

**Strategies for Utilization of
By-product Resources as Ruminant Feeds**

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**Strategies for Utilization of
By-product Resources as Ruminant Feeds**

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Abstract

A series of studies was conducted to evaluate the utilization of by-product resources as ruminant feeds. In Chapter 2 and 3, the nutritive value of two types of total mixed ration (TMR) silage containing different combinations of local food by-products such as noodle waste, soybean curd residue, soy sauce cake and potato waste was evaluated by determining their fermentation quality, digestibility and nitrogen utilization in wethers, and growth performance in fattening heifers. The TMR silage was well fermented and did not negatively affect digestibility or nitrogen utilization in wethers, or growth performance in fattening heifers. In Chapter 4, an experiment was conducted to determine the effect of feeding red winery wastes (i.e. winery sediment and grape pomace) on ruminal fermentation, antioxidant status and oxidative stress in wethers. Feeding winery wastes decreased ruminal ammonia production and also reduced peroxidation in wethers. In Chapter 5, the effect of lowering dietary cation-anion difference (DCAD) by feeding amino acid fermentation by-products on beef tenderness was evaluated. Lowering DCAD in fattening beef diets could improve calpain, intracellular

calcium-dependent protease activities although beef tenderness measured by shear force was not enhanced. Overall, by-product resources have a potential to maintain or improve animal performance and well-beings, to meet consumers' demands and to enhance the sustainability of livestock and food production.

Key words: animal productivity, by-product, product quality, ruminant, well-being

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List of Publications

Chapter 2, 3, 4 and 5 are the peer reviewed version of the following articles, respectively:

Chapter 2

Ishida K, Yani S, Kitagawa M, Oishi K, Hirooka H, Kumagai H. 2012.

Effects of adding food by-products mainly including noodle waste to total mixed ration silage on fermentation quality, feed intake, digestibility, nitrogen utilization and ruminal fermentation in wethers.

Animal Science Journal **83**, 735-742. DOI: 10.1111/j.1740-0929.2012.01016.x

Chapter 3

石田恭平, Srita Yani, 北川政幸, 岡野寛治, 大石風人, 広岡博之, 熊谷元. 2012.

麺類規格外品を主とする食品製造副産物を用いた発酵 TMR の飼料特性と黒毛和種未經産肥育雌牛の発育成績, 血液性状およびルーメン発酵に及ぼす影響
肉用牛研究会報 **93**, 10-18.

Chapter 4

Ishida K, Kishi Y, Oishi K, Hirooka H, Kumagai H. 2015.

Effects of feeding polyphenol-rich winery wastes on digestibility, nitrogen utilization, ruminal fermentation, antioxidant status and oxidative stress in wethers.

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Chapter 5

Ishida K, Hirano K. 2018.

Effect of negative dietary cation-anion differences on carcass characteristics and beef tenderness of Japanese Black steers.

Animal Science Journal **89**, 132-139. DOI: 10.1111/asj.12918

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Chapter 1

General introduction

The world population reached seven billion in 2011 and is expected to keep growing in the future (United Nations 2017). Food production needs to be increased and improved to meet demands of the growing population. The population and income growth as well as urbanization especially in developing countries have driven up meat and milk consumption (Delgado 2003). Therefore, sustainable livestock production systems to adapt to changes in people's diets have to be established.

Feeds account for the majority of production costs regardless of livestock species or their production systems (MAFF 2018). That means stable supply of feeds is essential for sustainable livestock production. Traditionally, most feeds (i.e. grains and/or forages) have been produced on-farm, but the trend for intensive production with increased animal numbers has made it infeasible for many operations and forced those producers to purchase feeds from off-farm (Bradford & Mullins 2012). In recent years, grains are used for competitive purposes like human foods, biofuels and livestock feeds, which have resulted in significantly higher feed costs. These trends, however, have been accompanied by an increasing supply of by-products from food or biofuel production (Maghsoud *et al.* 2008; Bradford & Mullins 2012).

By-product resources are generally derived from harvesting, processing or storage of commodities (DePeters *et al.* 2000; Maghsoud *et al.* 2008). In many cases, the fiber component is of little value for manufacturing and thus many by-products are relatively high in fiber, which is suitable for ruminant feeds (Bradford & Mullins 2012). Also some by-products could be good sources of

energy and/or protein as well as functional components for ruminants (Westendorf 2000; Sugiura *et al.* 2009). By-products have been fed to ruminants for centuries and numerous studies to evaluate by-product feeds have been conducted. Recently many by-products originally from human food or biofuel industries are commercially available in the market of ruminant feed ingredients (e.g. soybean meal, wheat bran, sugar beet pulp, rice straw, distillers grain, etc.). In addition of the nutritional potential, costs and environmental impacts also have led to increase interests in by-products as ruminant feeds (Westendorf 2000; Maghsoud *et al.* 2008; Oishi *et al.* 2011). Previous analysis by VandeHaar (1998) suggested that dairy operations using significant quantities of by-products required less amount of land compared with conventional feeding system and could produce nutrients for human consumption more efficiently. The utilization of by-product feeds can improve economic and environmental sustainability of ruminant agricultural industries.

One of the biggest challenges in using by-products as ruminant feedstuffs is in assessing their relative value compared with conventional feeds (Bradford & Mullins 2012). In general, fibrous by-products are not widely used in other industries than ruminant feeding, and thus those are obtained at relatively lower costs. However, Bradford and Mullins (2012) stated that market prices of by-product feeds could deviate from their nutritional values. For instance, some wet by-products with short shelf life can lead to increase the cost for transportation or feed shrinkage, which might decrease cost-efficiency

compared with conventional feedstuffs.

Drying can be a reasonable method to make it easy and industrial to handle by-product resources. Most of by-product ingredients broadly distributed or used at feed mills are generally low in moisture. However, drying needs specific facilities or equipment and additional energy costs, and also can create a dusty product, which may result in more difficult handling. Ensiling can be another method to handle especially for wet by-products. Acids generated during ensiling process prevent growth of undesirable microorganisms and keep stability of feeds with decreasing pH (Danner *et al.* 2003). If wet by-products were ensiled with dry feeds, the risk of effluent production or feed sorting would be minimized (Xu *et al.* 2007; Bradford & Mullins 2012). Moreover, some unpalatable by-products could be fed with incorporating with other ingredients by masking the unpalatability (Horst *et al.* 1994; Xu *et al.* 2007).

Local use of by-product resources to avoid long-distance transportation can be a possible alternative. In many cases, by-products vary greatly in quantity and quality depending on their sources (Stock *et al.* 1999). Therefore, using local by-products from single or limited sources could minimize fluctuation in nutritional values and costs. In some countries where most feeds are imported costly, such as Japan, local feeds could contribute to decrease feed costs and also to increase the self-sufficiency of feed (Sugiura *et al.* 2009). Moreover in recent years, consumers have preferred livestock products that can be identified by farmers and have begun to pay attention to what was fed

to these animals. A survey by the Ministry of Agriculture, Forestry and Fisheries of Japan (MAFF 2017) demonstrated that more than 60% of consumers put importance on domestic foods and over 40% put on locally-grown foods when purchasing. Feeding local by-products to local animals could give consumers a will to pay additional price to these products, which in turn could support profitability of farmers.

When incorporating a new feedstuff into a diet, it is common to directly replace an existing ingredient. Many by-products have been evaluated as isonutritional and cost-effective alternatives of conventional feedstuffs. It has been considered that fibrous by-products (e.g. soybean hulls, wheat middlings, sugar beet pulp, etc.) have a potential to maintain or improve performance of cattle and reduce feed costs when replacing grains or forages (Grant 1997; Bradford & Mullins 2012). Stock *et al.* (1999) also mentioned that grain milling by-products such as corn gluten feed, hominy feed or distillers grain are good sources of energy and protein for cattle. In the study by Padunglerk *et al.* (2017), it was concluded that monosodium glutamate by-product could be used in dairy cow diet as a replacement for soybean meal at a lower feed cost. On the other hand, by-products with some functional components could be used as feed additives, not as replacements of conventional feeds. In the previous study by Shabtay *et al.* (2008), feeding pomegranate peel to bull calves promoted significant increases in feed intake and α -tocopherol concentration in the plasma, which suggested that pomegranate peel would be a health-promoting constituent of cattle diet.

Veskoukis *et al.* (2012) also demonstrated that extracts from polyphenol-rich grape pomace exhibited protective activity against oxidative damage. Consumers have demanded healthier food and have paid more attention to the hedonistic aspect of products (Vasta *et al.* 2008). For instance, the use of synthetic additives has begun to be resisted because of consumers' concerns about food safety (Greathead 2003; Brooker & Acamovic 2005; Gladine *et al.* 2007a). In addition, meat and milk with favorable characteristics, such as fatty acid profiles, taste and flavor, color or tenderness (Vasta *et al.* 2008), have been purchased at premium prices. Thus, using by-product feeds to produce healthy and high-quality livestock products would fit with consumers' needs.

By-product resources present opportunities to feeds as replacements of common feedstuffs or as additives to support quality of products, which in turn promote the sustainability of ruminant agriculture. Overall objective in this study, therefore, was to evaluate the utilization of by-product resources as ruminant feeds. In Chapter 2, an experiment was conducted to evaluate the nutritive value of two types of total mixed ration (TMR) silage, respectively containing different combinations of local food by-products by assessing their fermentation quality, and their intake, digestibility and ruminal fermentation in wethers. In Chapter 3, the effect of feeding TMR silage containing local food by-products to fattening beef cattle on their growth performance and ruminal fermentation was determined. In Chapter 4, a study was conducted to determine the effect of feeding red winery wastes

on digestibility, nitrogen utilization, ruminal fermentation, antioxidant status and oxidative stress in wethers. In Chapter 5, the effect of lowering dietary cation-anion difference (DCAD) by feeding amino acid fermentation by-products on carcass characteristics and beef tenderness was evaluated.

Chapter 2

Effects of adding food by-products

mainly including noodle waste to total mixed ration silage
on fermentation quality, feed intake, digestibility, nitrogen
utilization and ruminal fermentation in wethers

2.1 Introduction

In almost all developed or developing countries, several factors have recently led to increased interests in using food by-products as animal feeds. These factors include the increased necessity of pollution abatement, the increasing costs of waste disposal, the goal of feeding livestock without competing with human nutrition and changes in perception of the value of economical feed (Maghsoud *et al.* 2008). In Japan, numerous food by-products are produced by the food processing industries, but most of them are incinerated or buried in landfills, leaving the country to depend mostly on imported feeds for its livestock. The food by-products thus produced and discarded include potato waste, soy sauce cake, soybean curd residue and noodle waste, all of which contain high crude protein (CP) and/or total digestible nutrients (TDN). Using these by-products as livestock feeds could provide competitive alternatives to traditional feed sources, and possibly reduce costs and environmental impact (Westendorf 2000; Oishi *et al.* 2011). Moreover, it is important to utilize food by-products in order to raise the self-sufficiency rate and ensure the stable supply of feeds (Sugiura *et al.* 2009).

Potato waste, a potato hash containing both skins and solids, is a by-product from the production of snacks or potato starch. Potato waste is a quantitatively important energy source in animal feeds, and at the same time, this application of potato waste solves a potentially massive disposal problem for the food processing industry (Nelson 2010). Soy sauce cake is a

dried pomace derived from soy sauce production. Chiou *et al.* (1995) reported that soy sauce cake contained high levels of CP and/or energy and possessed protein characteristics similar to corn silage, and thus could be a good dietary source for ruminants. Soybean curd residue contains particularly high CP, and is therefore a good source of protein for ruminants (Chiou *et al.* 1995). Chiou *et al.* (1998) reported that the partial substitution of soybean curd residue for soybean meal (10% on a dry matter (DM) basis) in cattle feed resulted in a better lactation performance. Noodle waste is a below-standard product of wheat noodles (udon noodle) or buckwheat noodles. Wheat is generically used in ruminant feeding, and the suitability of whole buckwheat silage or buckwheat grain as a diet ingredient has been demonstrated in dairy cows (Amelchanka *et al.* 2010). However, the use of noodles made from wheat or buckwheat in ruminant diets is less common, whereas broken noodles (extruded wheat flour) are common ingredient in swine diets in Asia (Hongtrakul *et al.* 1998).

Food by-products such as potato waste, soybean curd residue and noodle waste, however, are readily damaged due to their high moisture content. Although these local food by-products have nutritive value, they are difficult to incorporate into a commercial feeding program because of the preservation problem (Westendorf *et al.* 2000). Ensiling can be considered as a method to use wet by-products more effectively (Kayouli & Lee 1999). If high moisture by-products are ensiled without any additives, however, substantial financial resources are wasted on transporting water (Wang &

Nishino 2008). Therefore, ensiling wet by-products with dry feeds as a total mixed ration (TMR) is a suitable method to minimize the risk of effluent (Xu *et al.* 2007). In addition, unpalatable by-products could be used as ingredients in TMR silage if their flavor and odor could be altered by fermentation (Xu *et al.* 2007; Cao *et al.* 2009). In Japan, wet by-products such as soybean curd residue are often used as ingredients in TMR silage (Wang & Nishino 2009), especially in dairy cattle farming.

Nonetheless, there has been only limited research on the use of TMR silage produced from various food by-products for purposes of beef fattening. Thus, a pilot study was conducted to evaluate the nutritive value of two types of TMR silage, respectively containing different combinations of potato waste, soy sauce cake, soybean curd residue and noodle waste, for beef fattening by assessing their fermentation quality, and their intake, digestibility and ruminal fermentation in wethers.

2.2 Materials and Methods

Silage preparation of TMR

Two types of TMR silage (T1 and T2) were produced by mixing food by-products with 41.67% DM commercial concentrate (CC) as a dry feed. Four food by-products, potato waste, soy sauce cake, soybean curd residue and noodle waste, were obtained from commercial food-processing factories

in Nantan District of Kyoto Prefecture. The commercial concentrate included 38% barley grains, 32% flaked corn, 14% wheat bran, 9% rice bran, 5% soybean meal, 1% calcium carbonate, 1% salts and 0.05% vitamin and mineral mixture. The chemical composition of each food by-product is shown in Table 2-1.

The TMR silage was formulated to satisfy the minimum levels of the CP, TDN, calcium (Ca) and phosphorus (P) requirements as nutrient contents of feed for Japanese Black heifers weighing 450 kg and gaining 0.6 kg per day according to the Japanese Feeding Standard for Beef Cattle (National Agriculture and Food Research Organization 2009). T1 was formulated to use all of the available food by-products, and T2 was formulated to put a higher priority on fitting the chemical composition to that of CC. The proportions of ingredients in the TMR silage are shown in Table 2-2.

The TMR silage was ensiled and fermented in oil drums (200 L capacity), and then stored outdoors for one month from August 22 to September 24, 2009. During the experiments, the TMR silage was repackaged in amounts of about 8 kg in plastic bags and stored at 4°C.

***In vivo* digestibility experiment**

A 4 × 4 Latin square design experiment was conducted at the animal shelter at Kyoto University. Four wethers with initial body weight (BW) of 43.4 ± 1.6 kg, which had ruminal cannulae, were used in this experiment. The wethers were individually housed in metabolic cages. An adaptation period (9 days)

was followed by a sample collection period (6 days). The wethers used in the experiment were managed according to the guidelines of the Kyoto University Animal Ethics Committee.

The experimental treatments consisted of TRE1, TRE2, CTL and TFH (only a basal diet: tall fescue hay). In the TRE1, TRE2 and CTL treatments, T1, T2 or CC were combined with TFH in a 1:1 ratio on a DM basis before feeding. The wethers were given the experimental diets at 2% of BW on a DM basis in two equal portions fed at 09.30 and 16.30 hours. The amounts of the diets offered to and refused by the wethers were recorded daily. The wethers had *ad libitum* access to water and mineral blocks throughout the experiment.

During the sample collection period, feed residue and feces were obtained and weighed daily before the morning feeding. These samples were respectively mixed per wether in each treatment. All samples of diets, residue and feces were dried at 60°C for 48 h and ground with a Willey mill to pass a 1 mm screen for chemical analyses. Urine was collected into vessels containing 20% sulfuric acid to prevent the loss of ammonia nitrogen (N). The volume of urine was measured daily, and 50 mL representative samples were collected. The urine samples were mixed per wether per treatment according to the original excretion ratio and stored at -20°C until analysis. The digestibility of each experimental diet was calculated by subtracting the digestibility of TFH according to the indirect method as described by the National Agriculture and Food Research Organization (2010).

On the last day (6th day) of the collection period, ruminal fluid was

collected via the rumen cannula of each wether at 0, 1, 4 and 7 h after the morning feeding. The ruminal fluid was filtered through four layers of gauze, and immediately analyzed for ruminal pH using a glass electrode pH meter (Horiba Ltd., Kyoto, Japan).

Chemical analyses

Diet samples were analyzed for DM, CP, ether extract (EE) and crude ash (methods 930.15, 925.04, 976.05, 920.39 and 942.05, respectively) according to the Association of Official Analytical Chemists (AOAC 2000). The organic matter (OM) was calculated as weight loss through ashing. Neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash (aNDFom) and acid detergent fiber expressed exclusive of residual ash (ADFom) were analyzed according to Van Soest *et al.* (1991). Samples of residue and feces were analyzed for DM, CP, OM, aNDFom and ADFom as stated above. Samples of urine were analyzed for urinary N using the Kjeldahl procedure described by AOAC (2000). Fermentation products of the TMR silage were determined from cold water extracts. Wet TMR silage (45 g) was homogenized with 140 mL of distilled water and the pH was measured using a glass electrode pH meter (Horiba Ltd.). Lactic acid was analyzed according to Barnett (1951). The NH₃-N was determined by the microdiffusion method (Conway 1962). The Ca and P contents in food by-products were determined by the atomic absorption analysis (Price 1979) and the colorimetric method of Gomori (1942), respectively.

Statistical analyses

Data on intake, apparent digestibility and nitrogen balance were analyzed using the MIXED procedure of Statistical Analysis System (SAS 1998). The mathematical model was $Y_{ijk} = \mu + T_i + P_j + A_k + e_{ijk}$, where μ = the overall mean, T_i = the fixed effect of treatment, P_j = the fixed effect of period, A_k = the random effect of animal and e_{ijk} = residual error. Ruminant pH was analyzed by the MIXED procedure (SAS 1998) for repeated measures according to the method of Atkinson *et al.* (2007). The model included treatment, period, sampling time, animal and the treatment $\times$ sampling time interaction. The three way interaction of treatment $\times$ period $\times$ animal was used to specify variation among animals using RANDOM statement (SAS 1998). An autoregressive covariance structure (AR1) was determined to be most appropriate based on Akaike's criterion. The Tukey-Kramer test was used to detect differences between the means (Kramer 1956).

2.3 Results

Chemical composition and fermentation characteristics of TMR silage

Table 2-3 shows the chemical composition of the experimental diets and fermentation characteristics of the TMR silage. Among the experimental diets, T1 and T2 had lower DM ($P < 0.01$), aNDFom ($P < 0.01$), ADFom ($P <$

0.05) and crude ash contents ($P < 0.01$), but higher CP ($P < 0.01$), OM ($P < 0.01$) and nonfibrous carbohydrate (NFC) contents ($P < 0.01$). The EE content was similar for T1 and CC, while was lower for T2 ($P < 0.01$). As for the fermentation characteristics, The TMR silage was well fermented, as indicated by the high lactic acid contents, the low pH values and the low $\text{NH}_3\text{-N}$ /total N levels.

Table 2-4 shows the chemical composition of the experimental treatments (TRE1, TRE2 and CTL). The DM, aNDFom and ADFom contents were lower for TRE1 and TRE2 than for CTL ($P < 0.01$). On the other hand, TRE1 and TRE2 had higher CP, OM and NFC contents than CTL ($P < 0.01$). The EE content was similar for TRE1 and CTL, while was lower for TRE2 ($P < 0.01$).

Voluntary feed intake and digestibility

The DM, CP, OM, aNDFom and ADFom intakes and apparent digestibility in each treatment are presented in Table 2-5. The DM, OM, aNDFom and ADFom intakes did not significantly differ among TRE1, TRE2 and CTL. The CP intake was higher for TRE1 and TRE2 than for CTL ($P < 0.05$). In TFH treatment, the intakes of DM, CP, OM, aNDFom and ADFom were 44.75, 2.49, 41.75, 31.91 and 19.18 g/day $\cdot \text{BW}^{0.75}$, respectively.

The DM and OM digestibility had a tendency to be higher for TRE1 than for CTL ($P = 0.083$, $P = 0.069$). The aNDFom digestibility for TRE2 was lower than those for CTL ($P < 0.05$). The CP and ADFom digestibility did not significantly differ among TRE1, TRE2 and CTL. The apparent digestibility

of DM, CP, OM, aNDFom and ADFom in TFH was 46.82, 38.93, 46.95, 50.87 and 46.65 %, respectively.

The digestibility of DM, CP and OM in each experimental diet estimated by subtracting the digestibility of TFH using the indirect method is shown in Table 2-6. The DM and OM digestibility for T1 was higher than for T2 and CC ($P < 0.05$), while CP digestibility was similar among the experimental diets.

Nitrogen balance

The nitrogen balance of each treatment is shown in Table 2-7. The N intakes of TRE1 and TRE2 were higher than that of CTL ($P < 0.05$). Fecal and urinary N did not significantly differ among the treatments. The averaged urine volumes were 2220, 2305 and 1474 mL/day for TRE1, TRE2 and CTL, respectively. The urine volumes of TRE1 and TRE2 were not significantly different from that of CTL. The retained N and the retention rate of N were similar among TRE1, TRE2 and CTL. In TFH, retained N was $-0.06 \text{ g/day} \cdot \text{BW}^{0.75}$ and retention rate was -15.18% , which indicated the negative balance of nitrogen.

Ruminal fermentation

The ruminal pH values at 0, 1, 4 and 7 h after feeding are shown in Table 2-8. There were significant effects of treatment, sampling time and the treatment $\times$ sampling time interaction on ruminal pH ($P < 0.01$). A similar ruminal pH

was observed in TRE1, TRE2 and CTL throughout the sampling time. In TFH, ruminal pH was 6.72, 6.67, 6.48 and 6.98 at 0, 1, 4 and 7 h after feeding, respectively.

Ruminal pH in TRE1 and TRE2 reached the minimum pH levels of 6.26 and 6.25, respectively, at 1 h after feeding. In CTL, ruminal pH was lowest at 4 h.

2.4 Discussion

Chemical composition and fermentation characteristics

The DM contents of T1 and T2 were lower than that of CC (Table 2-3). When formulating the TMR silage, however, estimated DM contents calculated by the chemical analyses of the ingredients were 37.7 and 40.7% for T1 and T2, respectively, which were higher than measured values. Previous studies on the effects of air exposure of silage on fermentative losses of nutrients (Woolford 1990; Tabacco *et al.* 2011) reported that the DM content decreased and the CP content increased with increasing duration of exposure to air. In the present study, the CP contents on a DM basis were higher for T1 and T2 than for CC (Table 2-3), which might have been due to the DM losses by air exposure after repackaging. The lower aNDFom and ADFom concentrations of the TMR silage were likely due to the high noodle waste content in the ingredients (Table 2-1).

McDonald and Whittenbury (1973) classified the fermentation quality of silage based on the pH and -N/total N levels. According to their classification system, the fermentation quality of T1 and T2 in this study can be evaluated as good ($\text{pH} \leq 4.2$) or excellent (ammonia-N/total N $\leq 12.5\%$) as shown in Table 2-3.

Evaluation of the nutritive value of TMR silage by the *in vivo* digestibility experiment

In TRE1, TRE2 and CTL, the wethers ate the offered diets almost completely in a short time and the DM intake was similar (Table 2-5), implying that the palatability of the TMR silage by wethers was as high as CC. However, Chiou *et al.* (1998) reported that 10% soybean curd residue in TMR depressed the DM intake due to its high water content. Although Lahr *et al.* (1983) also demonstrated that DM intake decreased with the increase of dietary water content, such a result was not observed in this study despite lower DM contents in TRE1 and TRE2 (Table 2-4). Xu *et al.* (2007) and Cao *et al.* (2009) mentioned that ensiling unpalatable wet by-products with dry feeds could regulate the water contents and alter their flavor and odor. In the present study, ensiling these high moisture by-products with CC as TMR might have a positive effect on the feed intake by wethers. The higher CP intake for TRE1 and TRE2 than for CTL (Table 2-5) might have been attributable to the higher CP contents of T1 and T2 as shown in Table 2-3.

Stanhope *et al.* (1980) reported that DM digestion was not affected by

addition of potato waste. In this study, the DM digestibility was not different among TRE1, TRE2 and CTL (Table 2-5), but was higher for T1 than for CC (Table 2-6), which was inconsistent with the previous result. On the other hand, Cao *et al.* (2009) reported that the DM and OM digestibility of TMR silage containing 30% dried soybean curd residue was high because of the low aNDFom content of this silage. The higher DM and OM digestibility of T1 in the present study might be due to the lower aNDFom contents of T1 than CC.

These results indicated that feeding the TMR silage to wethers had negative effects neither on feed intake nor on digestion, except that the aNDFom digestibility of TRE2 was lower than that of the other treatments (Table 2-5). Ben Salem *et al.* (1996) reported that a high soluble carbohydrates content depressed ruminal cellulolytic activity. Our study showed that food by-products such as potato waste and noodle waste were rich in easily fermentable carbohydrates. That the NFC content was highest in TRE2 (Table 2-4) might explain the lower apparent aNDFom digestibility.

Although the CP intake was higher for TRE1 and TRE2 than for CTL, the urinary N levels and the retention N of TRE1 and TRE2 were similar to those of CTL (Table 2-7). Ruminants have an ability to recycle urea N into the rumen for microbial protein synthesis with carbohydrates and proteins, and this ability leads to a reduction of urinary N excretion (Benlamlih & Pomyers 1989). Microbial protein synthesis is enhanced when easily fermentable carbohydrates are fed because total volatile fatty acid

production increases (Hoover & Stokes 1991; Firkins 1996; Hristov & Ropp 2003). Reducing N losses through this system will improve N utilization (Osuji *et al.* 1993; Shem *et al.* 2003). The rich, easily fermentable carbohydrates in potato waste and noodle waste in T1 and T2 might have promoted microbial protein synthesis in the rumen, which in turn could have inhibited N excretion into the urine and maintained the amount and rate of N retention. The results indicated that the TMR silage could be a good source of carbohydrates and proteins. On the other hand, a negative balance was observed in TFH, which indicated that TFH did not meet the nitrogen requirement for wethers.

Previous studies showed that high salt loading induced an increase in water intake both in sheep and cattle (Kelly *et al.* 1955; Winchester & Morris 1956; Peirce 1957). In addition, Weeth and Lesperance (1965) demonstrated that salt loading to cattle increased not only water intake but also urine volume. In the present study, the numerically increased urine output in TRE1 and TRE2 treatments might have been due to their high salt content from the dietary ingredients, particularly soy sauce cake. According to the Standard Tables of Feed Composition in Japan (National Agriculture and Food Research Organization 2010), soy sauce cake contains 4.43% sodium (11.26% salt) on a DM basis. Oishi *et al.* (2011) restricted the amount of soy sauce cake when formulating TMR with food by-products due to its high level of salt. The intake of salt in this study, however, was below the maximum tolerable level (9% DM) of the National Research Council (1980) because the

calculated amounts of salt consumed by the wethers were 4.8 and 8.1 g/day for TRE1 and TRE2, respectively. In fact, feeding the TMR silage to wethers had adverse effects neither on feed intake nor on digestion, although Masters *et al.* (2005) reported that increasing sodium in the diet significantly decreased feed intake and digestibility in sheep.

Ruminal pH has been shown to decline after feeding because the production of organic acid is increased by fermentation of the diet in the rumen, and to be immediately decreased after feeding easily fermentable carbohydrates, due to the enhanced production of organic acid (Allen 1997; Russell & Rychlik 2001). Maghsoud *et al.* (2008) mentioned that the high NFC content of noodle waste led to high propionate production, which resulted in decreasing ruminal pH. In addition, Baldwin *et al.* (1962) showed that lactate ingested from diets was converted into propionate by the rumen microflora. In the present study, the ruminal pH was affected by the interaction between treatment and time after feeding (Table 2-8), and the ruminal pH in TRE1 and TRE2 immediately decreased compared to that in CTL, probably due to the lactic acid concentration in the TMR silage or NFC content in T1 and T2 (Table 2-3) due to the inclusion of potato waste or noodle waste. In addition, increasing chewing activities and saliva flow can decrease the acid production in rumen because saliva flow has a dilution effect (Sudweeks 1977). The amount of the diets was restricted to 2 % of BW on a DM basis in this study, and the wethers consumed the offered diets in a short time. The time spent eating TRE1 or TRE2 diets might have also

enhanced the acid production and affected the ruminal pH levels.

However, minimum pH was similar among the TRE1, TRE2 and CTL in the present study. This result was not consistent with that of the previous study by Krause *et al.* (2002), who reported that the level of ruminally fermentable carbohydrates affected mean ruminal pH and minimum pH, but did not affect the time post feeding for minimum pH. Maekawa *et al.* (2002) showed that nadir ruminal pH value was affected by the feeding management (i.e. whether feeding a TMR or separate ingredients). In this study, T1, T2 or CC was mixed with TFH when offering TRE1, TRE2 or CTL diets. The same feeding management could explain the similar minimum pH values in rumen fluid among TRE1, TRE2 and CTL.

Two types of TMR silage including potato waste, soy sauce cake, soybean curd residue and noodle waste were well preserved with high fermentation quality and high CP contents. Feeding the TMR silage had few negative effects on the intake and digestibility of DM and OM, CP digestibility and N balance, and increased CP intake. Meanwhile, the TMR silage immediately decreased ruminal pH after feeding, which might have been due to the high ruminal degradability resulting from much NFC and less aNDFom.

In summary, it may be concluded that the two types of TMR silage containing various food by-products can be utilized as a substitute for a barley-and-corn-based concentrate for beef fattening. Further studies will be needed to increase the availability of food by-products as animal feeds.

Table 2-1 Chemical composition of food by-products (%)

Component	Potato waste	Soy sauce cake	Soybean curd	
			residue	Noodle waste‡
DM	15.90	66.30	29.30	25.20
CP†	6.92	26.09	37.06	13.10
EE†	5.66	32.13	15.36	1.19
NFC†	64.56	3.74	45.01	83.39
TDN†	71.30	99.80	96.10	80.90
aNDFom†	19.70	24.92	23.30	1.14
ADFom†	17.40	23.57	15.27	0.78
Crude Ash†	3.15	13.12	4.44	1.19
Ca†	0.19	0.23	0.34	-
P†	0.13	0.18	0.44	0.08

† On a DM basis.

‡ Contains udon noodle mainly.

ADFom, acid detergent fiber expressed exclusive of residual ash; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; Ca, calcium; CP, crude protein; DM, dry matter; EE, ether extract; NFC, nonfibrous carbohydrate (100 - CP - ether extract - aNDFom - crude ash); P, phosphorus; TDN, total digestible nutrients.

Table 2-2 Proportions of ingredients in the TMR silage (% DM)

Ingredient	Diet	
	T1	T2
Commercial concentrate [†]	41.67	41.67
Potato waste	2.45	-
Soy sauce cake	6.13	13.30
Soybean curd residue	7.42	-
Noodle waste	42.32	45.04

[†] Contains (% as fed basis) 38.0 barley grains, 32.0 flaked corn, 4.0 wheat bran, 9.0 rice bran, 5.0 soybean meal, 1.0 calcium carbohydrate, 1.0 salts, 0.05 vitamin.

DM, dry matter; T1 and T2, TMR silage.

Table 2-3 Chemical composition of the experimental diets and fermentation quality of the TMR silage

Item	Diet				SEM
	T1	T2	CC	TFH	
Chemical composition (%)					
DM	36.31 ^a	39.68 ^a	85.77 ^b	85.76 ^b	0.99
CP [†]	16.34 ^c	15.96 ^c	13.42 ^b	5.59 ^a	0.14
OM [†]	96.97 ^b	96.56 ^b	92.67 ^a	93.13 ^a	0.32
EE [†]	7.51 ^c	5.46 ^b	7.27 ^c	1.04 ^a	0.23
NFC [†]	62.88 ^c	65.28 ^c	52.10 ^b	15.24 ^a	0.70
aNDFom [†]	10.24 ^a	9.86 ^a	19.88 ^b	71.26 ^c	0.37
ADFom [†]	4.62 ^a	4.23 ^a	7.36 ^b	42.85 ^c	0.57
Crude ash [†]	3.03 ^a	3.44 ^a	7.33 ^b	6.87 ^b	0.45
Fermentation quality					
pH	3.71	3.67	-	-	0.02
Lactic acid (%)‡	2.78 ^b	2.37 ^a	-	-	0.08
Ammonia-N/total N (%)‡	3.48 ^b	2.07 ^a	-	-	0.17

Means in the same row with different superscripts differ significantly ($P < 0.05$).

[†] On a DM basis. [‡] On a FM basis.

ADFom, acid detergent fiber expressed exclusive of residual ash; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; CC, commercial concentrate; CP, crude protein; DM, dry matter; EE, ether extract; FM, fresh matter; NFC, nonfibrous carbohydrate (100 - CP - EE - aNDFom - crude ash); N, nitrogen; OM, organic matter; SEM, standard error of the means; T1 and T2, TMR silage; TFH, tall fescue hay.

Table 2-4 Chemical composition of the experimental treatments fed to wethers (%)

Item	Treatment			SEM
	TRE1 [†]	TRE2 [†]	CTL [†]	
DM	61.04 ^a	62.72 ^a	85.76 ^b	0.39
CP [‡]	10.97 ^b	10.77 ^b	9.50 ^a	0.07
OM [‡]	95.05 ^b	94.85 ^b	92.90 ^a	0.07
EE [‡]	4.27 ^b	3.25 ^a	4.16 ^b	0.12
NFC [‡]	39.06 ^b	40.26 ^a	33.67 ^c	0.28
aNDFom [‡]	40.75 ^a	40.56 ^a	45.57 ^b	0.15
ADFom [‡]	23.74 ^a	23.54 ^a	25.11 ^b	0.12
Crude ash [‡]	4.95 ^a	5.16 ^a	7.10 ^b	0.07

Means in the same row with different superscripts differ significantly ($P < 0.05$).

[†] TRE1, TRE2 and CTL: fed T1, T2 and CC with TFH by 1:1 on a DM basis, respectively.

[‡] On a DM basis.

ADFom, acid detergent fiber expressed exclusive of residual ash; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; CC, commercial concentrate; CP, crude protein; DM, dry matter; EE, ether extract; NFC, nonfibrous carbohydrate (100 - CP - EE - aNDFom - crude ash); OM, organic matter; SEM, standard error of the means; T1 and T2, TMR silage; TFH, tall fescue hay; TRE1, TRE2 and CTL, the experimental treatments.

Table 2-5 Effect of feeding the experimental diets on intake and apparent digestibility in wethers

Item	Treatment			SEM
	TRE1 [†]	TRE2 [†]	CTL [†]	
Intake (g/day/BW ^{0.75})				
DM	50.58	49.62	49.27	1.42
CP	5.59 ^b	5.31 ^b	4.63 ^a	0.10
OM	48.09	47.05	45.78	1.32
aNDFom	20.39	20.35	22.77	0.98
ADFom	11.87	11.81	12.59	0.50
Apparent digestibility (%)				
DM	68.50	66.63	65.77	0.89
CP	71.62	68.30	67.99	1.22
OM	69.19	67.11	66.20	0.94
aNDFom	50.05 ^{ab}	46.94 ^a	52.47 ^b	1.29
ADFom	48.46	47.71	47.04	1.14

Means in the same row with different superscripts differ significantly ($P < 0.05$).

[†] TRE1, TRE2 and CTL: fed T1, T2 and CC with TFH by 1:1 on a DM basis, respectively.

ADFom, acid detergent fiber expressed exclusive of residual ash; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; BW, body weight; CC, commercial concentrate; CP, crude protein; DM, dry matter; OM, organic matter; SEM, standard error of the means; T1 and T2, TMR silage; TFH, tall fescue hay; TRE1, TRE2 and CTL, the experimental treatments.

Table 2-6 Estimated digestibility of the experimental diets (%)[†]

Item	Diet			SEM
	T1	T2	CC	
DM	87.61 ^b	84.72 ^a	83.61 ^a	2.96
CP	82.06	78.43	80.31	1.28
OM	89.96 ^b	87.10 ^a	86.47 ^a	1.45

Means in the same row with different superscripts differ significantly ($P < 0.05$).

[†] Estimated by the indirect method.

CC, commercial concentrate; CP, crude protein; DM, dry matter; OM, organic matter; SEM, standard error of the means; T1 and T2, TMR silage; TFH, tall fescue hay.

Table 2-7 Effect of feeding the experimental diets on nitrogen balance in wethers

Item	Treatment			SEM
	TRE1 [†]	TRE2 [†]	CTL [†]	
N balance (g/day/BW ^{0.75})				
Intake N	0.89 ^b	0.85 ^b	0.74 ^a	0.02
Fecal N	0.25	0.27	0.24	0.01
Urinary N	0.42	0.38	0.34	0.03
Retention N	0.22	0.20	0.16	0.03
Retention rate (%)	24.70	23.94	21.71	4.37

Means in the same row with different superscripts differ significantly ($P < 0.05$).

[†] TRE1, TRE2 and CTL: fed T1, T2 and CC with TFH by 1:1 on a DM basis, respectively.

BW, body weight; CC, commercial concentrate; N, nitrogen; SEM, standard error of the means; T1 and T2, TMR silage; TFH, tall fescue hay; TRE1, TRE2 and CTL, the experimental treatments;

Table 2-8 Effect of feeding the experimental diets on ruminal pH

	Treatment	Time after feeding (h)				SEM	<i>P</i> -value		
		0	1	4	7		Treatment	Time	Treatment × time
Ruminal pH	TRE1†	7.14 ^b	6.26 ^a	6.55 ^a	6.72 ^{ab}	0.12	**	***	**
	TRE2†	7.08 ^b	6.25 ^a	6.47 ^a	6.67 ^{ab}				
	CTL†	6.92 ^b	6.55 ^{ab}	6.29 ^a	6.48 ^{ab}				

Means in the same row with different superscripts differ significantly ($P < 0.05$).

** $P < 0.01$, *** $P < 0.001$.

† TRE1, TRE2 and CTL: fed T1, T2 and CC with TFH by 1:1 on a DM basis, respectively.

CC, commercial concentrate; SEM, standard error of the means; T1 and T2, TMR silage; TFH, tall fescue hay; TRE1, TRE2 and CTL, the experimental treatments.

Chapter 3

Effects of adding food by-products
mainly including noodle waste to total mixed ration silage
on its feed characteristics, and growing performance,
blood metabolite concentrations and ruminal fermentation
in Japanese Black fattening heifers

3.1 Introduction

Interests in using food by-products as livestock feeds have recently increased because of the necessity of pollution abatement, the increasing costs of waste disposal, the goal of feeding livestock without competing with human nutrition and changes in perception of the value of economical feed (Maghsoud *et al.* 2008). In Japan, numerous food by-products are produced by the food processing industries, but most of them are incinerated or buried in landfills, leaving the country to depend mostly on imported feeds for its livestock. Those food by-products include potato waste, soy sauce cake, soybean curd residue and noodle waste, all of which contain high crude protein (CP) and/or total digestible nutrients (TDN). Using these by-products as livestock feeds could provide good alternatives to conventional feed sources, and possibly reduce costs and environmental impact as well as ensure self-sufficient feed supplies (Westendorf 2000; Sugiura *et al.* 2009; Oishi *et al.* 2011).

Feeding by-products to livestock is nothing new. Soybean curd residue contains particularly high CP, and is therefore a good source of protein for ruminants (Chiou *et al.* 1995; Sugiura *et al.* 2009). Chiou *et al.* (1995) also mentioned that soy sauce cake derived from soy sauce production would contain high level of CP and/or energy. Potato waste, a by-product from the production of potato starch, could be a good energy source for ruminant feed (Nelson 2010). Noodle waste is a below-standard product of wheat noodles

(udon) or buckwheat noodles (soba). Wheat is generically used in ruminant feeding, and the applicability of buckwheat grain as a feed ingredient has been demonstrated in dairy cows (Amelchanka *et al.* 2010). However, using noodle waste in ruminant diets is less common, whereas noodles are common ingredient in swine diets in Asia (Hongtrakul *et al.* 1998).

Although they have a nutritive potential as ruminant diets, the preservation of those food by-products is problematic because their high moisture content can lead to feed spoilage. Drying can prevent feed shrinkage, but it needs additional costs for equipments and energy. Also some sticky by-products like noodle waste may chunk during drying process, which makes their handling more difficult. Therefore, ensiling those wet by-products with dry ingredients as total mixed ration (TMR) silage is a suitable method to prevent spoilage (Xu *et al.* 2007).

In Japan, feeding TMR silage to fattening beef cattle is not common compared with dairy cow feeding. Our previous study have demonstrated that feeding the TMR silage containing noodle waste, soybean curd residue, soy sauce cake and potato waste had no adverse effects on intake, nutrient digestibility or nitrogen (N) balance in wethers compared with feeding a barley-and-corn-based concentrate for beef fattening (Ishida *et al.* 2012a). In this study, therefore, we evaluated the applicability of TMR silage containing various food by-products by determining its fermentation quality, digestibility, and growth performance, blood metabolite concentrations and ruminal fermentation in fattening heifers.

3.2 Materials and Methods

The animals in the study were managed according to the guidelines of the Kyoto University Animal Ethics Committee.

Feed preparation

Four food by-products, noodle waste, soybean curd residue, soy sauce cake and potato waste, were obtained from commercial food-processing factories in Nantan District of Kyoto Prefecture. The chemical compositions of the obtained food by-products are shown in Table 3-1. The TMR silage was produced by mixing 38.6% noodle waste, 6.8% soybean curd residue, 5.6% soy sauce cake, 2.2% potato waste, 38.0% commercial concentrate mix for beef fattening and 8.9% wheat straw on a dry matter (DM) basis (Table 3-2). The amount of food by-products in the TMR silage was chosen to be similar CP, TDN, Ca and P contents to the concentrate mix. After mixing all ingredients, the TMR silage was ensiled in flexible container bag silos (700KHR; Fukunaga Engineering, Osaka, Japan) for one month.

***In vivo* digestibility experiment**

Three wethers (averaged body weight (BW): 46.4 kg) were used to determine the TDN content of the TMR silage. In this experiment, 9-day adaptation period was followed by 5-day sample collection period. The wethers were

individually housed in metabolic cages at the animal shelter at Kyoto University. The wethers were fed the TMR silage and tall fescue straw at 1% of BW on a DM basis respectively. The diets were offered twice a day in equal portions at 09.30 and 16.30 hours. The diets offered and refused for each wether were recorded daily. The wethers had free access to water and mineral blocks.

In the sample collection period, all feces from each wether were obtained and weighed daily before morning feeding, and were dried at 60°C for 48 h. Dried feces were sub-sampled for each wether based on daily amount. The composite samples of diets, refusals and feces were ground with a Willey mill to pass a 1-mm screen.

Samples were analyzed for DM, CP, ether extracts (EE) and ash contents according to the Association of Official Analytical Chemists (AOAC 2000). The organic matter (OM) was calculated as weight loss through ashing. Total tract digestibility of DM, CP, OM and crude fat was calculated for each wether based on the nutrient composition of the diet offered and refused. The digestibility of the TMR silage itself was determined by subtracting the digestibility of tall fescue straw according to the indirect method as described by the National Agriculture and Food Research Organization (Japan Grassland Farming Forage Seed Association 2009), and TDN content of the TMR silage was calculated. The TDN content of the concentrate mix was estimated according to the Standard Tables of Feed Composition in Japan (NARO 2010).

Feeding experiment

A switch back experiment was conducted with six 31.3 month old Japanese Black fattening heifers (averaged BW: 564 kg). The animals were divided into two groups with similar averaged BW between the groups, and were allotted to one of the two treatments (Table 3-3): 1) control (CC; feeding commercial concentrate at 1.4% of BW and wheat straw at 0.3% of BW on a DM basis), 2) experimental treatment (TMR; replacing 40% of commercial concentrate in CC with food by-products in the TMR silage, i.e. feeding food by-products at 0.56% of BW on a DM basis). One-week adaptation periods were followed by 6-week experimental periods. The heifers were fed twice daily at 9.30 and 15.30 hours with an individual feeding system (DF-6; Balance System Ltd., Chiba, Japan). The diets were offered to meet DM and energy requirements for targeted BW gain 0.6 kg/d for Japanese Black heifers according to the Japanese Feeding Standard for Beef Cattle (NARO 2009). The amounts of the diets offered and refused were recorded every day.

In the experimental periods, feed refusals were collected individually and composited every week. The composited refusals were dried at 60°C for 48 h. Dried samples were sub-sampled for each heifer based on weekly amount and were ground with a Willey mill to pass a 1-mm screen. Body weight was measured at the beginning, 21 and final day of each experimental period. At 0 and 4 h after morning feeding on the last day of the experimental period, ruminal fluid was collected with oral catheter. Ruminal fluid was filtered

through four layers of gauze and measured for pH using a glass electrode pH meter (Horiba Ltd., Kyoto, Japan). Blood samples were collected from each heifer via jugular vein puncture into vacuum tubes containing heparin sodium before morning feeding on the last day of the experimental period. The collected blood was centrifuged at $2,300 \times g$ for 10 min and the plasma was stored at -20°C until analysis.

Chemical Analyses

Diet and refusal samples were analyzed for DM, CP, EE and ash contents (AOAC 2000), and calculated for OM as weight loss through ashing. Neutral detergent fiber (aNDFom) and acid detergent fiber (ADFom) expressed exclusive of residual ash were also determined by the method of Van Soest *et al.* (1991). Non-fibrous carbohydrate (NFC) was calculated as $\text{NFC} = 100 - \text{CP, \%DM} - \text{EE, \%DM} - \text{aNDFom, \%DM} - \text{ash, \%DM}$ (NRC 2001). The Ca and P contents in food by-products were determined by the atomic absorption analysis (Price 1979) and the colorimetric method of Gomori (1942), respectively.

Wet TMR silage (approximately 40 g) was homogenized with 140 mL of distilled water and was stored overnight. The slurry was filtered through two layers of gauze, and pH of the filtrate was measured using a glass electrode pH meter (F-52; Horiba Ltd., Kyoto, Japan). Ammonia-N was determined by the microdiffusion method (Conway 1962). Lactic acid was analyzed according to Barnett (1951). Concentrations of volatile fatty acids

in the TMR silage were determined by gas chromatography (GC-14B; Shimadzu Corporation, Kyoto, Japan) with a glass column (hermon-3000-3% Shimalite TPA 60/80, 3.2 mm $\phi \times 2.1$ m; Shimadzu Corporation, Kyoto, Japan). Column temperature was set at 120°C and injection temperature was set at 200°C. To evaluate the quality of the TMR silage, V-score was calculated based on Ammonia-N/total N and VFA concentrations (Takahashi *et al.* 2005).

Concentrations of glucose, nonesterified fatty acid (NEFA), albumin, blood urea nitrogen (BUN), total cholesterol in plasma were analyzed using diagnostic kits (Glucose-HR II, NEFA-HR, Albumin-HR II, L type Wako UN, L type Wako CHO·H; Wako Pure Chemical Industries, Ltd., Osaka, Japan). Total plasma protein concentrations were measured using a refractometer (SPR-Ne; Atago Co. Ltd., Tokyo, Japan). Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities were determined by fluorescence analysis according to Huang *et al.* (2006).

Statistical analysis

Data obtained in the feeding experiment was analyzed using GLM procedure of Statistical Analysis System (SAS 1998). The mathematical model was $Y_{ijk} = \mu + T_i + P_j + G_k + e_{ijk}$, where μ = the overall mean, T_i = the fixed effect of treatment, P_j = the fixed effect of period, G_k = the random effect of group and e_{ijk} = residual error. Significance was declared at $P < 0.05$.

3.3 Results

Feed characteristics

Chemical compositions of the diet and fermentation characteristics of the TMR silage are shown in Table 3-4. The silage had relatively lower CP, EE, aNDFom and ash contents, while higher OM and ADFom compared with the commercial concentrate mix. Propionic acid or butyric acid was not detected in the TMR silage and its V-score was 92.6.

Apparent digestibility of the TMR silage is shown in Table 3-5. The DM, CP and OM digestibility of the TMR silage were numerically lower than those of the commercial concentrate mix determined in our companion study (83.61, 80.31 and 86.47%, respectively; Ishida *et al.* 2012a).

Growth performance, rumen pH and blood components

Nutrient consumptions of the heifers in the feeding experiment were not different between the treatments (Table 3-6). The BW, daily gain or feed conversion ratio did not significantly differ between the treatments.

Rumen pH before and after morning feeding was similar in CC and TMR, and there is no significant differences in pH changes between the treatments (Table 3-7). Any measured blood components were not different between the treatments.

3.4 Discussion

Silage is produced as a result of fermentation of sugar in the ingredients by epiphytic microbes especially lactic acid bacteria. This fermentation generates mainly lactic acid which decreases pH and prevents spoilage (Ohyama *et al.* 1975; Davies *et al.* 1998; Cai *et al.* 1999). In addition to lactic acid bacteria and anaerobic condition, therefore, plenty of sugar is needed to produce high-quality silage (Davies *et al.* 1998; Takahashi *et al.* 2005). According to the classification based on pH and ammonia-N/total N described by McDonald and Whittenbury (1973), the TMR silage in this study (Table 3-4) was evaluated as good ($\text{pH} \leq 4.2$) or excellent (ammonia-N/total N $\leq 12.5\%$). In addition, V-score of the TMR was evaluated as good (> 80 ; Japan Grassland Farming Forage Seed Association 2009). These results suggested that the TMR silage was well fermented with good quality. High NFC in potato waste and noodle waste (Table 3-1) would contribute to appropriate fermentation. In general, soy sauce cake contains high sodium (11.26% sodium chloride on a DM basis; NARO 2010). Shockey and Borger (1991) demonstrated that adding 4% salt (sodium chloride) to silage inhibit growth of lactic acid bacteria. In this study, however, salts from soy sauce cake in the TMR silage (Table 3-2) was calculated to be about 0.6%DM, which could not lead to decrease fermentation quality. It also should be noted that 0.6% salt was below maximum tolerance level for beef

cattle (9%DM; NARO 2009).

Addition of straw to silage can decrease digestibility because of decreased CP and NFC and increased less digestible fiber contents (Phillips & Pendlum 1984; Jakhmola *et al.* 1990). In this study, CP and TDN concentrations in the TMR silage was lower than the commercial concentrate mix (Table 3-4) although the ingredient proportions of food by-products were formulated to be similar in CP and TDN contents. It is suggested that wheat straw included in the TMR silage diluted CP and digestible energy values. In our companion study where we formulated the TMR silage with the same ingredients except for wheat straw, DM and OM digestibility was higher and CP digestibility was similar compared with the concentrate mix (Ishida *et al.* 2012a). Radunz *et al.* (2003) reported that feeding 400 g/kg potato waste in corn-based concentrate decreased DM intake because of high moisture content of the by-product. In this study, however, similar DM and TDN intake was recorded between the treatments (Table 3-6) likely because wheat straw was added to reduce moisture content in the TMR silage. Those results demonstrate that adding wheat straw to the TMR silage in this study could maintain nutrient consumptions although digestibility was decreased. Numerical difference of daily gain between the treatments (Table 3-6) was likely due to the difference of digestibility between the concentrate mix and the TMR silage since nutrient intakes were similar.

Nelson *et al.* (2000) reported that steers fed 10% or 20% potato by-products in a barley- or corn-based diet did not affect feed efficiency compared with

those diets without potato by-products, which is consistent with our result of feed conversion ratio (Table 3-6). However, Radunz *et al.* (2003) noted that increasing up to 30% potato waste could decrease feed efficiency. In this study, potato waste which was the wettest ingredient (Table 3-1) was included at 2.2% DM in the TMR silage. This small proportion might minimize negative effects of potato waste on intake, daily gain or efficiency. Based on these results, we can conclude that feeding the TMR silage containing food by-products to fattening heifers did not adversely affect their growth performance in this study.

Blood metabolite concentrations fluctuate with nutritional and physiological status, and thus can be used as indicators of animal health and adequacy of feeding management (Russel & Wright 1983). Elevation of blood total cholesterol concentrations in Japanese Black is normally observed in fattening period (Matsuzaki *et al.* 1997). Previous studies have shown that total cholesterol concentrations in late fattening period are positively correlated to some carcass traits (Ito & Hirooka 2003; Kato *et al.* 2011). In this study, blood total cholesterol concentrations were similar between the treatments (Table 3-7) and relatively higher than those observed in the previous study (approximately 120 mg/dL; Matsuzaki *et al.* 1997). Several studies in sheep and cattle have reported elevated plasma glucose concentrations when feeding high-starch diets (Evans *et al.* 1975; Jenny & Polan 1975). Dietary starch content was not measured in this study, but high NFC in noodle waste or potato waste might have contributed to numerically

high plasma glucose concentrations in the TMR treatment. Blood NEFA is elevated when feed intake is restricted and body fat reserves are mobilized (Bell 1995). In addition increased plasma albumin concentrations reflect the utilization of body protein reserves to meet energy requirements (Blum *et al.* 1985). Similar blood NEFA and albumin concentrations between the treatments were observed in this study, and those levels were not too high relative to the previous study (approximately 0.2 mEq/L in NEFA and 3.5 g/dL in albumin; Matsuzaki *et al.* 1997). That might suggest that body fat or protein mobilization did not occur since heifers had as much intake as they needed. Plasma total protein and BUN concentrations increase with increased protein supply, and thus they are used as indicators of protein status and/or N efficiency (Jahoor *et al.* 1999; Huntington *et al.* 2001; Kohn *et al.* 2005). The total protein and BUN concentrations in the TMR treatment were not different from those in CC, which could reflect similar CP intake between the treatments (Table 3-6). We have demonstrated that the TMR silage including food by-products did not negatively affect N balance in wethers (Ishida *et al.* 2012a), which would agree with the result in the present study. Activities of GOT and GPT are evaluated as indicators of health disorders especially in liver function. In this study, blood GOT or GPT activities did not differ between the treatments, which suggested that feeding the TMR silage had no negative effects on liver function of the heifers. Overall, similar blood components indicated that the TMR silage fed in this experiment did not affect nutritional and physiological status in heifers.

Ruminal pH fluctuates in a 24 h period, but mean pH is generally higher than 5.5, often in the range of 5.8 to 6.5 in grain adapted cattle (Nagaraja & Titgemeyer 2007). Most of measured ruminal pH in this study, however, was out of this range especially before morning feeding (Table 3-7). Saliva which has a high buffering capacity (Owens *et al.* 1998) might be contaminated because ruminal fluid was taken through oral catheter in this study. In general, ruminal pH immediately declines after feeding easily fermentable carbohydrates, due to the enhanced production of organic acid (Allen 1997; Russell & Rychlik 2001; Wang *et al.* 2009). Maghsoud *et al.* (2008) mentioned that the high NFC content of noodle waste would lead to high propionate production, which resulted in decreasing ruminal pH. In the present study, ruminal pH in the TMR treatment either before or after feeding was not significantly different from that in CC. This result is consistent with our companion study where the TMR silage mainly including noodle waste was fed to wethers (Ishida *et al.* 2012a). It is suggested that the TMR silage including 38.6% noodle waste did not differ ruminal fermentation of the heifers compared with when feeding a barley-and-corn-based concentrate mix.

In summary, the present results showed that the TMR silage including noodle waste, soybean curd residue, soy sauce cake and potato waste was well preserved with high fermentation quality, and had no adverse effects on growth performance, blood components or ruminal fermentation in heifers. It may be concluded that the TMR silage including various food by-products

can be used as a substitute for a barley-and-corn-based concentrate for beef fattening.

Table 3-1 Chemical composition of the food by-products (%)

	Soybean curd			
	Noodle waste [†]	residue	Soy sauce cake	Potato waste
DM	25.20	29.30	66.30	15.90
CP*	13.10	37.06	26.09	6.92
EE*	1.19	15.36	32.13	5.66
NFC*	83.39	45.01	3.74	64.56
TDN*	80.90	96.10	99.80	71.30
aNDFom*	1.14	23.30	24.92	19.70
ADFom*	0.78	15.27	23.57	17.40
Ash*	1.19	4.44	13.12	3.15
Ca*	-	0.34	0.23	0.19
P*	0.08	0.44	0.18	0.13

* On a DM basis.

[†] Contains udon noodle mainly.

ADFom, acid detergent fiber expressed exclusive of residual ash; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; Ca, calcium; CP, crude protein; DM, dry matter; EE, ether extract; NFC, nonfibrous carbohydrate; P, phosphorus; TDN, total digestible nutrients.

Table 3-2 Proportions of ingredients in the TMR silage (% DM)

	Proportion
Noodle waste	38.6
Soybean curd residue	6.8
Soy sauce cake	5.6
Potato waste	2.2
Commercial concentrate [†]	38.0
Wheat straw	8.9

[†] Contains (% as fed basis) 38.0 barley grains, 32.0 flaked corn, 14.0 wheat bran, 9.0 rice bran, 5.0 soybean meal, 1.0 calcium, carbohydrate, 1.0 salts, 0.05 vitamin.

DM, dry matter.

Table 3-3 Proportions of feed ingredients in the treatment groups (% of BW on DM)

	Treatment [†]	
	CC	TMR
Commercial concentrate	1.40	0.44
Wheat straw	0.30	0.21
TMR silage	-	1.05 [‡]

[†] The dietary treatments are CC (contains no TMR silage) and TMR (contains 1.05% TMR silage on a DM basis).

[‡] Contained 0.56% food by-products (amount to 40% of commercial concentrate in CC).

BW, body weight; DM, dry matter.

Table 3-4 Chemical composition of the experimental diets and fermentation quality of the TMR silage

	Commercial concentrate	Wheat straw	TMR silage
Chemical composition (%)			
DM	85.77 ± 0.19	84.11 ± 0.18	42.32 ± 1.63
OM*	92.67 ± 0.24	96.19 ± 0.69	96.48 ± 0.16
CP*	13.42 ± 0.14	1.49 ± 0.01	12.49 ± 0.58
EE*	7.27 ± 0.11	1.21 ± 0.03	3.45 ± 0.47
NFC*	52.13 ± 1.57	10.66 ± 0.10	62.71 ± 3.48
TDN*	83.47 [†]	44.30 [†]	79.88 ± 6.73 [‡]
aNDFom*	19.88 ± 1.08	82.83 ± 0.80	17.84 ± 3.39
ADFom*	7.36 ± 0.66	64.86 ± 0.43	10.42 ± 1.71
Ash*	7.33 ± 0.24	3.81 ± 0.69	3.51 ± 0.16
Fermentation quality			
pH	-	-	4.18 ± 0.09
Lactic acid** (%)	-	-	2.34 ± 0.13
Acetic acid** (%)	-	-	0.92 ± 0.13
Propionic acid** (%)	-	-	ND
Butyric acid** (%)	-	-	ND
Ammonia-N/Total N** (%)	-	-	0.65 ± 0.20
V-score	-	-	92.6 ± 0.9

Values are means ± standard deviations.

* On a DM basis. ** On a FM basis.

[†] Calculated using Standard Tables of Feed Composition in Japan (NARO 2010).

[‡] Calculated using the estimated digestibility: TDN = digestible OM + 1.25 × digestible EE.

ADFom, acid detergent fiber expressed exclusive of residual ash; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; CP, crude protein; DM, dry matter; EE, ether extract; FM, fresh matter; N, nitrogen; ND, not detected; NFC, nonfibrous carbohydrate; OM, organic matter; TDN, total digestible nutrient.

Table 3-5 Digestibility of the TMR silage estimated by the indirect method (%)

	Digestibility
DM	77.87 ± 7.52
OM	78.43 ± 6.89
CP	69.37 ± 6.74
EE	97.62 ± 2.52

Values are means ± standard deviations.

CP, crude protein; DM, dry matter; EE, ether extract; OM, organic matter.

Table 3-6 Effect of feeding the TMR silage on performance of the heifers

	Treatment [†]		SEM
	CC	TMR	
Feed intake			
DM (kg/day)	7.37	7.62	0.33
OM (kg/day)	7.16	7.51	0.33
CP (kg/day)	0.820	0.842	0.04
TDN (kg/day)	5.81	5.97	0.28
Growth performance			
Initial BW (kg)	580.5	581.8	17.8
Final BW (kg)	606.5	602.3	16.8
ADG (kg/day)	0.62	0.49	0.07
Feed conversion ratio	13.50	16.45	2.22

[†] The dietary treatments are CC (contains no TMR silage) and TMR (contains 1.05% TMR silage on a DM basis).

ADG, average daily gain; BW, body weight; CP, crude protein; DM, dry matter; OM, organic matter; SEM, standard error of the means; TDN, total digestible nutrients.

Table 3-7 Effect of feeding the TMR silage on blood metabolite concentration and ruminal pH

	Treatment [†]		
	CC	TMR	SEM
Blood metabolites			
Glucose (mg/dL)	78.50	81.67	2.96
NEFA (mEq/L)	0.22	0.20	0.05
Total protein (g/dL)	7.62	7.67	0.13
Albumin (g/dL)	3.83	3.85	0.05
BUN (mg/dL)	13.58	14.77	1.07
Total cholesterol (mg/dL)	191.00	191.50	13.46
GOT (IU/L)	62.33	63.67	5.28
GPT (IU/L)	26.00	25.00	1.83
Ruminal pH			
0 h after feeding	7.78	8.06	0.13
4 h after feeding	7.27	7.46	0.17

[†] The dietary treatments are CC (contains no TMR silage) and TMR (contains 1.05% TMR silage on a DM basis).

BUN, blood urea nitrogen; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; NEFA, nonesterified fatty acid; SEM, standard error of the means.

Chapter 4

Effects of feeding polyphenol-rich red winery wastes
on digestibility, nitrogen utilization, ruminal fermentation,
antioxidant status and oxidative stress in wethers

4.1 Introduction

Annual global wine production has stood at more than 26.5 billion liters in recent years (OIV 2007). Despite occasionally unfavorable climate conditions, such as El Niño in 1998, global wine production has never dropped because areas devoted to the cultivation of wine has expanded. In particular, wine production in North America, Asia and Africa has grown rapidly, while the yields in Europe remain at the highest level in the world (OIV 2007).

There are various kinds of winery wastes: stalks, winery grape pomace and winery sediment. Winery grape pomace is produced in the process of crushing the grapes to extract their juice after fermentation. Winery sediment (the remaining protein in the fermented wine) is produced by removal with fining agents such as bentonites, albumens or gelatins. Although these winery wastes (stalks, grape pomace and sediment) may account for approximately 300 g/kg (50 g/kg, 200 g/kg and 50 g/kg, respectively) of the grapes for wine production (Makris *et al.* 2007), most of them are incinerated or buried in landfills. Using these wastes as livestock feed might possibly reduce both livestock production costs and the environmental impact of waste disposal from wine production.

In recent years, consumers have begun to resist the use of non-plant xenobiotics in feed because of concerns about safety and toxicity (Greathead 2003; Brooker & Acamovic 2005; Gladine *et al.* 2007a). Thus, natural agents derived from plants are considered of great interest for use as dietary

supplements. It has been reported that both winery sediment and grape pomace possess high phenolic compounds, such as polyphenols (Lu & Foo 1999; Negro *et al.* 2003; González-Paramás *et al.* 2004; Bustamante *et al.* 2008). Vinson *et al.* (2001) mentioned that antioxidative capacity of plant-derived polyphenols was stronger than that of vitamins. Oliveira *et al.* (2010) also demonstrated that feeding polyphenols from pomegranates enhanced cytokine production in calves, which might benefit their immune competence. In the *in vitro* studies by Veskoukis *et al.* (2012), it was demonstrated that the polyphenol-rich grape pomace extract exhibited significant protective activity against DNA damage induced by oxidants. Sgorlon *et al.* (2006) reported that polyphenols extracted from grape skin were effective in inducing the specific transcriptional activity of genes involved in antioxidative defenses in sheep. These previous studies indicated that polyphenols might be good sources of natural antioxidants, and thus winery wastes can be potential sources of natural antioxidants to provide some benefits to livestock.

Although they can be good sources of natural antioxidants, winery wastes have not been commonly used as dietary antioxidants for livestock and there is little information about their bioavailability and *in vivo* antioxidant activities. Veskoukis *et al.* (2012) suggested that the *in vitro* antioxidant activities did not necessarily translate to *in vivo*. In addition, antioxidative additives have different effects on ruminants than on monogastrics because of bacterial fermentation in the rumen. It is therefore important to evaluate

the availability of winery sediment and grape pomace as ruminant feeds possessing antioxidant activities. For this purpose, a study on winery sediment and grape pomace as additives to feeds was conducted to determine feed intake, digestibility, nitrogen balance, ruminal fermentation, plasma total antioxidant status (TAS) and urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) excretion in wethers. It should be noted that the TAS is the sum of water-soluble antioxidant substances (Yuki *et al.* 2010), and urinary 8-OHdG has been used as a biomarker of oxidative stress, especially of DNA damage *in vivo* (Shigenaga *et al.* 1989; Koike *et al.* 2010).

4.2 Materials and Methods

All animals used in the experiment were managed according to the guidelines of the Kyoto University Animal Ethics Committee.

Preparation of diets

Two types of winery wastes, winery sediment and fresh grape pomace, were obtained from a commercial winery. The winery wastes were all from a red cultivar (Cabernet Sauvignon) grown in Maipo Valley, Chile. The winery grape pomace, which contained peels, seeds and stalks, was collected in the process of crushing for their juice after fermentation. The sediment was obtained by removing it from wine by bentonite fining.

The winery sediment was filtered through four layers of gauze to reduce

their moisture contents, and the residue was used in the experiments. During the experiments, the remaining winery sediment and grape pomace were stored at 4°C.

Animals, treatments and experimental design

A 4 × 4 Latin square design experiment was conducted at the animal shelter at Kyoto University, Japan. Four wethers with ruminal canulae whose initial body weight (BW) was 42.5 ± 6.8 kg were used in this experiment. The wethers were housed individually in metabolic cages. An adaptation period (9 days) was followed by a sample collection period (5 days). Each wether was assigned to one of four treatments: (i) 75 g/kg winery sediment (WS) on a dry matter (DM) basis; (ii) 166 g/kg DM winery grape pomace (WP); (iii) control diet (CD; 17 g/kg DM soybean meal); and (iv) only tall fescue hay (TFH; no additive). In the WS, WP and CD treatments, each additive was combined with the commercial total mixed ration (TMR) (314 g/kg corn grains, 246 g/kg barley grains, 156 g/kg wheat bran, 100 g/kg brewers' grains, 50 g/kg Italian rye grass, 50 g/kg straw, 50 g/kg vitamin premix, 23 g/kg soybean meal, 9 g/kg calcium carbonate and 2 g/kg salts) and tall fescue hay before feeding. In the TFH treatment, no concentrate was included to examine an effect of the high concentrate diets such as WS, WP and CD on susceptibility to oxidative stress of the wethers. The ingredient proportions for WS, WP and CD were calculated to be isonitrogenous after the chemical analysis of each dietary ingredient for its crude protein (CP) content as shown in Table 4-1.

The winery sediment, which contained high CP, was added as a protein source by replacing TMR for WS. In WP, winery grape pomace was substituted for tall fescue hay because of its high fiber content. The roughage-concentrate ratio was set to be 1:3 in WS, WP and CD. The wethers were given the experimental diets at about 20 g/kg of BW on a DM basis to promote continuous intake of the winery wastes. The diets were fed in two equal portions at 09.30 and 16.30 hours and the amounts offered to and refused by the wethers were recorded daily. The wethers had *ad libitum* access to water throughout the experiment.

Sampling

During the sample collection period, refused feed and feces were obtained and weighed daily before the morning feeding. These samples were accumulated separately throughout the period for each wether. All samples of feed, refused feed and feces were dried at 60°C for 48 h and ground with a Wiley mill to pass through a 1 mm screen for chemical analyses. Urine was collected into vessels containing 20% sulfuric acid to prevent the loss of ammonia nitrogen (NH₃-N). The volume of urine was measured daily, and 50 mL representative samples were collected. The urine samples were mixed per wether per treatment according to the original excretion quantities and stored at -20°C until analysis.

On the last (5th) day of the collection period, ruminal fluid (approximately 100 mL) was collected via the rumen cannula of each wether at 0 and 4 h

after the morning feeding. The ruminal fluid was filtered through four layers of gauze, and immediately analyzed for ruminal pH using a glass electrode pH meter (Horiba Ltd., Kyoto, Japan). Ruminal fluid was centrifuged at $500 \times g$ for 5 min and the supernatants were stored at -20°C until analysis.

At 0, 2 and 4 h after feeding on the last day of the sample collection period, blood samples (approximately 10 mL) were collected from each wether via jugular vein puncture into vacuum tubes containing heparin sodium. The collected blood was centrifuged at $1460 \times g$ for 10 min and the plasma was stored at -20°C until analysis.

Chemical analyses

Diet samples were analyzed for DM, CP, ether extract (EE) and crude ash (methods 930.15, 976.05, 920.39 and 942.05, respectively) according to the Association of Official Analytical Chemists (AOAC 2000). Organic matter (OM) was calculated as weight loss through ashing. Neutral detergent fiber assayed with a heat-stable amylase and expressed exclusive of residual ash (aNDFom), as well as acid detergent fiber expressed exclusive of residual ash (ADFom), were analyzed according to Van Soest *et al.* (1991).

Total polyphenols and antioxidant activities of each diet were determined from methanol extracts. Wet dietary samples (1.0 g on a DM basis) were crushed and homogenized with methanol/water (80/20, v/v), and then centrifuged at $8000 \times g$ for 15 min. The supernatants were diluted to 250 mL with methanol/water (80/20, v/v) and utilized in the analytical

determinations. Total polyphenols were determined by the colorimetric method employing the Folin-Denis assay (Swain & Hills 1959), and the results were expressed as mg caffeic acid equivalents (CAE) per g of dry weight. The free-radical scavenging activity of the extracts was evaluated spectrophotometrically using the free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method of Brand-Williams *et al.* (1995). The results were expressed as $\mu\text{mol/L}$ Trolox equivalents (TE) per g of dry weight.

The samples of refused feed and feces were analyzed for DM, CP and OM as stated above. After determining the DM digestibility, total digestible nutrients (TDN) intake was calculated using the regression equation, TDN content = $5.81 + 0.869 \times \text{digestible DM}$, reported by Heaney and Pigden (1963). Urine samples were analyzed for urinary nitrogen (N) using the Kjeldahl procedure described by AOAC (2000). Ruminal $\text{NH}_3\text{-N}$ was determined by the microdiffusion method (Conway 1962). The metabolite concentrations of the blood plasma samples collected at 0 h after feeding were analyzed. Concentrations of glucose, nonesterified fatty acid (NEFA), albumin, blood urea nitrogen (BUN), total cholesterol, phospholipids and inorganic phosphorus (IP) were analyzed using diagnostic kits (Glucose-HRII, NEFA-HR, Albumin-HRII, L type Wako UN, L type Wako CHO·H, L type Wako Phospholipids and Inorganic phosphorus-HRII; Wako Pure Chemical Industries, Ltd., Osaka, Japan). Total protein was determined by a refractometer (SPR-Ne; Atago Co., Ltd., Tokyo, Japan). Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities

were detected by fluorescence analysis according to Huang *et al.* (2006).

To evaluate antioxidant activities and oxidative stress in the wethers, total antioxidant status (TAS) in plasma and the amount of 8-OHdG in urines were also determined. The TAS levels were measured colorimetrically in the plasma samples collected at 0, 2 and 4 h after feeding according to the method of Rice-Evans and Miller (1994) that detects a reduction in the generation of the 2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical cation by antioxidant substances.

Urinary 8-OHdG concentrations were analyzed by enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies specific to 8-OHdG, which was established as described by Yoshida *et al.* (2002). The acidified urine samples were preliminary diluted with phosphate-buffered saline to adjust the pH range to 6-8.

Statistical analyses

Data on intake, apparent digestibility, N balance, blood metabolite concentrations and urinary 8-OHdG levels were analyzed using the MIXED procedure of Statistical Analysis System (SAS 1998). The mathematical model was $Y_{ijkl} = \mu + T_i + P_j + A_k + e_{ijkl}$, where μ = the overall mean, T_i = the fixed effect of treatment, P_j = the fixed effect of period, A_k = the random effect of animal and e_{ijkl} = residual error. Data on ruminal fermentation and TAS in plasma were analyzed by the MIXED procedure (SAS 1998) for repeated measures according to the method of Atkinson *et al.* (2007). The model

included the treatment, period, sampling time, animal and treatment $\times$ sampling time interaction. The three-way interaction of treatment $\times$ period $\times$ animal was used to specify variations among animals using the RANDOM statement (SAS 1998). An autoregressive covariance structure was determined to be most appropriate based on Akaike's criterion. The Tukey-Kramer test was used to detect differences between the means (Kramer 1956). Data on intake, apparent digestibility, N balance, ruminal fermentation (pH and $\text{NH}_3\text{-N}$) and blood metabolite concentrations were compared among WS, WP and CD, and TAS and oxidative stress marker (8-OHdG) were among four treatments, including TFH. A difference at $P < 0.05$ was considered significant and at $0.05 < P < 0.10$ was designated as a tendency.

4.3 Results

Chemical composition and antioxidant activities of winery wastes

Table 4-1 shows the chemical composition and antioxidant activities of the experimental diets. Winery sediment had higher CP than winery grape pomace and TMR. The winery grape pomace had the highest EE content among the experimental diets. Winery sediment and pomace had higher polyphenol contents than TMR, soybean meal and tall fescue hay, all of which had similar polyphenol contents. The DPPH radical scavenging activities of the sediment and pomace were over five times higher than those

of the other experimental diets.

Table 4-2 shows the chemical composition and antioxidant activities of the experimental treatments (WS, WP and CD). Polyphenols and DPPH radical scavenging activity were highest for WP, followed by WS.

Feed intake and digestibility

The intakes and digestibility of DM, OM and CP in each treatment are presented in Table 4-3. The DM and CP intakes did not significantly differ among WS, WP and CD, although the OM intake was lower for WS than for WP ($P = 0.030$).

The estimated TDN intakes were 523, 488, 546 and 334 g/day in WS, WP, CD and TFH, respectively. These intakes amounted to 128, 119, 133 and 81% of TDN requirement for maintenance of mature ewes weighing 40 kg according to the National Research Council (2007).

The CP digestibility was lower for WP and tended to be lower for WS than for CD ($P < 0.01$ and $P = 0.052$). The DM and OM digestibility tended to be lower for WP than for CD ($P = 0.087$ and $P = 0.053$), while those in WS were similar to those in WP and/or CD.

Nitrogen balance

The N balance of each treatment is shown in Table 4-4. Fecal N excretion and proportion of fecal N to N intake were higher for WP than for CD ($P < 0.05$), although those in WS did not significantly differ from CD. Urinary N,

retained N and proportion of urinary N or N retention to N intake did not significantly differ among WS, WP and CD.

Ruminal pH and NH₃-N concentrations

The ruminal pH and NH₃-N levels at 0 and 4 h after feeding are shown in Table 4-5. Sampling time significantly affected ruminal pH ($P < 0.001$) and NH₃-N ($P < 0.01$). Treatment $\times$ sampling time interaction was also significant in NH₃-N ($P < 0.01$). At 4 h after feeding, ruminal pH was similar among WS, WP and CD.

At 4 h after feeding, the ruminal NH₃-N levels were lower for WP and tended to be lower for WS than for CD ($P < 0.05$, $P = 0.089$), whereas similar levels were observed before feeding.

Antioxidant status and oxidative stress marker

Table 4-6 shows the TAS in plasma collected at 0, 2 and 4 h after feeding. There were no significant effects of treatment, sampling time or the treatment $\times$ sampling time interaction on TAS, whereas period had a significant effect ($P < 0.01$). In TFH, the TAS was 796.3, 802.8 and 808.8 $\mu\text{mol/L}$ at 0, 2 and 4 h after feeding, respectively. Similar TAS levels were indicated among the treatments throughout the sampling time.

Other blood metabolites, such as glucose, NEFA, albumin, BUN, total cholesterol, phospholipids and IP, did not differ among the treatment (data was not shown).

Table 4-7 shows the amount of 8-OHdG excretion obtained by multiplying urinary 8-OHdG concentration by its urine volume. The urinary 8-OHdG excretions per day were lower for WS and tended to be lower for WP than for CD ($P < 0.05$, $P = 0.059$). In TFH, 8-OHdG excretion was lower compared to CD ($P < 0.05$), and similar to WS and WP.

4.4 Discussion

Chemical composition and antioxidant activities

Wine usually contains proteins that originate from grapes and yeasts (Yokotsuka *et al.* 1991; Kwon 2004). Grape- or yeast-derived proteins removed from wine by bentonite fining might be responsible for the higher CP content in the sediment (Table 4-1). In contrast, the EE content in our study was higher than the 77 g/kg reported by Swanson *et al.* (2001) and the 70 g/kg by Baumgärtel *et al.* (2007). These differences might result from the proportions of the pomace fractions (i.e. peels, seeds and stalks) and the grape varieties (Baumgärtel *et al.* 2007; Spanghero *et al.* 2009; Basalan *et al.* 2011). In particular, the proportion of seeds might greatly affect the EE content (Basalan *et al.* 2011).

Bustamante *et al.* (2008) reported that winery sediment contained 7.5 mg/g polyphenols on average, lower than in the present study (Table 4-1). Winery sediment contains ellagic acid resulting from the hydrolysis of larger ellagitannins, polyphenols that are common in wines aged in wooden casks

(Lee & Talcott 2002). Ellagic acid and ellagitannins can scavenge both superoxide and peroxy radicals (Puech *et al.* 1999). Lee and Talcott (2002) also noted that anthocyanins might constitute other phenolic compounds in winery sediment and possess various antioxidant properties; for example, they prevent lipid peroxidation and scavenge superoxide anion (Sichel *et al.* 1991; Tamura & Yamagami 1994; Wang *et al.* 1997). In the present study, polyphenols might have contributed to the high DPPH radical scavenging activity of winery sediment (Table 4-1). The predominant polyphenols in grape pomace are anthocyanins and proanthocyanidins that have been known to exhibit high levels of antioxidant activities (Ricardo da Silva *et al.* 1991; Negro *et al.* 2003; Makris *et al.* 2007). Therefore, the high level of DPPH radical scavenging activity of winery grape pomace in this study might be attributable to the abundance of anthocyanins and proanthocyanidins contained in winery grape pomace.

Feed intake and digestibility

The wethers ingested the offered diets almost completely and there were few refusals in WS, WP and CD in spite of the fact that winery wastes contain high levels of tannins (Lee & Talcott 2002; Besharati & Taghizadeh 2009; Alipour & Rouzbehan 2010), which are known to be unpalatable to ruminants (Mangan 1988; Provenza *et al.* 2000; Makkar 2003). The similar levels of intake among these treatments (Table 4-3) implied that the addition of 75 g/kg of winery sediment or 166 g/kg of winery grape pomace had no

negative effects on feed intake.

It has been reported in general that tannins and other polyphenols such as anthocyanins or proanthocyanidins have the property of binding to protein, cellulose, hemicellulose and pectin (McSweeney *et al.* 2001; Singh *et al.* 2003; Melaku 2004). Abarghuei *et al.* (2010) showed that 762 g/kg grape pomace on a DM basis reduced DM and OM digestibility. Nevertheless, in the present study, reduction of DM and OM digestibility was not observed in WS (Table 4-3). The difference from the previous results might be explained by the lower amounts of winery sediment in the feed in the present study. On the other hand, declining trend of DM and OM digestibility was observed in WP as in the previous study. When the amount of winery grape pomace was increased, the negative impact on digestibility might be more significant.

Ruminal fermentation and nitrogen utilization

Previous studies showed that adding grape pomace to feed adversely affected CP digestibility in ruminants (Baumgärtel *et al.* 2007; Besharati & Taghizadeh 2009; Abarghuei *et al.* 2010). In the present experiment, as in the previous ones, adding winery sediment and grape pomace to feed reduced CP digestibility (Table 4-3). Tannins and other polyphenols in grape wastes have the property of binding to proteins to prevent their degradation in the rumen, and these complexes would decrease CP digestibility and ruminal NH₃-N production (Hagerman *et al.* 1992; Singh *et al.* 2003; Melaku 2004; Min *et al.* 2005). Indeed, both *in vitro* (Alipour & Rouzbehan 2010) and *in*

vivo (Besharati & Taghizadeh 2009; Abarghuei *et al.* 2010), feeding winery grape pomace decreased $\text{NH}_3\text{-N}$ concentrations in the rumen because the tannin slowed the rate of fermentation (i.e. it inhibited the activities of proteolytic microbes). Therefore, the results of the present study (Table 4-5) might have been attributable to the inhibition of N absorption by tannins and other polyphenols contained in winery sediment or grape pomace.

Abarghuei *et al.* (2010) reported that retained N was decreased in sheep fed grape pomace because of the lower microbial protein synthesis in the rumen and the increased fecal N losses. However, retained N in this study (Table 4-4) was not negatively affected by feeding winery wastes despite the increased fecal N losses in WP. It has been reported that tannins inhibit the absorption of N from the rumen and shift N excretion from urine toward feces (Vaithiyanathan *et al.* 2007; Waghorn 2008). Therefore, in the present study, compensation between urinary and fecal N excretion could explain the similar N retention among WS, WP and CD.

Antioxidant status and oxidative stress

Fang *et al.* (2002) noted that an increase in dietary energy intake enhances mitochondrial free-radical production and causes oxidative stress. Thus, the wethers in WS, WP and CD might be susceptible to oxidative stress because diets fed to these wethers contained more energy than the TFH.

Plasma antioxidant levels are a useful indicator of the fate of supplemental antioxidants in animal diets (O'Grady *et al.* 2006). Polyphenolic compounds

can reach the plasma and target organs after being absorbed in the intestine, and thus increased concentrations of plasma polyphenols are often accompanied by increased TAS in plasma (Alía *et al.* 2003). When plant extracts rich in polyphenols were given as a single acute dose (100 g/kg of feed intake on a DM basis) directly into the rumen of sheep that had been made highly susceptible to lipoperoxidation, the extracts, especially of grape, efficiently enhanced TAS in plasma (Gladine *et al.* 2007b). However, in the present study the supplementary winery sediment and winery grape pomace did not improve TAS (Table 4-6). When a low level of antioxidants is offered, their contribution to TAS is probably negligible compared to endogenous antioxidative defenses (Gladine *et al.* 2007a,b). Therefore, the amount of winery wastes and/or the experimental period may be the limiting factors in the improvement of TAS.

Iwasawa *et al.* (2010) reported that the urinary 8-OHdG concentration in mice decreased after feeding kiwi fruit rich in polyphenols. Similar results were obtained from the studies by Fukuda *et al.* (2004) who examined walnut polyphenols for mice, and Mizutani *et al.* (2001) who evaluated resveratrol, a polyphenol contained in red wine, in rats. Those indicate that feeding with polyphenols can affect urinary 8-OHdG excretion. The lower urinary 8-OHdG excretion in TFH than in CD showed that the wethers in CD might be subjected to oxidative stress by its high energy content. On the other hand, the results in WS and WP indicated that polyphenols from winery wastes might alleviate oxidative stress arising from an increase in

dietary energy intake to the level of TFH. It should be noted that the antioxidant activities of winery wastes corresponded *in vivo* in this study, as opposed to Veskoukis *et al.* (2012) who reported that grape pomace extract possessing antioxidant activities did not affect *in vivo* response in rats. Gladine *et al.* (2007b) stated that polymeric proanthocyanidins (the main polyphenols in grape) were not bioavailable for monogastrics, but ruminants can make polymeric proanthocyanidins bioavailable by hydrolyzing proanthocyanidin polymers into bioavailable and bioactive monomers by rumen micro-organisms. Therefore, it is assumed that ruminants can benefit from strong antioxidant activities of polyphenols, especially in winery sediment. On the other hand, the TAS was not affected by feeding winery wastes in spite of the *in vivo* response indicated by 8-OHdG. Generally, blood is protected by water-soluble antioxidants and cell membranes by lipid-soluble antioxidants (Sies 1997). Some phenolic compounds in grape are watersoluble, and some are lipid-soluble (Shi *et al.* 2003). Therefore, it is inferred that the different responses between the TAS and 8-OHdG in this study might be due to the difference in the fraction of polyphenols acting on each, although in the study on humans, Chen *et al.* (2008) reported that consumption of polyphenols in purple sweet potato leaves decreased urinary 8-OHdG excretion but did not affect the TAS, as in the present study.

The urinary 8-OHdG excretion was more significantly affected in WS than those in WP despite concentration of polyphenols in WP being higher than in WS. The result might be attributed to a difference in amount of polyphenol

absorption. As noted above, some polyphenols have the binding property to proteins (Hagerman *et al.* 1992; Singh *et al.* 2003; Melaku 2004; Min *et al.* 2005). The higher fecal N excretion in WP (Table 4) suggested that more polyphenols were excreted with proteins, and thus absorption of polyphenols was lower in WP compared with WS. The difference in absorption might be due to a variation in components of the polyphenols between winery sediment and grape pomace. Studies to identify the polyphenol components should be conducted to investigate the difference in the effects of polyphenols between winery sediment and grape pomace.

Conclusions

When fed winery sediment or winery grape pomace rich in polyphenols, wethers experienced few negative effects on intake, although CP digestibility was depressed because of their tannins and/or other phenolic compounds. The winery wastes did not affect the retained N or proportion of N retention to N intake despite the increased fecal N losses in WP. Polyphenols in winery wastes decreased redundant ruminal $\text{NH}_3\text{-N}$ production, likely due to their modulation of microbial activity in the rumen. Although TAS in plasma was not affected, the addition of winery wastes reduced urinary 8-OHdG excretion.

The present results indicate that winery sediment and grape pomace can alter nitrogen metabolism and act as new antioxidants for ruminants. Further studies are needed to determine the optimum feeding amount by

better understanding the mechanisms underlying the action of polyphenols and their composition contained in winery wastes.

Table 4-1 Chemical composition of the feedstuffs used in the experimental diets

Item	Winery Sediment	Winery grape pomace	TMR	Soybean meal	Tall fescue hay
Chemical composition (%)					
DM	31.2	43.9	87.0	86.6	85.2
OM [†]	78.6	91.8	94.5	92.2	91.4
CP [†]	19.8	9.5	14.1	51.2	4.9
EE [†]	2.8	8.5	3.8	0.4	1.1
NFC [†]	49.6	26.3	47.7	30.7	13.3
aNDFom [†]	6.4	47.4	28.9	9.9	72.2
ADFom [†]	4.3	44.0	11.0	6.9	43.5
Crude ash [†]	21.4	8.2	5.5	7.8	8.6
Polyphenols [†] (mg CAE/g)	12.7	10.0	1.9	2.2	1.9
DPPH radical scavenging activity (μmol TE/g)	33.4	22.5	4.3	3.5	2.0

[†] On a DM basis.

ADFom, acid detergent fiber expressed exclusive of residual ash; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; CAE, caffeic acid equivalents; CP, crude protein; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DM, dry matter; EE, ether extract; NFC, nonfibrous carbohydrate; OM, organic matter; TE, Trolox equivalents; TMR, total mixed ration.

Table 4-2 Proportions of feed ingredients and chemical composition in the experimental treatments

Item	Treatment [†]		
	WS	WP	CD
Proportions of feed ingredients (% DM)			
Tall fescue hay	25.0	8.4	25.0
TMR	67.5	75.0	73.3
Winery sediment	7.5	-	-
Winery grape pomace	-	16.6	-
Soybean meal	-	-	1.7
Chemical composition (%)			
DM	82.4	79.7	86.6
OM [‡]	92.5	93.8	93.7
CP [‡]	12.2	12.6	12.4
EE [‡]	3.1	4.4	3.1
NFC [‡]	39.2	41.3	38.8
aNDFom [‡]	38.0	35.6	39.4
ADFom [‡]	18.6	19.2	19.1
Crude ash [‡]	7.5	6.2	6.3
Polyphenols [‡] (mg CAE/g)	2.7	3.2	1.9
DPPH radical scavenging activity (μmol TE/g)	5.9	7.1	3.7

[†] On a DM basis.

[‡] The dietary treatments were WS (contained winery sediment), WP (contained winery grape pomace) and CD (control diet).

ADFom, acid detergent fiber expressed exclusive of residual ash; aNDFom, neutral detergent fiber assayed with a heat stable amylase and expressed exclusive of residual ash; CAE, caffeic acid equivalents; CP, crude protein; DM, dry matter; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EE, ether extract; NFC, nonfibrous carbohydrate; OM, organic matter; TE, Trolox equivalents; TMR, total mixed ration.

Table 4-3 Effect of winery waste addition on intake and apparent digestibility in wethers

Item	Treatment [†]				SEM	<i>P</i> -value
	WS	WP	CD	TFH		
Intake (g/day/BW)						
DM	20.80	20.94	20.84	19.49	0.11	NS
OM	19.28 ^a	19.64 ^b	19.53 ^{ab}	17.81	0.10	*
CP	2.56	2.65	2.61	1.00	0.02	NS
Apparent digestibility (%)						
DM	70.10	68.75	71.09	44.38	1.11	0.10
OM	72.28	70.54	73.29	43.96	1.10	0.06
CP	70.61 ^{ab}	65.69 ^a	75.14 ^b	32.42	1.55	*

Means in the same row with different superscripts differ significantly among WS, WP and CD ($P < 0.05$).

* $P < 0.05$, NS, not significant among WS, WP and CD.

[†] The dietary treatments were WS (contained winery sediment), WP (contained winery grape pomace), CD (control diet) and TFH (tall fescue hay).

BW, body weight; CP, crude protein; DM, dry matter; OM, organic matter; SEM, standard error of the means.

Table 4-4 Effect of winery waste addition on nitrogen balance in wethers

Item	Treatment [†]				SEM	<i>P</i> -value
	WS	WP	CD	TFH		
N balance (g/day/BW ^{0.75})						
Intake N	1.06 ^a	1.09 ^b	1.07 ^{ab}	0.41	0.02	*
Fecal N	0.31 ^{ab}	0.37 ^b	0.27 ^a	0.28	0.02	*
Urinary N	0.53	0.51	0.59	0.18	0.05	NS
Retention N	0.21	0.21	0.22	-0.05	0.05	NS
Proportion to N intake (%)						
Fecal N	29.4 ^{ab}	34.3 ^b	24.9 ^a	67.6	4.4	*
Urinary N	50.6	46.8	55.0	45.0	2.6	NS
Retention N	20.0	18.9	20.1	-12.6	4.3	NS

Means in the same row with different superscripts differ significantly among WS, WP and CD ($P < 0.05$).

* $P < 0.05$, NS, not significant among WS, WP and CD.

[†] The dietary treatments were WS (contained winery sediment), WP (contained winery grape pomace), CD (control diet) and TFH (tall fescue hay).

BW, body weight; N, nitrogen; SEM, standard error of the means.

Table 4-5 Effect of winery waste addition on ruminal fermentation in wethers

		Time after feeding (h)			<i>P</i> -value		
	Treatment [†]	0	4	SEM	Treatment	Time	Treatment × Time
Ruminal pH	WS	6.7 ^b	5.8 ^a	0.1	NS	***	NS
	WP	6.8 ^b	5.8 ^a				
	CD	6.5 ^b	5.8 ^a				
	TFH	7.1	6.9				
NH ₃ -N (mg/L)	WS	142.3	119.8 ^{AB}	16.0	NS	**	**
	WP	165.1 ^b	98.5 ^{a, A}				
	CD	161.9	168.0 ^B				
	TFH	29.9	21.5				

^{a, b} Means in the same row with different superscripts differ significantly among WS, WP and CD ($P < 0.05$).

^{A, B} Means in the same column with different superscripts differ significantly among WS, WP and CD ($P < 0.05$).

** $P < 0.01$, *** $P < 0.001$, NS, not significant among WS, WP and CD.

[†] The dietary treatments were WS (contained winery sediment), WP (contained winery grape pomace), CD (control diet) and TFH (tall fescue hay).

NH₃-N, ammonia nitrogen; SEM, standard error of the means.

Table 4-6 Effect of winery waste addition on total antioxidant status in plasma of wethers

		Time after feeding (h)				<i>P</i> -value		
	Treatment [†]	0	2	4	SEM	Treatment	Time	Treatment × Time
Total antioxidant status (μM)	WS	818.5	817.5	819.0	18.2	NS	NS	NS
	WP	825.0	842.8	825.8				
	CD	840.0	844.0	828.5				
	TFH	796.3	802.8	808.8				

[†] The dietary treatments were WS (contained winery sediment), WP (contained winery grape pomace), CD (control diet) and TFH (tall fescue hay).

NS, not significant among WS, WP, CD and TFH.

SEM, standard error of the means.

Table 4-7 Effect of winery waste addition on urinary 8-OHdG excretion in wethers

Item	Treatment [†]				SEM	<i>P</i> -value
	WS	WP	CD	TFH		
8-OHdG excretion (µg/day)	26.55 ^a	27.93 ^{ab}	37.61 ^b	23.38 ^a	3.81	*

Means in the same row with different superscripts differ significantly among WS, WP, CD and TFH ($P < 0.05$).

* $P < 0.05$ among WS, WP, CD and TFH.

† The dietary treatments were WS (contained winery sediment), WP (contained winery grape pomace), CD (control diet) and TFH (tall fescue hay).

8-OHdG, 8-hydroxy-2'-deoxyguanosine; SEM, standard error of the means.

Chapter 5

Effects of lowering dietary cation-anion difference
by amino acid fermentation by-products
on carcass characteristics and beef tenderness
of Japanese Black steers

5.1 Introduction

Beef tenderness has been one of the most important traits impacting on consumer palatability and satisfaction (Dransfield *et al.* 1984; Morgan *et al.* 1991). Miller *et al.* (2001) reported that consumers could perceive differences in beef tenderness and were willing to pay more for more tender beef.

Postmortem protein degradation is involved in beef tenderness (Huff-Lonergan *et al.* 1996). Previous studies have shown that the calpain, intracellular calcium-dependent protease, play an important role in postmortem myofibrillar proteolysis and subsequent tenderization of beef (Koohmaraie *et al.* 1987; Edmunds *et al.* 1991; Koohmaraie & Geesink 2006; Lian *et al.* 2013). In fact, injection of calcium chloride into carcasses or beef cuts has improved tenderness by activating postmortem proteolysis (Kerth *et al.* 1995; Wulf *et al.* 1996). Duckett *et al.* (2001) also demonstrated that oral administration of Ca propionate to cattle before slaughter increased calpain activities and consequently improved longissimus muscle tenderness. Those previous results indicate that controlling Ca status of beef cattle can alter postmortem proteolysis and contribute to beef tenderness.

Manipulation of dietary cation-anion differences (DCAD) has been extensively studied for periparturient dairy cows, and negative DCAD can potentially prevent peripartum hypocalcemia by acidifying body fluid and enhancing responsiveness to Ca-homeostatic hormones and Ca mobilization (Block 1994). In a meta-analysis of previous studies, negative DCAD

increased plasma ionized Ca concentration in nonlactating dairy cows (Charbonneau *et al.* 2006). In Korean beef cattle, a negative DCAD diet of -10 mEq/100g of dry matter (DM) with 25-hydroxyvitamin D₃ for 14 d before slaughter was reported to increase mRNA and protein concentrations of calpain, and numerically improve longissimus muscle tenderness (Cho *et al.* 2006). On the other hand, Schoonmaker *et al.* (2013) demonstrated that a negative DCAD of -16 mEq/100g DM for 14 d did not affect Ca influx into muscle. When prepartum cows were provided a negative DCAD diet (-16 mEq/100g DM) for 21 or 42 d, postpartum blood Ca concentration was greater for cows fed for 42 d (Weich *et al.* 2013). Based on those previous results, we hypothesized that extended negative DCAD feeding would have more obvious effect on Ca status of cattle and muscle calpain activity.

Japanese Black cattle are characterized by their ability to deposit a lot of intramuscular fat (Zembayashi *et al.* 1988). It has been reported that intramuscular fat content and tenderness are correlated, and that muscles with high marbling have a lower shear value (Mitsumoto *et al.* 1992; Ueda *et al.* 2007). Ueda *et al.* (2007) suggested that tenderness of longissimus muscle of Japanese Black cattle was affected by not only the chemical composition but also by protease activity. But there is little information about calpain activity and its effect on beef tenderness of Japanese Black cattle. Thus, our study was conducted to investigate the effects of prolonged DCAD manipulation on calpain activity and beef tenderness in Japanese Black steers by feeding a negative DCAD diet for 70 d before their slaughter.

5.2 Materials and Methods

The animals in the study were managed according to the guidelines of the Committee of Animal Use, Care and Experiments at Central Research Institute for Feed and Livestock, ZEN-NOH.

Animals and treatments

Thirty six Japanese Black steers (initial age of 27.6 ± 0.4 mo and body weight (BW) of 740.8 ± 39.9 kg) were used in this study. The animals were blocked by initial BW to three groups and randomly assigned to pens within group to be six steers per pen.

The pens within group were randomly allotted to one of two treatments (3 pens/treatment; 18 steers/treatment) : 1) control (CON; targeted DCAD of +6.1 mEq/100g DM) by offering no additives, or 2) negative DCAD manipulation (NEGD; targeted DCAD of -8.5 mEq/100g DM) by offering 0.43 kg DM/d/head of Bio-Chlor (Church & Dwight Co., Inc., Princeton, NJ, USA) containing amino acid fermentation by-products for 70 d before slaughter. The feeding amount of Bio-Chlor to manipulate DCAD to -8.5 in NEGD was chosen according to data of Luebke *et al.* (2011) who reported that DCAD between -8 and -45 had similar effects on urine pH and that DCAD between +40 and -8 did not affect dry matter intake (DMI). All steers were fed the

commercial concentrate mixture and rice straw separately twice a day for *ad libitum* intake during the experimental period of 70 d before slaughter. The steers also had free access to water. The Bio-Chlor was offered once a day, approximately 30 min before the concentrate feeding in the morning, by putting separate from the concentrate mix or rice straw in the troughs. The amounts of each diet offered and refused were recorded daily by pen to calculate DCAD based on the actual intake of the diets. All steers were weighed three times at 0 and 50 d after beginning the experiment and before slaughter, and the average BW for each treatment was calculated. Urine was collected from the steers approximately after 4 h of morning feeding on d 0, 22 and 50 via an attached funnel on the lower abdomen, and then analyzed for pH using a glass electrode pH meter (Horiba Ltd., Kyoto, Japan).

Feed samples were taken and analyzed for DM, crude protein (CP), crude fat and ash contents according to the Association of Official Analytical Chemists (AOAC 2000). Neutral detergent fiber (aNDFom) and acid detergent fiber (ADFom) expressed exclusive of residual ash were also determined by the method of Van Soest *et al.* (1991). Metabolizable energy (ME) was calculated according to the equation of National Research Council (NRC 2000). Mineral analyses (Na, K, and S) by wet chemistry were carried out for the feed samples using a Thermo ICAP 6300 Inductivity Coupled Plasma Radical Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) after microwave digestion. Chloride ion was also determined using Metrohm 716 Titrino Titration Unit (Metrohm Ltd., Herisau, Switzerland)

according to the method of Cantliffe *et al.* (1970). These mineral contents were used for calculation of DCAD as $(\text{Na} + \text{K}) - (\text{Cl} + \text{S})$ (mEq/100g DM).

Carcass characteristics

The steers were slaughtered at 29.9 mo of age at a public slaughterhouse, and approximately one gram of longissimus cervicis muscle (LCM) sample was obtained from neck of each carcass at 30 min after slaughter for calpain activity analysis. The LCM samples were frozen in liquid nitrogen immediately after sampling and then stored at -80°C until analysis. The carcasses were chilled for 24 h, and their traits were evaluated between sixth and seventh rib section on the left side by trained official meat graders according to the Japan Meat Grading Standard described by Japan Meat Grading Association (JMGA 1988). Carcass trait evaluation was based on a carcass weight, longissimus muscle area, rib thickness, subcutaneous fat thickness, beef marbling and beef color. Beef marbling was scored from 1 (poor) to 12 (very abundant), and beef color was from 1 (light) to 7 (dark) according to the Beef Marbling Standard (BMS) and the Beef Color Standard (BCS). After the carcass grade was recorded, a longissimus thoracis muscle (LTM) sample was obtained from each carcass. The LTM samples were stored at 4°C until further processed.

Chemical composition and calpain activity analyses

The LTM samples were cut and minced with a meat grinder for chemical

composition analyses approximately 24 h after sampling. The minced samples were oven-dried at 135°C for 2 h. Moisture content was calculated from difference of the weight before and after drying. The dried samples were analyzed for CP by Kjeldahl method and for crude fat by Soxhlet extraction method according to AOAC (2000). Meat color was measured five times with Spectrophotometer SE 2000 (Nippon Denshoku Industries Co. Ltd., Osaka, Japan) with D65 illuminant and 2° standard observer at different locations on the surface of LTM samples. The results were reported in the scale of lightness (L*), redness (a*) and yellowness (b*) according to Commission Internationale de l'Eclairage (CIE; International Commission on Illumination 1978). Color difference (ΔE) between the treatments was calculated by the equation of CIE (1978): $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$, where ΔL^* : difference of L* values, Δa^* : difference of a* values and Δb^* : difference of b* values. The ΔE value was evaluated according to the criteria used in the study by Sakowska *et al.* (2016): 0.0-0.5, not noticeable; 0.5-1.5, slightly noticeable; 1.5-3.0, noticeable; 3.0-6.0, very visible; > 6.0, extremely visible.

Calpain activity in LCM was analyzed using a commercial assay kit (PromoKine, Heidelberg, Germany) by fluorometry. Approximately 10 mg of LCM samples were gently homogenized in extraction buffer to extract cytosolic proteins. After centrifugation for one minute, the supernatant was transferred to a fresh tube on ice and diluted to the extraction buffer. Provided reaction buffer and calpain substrate was added to each lysate and

incubated at 37°C for an hour in the dark. Then the samples were read in a fluorometer equipped with a 400 nm excitation filter and a 505 nm emission filter. The results were expressed as relative fluorescent unit (RFU).

Warner-Bratzler shear force determination for tenderness

Warner-Bratzler shear force (WBSF) was determined on LTM steaks (2.54 cm thick) aged for one day using Warner-Bratzler Meat Shear Fixture (Instron, Norwood, MA, USA). Steaks were cooked at 190°C by an electric oven to a core temperature of 70°C and then chilled at 4°C overnight. Six to eight cores, each with a diameter of 1.27 cm were taken parallel to the muscle fibers from each steak. The steak cores were shorn once perpendicular to the muscle fibers using a Warner-Bratzler blade with a crosshead speed of 25 cm/min and the peak force was measured. The measured WBSF was averaged without the lowest and highest values.

Statistical analyses

The study was analyzed as a randomized complete block design with steer defined as the experimental unit except for feed intake data. Steer nested within treatment was designated as a random effect. In analysis of feed intake data, pen was defined as the experimental unit and nested within treatment as a random effect. Data measured over time (i.e. feed intake, urine pH and performance) was analyzed for repeated measures using JMP® 11 (SAS Institute Inc., Cary, NC, USA), and a first-order autoregressive

covariance structure was determined to be the most appropriate according to Akaike's criterion. The model contained the fixed effects of treatment, time and treatment by time interaction. Other data (i.e. measurements after slaughter) was analyzed by analysis of variance using JMP® 11 (SAS Institute Inc., Cary, NC, USA), with the fixed effect of treatment. Correlation between BMS and fat content in LTM and WBSF value was determined using JMP® 11 (SAS Institute Inc., Cary, NC, USA). Significance was declared at $P < 0.05$ and tendency at $0.05 \leq P < 0.10$.

5.3 Results and Discussion

Feed intake, urine pH and feedlot performance

Feed intake and nutrient consumptions in each treatment are shown in Table 5-2. Based on the results, the actual DCAD values were +6.09 mEq/kg DM in CON and -8.27 in NEGD, which were close to the target (+6.1 in CON and -8.5 in NEGD). There was no significant difference in rice straw intake among the treatments. Intake of the concentrate mix, however, was significantly lower for NEGD than for CON ($P < 0.01$), which resulted in significantly lower intake of total DM and ME for NEGD ($P < 0.01$). Similarly, in dry cows, a negative DCAD diet containing anionic salts was consumed less than a no anionic salts added diet (Oetzel & Barmore 1993; Vagnoni & Oetzel 1998). Luebke *et al.* (2011) also reported that manipulating DCAD

more negatively than -8 mEq/100g DM decreased DMI. Potential reasons involved in reduction of feed intake observed in this study would include unpalatability of the anionic diet and/or control of feed intake in order to alleviate the acid load caused by negative DCAD (Oetzel & Barmore 1993; Horst *et al.* 1994; Vagnoni & Oetzel 1998). Using a negative DCAD diet as total mixed rations must be successful by masking the unpalatability as suggested by Horst *et al.* (1994). On the other hand, Cho *et al.* (2006) reported that feeding DCAD of -10 for 14 d did not have a negative effect on feed intake, and similar result was observed in the study by Schoonmaker *et al.* (2013) who determined the effect of DCAD of -16 for 14 d. Hersom *et al.* (2010) demonstrated that feeding a negative DCAD diet (-0.9 mEq/100g DM) for 43 d did not affect DMI before d 28 but decreased after d 28 compared with positive DCAD (+25 mEq/100g DM). It is suggested that extended feeding of a negative DCAD diet exacerbated its influence on feed intake, which would be another reason for reduction of feed intake in our study. The CP intake, however, was higher for NEGD than for CON ($P < 0.01$). The Bio-Chlor contained more CP than concentrate mix (Table 5-1), and its high CP could offset the reduction of DMI.

Manipulation of DCAD during the last 70 d did not affect average daily gain or final BW of the steers (Table 5-3) even though nutrient consumption was significantly different among the treatments (Table 5-2). From d 50 to final day of the experiment, steers on the CON and NEGD treatments gained 0.53 and 0.29 kg/d respectively, but this numerical difference was not

significant ($P = 0.11$). Hersom *et al.* (2010) similarly reported that daily gain of cows was not different between negative and positive DCAD although DMI was lower for cows consuming a negative DCAD diet. Gain: feed ratio was not significantly different among the treatments throughout the experiment. In NEGD, lower DMI seemed to cause the numerical decrease of BW gain after d 50, which resulted in the similar feed efficiency to CON.

Urine pH did not differ on d 0 among the treatments, but significantly declined on d 22 and 50 for steers consuming a negative DCAD diet (Figure 5-1; $P < 0.01$). Schoonmaker *et al.* (2013) also reported a decrease in urine pH after 7 d and 14 d of negative DCAD control. In this study, the steers in NEGD were acidifying after the beginning of the experiment. It could be considered that the acidogenic environment caused depression of DMI in this study as suggested by Oetzel and Barmore (1993) and Vagnoni and Oetzel (1998). Acidosis also leads to promote Ca entry into the exchangeable Ca pool by enhanced gastrointestinal absorption (Roche *et al.* 2007) and/or mobilization from bones (Block 1994). In fact, Cho *et al.* (2006) successfully increased blood Ca in beef cows by feeding a negative DCAD diet for 14 d.

Carcass characteristics

Cold carcass weight, LTM area, rib thickness, fat thickness, BMS and BCS No. were not different among the treatments (Table 5-4). Our results were consistent with the studies by Luebke *et al.* (2011) and Schoonmaker *et al.* (2013), in which they studied -16 mEq/100g DM DCAD diets for a long (145

and 196 d) and a short (14 d) period, respectively. On the other hand, Ross *et al.* (1994) reported that carcass marbling score increased by consuming cationic diets for 84 d. Schoonmaker *et al.* (2013) suggested that an increase in marbling of cattle fed positive DCAD could be a result of increased production of volatile fatty acid in the rumen due to the ability of sodium bicarbonate to increase ruminal pH and enhance fiber digestibility. In this study, feeding a negative DCAD diet for 70 d before slaughter resulted in acidogenic environment (Figure 5-1) and decreased DM and energy intake as shown in Table 5-2. Those might have been contraindicated to overall productivity such as carcass weight or marbling deposition if the steers had been fed a negative DCAD diet for a longer period.

Chemical composition and calpain activity of meat

Chemical composition and meat color (L^* , a^* and b^*) of LTM samples are shown in Table 5-5. Moisture, CP and fat contents of LTM did not significantly differ among the treatments. The L^* , a^* and b^* color values were not affected by DCAD manipulation, and ΔE between the treatments were 0.69, meaning slightly noticeable. Lansdell *et al.* (1995) reported that injection of calcium chloride into LTM did not affect visual color scores and colorimeter L^* , a^* and b^* values. It is consistent with the results of our study that negative DCAD manipulation did not affect BCS No. and colorimeter values.

The calpain activities in LCM samples are shown in Figure 5-2, in which

the data are expressed as relative value to the control. The calpain activity tended to be higher in NEGD compared with CON ($P = 0.09$). Acidogenic environment potentially contributes to improve calpain activity by suppressing the autolysis of the enzymes as suggested by Cho *et al.* (2006). Therefore, tendency for improvement of the calpain activities in the present study would be explained by acidification caused by feeding a negative DCAD diet. Cho *et al.* (2006) reported that DCAD manipulation with 25-hydroxyvitamin D₃ induced greater protein and mRNA expression of μ -calpain. In the present study, we revealed that DCAD manipulation, alone could affect calpain activity. However, just tendency, not significant effect to improve calpain activity was observed. The actual DCAD value calculated based on intake by the steers in this study (-8.3 mEq/100g DM; Table 5-2) was more positive than in the previous study (-10 mEq/100g DM; Cho *et al.* (2006)), and that might have diminished its effect on calpain activity. Walder *et al.* (2002) showed that calpain-3 gene expression was negatively correlated with body fat mass in both humans and mice. High fat content in LTM (Table 5-5) also might have compensated the DCAD effect on calpain activity, but further studies are needed to evaluate the relationship between calpain activity and fat content in cattle.

Beef tenderness

The WBSF values did not differ among the treatments (Figure 5-3), which meant that DCAD manipulation did not affect beef tenderness in this study.

It has been suggested that homeostatic mechanisms might be regulated in regard to changes in Ca metabolism and proteolytic enzymes (Schoonmaker *et al.* 2013). In the present study, the DCAD might not be negative enough in view of a relationship between DCAD and calpain enzyme as described above. If the DCAD was more negatively regulated, the calpain possibly would be more significantly activated and consequently have an effect on beef tenderness. However, manipulating DCAD more negatively has a potential to exacerbate depression of DMI (Luebke *et al.* 2011). When feeding a negative DCAD diet to beef cattle, it is necessary to evaluate the optimum DCAD value, feeding method and period to effectively promote calpain activity and not to adversely affect other productivity in consideration of a trade-off relationship between intake and calpain activity.

The WBSF values of CON (2.4 kg) were relatively low compared with those of control groups in the previous studies (8.4 kg; Cho *et al.* (2006) or approximately 3.5 kg; Schoonmaker *et al.* (2013)). It was suggested that baseline tenderness in this study was so high that little additional improvement could be made. Intramuscular fat content and tenderness are positively correlated because developed adipose tissues weaken structures of the intramuscular connective tissues (Jennings *et al.* 1978; Mitsumoto *et al.* 1992; Ueda *et al.* 2007). In our study, WBSF values were negatively correlated with BMS No. and fat content in LTM ($R = -0.48$, $P < 0.01$; $R = -0.63$, $P < 0.01$). Various factors also affect beef tenderness, for instance animal age, marbling score, length of aging, subprimal cut, degree of

doneness as well as protease activity (Jennings *et al.* 1978; Wulf *et al.* 1996). It is assumed that a multidimensional approach is needed to improve beef tenderness.

Conclusions

In our study, manipulating DCAD to -8.27 mEq/100g DM in comparison with a DCAD of +6.09 for Japanese Black steers for 70 d before slaughter declined urine pH and suppressed feed intake even though it did not negatively affect the growth performance or carcass evaluation. Feeding a more negative DCAD diet for a longer period than this study may increase the risk of productivity decline due to reduction of intake. Calpain activities in the muscles of the steers tended to be improved by lowering DCAD for 70 d, but that did not enhance beef tenderness evaluated by shear force. Further multidimensional studies will be needed to produce more tender beef to satisfy consumers of Japanese Black beef.

Table 5-1 Chemical composition of the feeds used in the study

Items	Concentrate [†]	Rice straw	Bio-Chlor [‡]
Chemical composition			
DM (%)	90.5	95.0	86.4
CP (% DM)	13.4	3.2	47.8
Crude fat (% DM)	3.7	1.0	2.1
Ash (% DM)	4.4	17.7	12.0
aNDFom (% DM)	23.0	64.9	19.5
ADFom (% DM)	9.2	40.5	6.9
ME (Mcal/kg)	3.11	1.35	3.26
Na (% DM)	0.29	0.04	1.31
K (% DM)	0.83	0.74	1.06
Cl (% DM)	0.57	0.18	8.52
S (% DM)	0.18	0.13	3.34
DCAD (mEq/100g DM)	5.9	7.1	-315.2

[†] Contained (% as fed) 26.1 flaked corn, 22.9 wheat bran, 18.7 ground barley, 18.0 flaked barley, 4.1 corn gluten feed, 3.0 soybean curd cakes, 2.3 ground corn, 2.0 molasses, 1.1 soybean meal, 0.7 rapeseed meal, 1.1 vitamins and minerals.

[‡] Church & Dwight Co., Inc., Princeton, NJ, USA.

ADFom, acid detergent fiber expressed exclusive of residual ash; aNDFom; neutral detergent fiber expressed exclusive of residual ash; CP, crude protein; DCAD, dietary cation-anion differences; DM, dry matter; ME, metabolizable energy.

Table 5-2 Effect of DCAD manipulation on feed intake of the steers

	Treatment		SEM	<i>P</i> value
	CON	NEGD		
Feed intake (kg DM/d/head)				
Concentrate	8.47 ^b	7.46 ^a	0.06	< 0.01
Rice straw	1.39	1.39	0.01	0.91
Bio-Chlor [†]	-	0.39	-	-
Nutrient intake				
DM (kg/d/head)	9.86 ^b	9.23 ^a	0.05	< 0.01
CP (kg/d/head)	1.18 ^a	1.21 ^b	0.01	< 0.01
ME (Mcal/d/head)	31.09 ^b	29.05 ^a	0.15	< 0.01
DCAD (mEq/100g DM) [‡]	6.09	-8.27	-	-

^{a,b} Means with different superscripts within a row differ significantly ($P < 0.05$).

[†] Church & Dwight Co., Inc., Princeton, NJ, USA.

[‡] Calculated based on the actual intake in each treatment.

CON, control; CP, crude protein; DCAD, dietary cation-anion differences; DM, dry matter; ME, metabolizable energy; NEGD, negative DCAD manipulation, SEM, standard error of the mean.

Table 5-3 Effect of DCAD manipulation on BW changes of the steers

	Treatment		SEM	<i>P</i> -value
	CON	NEGD		
BW (kg)				
Initial	734.8	746.8	6.7	0.31
d 50	763.4	777.8	7.2	0.33
Final	774.4	783.6	7.6	0.56
Daily Gain (kg/d)				
Initial to d 50	0.56	0.61	0.04	0.54
d 50 to final	0.53	0.29	0.13	0.11
Total	0.54	0.50	0.05	0.62
Gain: Feed (kg/kg)				
Initial to d 50	0.057	0.064	0.003	0.14
d 50 to final	0.053	0.024	0.011	0.25
Total	0.055	0.054	0.004	0.95

BW, body weight; CON, control; DCAD, dietary cation-anion differences; NEGD, negative DCAD manipulation; SEM, standard error of the mean.

Table 5-4 Effect of DCAD manipulation on carcass characteristics of the steers

	Treatment		SEM	<i>P</i> -value
	CON	NEGD		
Cold carcass weight (kg)	493.7	496.0	7.6	0.84
LTM area (cm ²)	53.9	56.1	1.6	0.35
Rib thickness (cm)	8.1	8.0	0.2	0.57
Fat thickness (cm)	2.3	2.5	0.2	0.44
BMS No. †	7.7	7.2	0.4	0.45
BCS No. ‡	3.9	3.9	0.1	1.00

† Scored from 1 (poor) to 12 (very abundant).

‡ Scored from 1 (light) to 7 (dark).

BCS No., beef color standard number; BMS No., beef marbling standard number; CON, control; DCAD, dietary cation-anion differences; LTM, Longissimus thoracis muscle; NEGD, negative DCAD manipulation; SEM, standard error of the mean.

Table 5-5 Effect of DCAD manipulation on chemical composition and meat color of the LTM

	Treatment		SEM	<i>P</i> -value
	CON	NEGD		
Chemical composition (%)				
Moisture	44.8	45.4	0.8	0.68
CP	12.8	13.2	0.2	0.37
Crude fat	42.2	41.2	1.0	0.67
Meat color [†]				
L*	50.6	51.3	1.1	0.60
a*	24.2	24.4	0.6	0.85
b*	13.9	14.1	0.5	0.47
Color difference (ΔE) [‡]		0.69	-	-

[†] L*: lightness, a*: redness, b*: yellowness.

[‡] 0.0-0.5, not noticeable; 0.5-1.5, slightly noticeable; 1.5-3.0, noticeable; 3.0-6.0, very visible; > 6.0, extremely visible (Sakowska *et al.* 2016).

CON, control; CP, crude protein; DCAD, dietary cation-anion differences; LTM, Longissimus thoracis muscle; NEGD, negative DCAD manipulation; SEM, standard error of the mean.

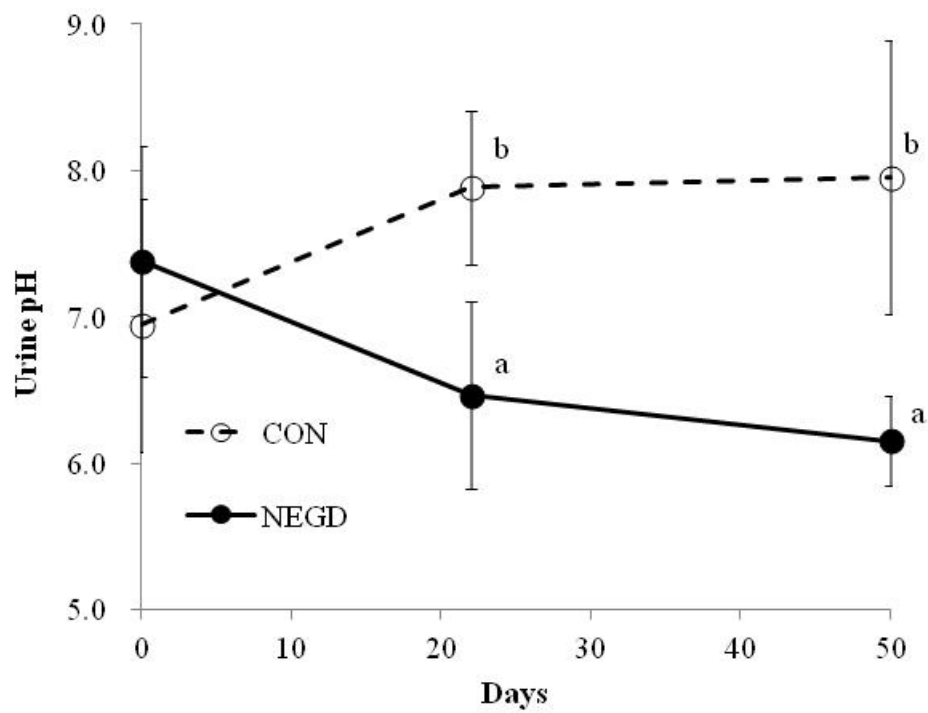


Figure 5-1 Effect of DCAD manipulation on urine pH. Points with different superscripts within days differ significantly ($P < 0.05$). There is a significant interaction between treatment and day ($P < 0.05$). CON, control; DCAD, dietary cation-anion differences; NEGD, negative DCAD manipulation.

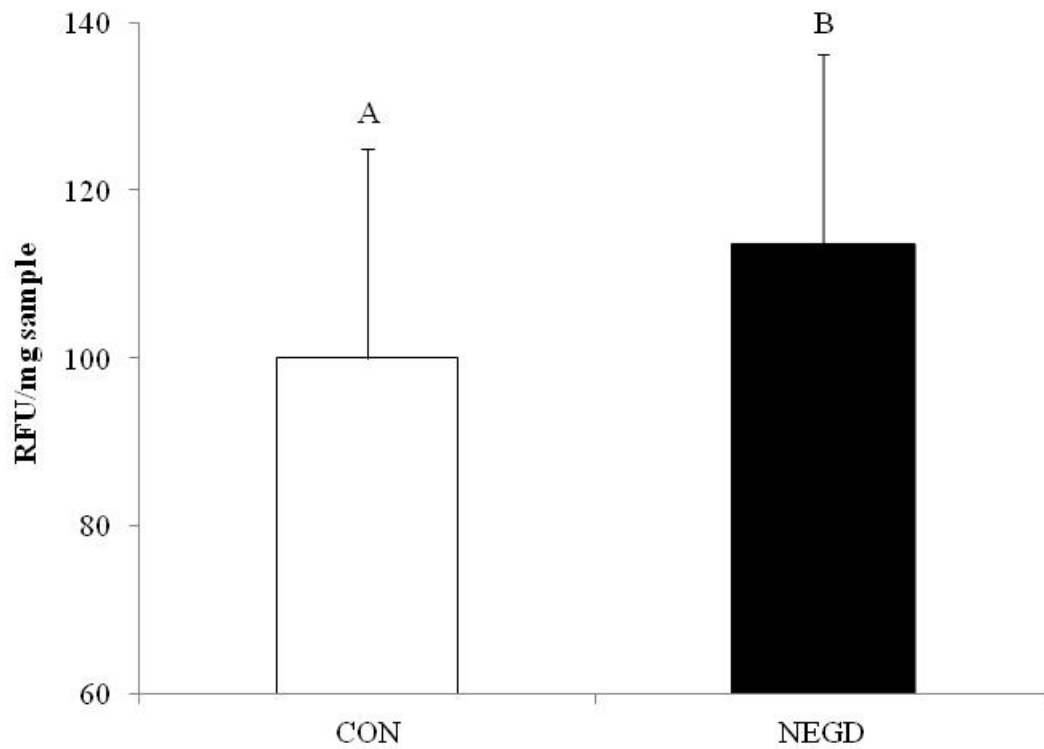


Figure 5-2 Effect of DCAD manipulation on calpain activity in the LCM. Data is expressed as relative value to the control. It has a tendency to differ between the treatments ($P = 0.09$). CON, control; DCAD, dietary cation-anion differences; LCM, Longissimus cervicis muscle; NEGD, negative DCAD manipulation; RFU, relative fluorescent unit.

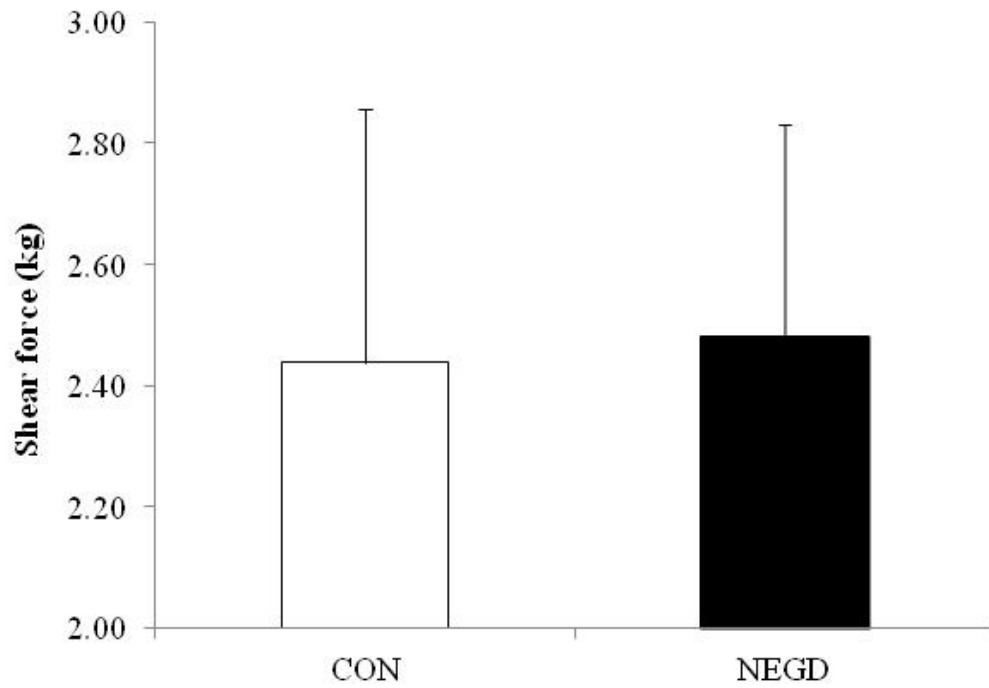


Figure 5-3 Effect of DCAD manipulation on WBSF in the LCM. CON, control; DCAD, dietary cation-anion differences; LCM, Longissimus cervicis muscle; NEGD, negative DCAD manipulation; WBSF, Warner-Bratzler shear force.

Chapter 6

General discussion

In this series of studies, we evaluated feeding by-product resources to ruminant from three viewpoints: 1) replacement of conventional feedstuff, 2) local use and 3) using as functional additive. In Chapter 2 and 3 where effects of adding local food by-products mainly including noodle waste to total mixed ration (TMR) silage were determined, it was demonstrated that the TMR silage was well preserved with high fermentation quality and did not negatively affect intake, digestibility, nitrogen balance and growth performance in wethers or fattening beef heifers compared with a conventional grain mix (Ishida *et al.* 2012a; Ishida *et al.* 2012b). These studies successfully showed a potential of local by-products as replacements of conventional feeds (viewpoint 1) and 2)). Yani *et al.* (2015) similarly reported that replacing a commercial concentrate with local food by-products including potato waste, soybean curd residue and noodle waste could be suitable for TMR silage in digestibility and nitrogen utilization. Sakai *et al.* (2015) also demonstrated that feeding TMR silage including brewers' grains from local breweries to lactating buffaloes increased milk yield likely due to high total digestible nutrients (TDN) intake. In Chapter 4, it was indicated that feeding polyphenol-rich winery wastes from a local red wine producer could decrease peroxidation in wethers (Ishida *et al.* 2015). To our knowledge, it is the first study on evaluation of feeding winery wastes (winery sediment and grape pomace) as antioxidants for ruminants, which could be a potential usage of local by-product resources (viewpoint 2) and 3)). In the study described in Chapter 5, it was shown that lowering dietary cation-anion

difference (DCAD) in fattening beef diets with a commercially available supplement containing amino acid fermentation by-products, Bio-Chlor could improve muscle calpain activities although beef tenderness did not change (Ishida & Hirano 2018). Manipulation of DCAD using Bio-Chlor has been a common nutritional strategy for periparturient dairy cows to prevent hypocalcemia (Block 1994), but it is relatively a new approach to improve beef tenderness. This study suggested another potential of by-product additives in producing high quality meat from our viewpoint 3). For years, by-products have been evaluated as alternatives of other feed ingredients as our viewpoint 1) (Westerndorf 2000; Sugiura *et al.* 2009; Bradford & Mullins 2012). On the other hand, using by-products as feed additives to improve health status of animals and/or quality of animal products has been less common so far, but will be getting more important when looking at increased consumers' needs for safe, healthy and high-quality foods.

There are several key points to consider when using by-product resources as ruminant feeds. One of the most critical points would be how to incorporate by-products into practical feeding systems. It was reported that annual production of by-products from food industries in Japan was approximately 20 million tons according to a survey by the Ministry of Agricultural, Fishery and Forestry of Japan (MAFF 2013). When looking at individual by-products, for instance, 30% of grapes for wine production are wasted (Makris *et al.* 2007), and about 1.1 kg of fresh soybean curd residue is produced from every kilogram of soybean for tofu or soy milk manufacturing (Khare *et al.* 1995).

Those values can be used to estimate the amount of by-products generated from each manufacturer. Meanwhile, some by-product resources are not used only for animal feeds but also for fertilizer, methanation or oil production (Sugiura *et al.* 2009) although others are not recycled (i.e. incinerated or buried in landfills). Thus it is needed to know the available amount of by-product resources for stable use as ruminant feeds.

Whether by-product resources can be used as raw materials depends on their nutritional values and stability of quality (Sugiura *et al.* 2009). It is challenging to use raw by-products which contain high moisture because they can deteriorate readily after being produced. Those wet resources can be dried or ensiled to enhance quality conservation (Kayouli & Lee 1999; Xu *et al.* 2007; Sugiura *et al.* 2009; Bradford & Mullins 2012) although each has both pros and cons. Drying allows by-product feeds to be distributed broadly because of less expensive transportation on a dry matter (DM) basis, but needs additional equipment and also may create dusty or chunky products that make their handling more difficult (Bradford & Mullins 2012; Ishida *et al.* 2012b). On the other hand, ensiling or combining with dry ingredients can prevent problems of physical property and minimize feed sorting that could cause nutritional issues (Xu *et al.* 2007; Bradford & Mullins 2012; Ishida *et al.* 2012a; Ishida *et al.* 2012b; Yani *et al.* 2015), but those methods are not suitable for wide distribution. Therefore, the method for utilization of by-product resources should be considered based on the place where they are produced. Yani *et al.* (2015) has noted that the nutritional values of

by-products vary widely depending on regions, meaning local use also has a potential to minimize nutritional fluctuations.

Considering different application of commonly used by-product feeds could be another opportunity. Ishida and Hirano (2018) mentioned that feeding Bio-Chlor that is amino acid fermentation by-products usually fed to preparturient dairy cows had a potential to improve beef quality, which could be another way to use Bio-Chlor. When somehow incorporating by-products to ruminant diets, however, careful diet formulation is needed. In the study by Ishida and Hirano (2018), it was reported that feeding Bio-Chlor to beef cattle decreased feed intake and it could be a risk of suppressing growth performance. Using fibrous by-products in place of forages can allow for greater dry matter intake (DMI) but can decrease physical effectiveness of fiber, which may upset rumen function (Bradford & Mullins 2012). Farmer *et al.* (2014) reported that increasing wheat straw to maintain chewing and rumen function resulted in similar lactation performance when increasing fibrous by-products in the diet for dairy cows. In contrast, replacing concentrates with by-products can decrease energy density, which may not be able to meet requirements especially for peak lactation cows or fattening beef cattle. Feeding by-products can provide potential benefits but these are dependent on nutritional strategies (Bradford & Mullins 2012). In addition, it is important to guarantee feed safety to promote the use of by-product resources (Sugiura *et al.* 2009). Needless to say, it is not allowed to use feed ingredients which are not approved by the Minister.

Another important point to consider is what advantages of using by-product resources can be expected. It is assumed that one of the biggest interests from many farmers would be impacts of by-products on animal productivity. For dairy cows, many studies have shown that optimal feeding fibrous by-products can improve productivity (Belibasakis & Tsirgogianni 1996; Ipharraguerre & Clark 2003; Schingoethe *et al.* 2009; Weiss 2012). Gessner *et al.* (2015) reported that adding grape seed and marc extract to the diet for high producing cows increased milk yield. Benefits on productivity of beef cattle have also been indicated in numerous studies. Feeding bread by-products instead of corn grains improved feed: gain ratio of beef steers (Guiroy *et al.* 2000). Eun *et al.* (2009) also reported that supplementing dried distillers grains improved daily gain and feed: gain ratio of growing beef steers. On the other hand, several studies have not shown any positive effects of feeding by-products on animal productivity (Radunz *et al.* 2003; Xu *et al.* 2007; Ishida *et al.* 2012b). It should be noted that replacing concentrates with fibrous by-products might sacrifice productivity because of decreased energy supply (Bradford & Mullins 2012).

Obviously farmers also have interests in feed cost and cost effectiveness. In general, by-product resources can be purchased at a lower cost because their utility is limited (Bradford & Mullins 2012). Previous research supports that it is possible to replace expensive grains with lower price by-products without compromising productivity (Dann *et al.* 2008). Ranathunga *et al.* (2010) showed that including distillers grains and soybean hulls instead of

concentrates in lactation diet decreased feed cost and increased income over feed cost. Romero-Huelva *et al.* (2017) also achieved similar milk yield and composition at a lower feed cost by feeding the mixture of tomato fruits, citrus pulp, brewer's grain, and brewer's yeast compared with a grain-based concentrate. Additionally, Farmer *et al.* (2014) demonstrated that lower forage with high fibrous by-products would be a viable strategy under conditions of expensive or limited supplies of grains and high-quality forages. It should be noted that profitability of feeding by-product diets must be dependent on regional conditions of feed supply. Local use of by-product resources could be a reasonable strategy to minimize transportation cost and in turn total feed cost.

From a perspective of animal welfare which has become a big concern of consumers, advantages of feeding by-products for animal well-beings could be well accepted in societies. By-products containing plant-derived antioxidants can be potential candidates. Ishida *et al.* (2015) demonstrated polyphenol-rich winery wastes could potentially reduce oxidative stress of sheep. Shabtay *et al.* (2008) also indicated that pomegranate peel would be a health-promoting diet for cattle.

Quality of animal products is another significant interest from both farmers and consumers, meaning feeding by-product diets which allow to improve product quality could also be desirable. For instance, DCAD manipulation using amino acid fermentation by-products, Bio-Chlor could improve activity of calpain which is a protease related to beef tenderness (Ishida & Hirano

2018). Shibuya *et al.* (2014) reported that the extracts of tea waste decreased muscle Pgc-1 α expression, which suggested the by-product could be used to modulate proportions of muscle fiber types and consequently meat color or texture. Romero-Huelva *et al.* (2017) demonstrated that feeding the mixture of tomato fruits, citrus pulp, brewer's grain, and brewer's yeast promoted higher mono- and polyunsaturated fatty acids in milk, meaning healthier fatty acid profiles for consumers. Although product quality does not depend only on dietary factors, future work looking for by-product resources to produce higher quality products is needed because consumers are willing to pay more for the products with desired characteristics (Miller *et al.* 2001; Vasta *et al.* 2008), which in turn can support profitability of farmers.

Methane is one of greenhouse gases and potentially playing a major role in global climate changes (IPCC 2007). Enteric fermentation of ruminants is a source of agricultural methane emissions (approximately 80 tons per year; Beauchemin *et al.* 2009). Therefore research aimed at decreasing enteric methane emissions has intensified in the last decade and a variety of mitigation practices have been evaluated (Hristov *et al.* 2013). In fact several studies have indicated that some by-product resources have a potential of decreasing methane emissions. Adding glycerol, a by-product from biodiesel production to substrates effectively decreased *in vitro* total gas and methane production (Lee *et al.* 2011). Humer *et al.* (2018) also reported that *in vitro* formation of methane was lower in the diet including 45% of bakery by-products due to shifting ruminal degradation pattern. Similar results

have been seen in *in vivo* studies. Benchaar *et al.* (2013) examined the effects of feeding corn dried distillers grains with solubles to lactating dairy cows. When expressed relative to energy-corrected milk, methane production declined linearly as the amount of distillers grains increased in the diet. In the study by Moate *et al.* (2014) where the effects of dietary supplementation with grape marc were evaluated, feeding grape marc to late lactating cows decreased methane emission by approximately 20% compared with the conventional diet likely because condensed tannins and fats in grape marc could alter ruminal bacterial and archaeal communities. In addition to methane emission, excess nutrient excretion from animal wastes has become a concern regarding environmental pollution. Ishida *et al.* (2012a) suggested that easily fermentable carbohydrates in potato waste and noodle waste would have a potential to promote microbial protein synthesis in rumen, which in turn could inhibit nitrogen excretion. Nudda *et al.* (2016) observed that feeding a by-product from myrtle liquor production to dairy ewes decreased urinary nitrogen excretion. Appropriate strategy of diet formulation can provide economically and environmentally optimal rations using various by-product resources (Oishi *et al.* 2011).

The potential advantages of by-products in terms of consideration for local community, animal welfare or environment can be additional values for consumers (Dentoni *et al.* 2009; Sonoda *et al.* 2018). Carpio and Isengildina-Massa (2009) noted that consumers would be willing to pay premiums for locally-grown animal products. It is indicated that

locally-grown animals fed locally-derived diets could more strongly affect consumer preference. Several studies have suggested that animal welfare is used by consumers as an indicator of product attributes, just like safety and the impact on health, meaning ethical considerations seem to motivate the purchase of animal products (Harper & Makatouni 2002; Frewer *et al.* 2005; Sonoda *et al.* 2018). In addition to advantages for animal productivity or feed costs, consumer preference can be a huge driving force to use by-product resources. It is important to build a sustainable system to recycle by-products among industries. In Japan, certification of food by-product diets called “Ecofeed” has been established to promote the use of by-products (Sugiura *et al.* 2009). The stability of feed supply can directly enhance the sustainability of food production by improving farm profitability and long-term viability.

Conclusions

In the series of studies, the utilization of by-product resources as ruminant feeds was evaluated. The TMR silage including local food by-products such as noodle waste, soybean curd residue, soy sauce cake and potato waste was well fermented and did not negatively affect digestibility or nitrogen utilization in wethers, or growth performance in fattening heifers. These results suggest that the TMR silage can be used as a substitute for conventional concentrate mixes. Feeding polyphenol-rich red winery wastes, winery sediment or grape pomace decreased ruminal ammonia production

likely due to their inhibitive effect on microbial activities in the rumen and also reduced peroxidation in wethers, which indicates winery wastes can be used as new antioxidants for ruminants. Lowering DCAD in fattening beef diets with a commercially available additive containing amino acid fermentation by-products could improve muscle calpain activities. This would be another potential use of by-product additives in producing high quality products.

When using by-product resources as ruminant feeds, it is important to consider advantages and disadvantages between upstream and downstream in industries (i.e. feed production, transportation, formulation, animal production, and farmers' and consumers' preferences etc.). In conclusion, by-products represent opportunities to maintain or improve animal performance and well-beings, to reduce feed costs, to meet consumers' demands and to enhance the sustainability of livestock and food production system.

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