Microbiol. 63, 194-200.

Nollet, L., Mbanzamihigo, L., Demeyer, D., Verstraete, W., 1998. Effect of the addition of Peptostreptococcus productus ATCC 35244 on recuctive acetogenesis in the ruminal ecosystem after inhibition of methanogenesis by cellfree supermatant of Lactobacillus phantarum 80. Anim. Feed Sci. Technol. 71, 49-66.

Nombekela, S.W., Murphy, M.R., Gonyou, H.W., Marden, J.I., 1994. Dietary preferences in early lactation cows as affected by primary tastes and some common feed flavors. J. Dairy Sci. 77, 2393-2399.

NRC. 1985. Nutrient Requirements of Sheep. 6th rev. ed. Natl. Acad. Press, Washington, DC.

Ogino, A., Ishida, M., Ishikawa, T., Ikeguchi, A., Waki, M., Yokoyama, H., Tanaka, Y., Hirooka, H., 2008. Enviromental impacts of a Japanese dairy farming system using whole-crop rice silage as evaluated by life cycle assessment. Anim. Sci. J. 79, 727-736.

Ohajuruka, O.A., Wu, Z., Palmquist, D.L., 1991. Ruminal metabolism, fiber, and protein

digestion by lactation cows fed calcium soap or animal–vegetable fat. J. Dairy Sci. 74, 2601-2609.

Organization, N.A.R., 2001. Standard Tables of Feed Composition in Japan. Japan Livestock Industry Association, Tokyo, Japan, pp. 48-49, 72-79.

Orozco-Hernanoez, J.R., Brisson, G.J., Girard, V., 1995. Juice-extracted grass pellets and sodium bicarbonate for cows fed timothy silage of two chop lengths. J. Dairy Sci. 78, 2415-2423.

Orskov, E.R., McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to ate of passage. J. Agric. Sci. Camb. 92, 499-503.

Oshio, S., Tahata, I., Minato, H., 1987. Effects of diets differing in ratios of roughage to concentrate on microflora in the rumen of heifers. J. Gen. Appl. Microbiol. 33, 99-111.

Preston, T.R., 1982. Nutritional limitation associated with the feeding of tropical forages. J. Anim. Sci. 54, 877-884.

Prins, R.A., Van Nevel, C.J., Demeyer, D.I., 1972. Pure culture studies of inhibitors for methanogenic bacteria. Ant. Van. Leeuwen. 38, 281-287.

Provenza, F.A., 1995. Postingestive feedback as an elementary determinant of food preference and intake in ruminants. J. Range Manage. 48, 2-17.

Reed, J.D., 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. J. Anim. Sci. 73, 1516-1528.

Reynolds, C.K., Cammell, S.B., Humphries, D.J., Beever, D.E., 2001. Effects of postrumen starch infusion on milk production and energy metabolism in dairy cows. J. Dairy Sci. 84, 2250-2259.

Runpler, W.V., Jphnson, D.E., Bates, D.B., 1986. The effect of high dietary cation concentrations on methanogenesis by steers fed with or without ionophores. J. Anim. Sci. 62, 1737-1741.

Russel, J.B., Houlihan, A.J., 2003. Factores that alter rumen microbial ecology. Science 292, 1119-1122.

Russell, J.B., 1998. The importance of pH in the regulation of ruminal acetate to propionate ratio and methane production *in vitro*. J. Dairy Sci. 81, 3222-3230.

Russell, J.B., Hino, T., 1985. Regulation of lactate production in *Streptococcus bovis*: a spiraling effect that contributes to rumen acidosis. J. Dairy Sci. 68, 1712-1721.

Russell, J.B., Wallace, R.J., 1997. Energy-yielding and energy-consuming reactions. In: Hobson, P.N., Stewart, C.S., (Eds), The Rumen Microbial Ecosystem, 2nd edn. Blackie Academic and Professional, London, UK. pp. 246-282.

Sahoo, B., Saraswat, M.L., Haque, N., Khan, M.Y., 2000. Energy balance and methane procution in sheep fed chemically treated wheat straw. Small Rumin. Res. 35, 13-19.

Sahoo, B., Walli, T.K., 2008. Effects of formaldehyde treated mustard cake and molasses supplementation on nutrient utilization, microbial protein supply and feed efficiency in growing kids. Anim. Feed Sci. Technol. 142, 220-230.

SAS, 1995. SAS/STAT User's Guide, Version 6. SAS Institute, Cary, NC, USA, pp. 569-666.

Sato, S., 2005. Perspectives on the grazing system in Japan. Jpn. J. Grassl. Sci 51, 17-31.

Seki, M., Kimura, Y., Sunaga, S., Muroi, S., Koga, T., Ishizaki, S., Saito, K., Shimizu, K., Kato, Y., Uchida, T., Terada, F., 2000. Effect of by-products and corn grain processing on lactation and metabolic responses of dairy cows during early lactation period. Proceedings of Japanese Society for Animal Nutrition and metabolism 44,

141-154.

Shellito, S.M., Ward, M.A., Lardy, G.P., Bauer, M.L., Caton, J.S., 2006. Effects of concentrated separator by-product (desugared molasses) on intake, ruminal fermentation, digestion, and microbial efficiency in beef steers fed grass hay. J. Anim. Sci. 84, 1535-1543.

Shingfield, K.J., Jaakkola, S., Huhtanen, P., 2002. Effect of forage conservation method, concentrate level and propylene glycol on diet digestibility, rumen fermentation, blood metabolity concentrations and nutrient utilisation of dairy cows. Anim. Feed Sci. Technol. 97, 1-21.

Shioya, S., 2008. Futrue prospects of TMR center based on self-supplying feed. Jpn. J. Grassl. Sci. 54, 178-181.

Shioya, S., Cai, Y., 2004. Development of whole crop rice silage use for dairy and beef production. Proceedings of the Japan-Korea-China Symposium on Grassland Agriculture and Animal Production. Japannes Society of Grassland Science, pp. 51-54.

Shioya, S., Hosoda, K., Matsuyama, H., 2007. Feed characteristics of fermented TMR and future prospects. Proceedings of Japanese Society for Animal Nutrition and metabolism 51, 1-5.

Shioya, S., Nishida, T., Matsuyama, H., Hosada, K., Eruden, B., 2006. Feeding value of corn silage prepared by roll baler for chopped material. AAAP Animal Science Congress 12 th 2006 Congress Preceedings 676.

Shito, H., Yamana, N., 2002. Development of harvesting and ensiling technique for maize and sorghum by the roll baler of chopped material of prototype. Jpn. J. Grassl. Sci. 47, 610-614.

Shito, H., Yamana, N., 2003. Trial use of the round baler for chopped material for grass harvesting. Jpn. J. Grassl. Sci. 49, 514-515.

Shu, Q., Gill, H.S., Hennessy, D.W., Leng, R.A., Bird, S.H., Rowe, J.B., 1999. Immunisation against lactic acidosis in cattle. Res. Vet. Sci. 67, 65-71.

Silveira, C., Oba, M., Yang, W.Z., Beauchemin, K.A., 2007. Selection of barley grain affects ruminal fermentation, starch digestibility, and productivity of lactating dairy cows. J. Dairy Sci. 90, 2860-2869.

Smith, E.J., Henderson, A.R., Oldham, J.D., Whitaker, D.A., Aitchinson, K., Anderson, D.H., Kelly, J.M., 1993. The influence of an inoculant/enzyme preparation as an additive for grass silage offered in combination with three levels of concentrate supplementation on performance of lactating dairy cows. Anim. Prod. 56, 301-310.

Struble, M.B., Aomari, L.L., 2003. Position of the american dietetic association: addressing world hunger, malnutrition, and food insecurity. J. Am. Diet. Assoc. 103, 1046-1057.

Stumm, C.K., Gitzen, H.J., Vogels, G.D., 1982. Association of methanogenic bacteria with ovine rumen ciliates. Brit. J. Nutr. 48, 417-431.

Sutton, J.D., 1968. The fermentation of soluble carbohydrates in rumen contents of cows fed diets containing a large proportion of hay. Br. J. Nutr. 22, 689-712.

Sutton, J.D., Hart, I.C., Broster, W.H., Elliott, R.J., Schuller, E., 1986. Feeding frequency for lactating dairy cows: effects on rumen fermentation and blood metabolites and hormones. Br. J. Nutr. 56, 181-192.

Suzuki, N., Kaiser, H.M., 2005. Impacts of the doha round framework agreements on diry policies. J. Dairy Sci. 88, 1901-1908.

Takahashi, J., 2006. Emission of GHG from livstock production in Japan. In: Soliva, C.R.,

Takahashi, J., Kreuzer, M. (Eds), Greenhouse Gases and Animal Agriculture: An Update. International gongress Series 1293, pp. 13-20.

Takahashi, T., Horiguchi, K., Goto, M., 2005. Effect of crushing unhulled rice and the addition of fermented juice of epiphytic lactic acid bacteria on the fermentation quality of whole crop rice silage, and its digestibility and rumen fermentation status in sheep. Anim. Sci. J. 76, 353-358.

Teferedegne, B., McIntosh, F., Osuji, P.O., Odenyo, A., Wallace, R.J., Newbold, C.J., 1999. Influence of foliage from defferent accessions of the sub-tropical leguminous tree, Sesbania sesban, on ruminal protozoa in Ethiopia and Scottish sheep. Anim. Feed Sci. Technol. 78, 11-20.

Thomas, E.D., Mandebvu, P., Ballard, C.S., Sniffen, C.J., Carter, M.P., Beck, J., 2001. Comparison of corn silage hybrids for yield, nutrient composition, *in vitro* digestibility, and milk yield by dairy cows. J. Dairy Sci. 84, 2217-2226.

Tobisa, M., Nakano, Y., Paek, J., Mochizuki, T., Shimojo, M., Masuda, Y., 2005. Fermentation quality and palatability of rice plant silages as affected by mixing with phasey bean (*Macroptilium lathyroides* L. Urb.). Jpn. J. Grassl. Sci. 51, 274-280.

Tsuru, M., Momoze, Y., Takai, T., Nakayama, T., Watanabe, H., 2007. Fermentative quality of round bale silage made from densely planted forage sorghum (*soryghum bicolor* moench) using a flail-type harvester. Jpn. J. Grassl. Sci. 53, 11-15.

Urakawa, S., Yoshimura, Y., Hiraoka, H., Okumura, M., 2004. Development of a parallel unloading device for wrap silo mounting with a self-propelled bale wrapper. Jpn. J. Grassl. Sci. 50, 211-213.

Ushida, K., Jouany, J.P., 1996. Methane production associated with rumen-ciliated protozoa and its effect on protozoan activity. Lett. Appl. Microbiol. 23, 129-132.

Valdes, C., Newbold, C.J., Hillman, K., Wallace, R.J., 1996. Evidence for methane oxidation in rumen fluid *in vitro*. Ann. Zootech. 45 (Suppl.), 351.

Valdes, C., Newbold, C.J., Hillman, K., Wallace, R.J., 1997. Los microorganisms metanotrofos comeagentes modifactdores de la fermentacion ruminal. ITEA 18, 205-210.

Van kessel, J.S., Russell, J.B., 1996. The effect of pH on ruminal methanogenesis. FEMS microbiol. Ecol. 20, 205-210.

Van Nevel, C.J., Demeyer, D.I., 1992. Influence of antibiotics and a deaminase inhibitor on volatile fatty acids and methane production from detergent washed hay and soluble starch by rumen microbes *in vitro*. Anim. Feed Sci. Technol. 37, 21-31.

Van Nevel, C.J., Demeyer, D.I., 1995. Feed additives and other interventions for decreasing methane emissions. In: Walace, R.J., Chesson, A., (Eds.), Biotechnology in Animal Feeds & Animal Feeding. VCH Publishers Inc., New York, pp. 329-349.

Van Nevel, C.J., Demeyer, D.I., 1996. Control of rumen methanogenesis. Environ. Monit. Assess. 42, 73-97.

Van Nevel, C.J., Prins, R.A., Demeyer, D.I., 1974. Observations on the inverse relationship between methane and propionate in the rumen. Z. Tierphysiol. Tiererniihr. Futtermittelkde. 33, 121-125.

Van Soest, P.J., 1982. Nutritional Ecology of the Ruminant. O & B Books, Oregon, USA, pp. 40-41.

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

Vogels, G.D., Hoppe, W.F., Stumm, C.K., 1980. Association of methanogenic bacteria

with rumen ciliates. Appl. Environ. Microbiol. 40, 608-612.

Waghorn, G.C., 1991. Bloat in cattle 47. Relationships between intra-ruminal pressure, distension and the volume of gas used to simulate bloat in cows. N. Z. J. Agric. Res. 34, 213–220.

Waldo, D.R., Schultz, L.H., 1956. Lactic acid production in the rumen. J. Dairy Sci. 39, 1453-1460.

Waldo, D.R., Schultz, L.H., 1960. Blood and rumen changes following the intraruminal administration of glycogenic materials. J. Dairy Sci. 43, 469-505.

Wang, F., Nishino, N., 2008a. Ensiling of soybean curd residue and wet brewers grains with or without other feeds as a total mixed ration. J. Dairy Sci. 91, 2380-1387.

Wang, F., Nishino, N., 2008b. Resistance to aerobic deterioration of total mixed ration silage: effect of ration formulation, air infiltration and storage period on fermentation characteristics and aerobic stability. J. Sci. Food Agric. 88, 133-140.

Wang, F., Nishino, N., in press. association of *Lactobacillus buchneri* with aerobic stability of total mixed ration containing wet brewers grains preserved as a silage. Anim. Feed Sci. Technol. (2008), doi:10.1016/j.anifeedsci.2008.06.012.

Wang, Z.S., Goetsch, A.L., 1998. Intake and digestion by holstein steers consuming diets based on litter harvested after different numbers of broiler growing periods or with molasses addition before deep-stacking. J. Anim. Sci. 76, 880-887.

Warren, B.E., Farrell, D.J., 1990. The nutritive value of full-fat and defatted Australian rice bran. I. Chemical composition. Anim. Feed Sci. Technol. 27, 219-228.

Weinberg, Z.G., Ashbell, G., Chen, Y., 2003a. Stabilization of returned dairy products by ensiling with straw and molasses for animal feeding. J. Dairy Sci. 86, 1325-1329.

Weinberg, Z.G., Chen, Y., Gamburg, M., 2004. The passage of lactic acid bacteria from

silage into rumen fluid, *in vitro* studies. J. dairy Sci. 87, 3386-3397.

Weinberg, Z.G., Muck, R.E., Weimer, P.J., 2003b. The survival of lactic acid bacteria in rumen fluid. J. appl. Microbiol. 94, 1066-1071.

Wing, J.M., Van Horn, H.H., Sklare, S.D., Harris, B., JR., 1998. Effects of citrus molasses distillers solubles and molasses on rumen parameters and lactation. J. Dairy Sci. 71, 414-420.

Wolin, M.J., 1975. Interactions between the bacterial species of the rumen. In: McDonald, I.W., Warner, A.C.I., (Eds.), Digestion & Metablolism in the Ruminant. University of New England Publ. Unit, Armidale, Australia, pp. 134-148.

Wolin, M.J., 1979. The rumen fermentation: a model for microbial interactions in anaerobic ecosystems, in: Alexander M. (Ed.), Advances in Microbial Ecology, Plenum, New York & London, Vol. 3, PP. 49-77.

Wuisman, Y., Hiraoka, H., Yahaya, M.S., Takeda, M., Kim, W., Takahashi, T., Karita, S., Horiguchi, K., Takahashi, T., Goto, M., 2006. Effects of phenylalanine fermentation byproduct and sugarcane molasses on fermentation quality and rumen degradation of whole crop barley (*Hordeum vulgare* L.) silage *in situ*. Grassl. Sci. 52, 73-79.

Xu, C., Cai, Y., Fujita, Y., Kawamoto, H., 2003. Chemical composition and Nutritive value of tea grounds silage treated with lactic acid bacteria and acremonium cellulose. Anim. Sci. J. 74, 355-361.

Xu, C., Cai, Y., Kida, T., Matsuo, M., Kawamoto, H., Murai, M., 2004a. Silage preparation of total mixed ration with green tea grounds and its fermentation quality and nutritive value. Jpn. J. Grassl. Sci. 50, 40-46.

Xu, C., Cai, Y., Moriya, N., Ogawa, M., 2007a. Nutritive value for ruminants of green tea grounds as a replacement of brewers' grains in totally mixed ration silage. Anim.

Feed Sci. Technol. 138, 228-238.

Xu, C., Cai, Y., Murai, M., 2004b. Fermentation quality and nutritive value of total mixed ration silage with barley tea grounds. Anim. Sci. J. 75, 185-191.

Xu, C., Cai, Y., Zhang, J.G., Fukasawa, M., Moriya, N., 2008. Ensiling and subequent ruminal degradation characteristics of barley tea grounds treated with contrasting additives. Anim. Feed Sci. Technol. 141, 368-374.

Xu, C., Cai, Y., Zhang, J.G., Ogawa, M., 2007b. Fermentation quality and nutritive value of a total mixed ration silage containing coffee grounds at ten or twenty percent of dry matter. J. Anim. Sci. 85, 1024-1029.

Xu, C., Suzuki, H., Toyokawa, K., 2001. Characteristics of ruminal fermentation of sheep fed tofu cake silage with ethanol. Anim. Sci. J. 72, 299-305.

Xu, C., Toyokawa, K., 2005. Nutritive charactieristics and utilization of apple pomace and tofu cake. Jpn. J. Grassl. Sci 51, 220-225.

Yabuuchi, Y., Matsushita, Y., Otsuka, H., Fukamachi, K., Kobayashi, Y., 2006. Effects of supplemental lauric acid-rich oils in high-gran diet on *in vitro* rumen fermentation. Anim. Sci. J. 77, 300-307.

Yamamoto, Y., Deguchi, Y., Mizutani, M., Urakawa, S., Yamada, H., Hiraoka, H., Inui, K., Kouno, S., Goto, M., 2004. Improvement of fermentation quality and dry matter digestibility of rice whole crop silage treated with fermented juice of epipytic lactic acid bacteria and mechanical processing. Jpn. J. Grassl. Sci. 49, 665-668.

Yamamoto, Y., Inui, K., Urakawa, S., Hiraoka, H., Tomida, T., Tanaka, K., Nishikawa, S., Nakanishi, H., Maezawa, T., 2005. Nutritive value and utilization of nitrogen of fermented TMR based whole crop silage prepared in lap silo. Jpn. J. Grassl. Sci. 51 (special), 126-127.

Yan, T., Offer, N.W., Roberts, D.J., 1996. The effects of dietary mitrogen sources and levels on rumen fermentation, nutrient degradation and digestion and rumen microbial activity by wether sheep given a high level of molasses. Anim. Sci. 63, 123-131.

Yokota, H., Ohshima, M., 1997. Silage quality of napier grass (pennisetum purpureum schum.) ensiled with rice bran. Jpn. Anim. Sci. Technol. 68, 305-309.

Yoshida, N., M., T., Takemasa, Y., Takahashi, T., 1987. Forage paddy rice for whole crop utilization. Jpn. J. Grassl. Sci. 33, 109-115.

Zinn, R.A., 1987. Infuence of lasalocid and monensin plus tylosin on comparative feeding value of steam-flaked versus dry-rolled corn in diets for feedlot cattle. J. Anim. Sci. 65, 256-266.