Improvement of oat hay-based diet with supplementing leguminous forages for crossbred Simmental calves

(シンメンタール種交雑子牛に対するマメ科牧草の補助給与によるエンバク乾草主体飼料の改善)

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The United Graduate School of Agricultural Sciences
Tottori University, Japan

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A dissertation submitted to
The United Graduate School of Agricultural Sciences, Tottori University, in partial fulfillment of requirements for the Degree of Doctor of Philosophy in Global Arid Land Science

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Contents

Abbreviations

DM	dry matter
DMI	dry matter intake
MEI	metabolizable energy intake
CP	crude protein
NDF	neutral detergent fiber
ADF	acid detergent fiber
OM	organic matter
AH	alfalfa hay
CVH	common vetch hay
BUN	blood urea nitrogen
BW	body weight
GHG	greenhouse gas
CH_4	methane
CO_2	carbon dioxide
N_2O	nitrous oxide
CP	crude protein
GE	gross energy
DE	digestible energy
ME	metabolizable energy
HP	heat production
RE	retained energy
NE	net energy
N	nitrogen

MP	metabolizable protein
BWG	body weight gain
FCE	feed conversion efficiency
SEM	standard error of the mean
GDP	gross domestic product
NO_3^-	nitrate
SO_2	sulfur dioxide
CH_4	methane
Exp	experiment

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Chapter 1 General introduction

1.1 Background

1.1.1 Global importance of animal production and beef cattle feeding system

Since the late 1970s, increasing population, growth in the per-capita gross domestic product (GDP) and urbanization have combined to boost demand for animal-source foods in developing countries–a phenomenon that has been termed the ‘livestock revolution’ (Delgado et al., 1999). In 21−century, although the overall growth of agricultural production is slowing down, the fact that levels of food consumption are likely to increase (FAO, 2011a; Phillips, 2018). For example, the stocks and meat production of livestock, especially beef cattle are still increasing globally (Figure 1, FAO, 2019). In a study on the transformation in demand for animal-source foods from 1980 to 1990, Delgado et al. (1999) reported the total amount of meat consumed in the developing countries would grow 3 times compared with that in the developed countries, and they predicted it continues to grow at a rate of 2.8% for meat in developing countries up to 2020.

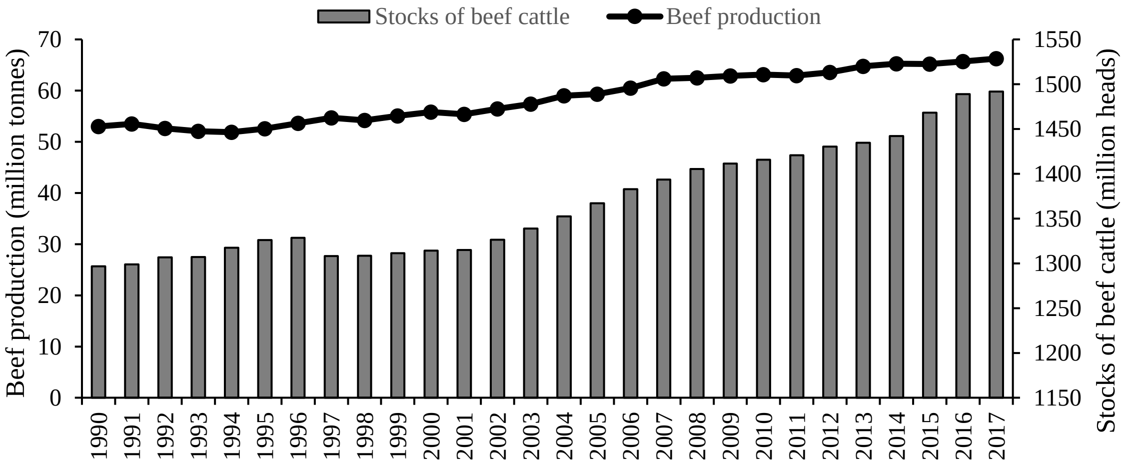


Figure 1 Stocks of beef cattle and beef production from 1990 to 2017, worldwide. Source: FAO (2019)

From 2000 to 2010, much of the forecast increase in demand had occurred, but it was in a rather patchy manner (FAO, 2011a). For example, meat consumption in China grew massively from an annual average of 9 kg per person to more than 50 kg per person in the space of 30 years (FAO, 2011a). Besides, beef production is rapidly increasing from 1990 in China due to the dramatic shifts in dietary preferences associated with rapid economic growth and improved living standards, rates of beef cattle production and beef consumption are increasing rapidly in China (Figure 2, FAO, 2019).

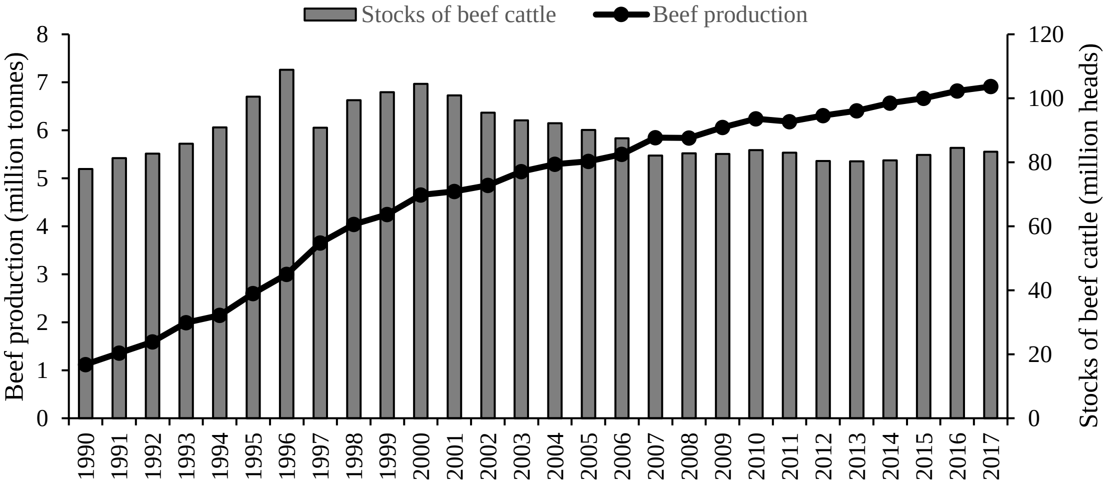


Figure 2 Stocks of beef cattle and beef production from 1990 to 2017, China. Source: FAO (2019)

According to *World Population Prospects* (UN, 2009), the population will grow to 9.1 billion in 2050, with most of the growth occurring in developing countries. Given that it will be a 40% increase in the world population, agricultural production will need to increase by 70% (nearly 100% in developing countries) by 2050 to raise average food consumption to 3130 kcal per person per day (FAO, 2011a). In developing countries, meat consumption has grown at over 5% per year during recent decades, which have been driven largely by China and to some extent Brazil. In agriculture production of developing countries, livestock is one of the fastest-growing sectors, potentially presenting opportunities for economic growth and poverty reduction in rural areas (FAO, 2011a). In 2010, the global ruminant population was estimated to be 3612 million, with cattle making up nearly 40% (FAOSTATE, 2012). Within the ruminant sector, the cattle sector is contributing about 79 % of total meat production from ruminants (FAO, 2013).

Another important issue in population growth is the distribution and the growth of urban areas (FAO, 2011a). The population living in urban areas is expected to rise from 3.3 billion in 2007 to 6.4 billion in 2050 according to the *2007 World Urbanization Prospect*. The level of urbanization is still expected to rise from 50% in 2008 to 70% in 2050 globally (UN, 2008), although there is considerable diversity in the levels of urbanization in different regions (FAO, 2011a). Especially, Africa and Asia urbanizing will be more rapidly than in other regions. In this case, there will be a shift in dietary preferences and demand for high-quality animal productions, such as beef production in these areas. Therefore, there is a demand to develop a more efficient beef cattle feeding system to meet the potential shortage of animal−source food supply in developing countries.

1.1.2 Environmental challenges for developing beef cattle feeding system

Globally, with the growth of livestock sector, particular ruminants, major concerns have been raised about the environmental consequences of such increases, such as its contribution to global warming and the low nitrogen utilization efficiency (NUE) on the pollution of surface water and potential protein wastage (Steinfeld et al., 2006). A report issued by IPCC (2006) has implied that there is an urgent need to better understand the sources of the ruminant’s greenhouse gas (GHG) emissions during enteric fermentation and related mitigation options, considering the important contribution of the livestock sector to total anthropogenic emissions. For example, in a study with an estimation method, livestock production contributes 8 to 18% of global GHG emissions, through the production of feed, growth of productive animals and the supporting herd, and the disposal of animal waste (O'Mara, 2011). Among the total GHG emissions, enteric fermentation represents 32−40% of total non−CO_2 emissions from agriculture emissions (Smith et al., 2013). It has been reported that the emissions of enteric CH_4 by ruminant livestock account for up to 28% of anthropogenic CH_4 emissions and an estimated 30−40% from agricultural sources (Beauchemin et al., 2008), and it had increased by 20% from 1970 to 2010 (IPCC, 2014) and 77% of enteric CH_4 production could be attributed to cattle (Steinfeld et al., 2006), followed by buffalo, sheep and goats (FAOSTAT, 2013). Except for the negative effect of enteric CH_4 emissions on global warming (Beauchemin and McGinn, 2006; Aboagye et al., 2018), it also represents a loss of dietary energy (Hristov et al., 2013), which could account for up to 12 % of gross energy (GE) intake (Yan et al., 2009)

In addition to GHG emissions from the livestock sector, negative effects of low NUE of ruminants with a range from 15 to 40% (Kohn et al., 2005) should also attract our attention, given that the stocks of ruminants are continuous increasing worldly (Figure 1). It has been reported that more than 70% of feed nitrogen (N) is excreted (such as in feces and urine) from livestock farming into the environment (Ghelichkhan et al., 2018), which is always perceived to be a major global environment problem (NRC, 2003). and the low NUE could contribute more ammonia (NH_3) emissions to the air and more manure N outputs to the soil (Montes et al., 2013; Aboagye et al., 2018), which not only indirectly contributes nitrous oxide (N_2O) emissions (Kebreab et al., 2009) but also lead to soil nitrification and acidification (Montes et al., 2013). In beef cattle feeding systems, approximately 60 to 80% of total N intake (NI) was excreted in the urine, which has great potential to aggravate NH_3 emissions, and only 20 to 40% was excreted in feces (Kebreab et al., 2009; Dong et al., 2014). In ruminant production systems, enteric CH_4 production is the largest contributor to GHG emissions followed by CH_4 from manure and in beef feedlot systems, N_2O from pen surface, and N_2O emissions from soils. Emissions from non-ruminant livestock systems are less than that of ruminants and are mostly CH_4 and N_2O from manure storage and land application (Hristov et al., 2013b).

Moreover, ruminant manure is a kind of nutrient resource that is easy to obtain and use, in which there are many essential elements required for plant growth and can be a significant source of nitrogen (N) in both intensive and subsistence animal production systems (Montes et al., 2013). However, unmanaged accumulation of organic wastes presents environmental and health concerns for humans and animals. Concerns include leaching of nitrate (NO_3^-) and pathogens to the groundwater, and deterioration of sensitive ecosystems, degradation of soil production potential through the accumulation of nutrients, salts, and metals, and emissions of gases considered a health and environmental risk (Steinfeld et al., 2006). For example, recent tropospheric satellite observations have demonstrated that NO_x emissions in China have accelerated impressively since 2000 (Irie et al., 2005) and emissions of N_2O increased by 45−75% from 1970 to 2010 (IPCC, 2014), which is also a component of GHG (Chadwick, 2005).

Therefore, the development of a diet that can improve the NUE and reduce enteric CH_4 emissions is on−demand and beneficial to both the animal husbandry and global environmental challenges (Yan et al., 2007; Waldrip et al., 2013; Aboagye et al., 2018).

1.1.3 Mitigations to reduce greenhouse gas emissions and improve nitrogen utilization efficiency

So far, there have been many ways to reduce GHG by supply-side mitigation options in the agriculture sector (IPCC, 2014). For example, in the livestock-feeding sector, dietary additives (such as bioactive compounds, fats), ionophores/antibiotics, propionate enhancers, nitrate and sulfate supplements to reduce CH_4 emissions from enteric fermentation. Besides, improving livestock productivity is another way to reduce GHG emissions (Yan et al., 2009). For example, feeding balanced rations also reduced enteric CH_4 emissions by 15−20 % per kilogram of milk produced and increased efficiency of microbial protein synthesis (FAO 2012; Garg et al. 2013).

Generally, higher NUE would stimulate livestock growth and reduce the influence of large N excretion on the environment. For the past 2 decades, NUE has been extensively studied in beef cattle (Yan et al., 2007; Dong et al., 2014). For example, increasing feeding level (FL, metabolizable energy [ME] intake divided by ME requirement for maintenance from the AFRC [1993]; Yan et al., 2007) and energy supplementation (Titgemeyer et al., 2012) could improve NUE. Higher diet quality (such as high ME; Zhao et al., 2016) also leads to high NUE.

1.2 Literature review

1.2.1 The forage evaluation in the beef cattle production system

Ruminant production systems throughout the world are based on forages, with grassland feeds being predominant (Givens, 2000). But, a wide range of crops in addition to perennial grasses and legumes can be used as forage crops. For example, the major forage crops are maize in most regions worldwide, which are not in competition with cash crops and food for direct human consumption, but also with low-cost production of DM and nutrients, such as straw and crop residues, could provide nutrients for ruminants because of the high yields of DM and energy that can be obtained (Givens, 2000). Forage crops must have particular features in order to warrant their inclusion in ruminant production systems, such as higher yield potential for maize than grass provides a major motivation for growing maize in many areas of Asia, North America and Europe. Likewise, high annual yields contribute to the use of sorghum and small-grain cereals (e.g. wheat, barley and oats) as forages.

In addition, estimates of the production of fibrous by-products from cereals and other crops are the potential forage for ruminant production systems. They have been some increases in by-product with further increases in global crop production (Givens, 2000). In theory, the total output of by-products could supply 84% of the energy and 74% of the CP required by the world's ruminants (Kossila, 1984). In many Asian countries, for example, India, Bangladesh, Pakistan, Myanmar and Indonesia, ruminant production is largely based on feeding of crop residues and agro-industrial by-products, such as straw. However, these resources need to be properly managed in smallholder systems. Straw worth millions of dollars is burned every year in many parts of Asia, causing environmental problems and soil degradation, in addition to the loss of this valuable feed resource. In India alone, the burning of crop residues releases CO_2, CH_4, N_2O and sulfur dioxide (SO_2), equivalent to 6.6 million tonnes of CO_2 annually (INCCA 2007). Improving the management of crop residues as animal feed should be one of the main priorities. There is an urgent need to optimize the use of limited feed resources, including straw for ruminant feeding.

Clearly, crop residues represent an underutilized feed source, although physical or chemical treatment may be necessary in order for fibrous by-products to make a major contribution to the energy requirements of productive livestock (Owen and Jayasuriya, 1989). Greatest reliance on straw occurs generally in areas where the number of ruminant animals is high in relation to the area of productive grassland and in which there is substantial production of cereals for human food. Normally, varieties of cereal will be selected on the basis of efficiency of grain production, but, in some situations, varieties may be used because of enhanced yield or quality of straw.

1.2.2 Feeding grass hay and its deficiency in the beef cattle production system

Forages represent a diverse range of feedstuffs that make a significant contribution to the overall nutritional economy of meat-, wool- and milk-producing ruminants (Givens, 2000). Undoubtedly, the principal forage is grass, which may be consumed by ruminants after conservation as silage or hay, such as forage maize silage or whole-crop wheat silage in the world, considering grasses and some forage crops may provide the high yields of DM and energy at low cost. However, grasses are not usually capable of achieving the required levels of animal production, due to limitations in feeding value (Givens, 2000). With the result that, higher levels of supplementary feeds are required with these grasses in order to achieve particular levels of animal performance. This contributes, for instance, to the substantial development of grain finishing of cattle in parts of northern Australia.

Animal production in extensive rangeland conditions in America, Africa, Australia, and northern Asia is almost entirely dependent on the native grassland vegetation, with animals often being exported to other agro−climatic zones for finishing. In some less developed areas, however, particularly in the north of Asia, low human population pressure and market opportunities for ruminant products have led to smallholder ruminant feeding systems. In these regions, imbalanced nutrition is common in most smallholder beef feeding systems as many farmers are unskilled in preparation and feeding of balanced diets (IPCC, 2014), which is one of the major factors responsible for low livestock productivity. Balanced nutrition is a concept for optimal mixed ratios of feeds, which is based on the physiological conditions of the animal and could contribute to improving animal output as well as to reducing both the cost of production and the emissions of GHG per unit of animal product produced (FAO, 2013). Previous studies reported that feeding of total mixed rations has also been shown to have several advantages such as a decrease in feed loss, higher nutrient availability, lower enteric CH_4 production and higher animal performance over feeding ingredients separately (FAO 2011b; FAO 2012), which is conventionally practiced in most Asian countries. As a result animal productivity is low, feed C and N get wasted and are not utilized efficiently in animal products, causing excessive release of GHG emissions. Smallholder production systems contribute over 65% of milk production and over 55% of meat production and hence targeting smallholder farmers should be the priority (FAO, 2012). Therefore, large-scale implementation of such an efficient feeding method can help improve the productivity of livestock and reduce the GHG emissions raised by smallholder farmers.

1.2.3 The associative effects of forages on digestive metabolism of beef cattle

Feeds are commonly evaluated as single entities despite the fact that most of the time an animal is fed a mixture of ingredients (Sandoval−Castro et al., 2002). Feeds other than forages, particular grains crops, are usually used because of forages are not capable of achieving the required levels of animal production, due to limitations in feeding value (Givens, 2000). For example, grain supplementary is required with grasses to achieve particular levels of animal performance, especially to the substantial development of grain finishing of cattle (Minson, 1990). However, the use of mixtures of forages may lead to positive or negative effects depend on the various components of the feeding value (Niderkorn and Baumont, 2009).

Usually, associative effects occur when the apparent digestibility of a mixture of forages does not equal to the sum of the separately determined digestibilities of its components (Huhtanen, 1991). Current feed evaluation systems usually assume that digestibility, energy and nitrogen values, as well as voluntary intake of individual forages, can be added and do not take into account possible interactions between the different forage components of a diet. But, these interactions can modify the metabolic processes in the gastrointestinal tract of ruminants, particularly in the rumen (Niderkorn and Baumont, 2009). As a result, digestibility and intake of a combination of forages can be higher (positive associative effects) or lower (negative associative effects) than the balanced median values calculated from forages taken separately (Niderkorn and Baumont, 2009). Previous studies showed that concentrate feed supplementation, which has high quantities of easily fermentable carbohydrates, could decrease the ruminal pH, and then negatively affects cellulolytic activity and subsequently digestibility of plant cell walls (Mould et al., 1983), protein supplementation of straw may improve its digestibility and voluntary intake, thus alleviating nitrogen deficiency and stimulating the growth of rumen microbes (Mawuenyegah et al., 1997). Therefore, there is a demand to clarify the associative effects of grass hay-based diet with other forages supplementation on the growth performance of beef cattle, which is common in most smallholder beef production sectors.

1.2.4 Characteristics and importance of oat, alfalfa, and common vetch

Oats (*Avena sativa* L.) is a crop of Mediterranean origin, which ranks around sixth in world cereal production, following wheat, maize, rice, barley, and sorghum. For now, it is a very important winter fodder on small farms (Suttie and Reynolds, 2004). Oat as green forage, hay and silage is highly palatable and a very good forage to ruminants (Kafilzadeh and Heidary, 2013). Due to some reasons, such as sensitive to hot, dry weather, world oat production is generally concentrated in higher-altitude regions. In China, it has a long history for cultivating oats as food and feedstuff (Suttie and Reynolds, 2004), and it was used to be valuable feed for horses and mules, now the whole crop or straw are mainly used as hay or silage for ruminants in north, northwestern and southwestern areas in China, such as Inner Mongolia, Gansu, Ningxia, Qinghai and Guizhou Provinces. In these regions, oats are an important feed resource for animal husbandry, especially as emergency or supplementary hay for winter and early spring. Previous studies reported that oat is reputed to be better suited for production under marginal environments, such as soils with low fertility, compared with other cereal crops (Kafilzadeh and Heidary, 2013). A mixture of oats and legumes could get a body weight gain (BWG) of 261 g/day more than those fed on rice straw (Luo et al., 2000), and oats could increase milk production in dairy cows compared with maize silage (Zheng et al., 2002). There was a higher intake, a similar digestibility and higher total digestible nutrients for oat forages compared to barley and wheat forages by steers when fed as silage (Mtimuni, 1976).

Alfalfa (*Medicago sativa* L.) a perennial forage legume, which is a nutritious and high yielding legume commonly fed to ruminants as hay or as silage (McMahon et al., 2000), and widely cultivated all over the world because of its resistance to drought and cold weather (Kobayashi et al., 2017). Generally, it can grow to a height of up to 1 m and has a deep root system, which could extend straight down into the soil to a depth of 6 m or more (Weaver, 1926). In early 2000, alfalfa was the most cultivated forage legume in the world and its worldwide production was around 436 million tons in 2006 (Reddy et al., 2014).

Common vetch (*Vicia sativa* L.), a new multi−purpose annual cereal legume for livestock feed (Huang et al., 2017), not only plays an important role in dryland mixed farming systems (Larbi et al., 2011) either for grazing (Rihawi et al., 2010) or cutting for hay (Assefa and Ledin, 2001), but also meet the structural forage deficit in winter that is linked to the seasonality of other feed sources (Graham and Vance, 2003). It originated from the arid areas of the Middle East and now widely cultivated in many regions of the world (Huang et al., 2017). Globally, the production area of the common vetch and other vetches is approximately 573,769 ha, equating to a crop production yield of 926,982 tonnes/year and an average annual yield of 1,616 kg/ha (FAOSTATE, 2013). In southwest China, especially the Tibetan plateau which has an average altitude of over 4000 m, shortage of feedstuffs in winter is one of the most challenges for local farmers to develop their animal husbandry. A mixture ration of oat hay (38%) and common vetch hay (18%) can be potential sources of roughage of total mixed ration silage to substitute whole-plant corn silage in Tibet (Chen et al., 2015). In Mediterranean regions, a ratio of common vetch and oat mixture at 45:55 could maximize DM and CP yield (Erol et al., 2009), and the increase of common vetch hay (CVH) in oat hay (OH)-CVH mixture has a positive effect on its nutritive value in a *nylon bag technique* in rumen fistulated wethers (Haj−Ayed et al., 2000).

1.2.5 Current research of beef cattle production using oat, alfalfa, and common vetch, and their deficiencies

In general, leguminous forage can offer higher nutritive value and raise the efficiency of conversion to livestock production compared with grass (Patra, 2010). Previous studies have shown that CP, digestible OM intake, and in vitro OM digestibility are significantly higher with oat−common vetch mixture diets than with oat−only diets for cattle (Assefa and Ledin, 2001). The addition of alfalfa to a grass hay basal diet also increased the digestibility of CP and the disappearance rate of dry matter (DM) and neutral detergent fiber (NDF) in the rumen in beef cattle diets (Bhatti et al., 2008). However, little information is available on N metabolism and partition of N excretion under oat−alfalfa or −common vetch mixture diets. What is more, too high a proportion of legumes in the diet may cause adverse effects. For example, although a high−alfalfa hay (AH) diet (34%) for growing Simmental calves could improve NUE, it led to a decreased DM and CP digestibility compared with a low proportion of AH (9%) in the diet (Kobayashi et al., 2018).

Alfalfa occupies the largest planted area of perennial legume crops in the world, and common vetch is one of the primary annual forage legume sources for ruminants in the arid and cold areas of the world (Kobayashi et al., 2018). Previous studies have shown that the addition of alfalfa to the grass hay basal diet also increased the digestibility of dry matter (DM), CP and digestible CP in sheep diets (Haddad 2000). The variation in livestock, such as animal type, weight, gender, and age, in different studies usually leads to differences in the results. Simmental cattle have historically been used for beef and are renowned worldwide for the rapid growth of their young. It has been extensively studied in various diets in the world (Mc Parland et al., 2007; Kobayashi et al., 2017).

Condensed tannins or bioactive plant metabolites, such as essential oils, flavonoids and saponins (Beauchemin and McGinn, 2006; Pen et al., 2008), extracted from leguminous forage, can not only reduce CH_4 emissions but also improve feed conversion efficiency (FCE) (Shibata and Terada, 2010; Liu et al., 2018). Changes in diet quality in terms of less grass forage and more leguminous forage, such as alfalfa (Beauchemin et al., 2008), or a higher grain diet (Mc Geough et al., 2010), have been shown to have an effect similar to that of leguminous forage extract addition to the diet. However, too much leguminous forage in the diet may cause adverse effects: for example, a low proportion of alfalfa hay (AH, 22%) for growing Simmental cattle slightly increased FCE and reduced CH_4 emissions, whereas CH_4 emissions significantly increased and FCE significantly decreased in cattle fed a high AH diet (44%) (Kobayashi et al., 2017). Hence, finding a diet with an appropriate proportion of legumes is necessary and beneficial for sustainable agronomic practice in dryland environments.

1.3 Objective

1.3.1 General objective

To clarify the effects of inclusion levels of leguminous forages on energy and nitrogen metabolism of crossbred Simmental calves

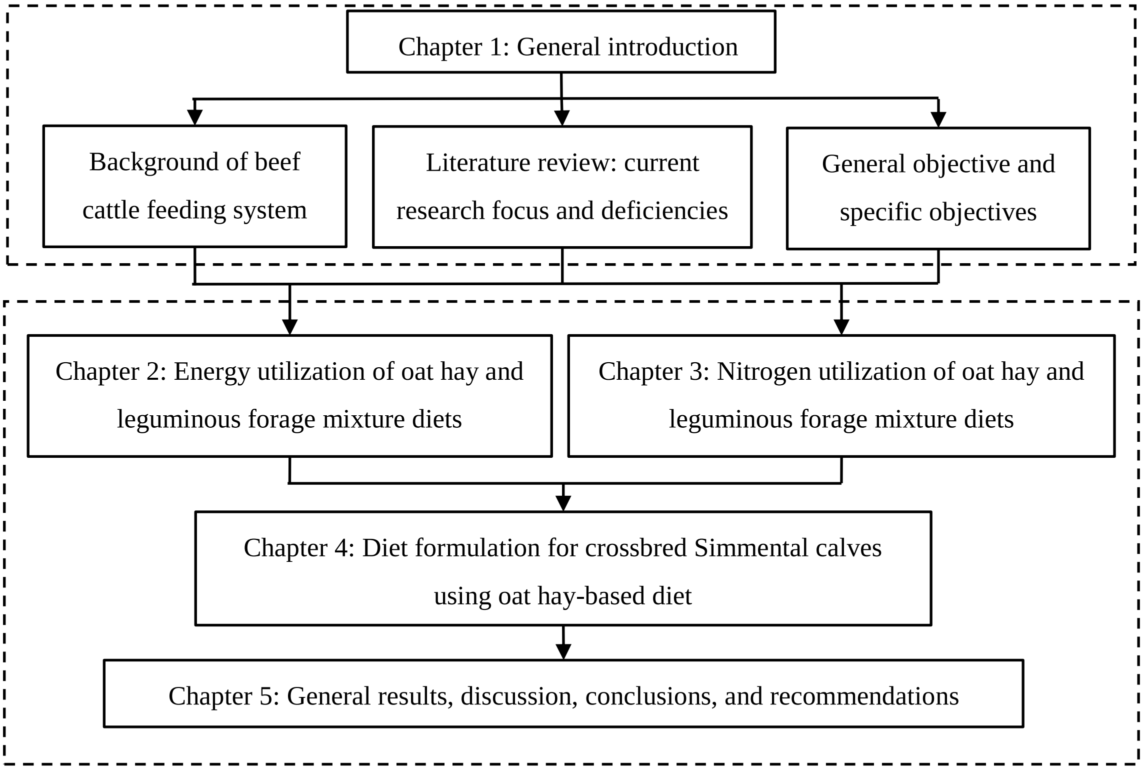
1.3.2 Specific objectives

(1) To clarify the optimal levels of alfalfa or common vetch substituting oat hay on nutrient digestibility and energy utilization of crossbred Simmental calves;

(2) To clarify the optimal levels of alfalfa or common vetch substituting oat hay on body weight gain and nitrogen utilization of crossbred Simmental calves;

(3) To formulate the oat hay-based diets with supplementing alfalfa or common vetch for crossbred Simmental calves.

1.4 Structure of the thesis



Chapter 2: Effects of oat hay and leguminous forages mixture diets on energy utilization

2.1 Introduction

This chapter was to set to determine the optimal levels of alfalfa or common vetch substituting oat hay on nutrient digestibility and energy utilization of crossbred Simmental calves

2.2 Materials and methods

This study was conducted at the Linze Grassland Agriculture Trial Station, Lanzhou University, China (latitude 39.24°N, longitude 100.06°E, 1390 m above sea level). The environment is characterized by a typical temperature continental climate, with annual precipitation of 121.5 mm and an average temperature of 7.7 ^{o}C, as derived from the agricultural meteorological station in Linze Grassland Agriculture Trial Station from 2006 to 2016 using a CR5000 datalogger (Campbell Scientific Inc., USA). In these experiments, AH was the second harvest and common vetch was harvested at the podding stage and prepared as common vetch hay (CVH). Oat hay (OH) was purchased from a large forage company (Sanbao Agricultural Company, Zhangye, Gansu, China), and the ingredients for the feed concentrate (maize, soybean meal, and wheat bran) were sourced locally. The chemical composition of the forage and the ingredients of the concentrate are shown in Table 1.

Table 1 Chemical composition of alfalfa hay, oat hay, common vetch hay, and ingredients of the concentrate used in the experimental diets.

Item[†]	Alfalfa Hay	Oat Hay	Common Vetch Hay	Soybean Meal	Wheat Bran	Maize
OM, g/kg DM	905	942	918	935	931	983
CP, g/kg DM	168	60	177	465	182	83
NDF, g/kg DM	458	559	413	166	454	100
ADF, g/kg DM	347	407	302	102	186	20
Ether extract, g/kg DM	22	18	23	26	55	44
GE, MJ/kg DM	17.9	16.8	17.7	19.6	19.4	18.5
MEC[§], MJ/kg DM	8.7	9.0	9.5	13.0	10.9	13.4
MPC[¶], g/kg DM	62	68	71	87	73	90

[†] OM, organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; GE, gross energy; MEC, metabolizable energy concentration; MPC, metabolizable protein concentration;

[§, ¶] They were calculated by the Agricultural and Food Research Council (1993) and the Chinese Feeding Standard for Beef Cattle (2004), see details in Methods and Materials.

2.2.1 Animals, treatments and diets

The Animal Ethics Committee of Lanzhou University approved all animal management and experimental procedures according to the rules and regulations of experimental field management protocols (file No. 2010-1 and 2010-2) in accordance with the Guides for Management of Laboratory Animals in Gansu Province, China (Gansu Provincial Department of Science & Technology, 2005). The target forage-to-concentrate ratio was fixed (60:40, DM basis) for all diets in all experiments. In experiment (Exp) 1, 16 crossbred male Simmental cattle (Simmental × local cattle) with an initial body weight (BW) of 134 ± 7.9 kg (mean ± standard deviation [SD], 5 months of age) were assigned to four diets with different OH-to-AH ratios (60:0, Diet-1; 52:8, Diet-2; 44:16, Diet-3;

and 36:24, Diet-4 on a DM basis of total feed supplied) in a randomized block design (four replicates per diet). In Exp 2, the same 16 crossbred male Simmental cattle with BW of 206 ± 16.5 kg (mean ± SD, 9 months of age) were also assigned to four diets with different OH-to-CVH ratios (60:0, Diet-1; 50:10, Diet-2; 40:20, Diet-3; and 30:30, Diet-4 on a DM basis of total feed supplied) in a randomized block design (four replicates per diet). There was a 2-month interval between the end of Exp 1 and the start of Exp 2, and the animals were allocated to their respective diets in Exp 1 and Exp 2 according to a completely independent randomized block design. There were no significant differences in the initial average BW among animals in the four diet groups in each experiment. The target daily BWG for each animal was set at 1.0 and 1.3 kg/day for Exp 1 and 2, respectively. Both feeding experiments lasted for 50 days, which included an initial 14 days of diet acclimation followed by 36 days of data collection.

The same amount of experimental diets was provided in each experiment, and the total amount of supplied diets was calculated based on the target BWG, the BW of cattle in each experiment, the published equations and values of the Agricultural and Food Research Council (AFRC, 1993), and the Chinese Feeding Standard for Beef Cattle (CFSBC, Ministry of Agriculture of the People’s Republic of China, 2004). All experimental diets were designed to provide sufficient metabolizable energy (ME) and metabolizable protein (MP) to meet the target BWG for each animal according to the published estimation equations and values of AFRC (1993) and BW of cattle (measured every 9 days). The diet composition required to fulfill the ME and MP requirements was calculated based on the tabulated values of digestible energy and ruminal CP degradation parameters for OH, AH, and concentrate ingredients established by CFSBC. The digestibility of ruminal CP and energy, and ruminal degradation parameters for CVH were obtained from Karabulut et al. (2007). The CP, ME, and MP levels of all diets in each experiment are shown in Table 2. Throughout the experimental period, all cattle were housed in individual pens and given free access to water and a mineral mixture, except for those cattle for data collection in the chamber. The daily mixed forage was divided into two equal parts and offered as separate meals twice a day (08:00 and 20:00 in Exp 1, 08:00 and 18:00 in Exp 2). The mixed concentrate was fed once a day (14:00 in Exp 1 and 13:00 in Exp 2).

Table 2 Composition of feed ingredients and target metabolizable energy concentration and metabolizable protein concentration of all diets in experiment 1 (Exp 1) and 2 (Exp 2).

Feed formula	Diet[†] (Exp 1)				Diet[†] (Exp 2)			
	AH0	AH8	AH16	AH24	CVH0	CVH10	CVH20	CVH30
Forage								
Leguminous forage (g/kg DM)	0	80	160	240	0	100	200	300
Oat hay (g/kg DM)	600	520	440	360	600	500	400	300
GE content (MJ/kg DM)	16.8	17.0	17.1	17.2	16.8	17.0	17.1	17.3
Concentrate								
Maize (g/kg DM)	48	78	110	140	55	69	81	95
Soybean meal (g/kg DM)	107	86	68	48	112	75	38	1
Wheat bran (g/kg DM)	245	236	222	212	233	256	281	304
GE content (MJ/kg DM)	19.4	19.3	19.2	19.1	19.3	19.3	19.2	19.2
Nutrient value[‡]								
CP (g/kg DM)	135	134	135	135	135	135	135	135
OM, g/kg DM	940	939	938	936	941	939	937	935
NDF, g/kg DM	469	457	442	429	465	456	448	439
ADF, g/kg DM	302	294	285	277	300	290	281	271
MEC[§](MJ/kg DM)	10.1	10.1	10.1	10.1	10.2	10.1	10.1	10.1
MPC[¶](g/kg DM)	99	97	95	94	100	96	91	86

[†] Experiment (Exp) 1, AH0, 60% oat hay; AH8, 52% oat hay and 8% alfalfa hay; AH16, 44% oat hay and 16% alfalfa hay; AH24, 36% oat hay and 24% alfalfa hay. Experiment (Exp) 2,

CVH0, 60% oat hay; CVH10, 50% oat hay and 10% common vetch hay; CVH20, 40% oat hay and 20% common vetch hay; CVH30, 30% oat hay and 30% common vetch hay.

[‡] CP = crude protein; OM = organic matter; NDF = neutral detergent fiber; ADF = acid detergent fiber; MEC = metabolizable energy concentration; MPC = metabolizable protein concentration.

[§, ¶] They were calculated by the Agricultural and Food Research Council (1993) and Chinese Feeding Standard for Beef Cattle (2004), see details in Methods and Materials.

In addition, 10 g/day of mineral mixture fed to each calf throughout the feeding period contained (minimum values in mg): manganese, 720; copper, 30; biotin, 0.05; folic acid, 0.4; vitamin B_1, 50; vitamin B_2, 2.5; vitamin B_6, 0.5; vitamin B_{12}, 0.1.

2.2.2 Measurement and sampling procedure

The amount of forage and concentrate offered and all leftovers were weighed three times per day prior to feeding (08:00, 14:00, and 20:00 in Exp 1; 08:00, 13:00, and 18:00 in Exp 2, respectively) throughout the experimental period. The difference between the feed refusals and the feed supplied was used to calculate forage daily DM intake (DMI) and concentrate DMI for each animal. After the 14-day acclimation period for target feeds, on day 15 of the experimental period, a randomly selected animal from each diet group was moved to one of four individual open-circuit respiration chamber for 9 days. On day 24, animals were moved to individual pens in a cowshed, and another four cattle, randomly selected from the remaining cattle of the four diet groups, entered the chambers and left on day 33; this continued until measurements were completed for all 16 cattle. The BW of all 16 cattle was measured in the morning when they were moved between the chambers and the cowshed. The BWG (kg/day) was calculated based on the difference between the beginning and the end of data collection period (36 days). Within the 9 days of measurements in the chamber, the cattle were acclimatized for the first 2 days. Digestibility data were collected over the following 4 days and gas exchange data (oxygen

[O_2] consumption, CH_4, and carbon dioxide [CO_2] emissions) were collected over the remaining 3 days. During the collection of digestibility data, the total weight of daily excreted feces and urine was recorded. Feces, which were excreted on a plastic mat placed under the cattle, were collected immediately with a shovel, placed in a plastic container, weighed, mixed, and sampled once per day. Ten-percent of each fecal samples was stored at −20 °C for later chemical analysis. Total urine was collected through a handmade urine bag into a bucket containing 200 mL 10% v/v sulphuric acid (H_2SO_4) to reduce the loss of ammonia, once per day. The pH of acidified urine was checked with a portable pH instrument (PHBJ-260, Shanghai INESA Scientific Instrument Co., Ltd). Twenty-percent of the daily urine was stored at −20 °C for chemical analysis.

The four indirect open-respiration calorimeter chambers used in the present study were equipped with a computer-controlled air-handling system with air conditioning units set to a temperature of 18 ± 1°C and relative humidity of 60 ± 10%. The calorimeter chambers were built with double Perspex walls fit with aluminum frames (Zhao et al., 2015), with a total volume of approximately 18 m^3 (4.2 m long, 1.95 m wide, and 2.2 m high). Each chamber was equipped with a gas flow meter (GFM57, Aalborg, Orangeburg, New York, USA) at the outflow site to record total airflow, and an engine to ensure a slight negative pressure within the chamber. All chambers were ventilated by suction pumps with a flow rate of 45–50 m^3/h. The exhaust air was removed for volume, temperature, and humidity analyses from the bottom, middle, and upper, respectively, of each chamber. During the 3 days of gas exchange measurements, the concentrations of CO_2, CH_4, and O_2, to determine atmospheric air entering and exhaust gas leaving each chamber, were measured every 16 min (4 min for each chamber and/or ambient air, the interval for each chamber) using a multi-gas analyzer (VA-3000, Horiba Ltd., Tokyo, Japan) in a general control room. The analyzer was calibrated using standard gases (O_2-free nitrogen [N_2] and a known quantity of CH_4, CO_2, and O_2 [span gas], Dalian Special Gases Co., Ltd., Liaoning, China) at the beginning of the gas exchange collection period in each experiment. This determined an absolute range of 0–500μL/L for CH_4, 0–2000μL/L for CO_2, and 0–25% v/v for O_2, and the linearity within this range. The rate of CH_4 recovery was determined by comparing the amount of CH_4 loss from a gas cylinder in the bottom of the chambers, and the CH_4 accumulations passing through the chambers (Livestock Research Group of the Global Research Alliance, 2014). The gas recovery rate was approximate 100 ± 2% for all chambers, as reported by Gerrits et al. (2018). Data were collected for each chamber for 4 min, which included the time for the gas mixture to flow from inside the chamber to the analyzer, and the time for gas concentration to stabilize. The concentrations of CO_2, CH_4, and O_2 were recorded during the last 3 s of this 4 min period. The O_2 consumption, and CH_4 and CO_2 emissions were calculated based on differences in the CO_2, CH_4, and O_2 concentrations between the air in and out of each chamber, and the total volume of gas exchange (flow rate × interval time). Each chamber was designed with a dedicated door, which was next to the animal trough. The staff only opened the door to feed the animal immediately upon completion of data collection in the chamber. This minimized the effects of feeding activity (less than 1 min) on the internal gas concentrations. CH_4 emission was expressed as the average CH_4 emission (g/day) from 3-days' measurement. Ruminal digestible organic matter intake (RDOMI, kg/day) was calculated by organic matter (OM) intake (kg/day) × OM digestibility (fraction) × 0.65 (ARC, 1980). CH_4:RDOMI (g/kg) was calculated by CH_4 emission (g/day) divided by RDOMI (kg/day).

2.2.3 Collection of ruminal fluid

Rumen fluid samples were taken from each cattle 4 h post forage feeding in the morning using stomach tubing on the two days of each experiment. The collected samples were immediately measured for pH using a portable pH meter (PHBJ−260, Shanghai INESA Scientific Instrument Co., Ltd), strained through two layers of muslin (mesh size 1 mm^2) and stored at −20 ℃ for volatile fatty acid (VFA) analysis. An additional 1 ml of strained rumen fluid was deproteinated by adding 0.2 ml metaphosphoric acid (215 g/L) and 0.1 ml internal standard (Crotonic acid), and the VFA concentrations were determined by a gas chromatograph (Trace1300, Thermo Ltd., Rodano-Milan, Italy) fitted with a polar capillary column.

2.2.4 Chemical analysis

After the chamber measurement, the stored feces samples were thawed at 4 ^{o}C for 12 h and fecal samples obtained from each animal over the 4 days were mixed. A portion of the thawed fecal sample was used to measure nitrogen (N) according to the Association of Official Analytical Chemists method 976.05 (AOAC, 1990). The CP concentration was calculated by multiplying the N concentration by 6.25. The remaining samples were oven-dried at 65°C for 48 h to measure the DM percentage and then ground to pass through a 1 mm screen. A portion of each dried sample, mixed forage and concentrate samples were used to measure ash by combustion using a muffle furnace at 550°C for 6 h (method 942.05; AOAC, 1990). The OM content (g/kg DM) was calculated by 1000 – ash content (g/kg DM). Another portion of each dried sample was finely ground and used to determine gross energy (GE), neutral detergent fiber (NDF), and acid detergent fiber (ADF). The

GE of the samples was determined with an automatic isoperibol calorimeter (6400, PARR Instrument Company, Moline, IL, USA). The NDF and ADF concentrations were analyzed sequentially in an ANKOM 2000 fiber analyzer (ANKOM Technology, Fairport, NY, USA) following the protocol described by Van Soest (1991). The ash was included to provide NDF and ADF analyses of all forage, concentrate, and feces samples. The α-amylase for NDF analysis was used only for concentrate samples. Urine samples taken from each animal over the 4 days were also thawed at 4 ^{o}C for 12 h and then mixed. Urinary energy (UE) was then determined by an automatic isoperibol calorimeter (see above), and N was determined by the Kjeldahl procedure as described previously by the AOAC (1990). For UE measurement, 4 mL of fully mixed urine was taken and absorbed on filter paper of known weight, and then the total energy of the filter paper with the urine sample was measured by automatic isoperibol calorimeter after drying. A further five samples of the same filter paper (known weight) were measured to determine energy content (MJ/kg), which was used to calculate the UE. CP, NDF, and GE were measured in the forage and concentrate of the diets using the above methods and instruments. The ether extract of forage and concentrate was analyzed using an ANKOM XT15 Extractor (ANKOM Technology, Fairport, NY, USA).

The total apparent tract digestibility of DM, OM, and NDF was calculated as: digestibility (%) = [nutrient intake (kg/day) – nutrient in feces (kg/day)]/nutrient intake (kg/day) × 100.

2.2.5 Energy balance

Digestible energy intake (DEI) was calculated as the difference between GEI and excreted fecal energy (FE). ME intake (MEI) was calculated as the difference between DEI, and the sum of UE and CH_4 energy (CH_4−E) output. Retained energy (RE) was calculated using the equation: MEI – heat production (HP). CH_4-E was calculated from CH_4 emissions (L/day) and the conversion coefficient (39.54 kJ/L; Brouwer 1965). CH_4 emissions in grams were also calculated from CH_4 emissions (L/day) and the conversion coefficient (0.716 g/L; Brouwer 1965). HP (kJ/day) was calculated with the equation: HP (kJ/day) = 16.18 × O_2 consumption (L/day) + 5.02 × CO_2 production (L/day) – 2.17 × CH_4 production (L/day) – 5.99 × N excretion (urinary N, g/day) (Brouwer, 1965).

2.2.6 Statistical analyses

One−way analysis of variance (ANOVA) was used to analyze the effects of diets on BWG, DMI, CH_4 emission, energy balance, and energy utilization efficiency. Differences among the means were considered to be significant at the $P \leq 0.05$ level on the basis of Tukey's test. The data of energy balance and energy utilization efficiency obtained from each experiment were subjected to the general linear models procedure for orthogonal polynomial analysis. The statistical program used in the current study was Statistical Package for the Social Sciences (SPSS), version 19.0 (SPSS Inc., Chicago, IL, USA).

2.3 Results

2.3.1 Effects of diets on dry matter intake, digestibility, body weight gain and feed conversion efficiency

In Exp 1, forage DMI increased from the Diet-1 group to the Diet-4 group, but differed significantly between the Diet-4 group and the Diet-1 group ($P<0.05$, Figure 3a). Concentrate DMI did not differ among the four diet groups. The total DMI was significantly higher ($P<0.05$, Figure 3a) in the Diet-4 group than in the Diet-1 and Diet-

2 groups. OM digestibility was relatively stable from the Diet-1 to the Diet-3 group (*P*>0.05, Figure 3b), while OM digestibility was significantly lower in Diet-4 group than in Diet-1 and Diet-3 groups (*P*<0.05, Figure 3b). No differences in DM or NDF digestibility were found between groups (*P*>0.05, Figure 3b). BWG was significantly higher in the Diet-4 group than in the Diet-1 group (*P*<0.05, Figure 3c), whereas no difference in FCE was observed among the four diet groups (*P*>0.05, Figure 3d).

In Exp 2, there were no differences in forage DMI, concentrate DMI, total DMI, BWG, or FCE (*P*>0.05, Figures 3e, 3g and 3h) among the four diet groups. The digestibility of DM, OM, and NDF was significantly lower in the Diet-4 group than in the Diet-2 group (*P*<0.05, Figure 3f).

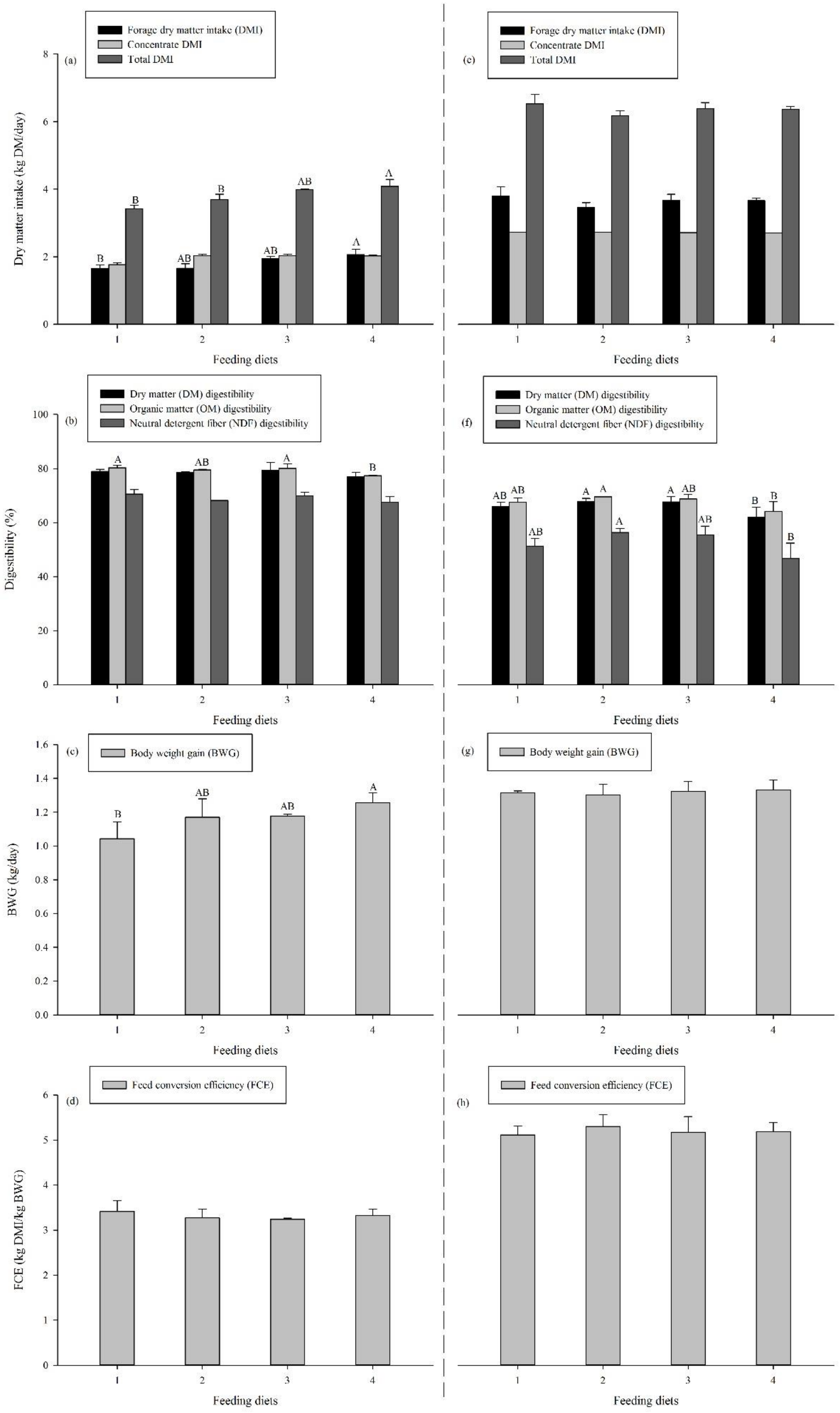

(a)
Forage dry matter intake (DMI)
Concentrate DMI
Total DMI
Dry matter intake (kg DM/day)
0
2
4
6
8
B
B
AB
B
AB
AB
AB
A
A
1
2
3
4
Feeding diets
(e)
Forage dry matter intake (DMI)
Concentrate DMI
Total DMI
1
2
3
4
Feeding diets
(b)
Dry matter (DM) digestibility
Organic matter (OM) digestibility
Neutral detergent fiber (NDF) digestibility
Digestibility (%)
0
20
40
60
80
100
A
AB
A
B
1
2
3
4
Feeding diets
(f)
Dry matter (DM) digestibility
Organic matter (OM) digestibility
Neutral detergent fiber (NDF) digestibility
AB
AB
AB
A
A
A
A
AB
AB
B
B
B
1
2
3
4
Feeding diets
(c)
Body weight gain (BWG)
BWG (kg/day)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
B
AB
AB
A
1
2
3
4
Feeding diets
(g)
Body weight gain (BWG)
1
2
3
4
Feeding diets
(d)
Feed conversion efficiency (FCE)
FCE (kg DMI/kg BWG)
0
1
2
3
4
5
6
1
2
3
4
Feeding diets
(h)
Feed conversion efficiency (FCE)
1
2
3
4
Feeding diets

Figure 3 Change of dry matter intake (DMI), digestibility, body weight gain (BWG) and feed conversion efficiency in experiment 1 and 2 (n = 4). Values are means and standard deviations, and the uppercase letters within the same indicator without common letters are significantly different ($P < 0.05$) in each Exp (Exp 1, a, b, c and d; Exp 2, e, f, g and h), respectively. No letters represent no significant difference.

2.3.2 Effects of diets on rumen fermentation

The mean ruminal pH was 6.60 and 6.28 across treatments in Exp 1 and 2, respectively (Table 3). The total VFA concentration increased in a linear manner from the Diet-1 group to the Diet-4 group in Exp 1 (P=0.002, Table 3), and was significantly higher in the Diet-2 and the Diet-4 groups than in the Diet-1 group (P<0.05, Table 3); there was no significant difference in the total VFA concentrations between groups in Exp 2. There was no significant difference in the mean acetate:propionate ratio between groups in either experiment; however, in Exp 1, the molar proportions of propionate in cattle fed diets including AH were significantly lower than in those fed diets without AH (P<0.05, Table 3). In Exp 2, the molar proportion of butyrate in cattle was significantly lower in the Diet-4 group than in the Diet-3 group (P<0.05, Table 3). Additionally, in Exp 1, the molar proportion of iso-valerate was significantly lower in the Diet-1 and the Diet-2 groups than in the Diet-3 group (P<0.05, Table 3). In Exp 2, the molar proportion of iso-valerate followed a parabolic trend from the Diet-1 group to the Diet-4 group (P=0.005, Table 3), and was significantly higher in the Diet-3 group than in the Diet-1 group (P<0.05, Table 3).

2.3.3 Effects of diets on enteric methane emissions

In Exp 1, CH_4 emission (g/day, Figure 4a), CH_4:DMI (Figure 4b) and CH_4:RDOMI (Figure 4c) did not differ between the Diet-1 and Diet-3 groups (P>0.05); however, emissions were significantly higher in the Diet-4 group compared with the Diet-3 group, regardless of whether CH_4 was expressed as a proportion of DMI or RDOMI (*P*<0.05, Figures 4b and 4c). In Exp 2, there were no differences in CH_4 emissions per day (Figure 4d) or CH_4:RDOMI (Figure 4f) among the four diets (*P*>0.05). However, CH_4:DMI was significantly lower in the Diet-1 and Diet-4 groups than in the Diet-2 group (Figure 4e). CH_4 emission, expressed as milligrams per 16 min per kilogram BW over 24 h post-feeding, is shown in Figures 5a (Exp 1) and 5b (Exp 2). There were intermittent peaks throughout the day for both experimental groups, which occurred a short time after feed supply. The peak of CH_4 emission (mg/kg BW/16 min) was higher following concentrate supply than following forage supply (Figures 5a and 5b).

Table 3 Effects of diet on ruminal fermentation parameters in Simmental crossbred cattle (4 replicates for each diet) for experiment 1 (Exp 1) and 2 (Exp 2).

Item	Diet[†] (Exp 1)				SEM[‡]	*P* Value	Polynomial contrast[‡]		Diet[†] (Exp 2)				SEM[‡]	*P* Value	Polynomial contrast[‡]	
	AH0	AH8	AH16	AH24			L	Q	CVH0	CVH10	CVH20	CVH30			L	Q
Total VFA, mmol/L	80.2^{b}	93.5^{a}	91.6^{ab}	98.3^{a}	3.91	0.004	0.002	0.006	95.9	96.9	93.5	92.4	2.83	0.252	NS	NS
pH	6.70	6.76	6.52	6.44	0.131	0.794	NS	NS	6.31	6.35	6.26	6.19	0.275	0.895	NS	NS
Molar proportions (mol/100 mol)																
Acetate	65.0	65.1	64.1	65.0	3.21	0.988	NS	NS	69.4	71.2	69.2	72.2	4.16	0.854	NS	NS
Propionate	20.5^{a}	18.1^{b}	18.5^{b}	17.9^{b}	0.84	0.011	0.009	0.023	16.9	15.9	15.3	16.7	2.38	0.985	NS	NS
Butyrate	11.4	13.6	11.7	12.9	2.03	0.682	NS	NS	10.8^{ab}	9.6^{ab}	11.6^{a}	8.1^{b}	1.02	0.040	NS	NS
Iso-butyrate	0.9	1.0	1.8	1.2	0.9	0.707	NS	NS	1.3	1.1	1.3	1.1	0.31	0.619	NS	NS
Valerate	1.0	0.9	1.1	1.4	0.37	0.597	NS	NS	0.7	0.7	0.8	0.7	0.129	0.707	NS	NS
Iso-valerate	1.3^{b}	1.3^{b}	2.7^{a}	1.6^{ab}	0.31	0.034	NS	NS	0.8^{b}	1.4^{ab}	1.7^{a}	1.3^{ab}	0.191	0.011	NS	0.005
Acetate:propionate ratio	3.2	3.6	3.5	3.6	0.29	0.280	NS	NS	4.1	4.5	4.5	4.4	0.25	0.592	NS	NS

[†] Experiment (Exp) 1, AH0, 60% oat hay; AH8, 52% oat hay and 8% alfalfa hay; AH16, 44% oat hay and 16% alfalfa hay; AH24, 36% oat hay and 24% alfalfa hay. Experiment (Exp) 2, CVH0, 60% oat hay; CVH10, 50% oat hay and 10% common vetch hay; CVH20, 40% oat hay and 20% common vetch hay; CVH30, 30% oat hay and 30% common vetch hay.

[‡] SEM, total standard error of means, NS = Not significantly different ($P > 0.05$), L = Linear, Q = Quadratic.

[a,b,c] Means within row with different superscript differ ($P < 0.05$).

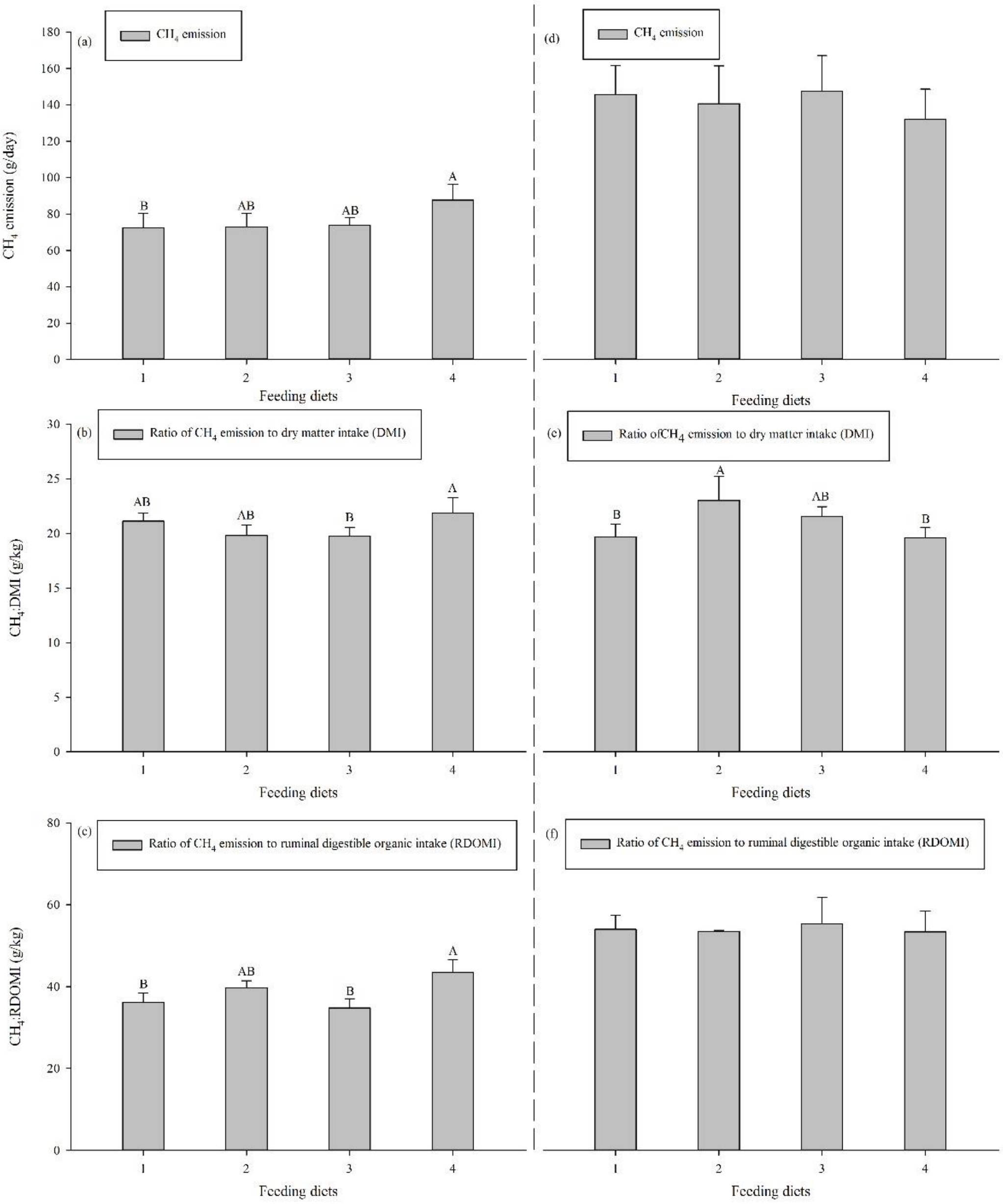


Figure 4 Methane (CH_4) emission (g/day), CH_4 emission:Dry matter intake ([DMI], g/kg), and CH_4:Ruminal digestible organic matter intake ([RDOMI], g/kg) among the four diets in experiment 1 and 2. Values are means and standard deviations. The uppercase letters within the same indicator without common letters are significantly different (*P* < 0.05) in each Exp (Exp 1, a, b, and c; Exp 2, d, e, and f). No letters represent no significant difference. RDOMI was calculated from organic matter intake (OMI) × organic matter (OM) digestibility × 0.65 (ARC 1980).

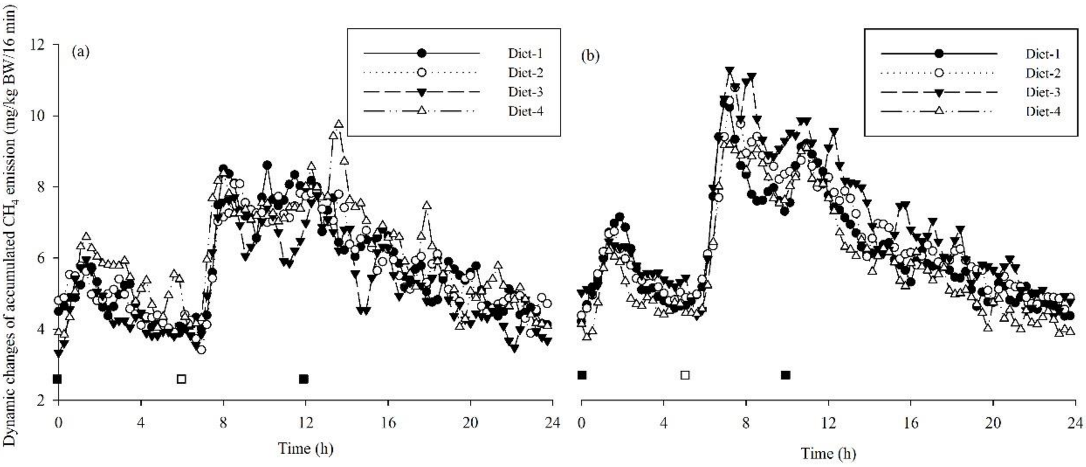


Figure 5 Dynamic changes of accumulated CH_4 emission (mg/kg body weight [BW]/16 min) during a 24 h period (starting from forage supplied offered at 08:00 hours and ending at 8:00 hours the next day) in experiment 1 (a) and 2 (b). The spots (■ and □ in [a] and [b]) represent the time when the forage and concentrate were supplied, respectively

2.3.4 Effects of diets on energy metabolism

Energy intake, output, and utilization efficiency are presented in Table 4. In Exp 1, GEI and FE increased linearly from the Diet-1 group to the Diet-4 group ($P<0.05$), and were significantly higher in the Diet-4 group than in the Diet-1 group ($P<0.05$). The highest MEI was observed in the Diet-3 group, and the lowest CH_4-E was observed in the Diet-1 group ($P<0.05$). There were no differences in DEI, UE, HP, and RE among the four diet groups ($P>0.05$). Regarding the energy utilization efficiency, no differences in DEI:GEI, MEI:GEI, UE:GEI, HP:GEI, or RE:GEI ($P>0.05$) were found between groups; however, CH_4-E loss (CH_4-E:GEI) and FE:GEI were significantly higher in the Diet-4 group than in the Diet-3 and Diet-2 groups, respectively ($P<0.05$).

In Exp 2, there were no differences in GEI between groups, however, DEI and MEI were significantly higher in the Diet-1 group than in the Diet-2 group ($P<0.05$). In addition, FE was significantly higher in the Diet-4 group than in the Diet-2 group

(P<0.05). There were no differences in other energy balance components (UE, CH_4-E, HP, and RE) between groups. The DEI:GEI and MEI:GEI were significantly lower in the Diet-4 group than in the Diet-1 group (P<0.05) whereas FE:GEI was significantly higher in the Diet-4 group than in the Diet-1 group (P<0.05). The lowest value of CH_4-E:GEI was observed in Diet-4 group, which was significantly lower than that observed in the Diet-2 group (P<0.05).

Table 4 Effects of diet on energy intake, energy excretion and energetic utilization in Simmental crossbred cattle (4 replicates for each diet) for experiment 1 (Exp 1) and 2 (Exp 2).

Item	Diet[†] (Exp 1)				SEM[‡]	*P* Value	Polynomial contrast[‡]		Diet[†] (Exp 2)				SEM[‡]	*P* Value	Polynomial contrast[‡]	
	AH0	AH8	AH16	AH24			L	Q	CVH0	CVH10	CVH20	CVH30			L	Q
Initial body weight (kg)	134	135	134	135	5.6	0.991	NS	NS	207	207	204	206	16.5	0.997	NS	NS
Actual forage to concentrate ratio	48:52	45:55	49:51	50:50	0.018	0.054	NS	NS	59:41	50:50	56:44	55:45	0.032	0.098	NS	NS
Feeding level[§]	1.73^{b}	1.90^{ab}	2.03^{a}	2.07^{a}	0.096	0.017	0.001	0.005	2.65	2.25	2.47	2.44	0.147	0.110	NS	NS
Energy balance[¶], MJ/day																
GEI	62.0^{b}	67.3^{ab}	72.4^{ab}	74.3^{a}	3.15	0.023	0.002	0.007	131.7	110.5	123.0	122.0	8.13	0.121	NS	NS
DEI	48.9	53.8	56.4	55.7	2.84	0.079	0.022	0.029	94.8^{a}	77.5^{b}	87.5^{ab}	79.7^{ab}	5.10	0.020	NS	NS
MEI	38.8^{b}	42.2^{ab}	45.7^{a}	43.6^{ab}	2.08	0.037	0.023	0.018	76.9^{a}	61.4^{b}	70.4^{ab}	62.4^{b}	4.67	0.019	0.079	NS
FE	13.2^{b}	14.9^{b}	16.0^{ab}	18.6^{a}	1.68	0.025	0.003	0.008	37.0^{ab}	33.0^{b}	35.5^{ab}	42.3^{a}	2.70	0.039	0.099	0.012
UE	6.4	7.5	6.9	7.2	0.47	0.133	NS	NS	9.8	8.3	9.0	10.0	1.04	0.469	NS	NS
CH_4-E	3.7^{b}	4.0^{ab}	3.9^{b}	4.8^{a}	0.28	0.008	0.007	0.012	8.0	7.8	8.2	7.3	0.73	0.647	NS	NS
HP	33.2	35.1	36.1	37.2	2.99	0.588	NS	NS	64.0	52.8	60.1	56.6	4.60	0.147	NS	NS
RE	5.6	7.1	9.6	6.4	4.67	0.845	NS	NS	12.9	8.5	10.2	5.9	2.88	0.149	0.049	NS
Energy utilization efficiency[¶], MJ/MJ																
DEI:GEI	0.786	0.800	0.781	0.751	0.0220	0.198	NS	NS	0.721^{a}	0.702^{ab}	0.712^{ab}	0.654^{b}	0.0192	0.033	0.005	0.019
MEI:GEI	0.622	0.628	0.632	0.588	0.0262	0.360	NS	NS	0.585^{a}	0.555^{ab}	0.572^{ab}	0.513^{b}	0.0218	0.044	0.013	0.051
FE:GEI	0.214^{ab}	0.200^{b}	0.219^{ab}	0.249^{a}	0.0140	0.044	0.032	0.016	0.279^{b}	0.298^{ab}	0.288^{ab}	0.346^{a}	0.0179	0.033	0.015	0.027
UE:GEI	0.104	0.112	0.096	0.098	0.0086	0.275	NS	NS	0.075	0.076	0.074	0.082	0.0139	0.879	NS	NS
CH_4-E:GEI	0.060^{ab}	0.060^{ab}	0.053^{b}	0.065^{a}	0.0031	0.046	NS	NS	0.061^{ab}	0.070^{a}	0.066^{ab}	0.060^{b}	0.0033	0.023	NS	0.017
HP:GEI	0.540	0.520	0.505	0.502	0.0495	0.855	NS	NS	0.487	0.478	0.488	0.462	0.0147	0.320	NS	NS
RE:GEI	0.082	0.108	0.127	0.087	0.0628	0.878	NS	NS	0.099	0.078	0.084	0.051	0.0266	0.371	0.084	NS

[†] Experiment (Exp) 1, AH0, 60% oat hay; AH8, 52% oat hay and 8% alfalfa hay; AH16, 44% oat hay and 16% alfalfa hay; AH24, 36% oat hay and 24% alfalfa hay. Experiment (Exp) 2, CVH0, 60% oat hay; CVH10, 50% oat hay and 10% common vetch hay; CVH20, 40% oat hay and 20% common vetch hay; CVH30, 30% oat hay and 30% common vetch hay.
[§] Feeding level, metabolizable energy (ME) intake divided by ME requirement for maintenance from the AFRC (1993).
[¶] GEI, gross energy intake; MEI, metabolizable energy intake; DEI, digestible energy intake; FE, fecal energy; UE, urinary energy; CH_4-E, methane energy; HP, heat production; RE, retained energy.
[‡] SEM, total standard error of means, NS = Not significantly different (P > 0.05), L = Linear, Q = Quadratic.
[a,b,c] Means within row with different superscript differ (*P* < 0.05).

2.4 Discussion

2.4.1 Body weight gain, dry matter intake, and feed conversion efficiency

In general, leguminous forage has a higher CP content and lower fiber content than grass forage (Haddad, 2000; Assefa & Ledin, 2001). In the present study, the increasing total DMI (Figure 3a), GEI (Table 4), and BWG (Figure 3c) from the Diet-1 group to the Diet-4 group in Exp 1 supported the finding by Haddad (2000), who reported that increasing the proportion of leguminous forage in the diet would reduce the NDF content per unit weight of feed and supplied more digestible nutrients, thus improving the digestible nutrient intake and growth performance of livestock. Although the Diet-1 group had the minimum forage DMI and the same concentrate DMI among the four diet groups in Exp 1, the BWG achieved with the Diet-1 indicated sufficient MP and ME in the concentrate for cattle growth. Thus, the increase in forage DMI (Figure 1a) may account for the corresponding increase in BWG from the Diet-1 group to the Diet-4 group in Exp 1 (Figure 3c). In contrast, no differences in DMI (Figure 3e) and BWG (Figure 3g) were found among the four diet groups in Exp 2.

The FCE (kg DMI/kg BWG) has a major impact on the cost of beef production, which varies both within and across breeds and ages (Garg et al., 2013). In our study, although there were no differences in the FCE among the four diets in each experiment, a higher FCE was observed in Exp 2 than in Exp 1 (Figures 3h and 3d). This may be due to the heavier BW of cattle in Exp 2, because the energy requirement for maintenance for ruminants with larger BWs is higher than that required for smaller ruminants (Estermann et al., 2002), as demonstrated by the increased HP (Table 4). Another reason may be to the digestibility of AH and CVH; however, the relatively lower OM digestibility in Exp

2 compared with Exp 1 (Figure 3f vs. 3b) was inconsistent with the results reported by Karabulut et al. (2007), who showed that the OM digestibility of CVH was significantly higher than that of AH. This could be attributed to the higher DMI and feeding level (ME intake/ME requirement for maintenance; AFRC [1993]) in Exp 2 compared with Exp 1, which increased the rate of passage through the rumen (Zhao et al., 2015) and then decreased digestibility, resulting a higher FCE. A previous study found an average FCE of 8.21 with a low energy diet, and 7.66 with a high energy diet in finishing Simmental steers (Mandell et al., 1998). The FCE values found in our study (average 3.31 in Exp 1 and 5.19 in Exp 2) were lower than those reported by Mandell and may be attributed to the higher proportion of concentrate (average 40% in our experimental diets vs. maximum value of 5.3% in Mandell et al. [1998]), which is the main source for BWG (Bailey, 1989).

2.4.2 Enteric methane emissions

Total DMI is the critical driver of daily CH_4 production (Yan et al., 2000; Wang et al., 2019). In Exp 1, the change in CH_4 emission (g/day) corresponded with the total DMI from the Diet-1 group to the Diet-4 group which supported the previous finding (Yan et al., 2000; Wang et al., 2019). In addition, the higher CH_4 emission (g/day) in Exp 2 than in Exp 1 also indicated that a higher DMI would lead to a higher CH_4 production.

CH_4 emission per unit of DMI or RDOMI was determined based on fermented carbohydrates, concentrate intake and feeding level (Johnson and Johnson, 1995). The average CH_4 emission per kg DMI in the present study was 28.5 L/kg, which was lower than the average value reported for beef cattle (37.5 L/kg, Yan et al., 2009). The lower value in the present study may be due to the higher proportion of concentrate compared with that used in diets by Yan et al. (2009), leading to relatively lower CH_4 emission. A more important reason may be the higher feeding level in our study; for example, when the ME requirement for maintenance was calculated with the AFRC guidelines (1993), the average feeding levels in our study were 1.93 and 2.45 for Exp 1 and Exp 2 (Table 4), respectively, which were higher than the average 1.57 reported by Yan et al. (2009). A feeding level more than twice the maintenance level would result in a shorter time for the retention of feed intake in the rumen, thereby reducing the CH_4 emission rate (Shibata &Terada, 2010).

In general, an appropriate proportion of leguminous forage in a diet is considered to be effective for mitigating CH_4 emission in beef cattle (Hess et al., 2004). This is because legumes are rich in secondary metabolites, such as saponins and tannins, which have potential to inhibit the activity of protozoa and methanogen in the rumen when their concentration reaches a dietary threshold (Beauchemin et al., 2008; Wang et al., 2019). Common vetch and alfalfa are saponin-containing plants (Evidente et al., 2011; Liu et al., 2018), and their extracts have been found to reduce CH_4 emission through a direct effect on methanogens (Pen et al., 2006). In Exp 2, this could explain the lower CH_4:DMI in the Diet-4 group compared with the Diet-2 group (Figure 4e). The variation of CH_4:DMI among the four diets indicated that 30% dietary CVH significantly decreased the CH_4 emission, which is consistent with the daily CH_4 emission (mg/kg BW/16 min) observed with Diet-4 group compared with Diet-2 and Diet-3 groups (Figure 5b). In Exp 1, the significantly higher CH_4:DMI and CH_4:RDOMI in the Diet-4 group compared with the Diet-3 group may be due to the relatively higher total DMI (Figure 3a) with the same NDF digestibility (Figure 4b) in the Diet-4 than in the Diet-3 group. This is because cellulose and hemicellulose (main components of the cell wall and NDF) are the main fermented nonstructural carbohydrates for CH_4 production (Zhao et al., 2015). This led to the presence of more fermented NDF in the rumen of animals in the Diet-4 group compared with those in the Diet-3 group, which negated the effect of saponin on CH_4 emission. This explains the relatively higher daily CH_4 emission (mg/kg BW/16 min) during a 24 h period in the Diet-4 group than in the Diet-3 group in Exp 1 (Figure 5a). Another possible reason is the increased populations of cellulolytic bacteria and methanogens (Hess et al., 2004). Although saponins have an inhibitory effect on the concentration of ciliate protozoa (Pen et al., 2006), they only associated with 9–25% of the total ruminal methanogens (Newbold et al., 1995). This could explain the same NDF digestibility but higher CH_4 emission in the Diet-4 group than in the Diet-3 group.

In ruminal fermentation, acetate fermentation usually accelerates CH_4 emission while propionate fermentation, which would compete with methane for available hydrogen, reduces CH_4 emissions (Pen et al., 2006). In Exp 1, the propionate proportion of the diets including AH was lower than that of diets containing no AH (Table 3). This might be due to the suppression of ruminal bacteria by some secondary metabolites of AH, which promoted lactate fermentation to propionate via the succinate fermentation pathway (Mackie et al., 1984). In Exp 2, there were no differences in the proportions of acetate and propionate; however, proportion of butyrate in the Diet-4 group was significantly lower than that in the Diet-3 group. This may be due to some other anti-nutrition factors of seeds in CVH (harvested at the podding stage), such as tannins, phenolics, trypsin inhibitors, and β-cyano-L-alanine (Huang et al., 2017); the concentration of these may reach reached a dietary threshold and subsequently have a negative effect on the activity of some enzymes on butyrate fermentation in the rumen. Furthermore, the limited values of infrequent sampling for each experiment, and the different type and origin of saponins from alfalfa and common vetch might have differential effects on rumen fermentation (Pen et al., 2006). Therefore, further research should elucidate the effects of legumes containing saponins on the rumen microbiome and microbial synthesis.

2.4.3 Energy metabolism and energy utilization efficiency

Forage composition affects energy metabolism and energy utilization efficiency (Win et al., 2015). In the present study, we hypothesized that dietary NDF concentration would decrease as the proportion of legumes increased. In Exp 1, the increasing forage DMI, with the same concentrate DMI, accounted for the linear increase in GEI from the Diet-1 group to the Diet-4 group (Table 4), considering the similar GE content of mixed forage among these four diets (Table 2). However, the linear increased FE and FE:GEI (Table 4) was likely attributed to the more NDF output, which is the primary source of FE (Hales et al., 2014). This is because the increased DMI led to an increase in NDF intake, which would result in more NDF output considering the similar NDF digestibility between these groups. In Exp 2, although no differences were found in the GEI (Table 4), the higher FE and FE:GEI in the Diet-4 group compared with other groups was due to lower apparent energy digestibility (DEI:GEI), which has a negative relationship with energy loss in feces (Hales et al., 2014). No differences in UE and UE:GEI were found among the four diet groups in each experiment, indicating that UE was not affected by increasing legume proportion. This is because UE loss was derived primarily from urinary N concentration (Hales et al., 2014), the differences of which were not significant in our study (unpublished data). Furthermore, the sum of UE and CH_4-E only occupied around 14−17% for both experiments, which was relatively stable (Table 4).

There were no differences in RE and RE:GEI among the diet groups in each experiment (Table 4). Nkrumah et al. (2006) reported that part of the variation in energy retention efficiency is associated with MEI:GEI above maintenance levels. In the present study, the change in RE and RE:GEI were consistent with MEI and MEI:GEI, respectively, regardless of Exp 1 or 2 (Table 4), which confirmed the previous finding (Nkrumah et al., 2006). In addition, an increase in RE usually corresponds with an increase in BWG, which involved the synthesis of protein and fat. Fat synthesis consumes more energy per unit gram than protein (Bailey et al., 1989). In a previous study, the ratio of retained N to N intake increased from the Diet-1 group to the Diet-4 group, which corresponded with the groups in the present study (Du et al., 2019), indicating an increase in protein synthesis in BWG. Nonetheless, an increase in the protein to fat ratio in the BWG might occur because the RE (P=0.049) and RE:GEI (P=0.084) tended to reduce from the Diet-1 group to the Diet-4 group (Table 3) under the similar BWG among the four diet groups in Exp 2.

2.5 Conclusions

The results of this study suggest that a 24% AH diet with an approximately 50:50 forage-to-concentrate ratio significantly improved BWG compared with a diet without AH, whereas BWG was not affected by CVH diets at a 60:40 forage-to-concentrate ratio. Additionally, inclusion of 16% AH in cattle diets resulted in lower CH_4 emissions (g/kg DMI) compared with a 24% AH diet. Conversely, a 30% CVH diet resulted in significantly lower CH_4 emissions (g/kg DMI) than a 10% CVH diet, with no significant difference observed at other levels. Our results suggest that strategic feed compositions containing alfalfa (16%) and common vetch (30%) are optimal, respectively, compared with 0, 8, or 24% AH, and 0, 10, or 20% CVH, which leads to lower CH_4 emission per unit DMI while maintaining BWG in crossbred Simmental beef cattle in dryland environment.

Chapter 3: Effects of oat hay and leguminous forages mixture diets on nitrogen utilization efficiency

3.1 Introduction

This chapter was to set to determine the optimal levels of alfalfa or common vetch substituting oat hay on body weight gain and nitrogen utilization of crossbred Simmental calves, the data of this chapter was from the same experiments in chapter one.

3.2 Materials and methods

3.2.1 Animals, treatments, and diets

Please see 2.2.1

3.2.2 Measurement and sampling procedure

Please see 2.2.2

3.2.3 Collection of ruminal fluid

Please see 2.2.3

3.2.4 Chemical analysis

Please see 2.2.4

3.2.5 Statistical analysis

One-way ANOVA was used to analyze the effects of diets on DMI, BWG, nutrient digestibility, N balance, and NUE. Linear regression analysis was used to investigate the relationship between ruminal ammonia N and UN, between ruminal ammonia N and the ratio of UN to NI, and between ruminal ammonia N and BUN. Differences among the means were considered significant at the $P \leq 0.05$ level on the basis of the Tukey’s test. All data obtained from each experiment were subjected to the General linear models procedure for orthogonal polynomial analysis. The statistical program used in the current study was IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY).

3.3 Results

3.3.1 Feed intake and nutrient digestibility

In Exp 1, forage DMI and total DMI of calves increased from the AH0 group to the AH24 group linearly ($P < 0.05$, Table 5), and total DMI was significantly higher (13.7%) in the AH24 group than in the AH0 group ($P < 0.05$, Table 5), corresponding to a greater BWG ($P < 0.05$, Table 5). No differences were found in the digestibility of DM and NDF ($P > 0.05$, Table 5) whereas OM digestibility was reduced 2.6% in the AH24 group than in the AH0 group ($P < 0.05$, Table 5). N digestibility tended to decrease linearly ($P < 0.05$, Table 5) and it was significantly reduced by 4.1% in the AH24 group compared with the AH0 group ($P < 0.05$, Table 5).

In Exp 2, there were no differences in forage DMI, concentrate DMI, total DMI, and BWG among the four diet groups ($P > 0.05$, Table 5). But, the digestibility of DM, OM, NDF, and N showed a quadratic trend from the CVH0 group to the CVH30 group ($P < 0.05$, Table 5) and were significantly higher in the CVH10 group than in the CVH30 group ($P < 0.05$, Table 5).

3.3.2 Nitrogen balance and nitrogen utilization efficiency

In Exp 1, although there were no differences in NI, manure N (MN), and retained N for the four diet groups ($P > 0.05$; Table 6), FN and UN of calves significantly differed between the AH0 group and the AH24 group ($P < 0.05$, Table 6): FN tended to increase linearly ($P < 0.05$) with increasing AH proportions and it was significantly higher in the AH24 group than in the AH0 group by 38% ($P < 0.05$), whereas UN tended to decrease with an increase in AH proportions and it was significantly lower in the AH24 group than in the AH0 group by only 8.3% ($P < 0.05$).

In Exp 2, no differences were found in NI and retained N among the four diet groups; but FN tended to increase quadratically ($P < 0.05$) and it was 18.8% higher in the CVH30 group than in the CVH10 group ($P < 0.05$, Table 6) despite there was no significant difference from CVH0 group to CVH20 group ($P > 0.05$, Table 6). UN and MN decreased from the CVH0 group to the CVH30 group linearly ($P < 0.05$) and they were significantly lower in the CVH30 group than in the CVH0 group by 19.3 and 10.8%, respectively ($P < 0.05$, Table 6).

The FN:NI and UN:NI ratios showed a tendency similar to those of FN and UN, respectively, in both Exp 1 and Exp 2 ($P < 0.05$; Table 6). What is more, in Exp 1, the FN:NI ratio increased by 4.1% from the AH0 group to the AH24 group whereas the UN:NI ratio decreased by 7.2%. In Exp 2, although the FN:NI ratio increased 3.6% from the CVH0 group to the CVH30 group, the UN:NI ratio decreased by 8.5%.

Table 5 Effects of diet on DMI, BWG, and nutrient digestibility in Exp 1 and 2 for crossbred Simmental calves

Item	Diet[†] (Exp 1)				SEM[‡]	*P*	Polynomial contrast[‡]		Diet[†] (Exp 2)				SEM[‡]	*P*	Polynomial contrast[‡]	
	AH0	AH8	AH16	AH24			L	Q	CVH0	CVH10	CVH20	CVH30			L	Q
BW and feed intake[§]																
Initial BW, kg	134	135	134	135	5.6	0.991	NS	NS	213	200	201	209	14.8	0.749	NS	NS
Forage DMI, kg/day	1.74^{ab}	1.63^{b}	2.01^{ab}	2.13^{a}	0.124	0.013	0.035	NS	4.22	3.83	4.05	4.03	0.716	0.958	NS	NS
Concentrate DMI, kg/day	1.69	1.80	1.74	1.76	0.082	0.598	NS	NS	2.73	2.73	2.73	2.73	–	1.000	NS	NS
Total DMI, kg/day	3.42^{b}	3.43^{b}	3.75^{ab}	3.89^{a}	0.145	0.028	0.044	NS	6.53	6.18	6.38	6.37	0.647	0.959	NS	NS
BWG, kg/day	1.04^{b}	1.17^{ab}	1.18^{ab}	1.26^{a}	0.057	0.019	0.002	0.08	1.32	1.29	1.32	1.33	0.039	0.797	NS	NS
Nutrient digestibility, %																
DM	78.9	78.7	79.4	77.0	1.17	0.493	NS	NS	69.3^{ab}	70.9^{a}	70.7^{a}	65.6^{b}	1.43	0.020	NS	0.036
OM	80.0^{a}	79.5^{ab}	80.1^{a}	77.4^{b}	0.70	0.017	NS	NS	70.6^{ab}	72.4^{a}	71.7^{ab}	67.5^{b}	1.51	0.046	NS	0.047
NDF	70.6	68.2	70.0	67.6	2.1	0.468	NS	NS	56.0^{ab}	60.5^{a}	59.6^{a}	51.7^{b}	1.84	0.005	NS	0.010
Nitrogen	86.2^{a}	85.5^{a}	84.4^{ab}	82.1^{b}	0.01	0.022	0.034	NS	78.9^{ab}	79.4^{a}	78.4^{ab}	75.3^{b}	1.15	0.030	0.002	<0.001

Superscripts in lower case letters mean significant statistical difference at $P \leq 0.05$

[†] Experiment (Exp) 1, AH0, 60% oat hay; AH8, 52% oat hay and 8% alfalfa hay; AH16, 44% oat hay and 16% alfalfa hay; AH24, 36% oat hay and 24% alfalfa hay. Experiment (Exp) 2, CVH0, 60% oat hay; CVH10, 50% oat hay and 10% common vetch hay; CVH20, 40% oat hay and 20% common vetch hay; CVH30, 30% oat hay and 30% common vetch hay.

[‡] SEM, total standard error of means, NS = Not significantly different ($P > 0.05$), L = Linear, Q = Quadratic, C = Cubic.

[§] BW, body weight, DMI, dry matter intake

Table 6 Effects of diet on N intake, N excretion, and N utilization efficiency (NUE) in Exp 1 and 2 for crossbred Simmental calves.

Item	Diet[†] (Exp 1)				SEM[‡]	*P*	Polynomial contrast[‡]		Diet[†] (Exp 2)				SEM[‡]	*P*	Polynomial contrast[‡]	
	AH0	AH8	AH16	AH24			L	Q	CVH0	CVH10	CVH20	CVH30			L	Q
N balance[§], g/d																
NI	83.2	83.9	85.3	86.4	2.61	0.633	NS	NS	145	139	141	139	3.1	0.273	NS	NS
FN	11.3^{b}	12.1^{b}	13.6^{ab}	15.6^{a}	0.88	0.006	<0.001	<0.001	30.5^{ab}	28.8^{b}	30.4^{ab}	34.2^{a}	1.55	0.041	NS	0.017
UN	50.3^{a}	48.7^{ab}	47.8^{ab}	46.1^{b}	0.98	0.017	<0.001	0.002	79.8^{a}	78.4^{a}	69.3^{ab}	64.4^{b}	3.29	0.004	<0.001	<0.001
MN	61.6	60.9	61.4	61.7	1.73	0.963	NS	NS	111^{a}	106^{ab}	100^{b}	99^{b}	3.2	0.013	<0.001	0.004
RN	21.6	23.0	24.0	24.7	1.87	0.443	0.021	NS	34.7	32.1	40.9	40.3	5.89	0.420	NS	NS
NUE, g/g																
FN:NI ratio	0.138^{b}	0.145^{ab}	0.156^{ab}	0.179^{a}	0.0120	0.022	0.034	NS	0.211^{ab}	0.206^{b}	0.216^{ab}	0.247^{a}	0.0115	0.030	0.002	<0.001
UN:NI ratio	0.611^{a}	0.583^{b}	0.565^{b}	0.539^{b}	0.0193	0.032	0.041	NS	0.552^{a}	0.566^{a}	0.495^{ab}	0.467^{b}	0.0303	0.009	0.011	0.030
MN:NI ratio	0.750	0.728	0.721	0.719	0.0201	0.492	NS	NS	0.762	0.773	0.711	0.713	0.0274	0.113	NS	NS
RN:NI ratio	0.251	0.272	0.279	0.282	0.0201	0.492	NS	NS	0.238	0.228	0.289	0.287	0.0274	0.113	NS	NS

Superscripts in lower case letters mean significant statistical difference at $P \leq 0.05$

[†] Experiment (Exp) 1, AH0, 60% oat hay; AH8, 52% oat hay and 8% alfalfa hay; AH16, 44% oat hay and 16% alfalfa hay; AH24, 36% oat hay and 24% alfalfa hay. Experiment (Exp) 2, CVH0, 60% oat hay; CVH10, 50% oat hay and 10% common vetch hay; CVH20, 40% oat hay and 20% common vetch hay; CVH30, 30% oat hay and 30% common vetch hay.

[‡] SEM, total standard error of means, NS = Not significantly different ($P > 0.05$), L = Linear, Q = Quadratic.

[§] NI = N intake; FN = fecal N; UN = urinary N; MN = manure N (FN + UN); RN = retained N.

3.3.3 Blood urea nitrogen and ruminal ammonia nitrogen

In Exp 1, BUN and ruminal ammonia N concentrations tended to decrease from the AH0 group to the AH24 group linearly ($P < 0.05$, Table 7) and they were significantly lower in the AH24 group than in the AH0 group by 36.7% and 17.3%, respectively ($P < 0.05$, Table 7). In Exp 2, BUN and ruminal ammonia N concentrations showed a quadratic tendency from the CVH0 group to the CVH30 group ($P < 0.05$, Table 7) and they were significantly greater in the CVH10 group than in the CVH30 group by 37.6 and 23.3%, respectively ($P < 0.05$, Table 7). There were no differences in glucose concentration among the four diet groups in both experiments (Table 7).

3.3.4 Relationship between ruminal ammonia nitrogen and urinary nitrogen output, the ratio of urinary nitrogen to nitrogen intake, and blood urea nitrogen

Ruminal ammonia N was positively significantly correlated with UN output, the ratio of UN to NI, and BUN pooled from each Exp ($P < 0.05$, Figure 6). In detail, there was a higher slope of the linear regression between UN output and ruminal ammonia N in Exp 2 than that in Exp 1 (Figure 6a). For linear regression between the ratio of UN to NI and ruminal ammonia N, there was a similar value for the slope of the linear regression between Exp 1 and 2, but the ratio of UN to NI was relatively higher in Exp 1 than it in Exp 2 (Figure 6b). The slope of the linear regression between BUN and ruminal ammonia N was higher in Exp 1 than in Exp 2 (Figure 6c).

Table 7 Effects of diets on composition of blood serum and ruminal ammonia concentration in Exp 1 and 2 for crossbred Simmental calves

Item (mmol/L)	Diet† (Exp 1)				SEM‡	*P*	Polynomial contrast‡		Diet† (Exp 2)				SEM‡	*P*	Polynomial contrast‡	
	AH0	AH8	AH16	AH24			L	Q	CVH0	CVH10	CVH20	CVH30			L	Q
BUN§	7.09^{a}	6.23^{ab}	5.02^{b}	4.49^{b}	0.569	0.007	<0.001	<0.001	6.90^{ab}	7.06^{a}	6.77^{ab}	5.13^{b}	0.557	0.034	0.019	<0.001
Glucose	6.53	6.71	6.77	6.98	0.374	0.690	NS	NS	6.27	6.34	6.40	6.29	0.187	0.900	NS	NS
Ruminal ammonia N	4.79^{a}	4.46^{ab}	4.23^{ab}	3.96^{b}	0.313	0.047	<0.001	0.003	4.63^{ab}	4.93^{a}	4.27^{ab}	4.00^{b}	0.228	0.016	0.002	0.002

Superscripts in lower case letters mean significant statistical difference at $P \leq 0.05$

† Experiment (Exp) 1, AH0, 60% oat hay; AH8, 52% oat hay and 8% alfalfa hay; AH16, 44% oat hay and 16% alfalfa hay; AH24, 36% oat hay and 24% alfalfa hay. Experiment (Exp) 2, CVH0, 60% oat hay; CVH10, 50% oat hay and 10% common vetch hay; CVH20, 40% oat hay and 20% common vetch hay; CVH30, 30% oat hay and 30% common vetch hay.

‡ SEM, total standard error of means, NS = Not significantly different ($P > 0.05$), L = Linear, Q = Quadratic.

§ BUN = blood urea N.

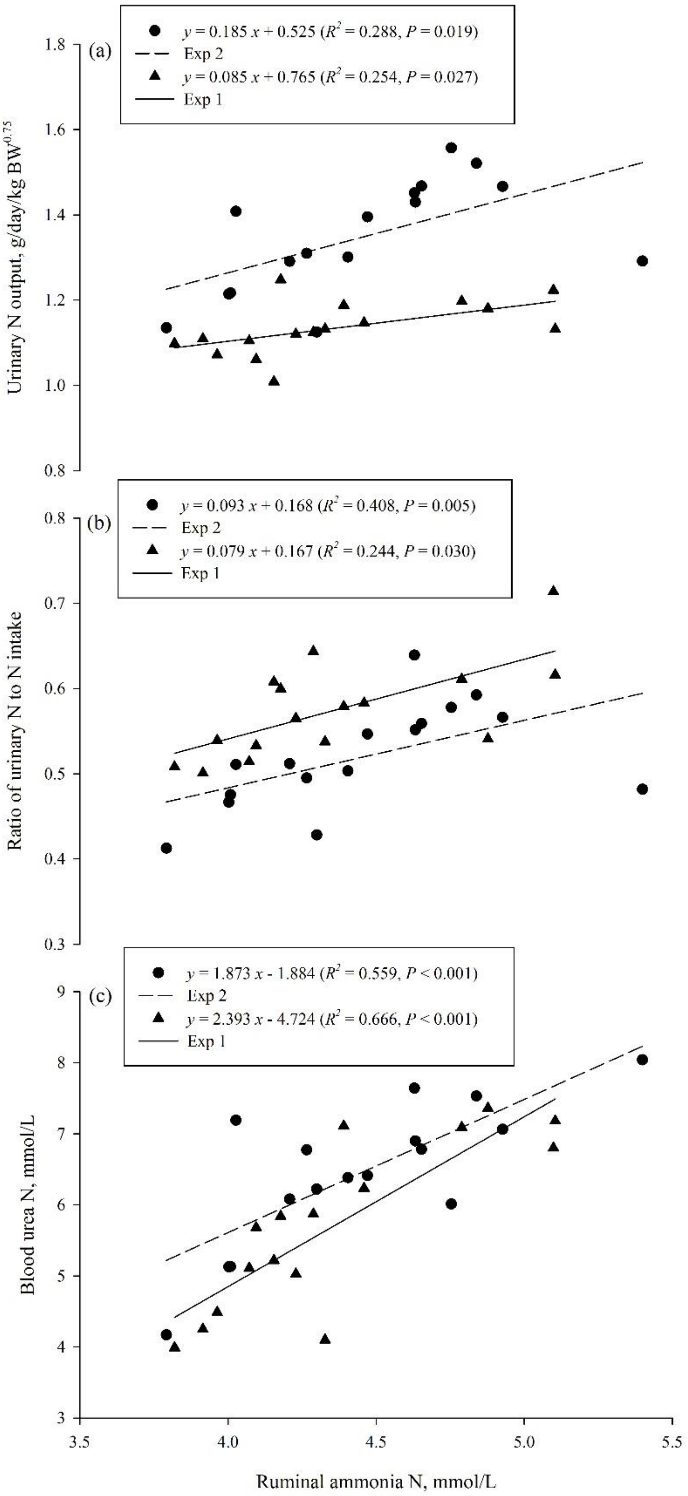


Figure 6 The relationship between ruminal ammonia N and urinary N output (a), the ratio of urinary N to N intake (b), and blood urea N (c) in crossbred Simmental calves. The data were pooled from both experiments. $P \leq 0.05$ means a significant difference.

3.4 Discussion

3.4.1 Feed intake and nutrient digestibility

Inclusion of legumes in diets affects the DMI through its influence on DM digestibility (McDonald et al., 2002). In Exp 1, the linearly increasing forage DMI with the same concentrate DMI from the AH0 group to the AH24 group led to the difference in the total DMI (Table 5). This was in agreement with the results of Osuji and Odenyo (1997) that supplement of leguminous forage in low-quality forages could increase total DMI. A more important reason is that legumes are more easily digested than grass, which may reduce the ruminal fill of livestock (Niederecker et al., 2018), thereby increased AH proportions in the diet, which promoted total intake (Bhatti et al., 2008; Zhao et al., 2015). The BWG linearly increased with increasing forage DMI in Exp 1 (Table 5). However, the DM digestibility slightly decreased from the AH16 group to the AH24 group, although a higher forage DMI with the same concentrate DMI was observed in the AH24 group than in the AH16 group (Table 5). The much higher total DMI, which is negatively correlated with digestibility (Zhao et al., 2017), could be partially responsible for the decreased digestibility in Exp 1. In Exp 2, there were no differences in feed intake (forage DMI and concentrate DMI), which is due to the limited supplementation of feed. Despite this, the DM digestibility also showed a quadratic trend from the CVH0 group to the CVH30 group (Table 5). This indicates that there is a tipping point in substituting AH/CVH for OH for digestibility, and an appropriate proportion of leguminous forage in the diet would be beneficial for feed utilization, which was in agreement with a previous study (Kobayashi et al., 2018).

The average lower nutrient digestibility, including DM, OM, and NDF, in Exp 2 than in Exp 1 was attributed to a higher FL in Exp 2 than in Exp 1 (average 1.91 vs. 2.44 in Exp 1 vs. Exp 2, respectively), which increased the fractional passage rate (AFRC, 1993); therefore, high FL depressed digestibility. The digestibility of a feed is influenced by the composition of other feeds consumed with it (Zhao et al., 2015). In Exp 1, the digestibility of DM, OM, and NDF were relatively stable from the AH0 group to the AH16 group and then decreased in the AH24 group, whereas in Exp 2, they showed parabolic tendencies from the CVH0 group to the CVH30 group (Table 5). The tendencies are probably due to an increasing proportion of maize in diets in both experiments (Table 2), which could provide more rapid fermentation of starch to VFA to depress rumen pH (McDonald et al., 2002). When the pH reached a threshold, it would inhibit microorganism activity and depress fiber digestibility (Zhao et al., 2015). Titgemeyer et al. (2012) also demonstrated that ruminal infusions of VFA could lead to slight decreases in fiber digestion and digestibility. Therefore, the present study found that 16% AH and 20% CVH were the highest levels of substitution for oat hay in the diet that did not suppress nutrient digestibility (DM, OM, NDF, and N).

3.4.2 Mitigation strategies to reduce nitrogen excretion

Nitrogen excretion in feces and urine represents a considerable N loss in livestock farming (Zhao et al., 2016). In the present study, MN output is in a range of 71 to 76%, pooled from both experiments, which was consistent with the results (74%) of Dong et al. (2014) in beef cattle but less than that (78%) reported by Yan et al. (2007) in growing to finishing beef cattle. This difference is likely due to the animal breeds, the forage-to-concentrate ratio, the different ingredients in the concentrate offered, and the various ages and BW. All of these factors likely affect N excretion per gram NI. Although the MN:NI ratio did not differ among the four diet groups in each experiment, the route of N excretion was altered. For example, more N was lost in feces than in urine (Table 6), which was in agreement with the study of Zhao et al. (2016) and Ghelichkhan et al. (2018). UN is usually more volatile than FN because most of the urinary urea N is inorganic N and can be rapidly hydrolyzed to ammonium and then converted to NH_3, which is more likely to lead to N lose from the farm system to the environment (Koenig and Beauchemin, 2018). In contrast, fecal NH_3 production is generally low due to slow mineralization rates of organic nitrogenous compounds (Kebreab et al., 2009). In the present study, the linearly increasing FN:NI ratio corresponded to a linearly decreasing UN:NI ratio (Table 6) in both experiments, which indicated that substitution of AH/CVH for OH could reduce UN loss and indirectly mitigate NH_3 emissions (Zhao et al., 2016).

Increasing FL (which is indicative of growth rate) could proportionally reduce N loss in urine more than in feces (Yan et al., 2007) because high feed intake can contribute to a high ruminal fractional outflow rate, which leaves less time for rumen microorganisms to ferment the feedstuffs, consequently leading to a reduction in ammonia N absorbed in the rumen and subsequently reducing N excreted in urine (Zhao et al., 2015). In Exp 1, the increased total DMI from the AH0 group to the AH24 group (Table 3) and the linearly decreased ruminal ammonia N concentration (Table 7) and UN:NI ratio (Table 6) support the previous findings of Zhao et al. (2015) and Yan et al. (2007). In Exp 2, although there was no difference in total DMI (Table 3), the ruminal ammonia N concentration and UN:NI ratio were still significantly lower in the CVH30 group than in the CVH10 group (Table 6). The low DM digestibility in the CVH30 group compared with that in the CVH10 group could be partially responsible for this.

3.4.3 Nitrogen metabolism, metabolizable energy supply, and nitrogen utilization efficiency

This study examined the effect of substitution of AH/CVH by OH on N metabolism and its partitioning, with the same NI (Table 6). In general, there is a positive relationship between N digestibility and the proportion of urine N loss per gram NI (Dong et al., 2014). A linearly decreased N digestibility from the AH0/CVH0 group to the AH24/CVH30 group in both Exp 1 and Exp 2 (Table 5), corresponding with a linearly decreasing UN output and UN:NI ratio (Table 6), was in line with the finding of Dong et al. (2014). This is likely due to high N digestibility usually being associated with a greater proportion of N absorbed as ammonia N from the rumen above the requirements of microbial activity. Therefore, higher N digestibility in the rumen would result in N being excreted more in urine than in feces (Zhao et al., 2016).

Nitrogen degradation and utilization efficiency in the rumen includes the supply of ME and the protein degradation rate (Patra, 2010; Prakash et al., 2013; Niederecker et al., 2018). In the present study, although dietary CP and ME concentrations of the four diets were set at the same level in each experiment, the actual energy concentration and the amount of degraded CP in the rumen varied with nutrient digestibility. In Exp 1, total VFA concentrations increased with increasing AH proportions (80.2 mmol/L in the AH0 group to 98.3 mmol/L in the AH24 group; Table 3), which suggested that there was increasing available energy for microorganisms in the rumen. But, total NI did not differ among the four diet groups (Table 6). Therefore, the ratio of actual energy to N supply in the rumen would increase with an increasing AH proportions in the diet in Exp 1. This trend, which is associated with a decreasing UN:NI ratio and increasing FN:NI ratio from AH0 group to AH24 group (Table 4), demonstrated that energy supplementation in the rumen decreased the proportion of N loss in the urine and increased FN output (Kebreab et al., 2009; Titgemeyer et al., 2012). Similar results were observed in Exp 2, although there was no significant difference in VFA concentrations (Table 3).

Generally, high ruminal ammonia N concentration for optimal OM degradation will result in more N loss through urine (Ipharraguerre and Clark, 2005). In Exp 1, a decreasing ruminal ammonia N concentration (Table 7) under a relatively stable OM digestibility from the AH0 group to the AH16 group (Table 5), associated with a decreasing UN:NI ratio from the AH0 group to the AH16 group (Table 6), supporting the previous finding. In Exp 2, although OM digestibility and ruminal ammonia N concentration and the UN:NI ratio showed parabolic trends, they were still consistent with the previous finding. There is usually a strong positive correlation between ruminal ammonia N and BUN (Kohn et al., 2005; Aboagye et al., 2018), and the same strong positive correlation was found in the present study (Figure 6). In addition, BUN could be used as an indicator of the protein status of the animal (Aboagye et al., 2018). In the present study, BUN decreased with increasing AH/CVH proportions, with the range of BUN being between 7.09 and 4.49 mmol/L. These values were higher than the average value of 4.04 mmol/L reported by Aboagye et al. (2018) for weaned crossbred steers fed alfalfa and barley silages. The optimal BUN concentration for protein deposition by beef steers is around 2.49 mmol/L (Johnson and Preston, 1995). Usually, the greater BUN values could be attributed to the age and breed of animals (Kohn et al., 2005). However, the higher BUN concentration coupled with small age of animals in the present study (Table 7) compared with a lower BUN coupled with older age of animals in the study of Aboagye et al. (2018) was unexpected (5 and 9 months of age in Exp 1 and 2 respectively vs. 12 months of age), given that older age of animals had a higher BUN than small age of animals. This might be due to that the lower ruminal ammonia N concentration for animals in the present study corresponded to greater post-ruminal MP, and greater intestinal absorption of amino acids, leading to an increase in BUN and then excreted in urine when dietary protein supply exceeded the requirement of animals (Reynolds and Kristensen, 2008; Aboagye et al., 2018). The higher BUN has been reduced by using less degradable protein sources in the feed (Ipharraguerre and Clark, 2005). However, less degradable protein in feed could also reduce the ruminal ammonia N concentration and thus decrease the available N supply for microbial growth (Ipharraguerre and Clark, 2005). In the present study, substituting less degradable protein sources in legumes for high degradable protein sources in the concentrate (Table 2) decreased ruminal ammonia N concentration. However, no reduction in BWG was observed (Table 5), which suggests that ruminal available N was adequate for microbial growth (Prakash et al., 2013), and the ammonia N concentration of the rumen fermentation was around 4.0 mmol/L in the current diets.

3.5 Conclusions

The results of this study suggested that up to 16% AH could be included in cattle diets at a forage-to-concentrate ratio at 60:40, because these values maintained optimal nutrient digestibility and reduced ruminal ammonia N concentration and UN output without negative effects on BWG. Additionally, 20% CVH in cattle diets not only could be used to reduce UN output and MN outputs but also to maintain BWG at high nutrient digestibility. The decreased UN:NI ratio in response to increasing AH/CVH proportions in the current study indicated additional environmental benefits, such as reducing volatile N excretion from urine, which may eventually impact N management on farms. Therefore, our results suggest an opportunity for strategic feeding containing alfalfa (16%) or common vetch (20%) to reduce the direct impact of N excretion on the environment while maintaining optimal nutrient digestibility and improving/maintaining BWG for crossbred Simmental beef cattle in dryland environments.

Chapter 4: Diet formulation for crossbred Simmental calves using oat hay-based diet

4.1 Introduction

Grass occupies an important role in the ruminant feeding system due to its high yields of DM at low cost, however, only grass is not capable of sustaining the required levels of animal production due to its low feeding value (Givens et al., 2000). Therefore, there has been an interest in supplementing legumes in a grass-based diet due to its rich protein and energy (Graham and Vance, 2003). However, until now, there is no available information on whether common vetch could substitute alfalfa in the ruminant feeding system, and the optimal proportions of common vetch to replace alfalfa. Therefore, the objective of this study is to investigate how CVH versus AH affect BWG, N metabolism (i.e., N digestibility, ruminal ammonia-N and BUN concentrations) and CH_4 emissions associated with ruminal fermentation parameters with two different proportions (20% [20] and 40% [40] of the total DM allowance) for growing beef cattle, at similar CP and predicted ME levels with a target BWG of 1.5 kg/d, in dryland environments.

4.2 Materials and methods

4.2.1 Animals, treatments and diets

The Animal Ethics Committee of Gansu Province, China, approved the experimental protocols (file No. 2010-1 and 2010-2). This experiment involved 16 crossbred male Simmental cattle (Simmental × Local cattle) with initial body weight (BW) of 216±24.4 kg (mean ± standard deviation, 10 months of age) at the start of the experimental period. The experiment was a randomized block experimental design with a 2×2 factorial arrangement of diets. All cattle were allocated to one of the 4 treatments. The forage to concentrate ratio was fixed (60:40, DM basis) for all diets. Dietary treatments were 2 kinds of legume (AH and CVH) and two different OH-to-AH/CVH ratios in the diet (40:20 or 20:40, DM basis) indicated as following: 20% CVH and 40% OH (CVH20); 40% CVH and 20% OH (CVH40); 20% AH and 40% OH (AH20); and 40% AH and 20% OH (AH40). All cattle were kept in individual pens in a cowshed for 2 weeks' diet adaptation.

The target BWG for each cattle was set at 1.5 kg/d. All experimental diets were formulated to provide sufficient ME and MP to meet the target BWG for a cattle according to the published estimation equations and values of Agricultural and Food Research Council (AFRC, 1993) and BW of cattle (measured every 8 days). The diet composition required to fulfill the ME and MP requirements were calculated based on the tabulated values of digestible energy and ruminal CP degradation parameters for OH, AH and concentrate ingredients established by the Chinese Feeding Standard for Beef Cattle (CFSBC, Ministry of Agriculture of the People’s Republic of ChinAH2004). The digestibility of ruminal CP and energy and ruminal degradation parameters for CVH were from Larbi et al. (2011). The CP, ME and MP level of all diets are shown in Table 8. Throughout this experimental period of 8 weeks, all cattle were given free access to water and 10 g/day of mineral mixture containing (minimum values in mg): manganese, 720; copper, 30; biotin, 0.05; folic acid, 0.4; vitamin B_1, 50; vitamin B_2, 2.5; vitamin B_6, 0.5; vitamin B_{12}, 0.1. The daily mixed forage was divided into two equal parts and offered as separate meals twice a day (08:00 and 19:00). The mixed concentrate was fed once a day (14:00).

Table 8 Composition of the feed ingredients and the target metabolizable energy concentration and metabolizable protein concentration of all diets.

Feed Formula	Experimental Diet [†]			
	CVH20	CVH40	AH20	AH40
Forage				
Leguminous forage (g/kg DM)	200	400	200	400
Oat hay (g/kg DM)	400	200	400	200
Concentrate				
Maize (g/kg DM)	30	80	48	120
Soybean meal (g/kg DM)	92	25	107	56
Wheat bran (g/kg DM)	278	295	245	224
Nutrient value [‡]				
CP (g/kg DM)	156.3	156.4	156.4	156.4
MEC [§](MJ/kg DM)	10.05	10.05	10.05	10.05
MPC [¶](g/kg DM)	102.9	94.6	106.1	101.4

[†] CVH20, 20% common vetch + 40% oat hay; CVH40, 40% common vetch + 20% oat hay; AH20, 20% alfalfa + 40% oat hay; AH40, 40% alfalfa + 20% oat hay. [‡] CP, crude protein, MEC, metabolizable energy concentration, MPC, metabolizable protein concentration.
[§,¶] These values were calculated by the Agricultural and Food Research Council (1993) and the Chinese Feeding Standard for Beef Cattle (2004); see details in Methods and Materials.

4.2.2 Chamber description

Please see 2.2.2

4.2.3 Collection of rumen fluid

Please see 2.2.3

4.2.4 Sample collection and procedure

The amount of forage and concentrate offered and all leftovers were weighed daily throughout the experimental period to calculate daily DM intake (DMI) for individual cattle. After the 14-day acclimation period for target feeds, on day 15 of the experimental period, a randomly selected cattle from each diet group were moved to the four chambers for 8 days. On day 22, they were moved out to the individual pens in the cowshed and another 4 cattle, randomly selected from the remaining cattle of these four diet groups, entered the chambers and left on day 30, it continued until all 16 cattle completed an 8-day's measurement on day 46. The moved-out 4 cattle from chambers on days 22, 30, 38 and 46 were reallocated to another diet group randomly which they did not belong. The 4 cattle, which left chambers on day 22, were removed into chambers on day 46 for measurement after 24–day acclimation period for diet in new diet groups. It continued until these 16 cattle completed measurements on day 78. In this case, there were 8 replicates for each diet group. The BW of all cattle was measured in the morning with an empty stomach to calculate BWG (kg/day) when exchanged cattle between chambers and cowshed. During the 8 days' measurements in the chamber, the cattle were kept for acclimation for the first 2 days. We collected the digestibility data over the following 3 days and gas exchange data (O_2 consumption, CH_4 and CO_2 emissions) over the remaining 3 days. During the digestibility data collection period, the total weight of daily excreted feces and urine was recorded. Feces, which were excreted on a plastic mat placed under the cattle, were collected immediately with a shovel into a plastic container and weighted, mixed, and sampled once per day. 10% of each feces sample was stored at −20 ℃ for later chemical analysis. Total urine was collected through a handmade urine bag into a bucket containing 200 mL 10% v/v H_2SO_4 to reduce ammonia loss once a day. Acidified urine was checked for pH with a portable pH instrument (PHBJ-260, Shanghai INESA Scientific Instrument Co., Ltd). 20% of the daily urine was stored at −20 ℃ for chemical analysis.

4.2.5 Energy balance

Please see 2.2.5

4.2.6 Chemical analysis

Please see 2.2.6

4.2.7 Statistical analysis

One-way analysis of variance (ANOVA) and generalized linear model analysis was used to investigate the effects of legume species (LS), legume proportion (LP), and their interaction (LS×LP) on DMI, BWG, nutrient digestibility, energy/N balance, and energy/N utilization efficiency. Differences among the means were considered to be significant at the $P \leq 0.05$ level on the basis of Tukey's test, unless otherwise stated. The statistical program used in the current study was IBM SPSS Statistics for Windows, version 19.0 (IBM Corp., Armonk, NY).

4.3 Results

4.3.1 Feed intake, apparent nutrient digestibility, and body weight gain

LS significantly influenced forage DMI and total DMI ($P < 0.05$, Table 9). In detail, forage DMI and total DMI of cattle were significantly higher when fed on CVH40 diet than AH20 and AH40 diets ($P < 0.05$, Figure 7a). But no significant differences were found in concentrate DMI under LS ($P > 0.05$, Table 9). In addition, there were no significant differences in forage DMI, concentrate DMI and total DMI of cattle under LP ($P > 0.05$, Table 9, Figure 7a).

LP significantly affected the nutrient digestibility of cattle, including the digestibilities of DM, OM, NDF, and apparent N ($P < 0.05$, Table 9). Specifically, the digestibilities of DM, OM, and NDF of cattle when fed on AH40 diet were significantly lower than AH20 diet ($P < 0.05$, Figure 7b). In the CVH diet groups, only NDF digestibility was significantly lower in the CVH40 diet group than in the CVH20 diet group ($P < 0.05$, Figure 7b).

Both LS and LP did not significantly influence BWG and FCE of cattle ($P > 0.05$, Table 9), but the interaction between LS and LP had a significant effect on FCE of cattle ($P < 0.05$, Table 9). In detail, the AH40 diet group had a significantly higher FCE than in the AH20 diet group ($P < 0.05$, Figure 7d) whereas there was no difference between CVH20 and CVH40 diet groups ($P > 0.05$, Figure 7d).

Table 9 A general linear model analysis of legume species (LS), legume proportion (LP), and their interaction effect on feed intake, digestibility, growth performance, and CH_4 emissions.

Item [†]	LS [‡]	LP [‡]	LS × LP [‡]
Dry matter intake (DMI)			
Forage DMI (g/kg $BW^{0.75}$/day)	5.783 [*]	0.932	0.498
Concentrate DMI (g/kg $BW^{0.75}$/day)	1.108	1.189	0.001
Total DMI (g/kg $BW^{0.75}$/day)	5.207[*]	0.109	0.598
Digestibility			
DM digestibility (%)	0.215	5.671 [*]	1.303
OM digestibility (%)	0.306	6.744 [*]	1.582
NDF digestibility (%)	1.177	18.476 [***]	0.001
Apparent N digestibility (%)	5.515[*]	5.949 [*]	0.265
Growth performance			
BWG (kg/day)	0.205	0.403	1.389
FCE (kg DMI/kg BWG)	0.077	2.515	5.796 [*]
CH_4 emissions			
CH_4 emissions (g/kg $BW^{0.75}$/24 h)	5.907 [*]	7.056 [*]	0.815
CH_4 emissions (g/kg DMI/24 h)	1.698	5.604 [*]	0.000

[†] DMI, dry matter intake; DM, dry matter; OM, organic matter; NDF, neutral detergent fiber; BWG, body weight gain; FCE, feed conversion efficiency (ratio of total DMI divided by the BWG).

[‡] values are the F value, [*] $P < 0.05$, [**] $P < 0.01$, and [***] $P < 0.001$.

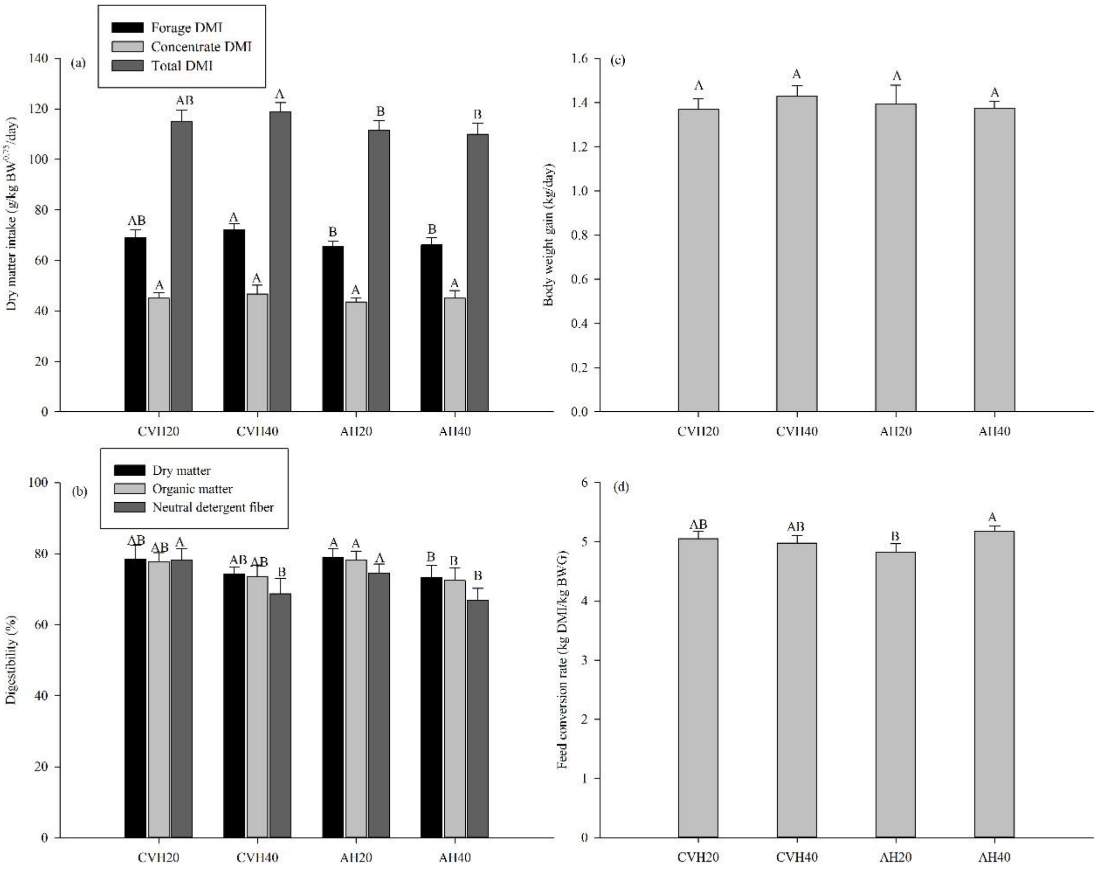


Figure 7 The dry matter intake (DMI, a), digestibility (b), body weight gain (BWG, c) and feed conversion efficiency (d) of cattle among the four diet groups. Values are presented as the mean ± standard deviation (SD). The uppercase letters within the same indicator without common letters are significantly different ($P < 0.05$).

4.3.2 Enteric methane emissions, energy balance, and energy utilization

CH_4 emissions, expressed on a milligram every 15 min per kilogram metabolic BW and gram per kilogram DMI over 24 h post-feeding, are shown in Figures 9a and 9b respectively. There were intermittent peaks throughout the day and it was apparent that the peaks occurred a short time after feed supply. Besides, the peak of CH_4 emissions (mg/kg BW$^{0.75}$ or g/kg DMI) were relatively higher after concentrate supply than forage supply (Figures 9a and 9b).

Both LS and LP could significantly affect CH_4 emissions (g/kg BW$^{0.75}$) in a 24 h ($P < 0.05$, Table 9). Individually, CVH diet groups had lower accumulated CH_4 emissions (g/kg BW$^{0.75}$) than AH diet groups (Figure 8c), and CVH40 and AH40 diet groups had relatively lower accumulated CH_4 emissions (g/kg $BW^{0.75}$) than CVH20 and AH20 diet groups, respectively (Figure 2c). In addition, accumulated CH_4 emissions (g/kg $BW^{0.75}$) were significantly lower in the CVH40 diet group than the AH20 diet group ($P < 0.05$, Figure 8c). For CH_4 emissions per kilogram DMI in a 24 h, LP had a significant effect ($P < 0.05$, Table 9). In detail, the CVH40 diet group had significantly lower accumulated CH_4 emissions (g/kg DMI) than the AH20 diet group ($P < 0.05$, Figure 8c).

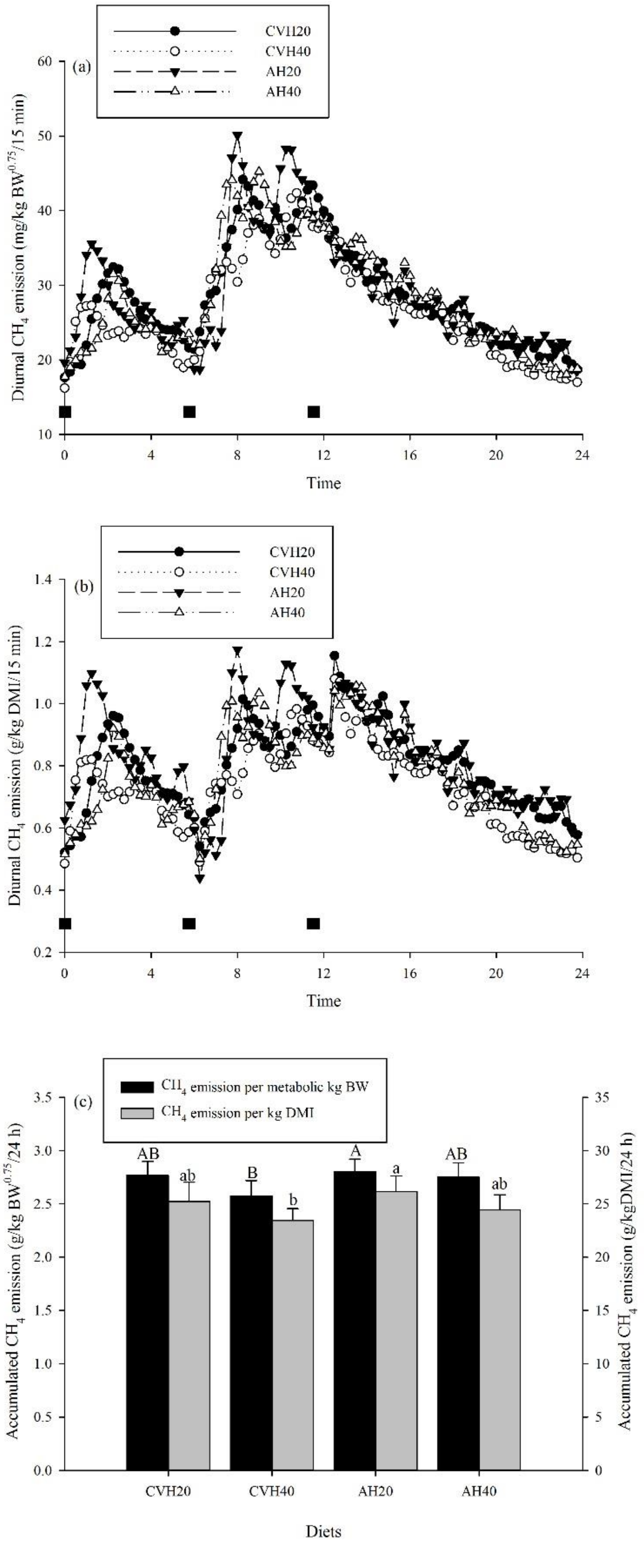


Figure 8 Diurnal CH_4 emissions (g/kg body weight $[BW]^{0.75}$/15min, a) and (g/kg dry matter intake [DMI]/15min, b), and accumulated CH_4 emissions (g/kg body weight $[BW]^{0.75}$, c) of cattle among the four diet groups.

Table 10 A general linear model analysis of legume species (LS), legume proportion (LP) and their interaction effects on energy balance/nitrogen balance and energy/nitrogen utilization efficiency.

Item [†]	LS [‡]	LP [‡]	LS × LP [‡]
Energy balance			
GE intake (MJ/kg BW$^{0.75}$/day)	1.302	2.783	0.126
ME intake (MJ/kg BW$^{0.75}$/day)	6.749*	1.132	0.127
FE output (MJ/kg BW$^{0.75}$/day)	0.042	13.739 **	1.054
UE output (MJ/kg BW$^{0.75}$/day)	4.675	1.584	1.992
CH_4-E (MJ/kg BW$^{0.75}$/day)	1.604	2.225	0.684
HP (MJ/kg BW$^{0.75}$/day)	6.208 **	1.198	0.170
RE (MJ/kg BW$^{0.75}$/day)	0.012	4.758	0.469
Energy utilization efficiency			
Ratio of ME intake to GE intake (MJ/MJ)	0.436	1.589	0.224
Ratio of FE output to GE intake (MJ/MJ)	0.392	8.630 *	0.504
Ratio of UE output to GE intake (MJ/MJ)	4.647	2.254	1.025
Ratio of HP to GE intake (MJ/MJ)	2.189	0.148	0.171
Ratio of CH_4-E to GE intake (MJ/MJ)	2.332	3.644	0.066
Ratio of RE to GE intake (MJ/MJ)	0.051	2.993	0.178
Nitrogen balance			
N intake (g/kg BW$^{0.75}$/day)	2.956	1.317	0.168
FN output (g/kg BW$^{0.75}$/day)	8.792 *	21.653 ***	0.207
UN output (g/kg BW$^{0.75}$/day)	9.602 **	0.046	0.176
RN (g/kg BW$^{0.75}$/day)	21.681 ***	3.876	3.038
N metabolism			
Ruminal ammonia N (mmol/L)	2.044	12.989 **	1.685
Blood urea N (mmol/L)	14.243 **	6.884 *	0.970
Urinary ammonia N (mmol/L)	0.241	1.420	0.140
Nitrogen utilization efficiency			
Ratio of FN output to N intake (g/g)	3.464	12.862 **	0.459
Ratio of UN output to N intake (g/g)	16.116 **	0.311	0.398
Ratio of RN to N intake (g/g)	5.992 *	4.759	1.252

[†] GE, gross energy; ME, metabolizable energy; FE, fecal energy; UE, urinary energy; CH_4-E, methane energy; HP, heat production; RE, retained energy; N intake, nitrogen intake; FN, fecal N; UN, urinary N; RN, retained N.

‡ Values are the F value, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

LS only significantly affected MEI and HP (P < 0.05, Table 10). In particular, CVH diet groups had higher MEI and HP than AH diet groups (Figures 10a and 10e). Within the legume diet groups, LP only significantly influenced FE output (P < 0.05, Table 10). CVH40 and AH40 diet groups had higher FE output than CVH20 and AH20 diet groups respectively (Figure 9d), whereas it only significantly differed between AH20 and AH40 diet groups (P < 0.05, Figure 9d). For energy utilization efficiency, LP only significantly influenced the ratio of FE to GEI (P < 0.05, Table 10). In detail, it was significantly higher in the AH40 diet group than CVH20 and AH20 diet groups (P < 0.05, Figure 9d).

4.3.3 Nitrogen balance, nitrogen metabolism, and nitrogen utilization efficiency

LS did not affect NI of cattle, but it significantly affected FN, UN and RN outputs in N balance (P < 0.05, Table 10). To be specific, although the UN output of CVH20 and CVH40 diet groups was significantly lower than in the AH40 diet group (P < 0.05, Figure 10b), they had relatively higher FN output, especially between CVH40 and AH20 diet groups (P < 0.05, Figure 10c). As a consequence, the RN of cattle in CVH20 and CVH40 diet groups was significantly higher than in the AH40 diet group (P < 0.05, Figure 10d). For the effect of LP on N balance, CVH40 and AH40 diet groups had relatively higher FE output than in CVH20 and AH20 diet groups respectively, but it only significantly differed between AH20 and AH40 diet groups (P < 0.05, Table 10, Figure 10c).

LP could significantly influence ruminal ammonia-N concentration (P < 0.05, Table 10) and it was significantly lower in the AH40 diet group than in AH20 and CVH20 diet groups (P < 0.05, Figure 10e). Both LS and LP significantly affected BUN concentration (P < 0.05, Table 10) and AH20 had a significantly lower BUN than in AH20, CVH20 and CVH40 diet groups (P < 0.05, Figure 10f). No differences were found for urinary ammonia-N concentration among the four diet groups (P > 0.05, Table 11).

LP significantly affected the ratio of FN to NI (P < 0.05, Table 10). Particularly, the AH20 diet group had a significantly lower value than in AH40 and CVH40 diet groups (P < 0.05, Figure 10c). Besides, LS significantly influenced the ratio of UN to NI (P < 0.05, Table 10), and the ratio of RN to NI (P < 0.05, Table 10). CVH40 diet group had a significantly lower UN:NI than AH20 and AH40 diet groups (P < 0.05, Figure 10b) and

CVH20 diet group had a significantly higher RN:NI than in AH40 diet group ($P < 0.05$, Figure 10d).

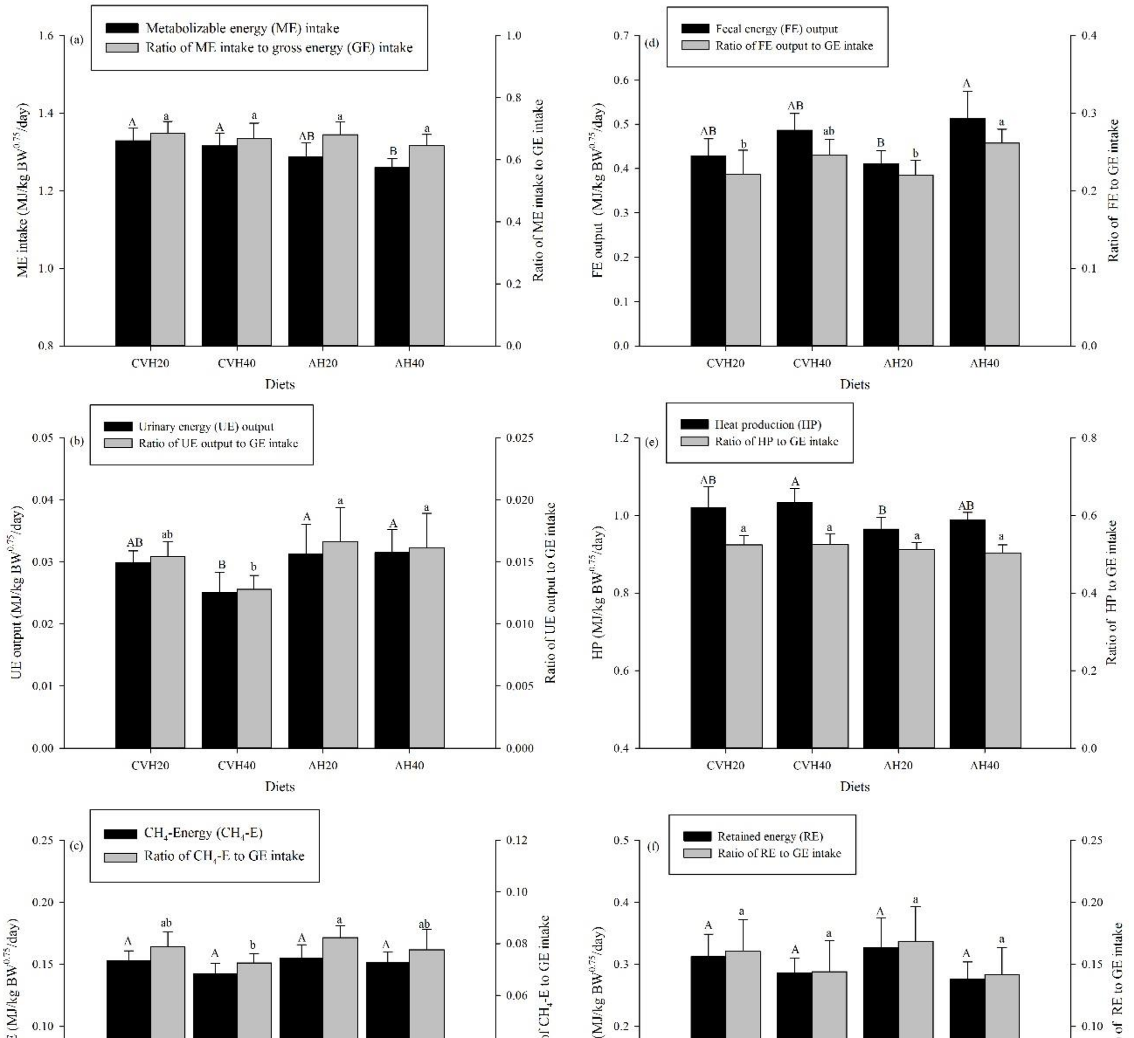


Figure 9 The energy balance and utilization efficiency of cattle among the four diet groups. Values are presented as the mean ± standard deviation (SD). Uppercase letters within the same indicator without common letters are significantly different ($P < 0.05$) among the four diet groups in energy balance, and lowercase letters within the same indicator without common letters are significantly different ($P < 0.05$) among the four diet groups in energy utilization. (a - f) represent ME intake, UE output, CH_4-E, FE output, HP, and RE, respectively, as well as their proportion of GE intake.

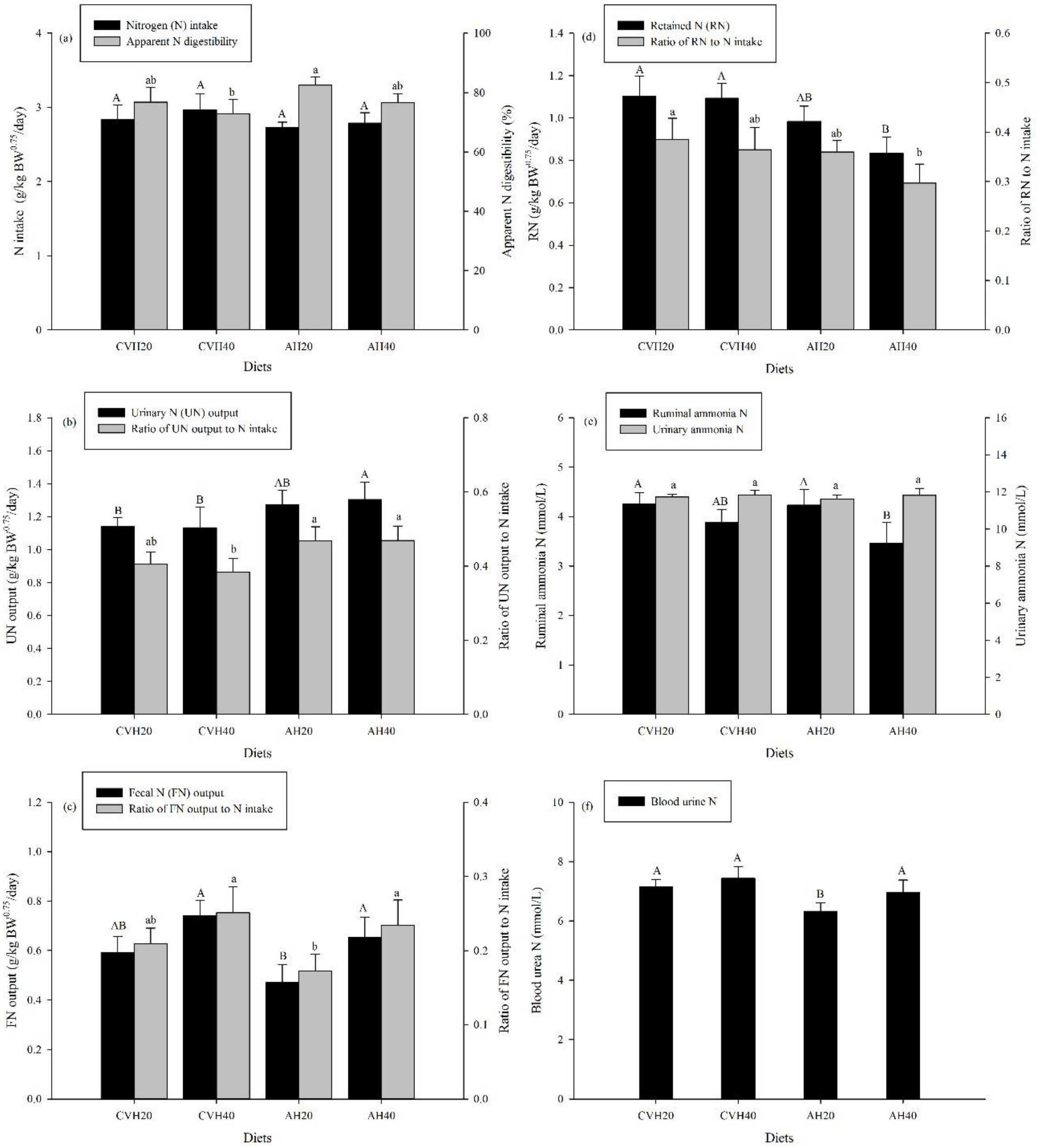


Figure 10 Nitrogen balance and utilization efficiency of cattle among the four diet groups. Values are presented as the mean ± standard deviation (SD). Uppercase letters within the same indicator without common letters are significantly different (*P* < 0.05) among the four diet groups in nitrogen balance and lowercase letters within the same indicator without common letters are significantly different (*P* < 0.05) among the four diet groups in nitrogen utilization. (a) represents N intake and apparent N digestibility; (b - d) represent UN output, FN output, and RN, respectively, as well as their proportion of N intake; (e) represents ruminal ammonia N and urinary ammonia N concentrations; (f) represents blood urea N.

4.3.4 Ruminal fermentation parameters

The total VFA and pH of ruminal fluid did not significantly differ among the four dietary treatments ($P > 0.05$, Table 11). But, the molar proportion of acetate was significantly lower in CVH40 and AH40 diet groups than CVH20 and AH20 diet groups respectively ($P < 0.05$, Table 11). Additionally, it was also significantly lower in CVH diet groups than AH diet groups ($P < 0.05$, Table 11). The molar proportions of propionate in CVH40 and AH40 diet groups were significantly higher than in CVH20 and AH20 diet groups respectively ($P < 0.05$, Table 11). As a consequence, the ratio of acetate to propionate was significantly lower in CVH40 and AH40 diet groups than in CVH20 and AH20 diet groups ($P < 0.05$, Table 11).

Table 11 Effects of different diets on the ruminal fermentation parameters in crossbred Simmental cattle.

Item	Experimental Diet †				Variance Analysis ‡		
	CVH20	CVH40	AH20	AH40	LS	LP	LS × LS
Total VFA, mmol/L	75.4 ± 6.73	72.5 ± 7.22	77.8 ± 3.32	75.7 ± 9.98	0.536	0.423	0.011
pH	6.07 ± 0.16	6.12 ± 0.25	6.05 ± 0.08	6.01 ± 0.06	0.686	0.009	0.293
Molar proportions (mol/100 mol)							
Acetate	72.3 ± 1.24	70.8 ± 0.56	73.8 ± 0.64	72.7 ± 1.13	11.967 **	6.503 *	0.122
Propionate	14.4 ± 0.24	15.7 ± 1.08	13.9 ± 0.76	15.2 ± 0.75	1.382	10.576 **	0.007
Butyrate	10.2 ± 1.11	10.2 ± 1.44	9.2 ± 0.72	8.9 ± 0.46	4.747	0.072	0.042
Iso-butyrate	1.1 ± 0.06	1.2 ± 0.19	1.1 ± 0.20	1.1 ± 0.15	0.173	0.640	0.539
Valerate	0.7 ± 0.12	0.6 ± 0.08	0.6 ± 0.18	0.6 ± 0.05	1.444	1.950	0.544
Iso-valerate	1.23 ± 0.09	1.44 ± 0.24	1.32 ± 0.23	1.37 ± 0.18	0.003	1.780	0.679
Acetate/propionate ratio	5.01 ± 0.11	4.53 ± 0.30	5.32 ± 0.30	4.78 ± 0.31	3.987	11.522 **	0.036

† CVH20, 20% common vetch + 40% oat hay; CVH40, 40% common vetch + 20% oat hay; AH20, 20% alfalfa + 40% oat hay; AH40, 40% alfalfa + 20% oat hay. Values are presented as the mean ± standard deviation (SD); LS, legume species; LP, legume proportion; LS × LP, interaction between LS and LP. Values are the F value; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

4.4 Discussion

4.4.1 Feed intake, nutrient digestibility, and body weight gain

In general, feed intake is restricted by the capacity of the rumen (Zhao et al., 2015) and NDF content, which is a measure of cell wall content, and digestibility of forage (Karabulut et al., 2007). In the present study, the higher forage DMI in CVH diet groups than AH diet groups ($P < 0.05$, Table 9, Figure 7a) could be attributed to the lower NDF content in CVH (Table 1). This indicates that feeds that are equal in digestibility but differ in NDF content would result in different intakes (Zhao et al., 2015). The similar DM digestibilities (Figure 7b) in CVH20 and AH20, CVH40 and AH40 (Figure 7b) but higher DMI in CVH diet groups confirm the above deduction.

The digestibility of a mixed feed is affected by its chemical composition (Zhao et al., 2015). For example, forage intake with increasing legume proportions could promote the passage rate of feedstuff in the rumen (McCaughey et al., 1999). Because legume has lower fiber content than grass, which reduces the retention time of forage in the rumen (Patra, 2010; Zhao et al., 2015). In the current study, the lower NDF digestibility in the diet with a higher legume proportion than in the lower legume proportion diet (Table 9, Figure 7b) confirms the above finding. Compared to grasses, the highly lignified cell walls could decrease cell wall digestion in legumes and then decrease OM digestion in the rumen (Archimède et al., 2011). The lower OM digestibility in higher legume proportion diets than lower ones support the previous finding (Figure 7b).

4.4.2 Enteric methane emissions and ruminal fermentation

There is a clear relationship between forage type, concentrate feed or starch intake, OM digestibility, and the pattern of ruminal fermentation (Hristov et al., 2013). In the present study, the lower CH_4 emissions in the diets with higher proportion legumes than lower ones, regardless of per kilogram metabolic BW or per kilogram DMI ($P < 0.05$, Table 9, Figure 8c), indicate that a diet with a higher proportion of legume could decrease CH_4 emissions. This is consistent with the finding of Lee et al. (2004) who reported an increasing percentage of white clover fed with perennial ryegrass could decrease CH_4 emissions. This may be attributed to the polyphenolic compound in legume, such as condensed tannins, which was negatively correlated with CH_4 emissions (Guglielmelli et al., 2011). For rumen fermentation, there is a negative relationship between CH_4 emissions and propionate formation in the rumen, which could depress the activity of methanogens (Hristov et al., 2013; Dong et al., 2019). In the present study, the lower ratios of acetate to propionate (Table 11), which corresponded with a lower CH_4 emission (Figure 8c), in a higher proportion of legume diet groups than lower ones were consistent with the above finding. In addition, there has been reported that lipid supplementation could reduce CH_4 emission (Beauchemin, 2008; Grainger and Beauchemin 2011). In the present study, legumes have higher crude fat (ether extract) concentration than grasses (Table 8), which led to a higher crude fat concentration per unit DM in the diet with a higher proportion of legume than lower ones. The lower CH_4 emissions in CVH40 and AH40 diet groups than in CVH20 and AH20 could also be explained in this regard. More importantly, feed intake is the single most important determinant of CH_4 emissions (Calabrò et al., 2006). In the present study, there was no difference in DMI between higher and lower proportion legume diets (Figure 7a), but the lower OM digestibility (Figrue 8b) of the diet with higher proportion of legumes and higher passage rate (McCaughey et al., 1999) leave less time for microorganisms to ferment feedstuff in the rumen (Archimède et al., 2011). Therefore, lower CH_4 emissions were observed in CVH40 and AH40 than CVH20 and AH20 (Figure 8c).

In addition to the effects of LP on CH_4 emissions, LS also affected CH_4 emissions, especially on the basis of per kilogram metabolic BW (Table 9). A relatively lower CH_4 emissions (g/kg $BW^{0.75}$) of CVH diet groups compared with AH diet groups at the same LP (Figure 8c) indicates that CVH had better potential to inhibit CH_4 emissions than AH. This might be due to the lower content of NDF and ADF in CVH than AH, which is in agreement with the finding of Beauchemin (2008), who reported that lower CH_4 emissions for animals fed legumes were often explained by the lower fiber content. Besides, the production of propionate over acetate in the rumen could also reduce CH_4 emissions in the rumen (Beauchemin, 2008). These changes of propionate and acetate were also confirmed in the present study that acetate molar proportion was lower in CVH diet groups than AH diet groups ($P < 0.05$, Table 11) although no differences in propionate molar proportion (Table 11). The ratio of acetate to propionate was around 4.77 in CVH diet groups (Table 11), which was higher than the result of Calabrò et al. (2006) who reported a value of 2.28 of OH-CVH mixture diet using an in vitro gas production technique. This could be attributed to differences between *in vivo* and *in vitro*. For example, increases in rumen propionate concentrations *in vivo* were lower than those observed *in vitro* (Fievez et al., 2003).

Increasing the inclusion of concentrate in diet, especially starch content, was regarded as another way to reduce CH_4 emissions (Yan et al., 2000; Grainger and Beauchemin, 2011). In the present study, CVH40 and AH40 diet groups corresponded with a relatively higher proportion of maize than CVH20 and AH20 diet groups (Table 9). As a consequence, lower CH_4 emissions were observed at CVH40 and AH40 diet groups, even if it only significantly differed between CVH20 and CVH40 diet groups per kilogram metabolic BW (Figure 8c). Besides, CH_4 emissions still tended to be lower in the higher proportion of maize diet groups per kilogram DMI although it did not differ significantly (Figure 8c). These suggested that starch intake could suppress CH_4 emissions (Archimède et al., 2011; Hristov et al., 2013).

4.4.3 Energy balance

In the ruminants, energy loses in the form of feces, urine and methane emissions (Chaokaur et al., 2015). In the present study, FE output and the ratio of FE output to GE intake were greater in CVH40 and AH40 diet groups than that in CVH20 and AH20 diet groups (Figure 9d). This could be explained by the higher passage rate of the diets with a higher proportion of legume in the rumen (McCaughey, 1999) and a decreased DM digestibility (Figure 7b). Because of the more DM excretion, the more FE loss. The ratio of UE output to GE intake, which ranged from 0.9% to 4.8 % in previous studies (Chaokaur et al., 2015; Zou et al., 2016), is an indispensable part of the energy loss and high UE loss is more common when animals fed silage diet (Kirkpatrick et al., 1997). In the present study, the mean 1.4% for the ratio of UE output to GE intake fell within the lower-range of the quoted studies, but LP did not significantly influence UE output and the ratio of UE output of GE intake (Figure 9b). The relatively lower values of the ratio of CH_4-E to GE intake in CVH40 and AH40 diet groups could be explained by the lower OM digestibility (Figure 7b), which reduced the retention time of feedstuff in the rumen.

ME intake, expressed as per kilogram of metabolic BW, was higher in CVH diet groups than AH diet groups (Table 9, Figure 9a), which could be attributed to higher forage DMI in CVH diet groups (Figure 7a) because CVH had a higher ME concentration (MEC) than AH (Table 1). But no differences were found for the ratio of ME intake to GE intake among the four diet groups (Figure 9a). Even so, the higher ratio of FE output to GE intake in the diet with higher proportion of legume (Table 10, Figure 9d), which accounted for the largest part of the feed energy that could not be utilized by the animals (Hernández-Ortega et al., 2011), were still tended to be lower in CVH40 and AH40 than that in CVH20 and AH20 diet groups (Figure 9a). Additionally, the ratio of ME intake to GE intake of crossbred Simmental cattle in the present study was around 0.67, which was higher than a report of 0.47 for mature Simmental cows (Estermann et al., 2002). This could be attributed to a higher OM digestibility (averaged 75.4%) in the present study compared to that (62.4%) of their study (Estermann et al., 2002). The higher ME intake (Table 10, Figure 9a) but no differences in RE (Figure 9f) in CVH diet groups compared to AH diet groups could be attributed to an increased HP for CVH diet groups than AH diet groups (Table 10, Figure 9e). This was consistent with the finding of Ferrell and Jenkins (1998) that HP increased with increasing ME intake for crossbred beef cattle.

4.4.4 Nitrogen balance, nitrogen metabolism, and nitrogen utilization efficiency

N excretion in feces and urine represents a considerable N loss from ruminant husbandry (Waldrip et al., 2013; Zhao et al., 2015). In the present study, N losses were affected by LS and LP, although LS and LP did not influence total N intake (Table 10). For example, the significant higher FN output and the ratio of FN output to N intake corresponded with a higher proportion of legume (CVH40 vs. CVH20, and AH40 vs. AH20, Figure 10c). These were likely caused by the decreased nutrient digestibilities (Figure 7b) as well as decreased apparent N digestibility (Figure 10a), which usually lead to more N excretion to feces. As a result, the higher FN output (Figure 10c) but no different UN output (Figure 10b) in the diet with a higher proportion of legume (Figure 10b) led to a reduced RN in the lower proportion of legume diets (*P* = 0.073, Table 10, Figure 10d). The UN, FN and RN outputs were influenced by LS (Table 10). The UN output in CVH diet groups was lower than AH diet groups (Figure 10b), whereas FN output was a reverse result (Figure 10c). The shift of N excretion from urine to feces in CVH diet groups than AH diet groups was regarded as an approach to reduce the impact of volatile N excretion on the environment (Yan et al., 2007). Because urinary urea is rapidly hydrolyzed to ammonium and then converted to ammonia which is readily volatilized and lost from the farm system to the environment (Koenig et al., 2018). In contrast, fecal ammonia production is generally low due to slow mineralization rates of organic nitrogenous compounds (Kebreab et al., 2009; Waldrip et al., 2013). As a consequence, the RN in CVH diet groups was higher than AH diet groups (Figure 10d). Therefore, the CVH diet has the greater potential to reduce the effect of volatile N excretion on the environment than the AH diet.

Generally, high ruminal ammonia-N concentration for optimal OM degradation will result in an increase in the loss of N through urine (Ipharraguerre and Clark, 2005). In the present study, ammonia-N concentration in the rumen tended to be lower with a higher proportion of legumes, especially in AH diets (Figure 10e). This difference could possibly be due to the relatively higher passage rate of feedstuff in the rumen with increasing legume proportions (McCaughey et al., 1999). As a result, it led to a lower OM digestibility (Figure 7b) and ammonia-N concentration in the diets with a higher proportion of legume than the lower ones.

In addition, BUN levels reflected the protein status of cattle and positively corresponded with the change in ammonia-N concentration in rumen fluid (Dong et al., 2014). In this study, BUN tended to be higher in the diets with a higher proportion of legume (Figure 10f), which was inconsistent with ruminal ammonia-N concentrations (Figure 10e). This might be attributed to the lowest pH in AH40 (Table 11), which depressed transport of ammonia across the rumen wall. Studies have shown that the permeability of the rumen wall for ammonia is pH-dependent and it has a positive correlation with pH (Abdoun et al., 2006). Additionally, although ruminal ammonia-N concentration tended to be lower in the diets with a higher proportion of legume than lower ones, there was no reduction in BWG (Figure 7c). This suggests that adequate ruminal available N was provided from the diet to maximize microbial fermentation in the rumen under a ruminal ammonia-N concentration of around 4.0 mmol/L.

4.5 Conclusions

The results of this study suggest that (1) higher proportion of legumes in the diet could reduce CH_4 emissions and minimize the impact of volatile N excretion to the environment; (2) increasing legume proportions in the diet could reduce nutrient digestibilities whereas the degree of reduction differs between common vetch hay and alfalfa hay; (3) Common vetch hay has greater potential to minimize the negative effects of CH_4 emissions and N excretion to the environment. Therefore, an opportunity for strategic feeding containing alfalfa hay (20%) and common vetch hay (40%) to reduce the direct impact of volatile N excretion and CH_4 emissions on the environment while maintaining BWG as well as nutrient digestibilities for crossbred Simmental cattle in dryland environments.

Chapter 5: General results, discussion, conclusions, and recommendations

5.1 General results

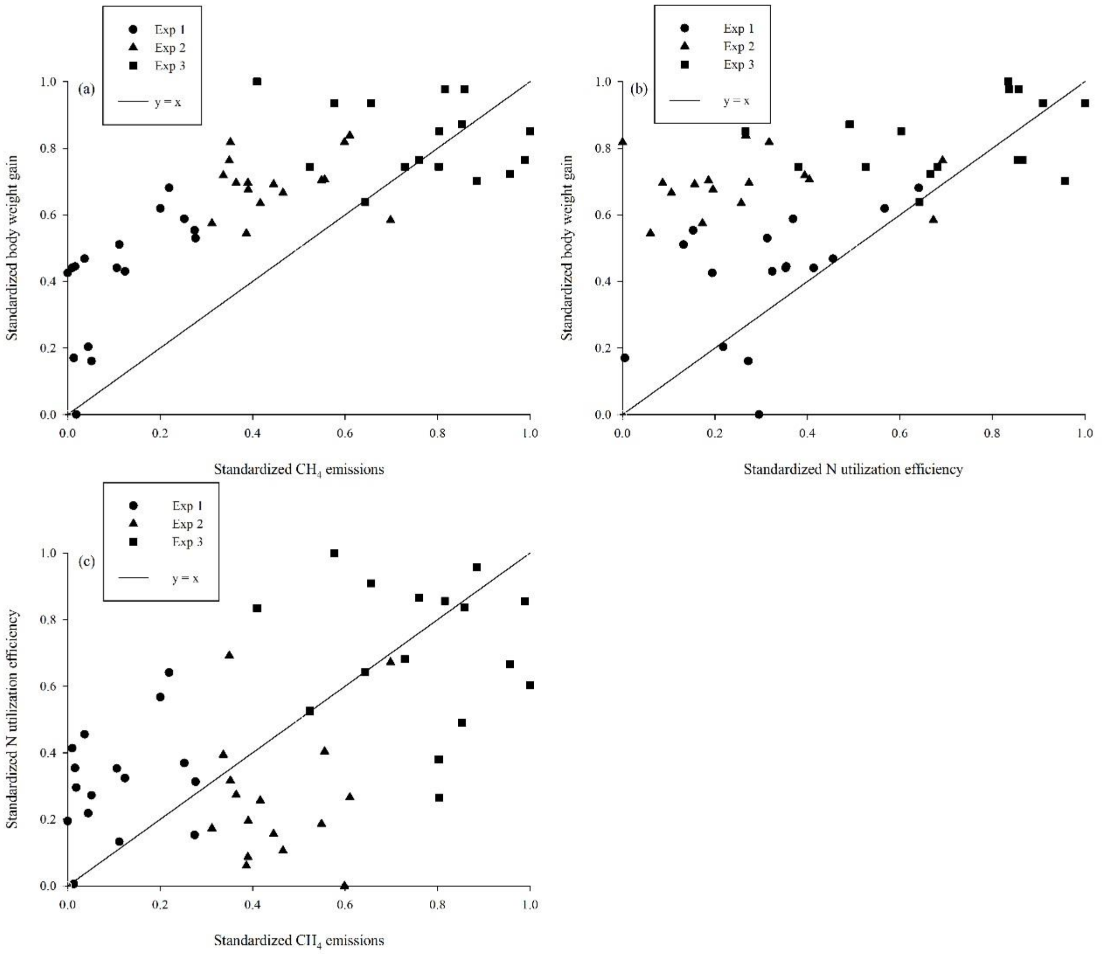


Figure 11. Relationship between standardized CH_4 emissions and standardized body weight gain (a), standardized N utilization efficiency and standardized body weight gain (b), and standardized CH_4 emissions and standardized N utilization efficiency (c). Data were pooled from three experiments (Exp)

All data of CH_4 emissions, BWG, and NUE from these three experiments were pooled and standardized to investigate their relationships (Figure 11). When the target BWG increased from 1.0 kg/day in Exp 1 to 1.3 kg/day in Exp 2, BWG increased more than CH_4 emissions, whereas the increments of BWG and CH_4 emissions were similar when the target BWG was set at 1.5 kg/day in Exp 3 (Figure 11a). In addition, BWG also increased more than NUE from 1.0 kg/day of target BWG in Exp 1 to 1.5 kg/day in Exp 3 (Figure 11b). The degree of increase of NUE and CH_4 emissions were similar from Exp 1 to Exp 3 whereas it showed differences between leguminous forage species (Figure 11c). For example, NUE increase more than CH_4 emissions for AH diets at a target BWG of 1.0 kg/day, however, CH_4 emissions tended to increase more than NUE for CVH diets at a target BWG of 1.3 kg/day (Figure 11c).

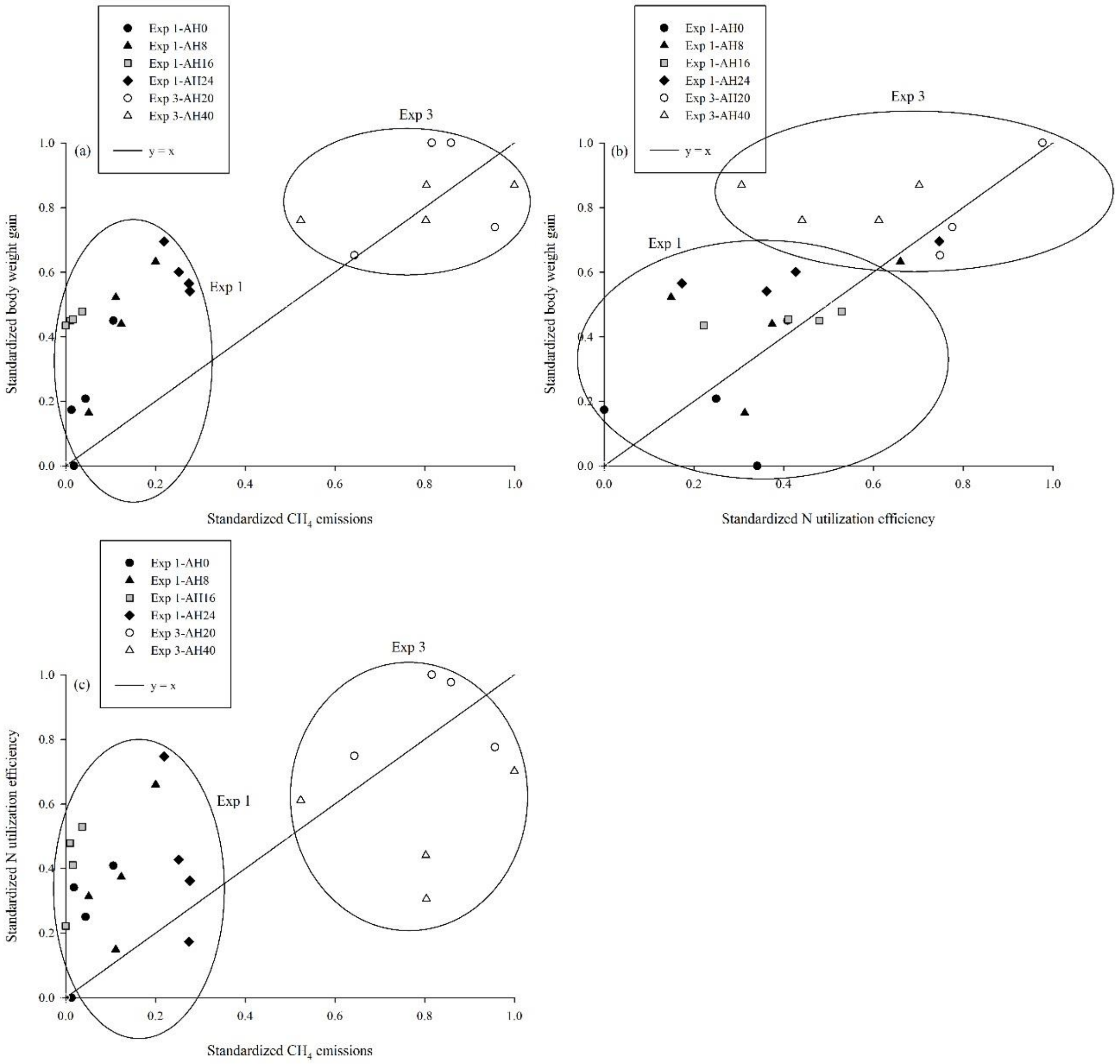


Figure 12 Relationship between standardized CH_4 emissions and standardized body weight gain (a), standardized N utilization efficiency and standardized body weight gain (b), and standardized CH_4 emissions and standardized N utilization efficiency (c) for alfalfa hay (AH) diets with different levels. Grey spot group (Exp 1-AH16) is the optimal proportion of AH in Exp 1.

For AH diet groups, CH_4 emissions increased with increasing target BWG from 1.0

kg/day in Exp 1 to 1.5 kg/day in Exp 3 (Figure 12a). In detail, BWG tended to increase more than CH_4 emissions with increasing AH in the diet when target BWG was 1.0 kg/day, however, there was not any advantage for BWG compared with CH_4 emissions when target BWG was 1.5 kg/day (Figure 12a). In detail, AH16 had a medium BWG while maintaining lower CH_4 emissions in Exp 1 whereas there was no difference for AH20 and AH40 diets in Exp 3 (Figure 12a). NUE tended to increase with increasing target BWG from 1.0 kg/day in Exp 1 to 1.5 kg/day in Exp 3 (Figure 12b). However, it differed with different levels of AH in the diet at the same target BWG (Figure 12b). For example, AH16 in Exp 1 or AH20 in Exp 3, which had relatively high NUE than AH24 and AH40 respectively, at the same target BWG (Figure 12b). Both NUE and CH_4 emissions increased with increasing target BWG from 1.0 kg/day in Exp 1 to 1.5 kg/day in Exp 3 (Figure 12c). But, there were differences with leguminous forage proportion within each Exp. For instance, there was a medium NUE for the AH16 while it could maintain the lowest CH_4 emissions in Exp 1(Figure 12c); AH40 tended to increase more CH_4 emissions than AH20 in Exp 3 (Figure 12c).

In CVH diet groups, both BWG and CH_4 emissions increased with increasing target BWG from 1.3 kg/day in Exp 2 to 1.5 kg/day in Exp 3 (Figure 13a). In Exp 2, CVH30 had the lowest CH_4 emissions while maintaining a higher BWG (Figure 13a). NUE also increased with increasing target BWG at 1.3 kg/day in Exp 2 to 1.5 kg/day in Exp 3 (Figure 13b). However, CVH40 had the more BWG than CVH20 at a similar NUE in Exp 3 (Figure 13b). In comparison with CH_4 emissions, increasing target BWG from 1.3 kg/day in Exp 2 to 1.5 kg/day in Exp 3 tended to increase more NUE (Figure 13c). In detail, CVH30 was the optimal one to keep a higher NUE while maintaining the lowest CH_4 emissions (Figure 13c).

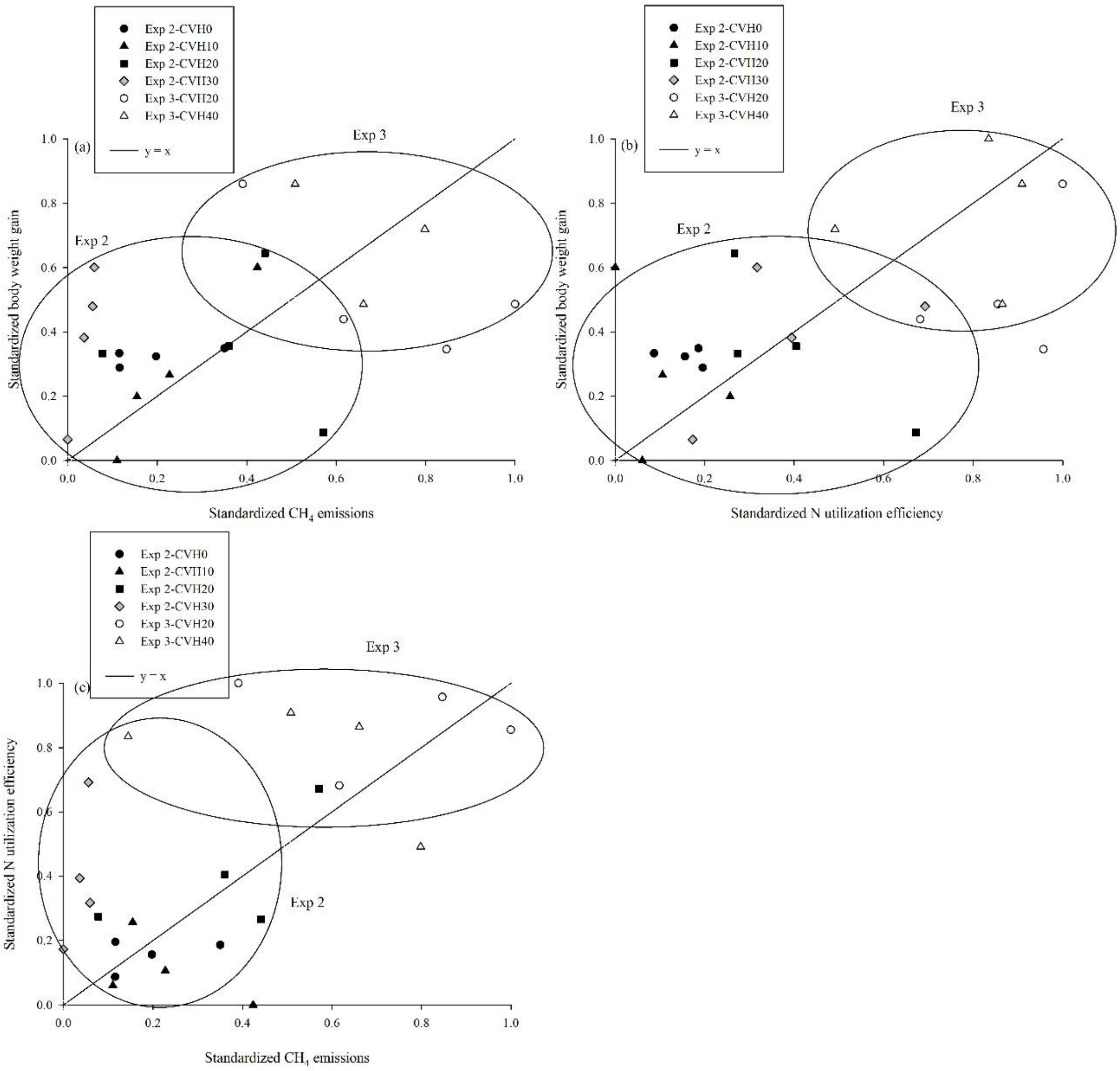


Figure 13 Relationship between standardized CH_4 emissions and standardized body weight gain (a), standardized N utilization efficiency and standardized body weight gain (b), and standardized CH_4 emissions and standardized N utilization efficiency (c) for common vetch hay (CVH) diets with different levels.

5.2 General discussion

Generally, although DMI is the single most important determinant of CH_4 production (Yan et al., 2000), improving livestock productivity, which usually corresponded with a higher DMI, was still regarded as a way to reduce CH_4 emissions (Yan et al., 2007). In our study, when the target BWG increased from 1.0 kg/day in Exp 1 to 1.5 kg/day in Exp 3, BWG increased more than CH_4 emissions. These results confirmed the previous finding. However, the degree of increment of BWG from Exp 1 to Exp 3 was likely a parabolic trend and there was no advantage for the target BWG of 1.5 kg/day, which could reduce CH_4 emissions efficiently compared to the target BWG of 1.0 and 1.3 kg/day (Figure 11a). This could be attributed to that although DMI was positively correlated to BWG, to some extent, the correlation was not linear. When the BWG beyond a threshold, such as 1.5 kg/day, the positive effects of high DMI for BWG would decrease and then CH_4 emissions would increase more than BWG. For NUE, increasing target BWG increased NUE. This was due to the generally increasing DMI, which corresponded with the increasing BWG from Exp 1 to Exp 3 in the present study. Usually, the higher DMI would lead to a higher passage rate of feedstuff in the rumen and then decreased the UN output while increasing FN output. However, the degree of decreasing the UN output was higher than the degree of increasing the FN output, which resulted in an increasing NUE eventually because of the UN and FN were the main pathways for N loss.

Leguminous forage not only is a good CP supplementation for grass hay-based diet, but also could be used to reduce CH_4 emissions, and improve NUE and BWG (Givens, 2000; Hess *et al.*, 2003). In the present study, BWG tended to increase more than CH_4 emissions and NUE with increasing leguminous forage in the diet no matter for AH diets in Exp 1 (Figures 13a and 13b) or CVH diets in Exp 2 (Figures 14a and 14b). These results were consistent with the previous finding. However, when the target BWG was set at 1.5 kg/day, improving livestock productivity was not so efficient, compared to a target BWG of 1.0 kg/day (Exp 1) and 1.3 kg/day (Exp 2), to reduce CH_4 emissions regardless of AH diets (Figure 12a) or CVH diets (Figure 13a) just as discussed above.

Appropriate leguminous forage proportion in the grass hay-based diet leads to a positive impact on growth performance and NUE of ruminants (Kobayashi et al., 2017, 2018). However, it differed with the leguminous forage species. For example, in our study, 16% AH in Exp 1 was the optimal one which not only increased more BWG but also maintained the lowest CH_4 emissions compared to other diets (Figure 12a). This may be due to the saponin concentration of AH, which could reduce CH_4 emissions. But its positive effects decreased at 24% AH, which corresponded with a higher DMI compare to 16% AH. When the target BWG was around 1.5 kg/day, 40% AH led to a lower NUE than 20% AH, which could be attributed to significant lower nutrient digestibility (Figure 7) and then increased more FN output which could not compensate the positive effects from reducing UN output (Figure 9). For CVH diets, 30% CVH could maintain a higher BWG (Figure 13a) while reducing CH_4 emissions compared to 0, 10, and 20% CVH diets at a target BWG of 1.3 kg/day. In addition, 40% CVH in the diet could increase more BWG while reducing CH_4 emissions than CVH20 at a similar NUE at the target BWG of 1.5 kg/day (Figures 14b and 14c). These results could be attributed to that increasing CVH proportions in the diet would decrease the DNF concentration and the retention time of feedstuff in the rumen, and then reduced the fermention time of microorganism on the feedstuff, which could decrease CH_4 emissions.

5.3 Key findings

In Chapter 2, appropriate levels of leguminous forage supplementation in oat hay-based diet could impact positive effects. For example, 24% AH diet had the significantly improved BWG compared to the diet without AH whereas only 16% inclusion of AH in cattle diets gave the lower CH_4 emissions than 24% AH diet; although BWG was not affected by CVH supplementation diets, 30% CVH diet had a significantly lower CH_4 emission than 0% and 20% CVH diets. These results suggested that oat hay based-diet with 16% AH or 30% CVH inclusion had the maximum positive effects on BWG and CH_4 emissions in crossbred Simmental calves.

In Chapter 3, NUE increased with supplementing leguminous forages whereas the growth rate of NUE reduced at 16% AH and 20% CVH diet compared to a much higher proportion of leguminous forages (such as 24% AH and 30% CVH). Nutrient digestibility had a parabolic trend in response to increasing AH and CVH proportions and their peak values were around 16% AH and 20% CVH. In addition, the decreased UN:NI ratio in response to increasing AH/CVH proportions in the current study indicated additional environmental benefits, such as reducing volatile N excretion from urine, which may eventually impact N management on farms. These results indicated that around 16% AH and 20% CVH could be used to be included in oat hay-based diet to improve NUE and reduce the direct impact of N excretion on the environment while maintaining optimal nutrient digestibility.

In Chapter 4, we focused on comparing the different effects of AH and CVH on NUE and CH_4 emissions, then formulated the diets which could not only improve NUE but also reduce CH_4 emissions. Firstly, there was one similar result that a higher proportion of leguminous forage in the diet could reduce CH_4 emissions and minimize the impact of volatile N excretion to the environment; besides, higher leguminous forage proportion could suppress nutrient digestibility whereas the degree of reduction differed between CVH and AH. Lastly, CVH has greater potential to mitigate CH_4 emissions and N excretion to the environment. Therefore, 20% AH and 40% CVH could be used to reduce the direct impact of volatile N excretion and CH_4 emissions on the environment while maintaining BWG as well as nutrient digestibility for crossbred Simmental cattle in dryland environments.

In an overall conclusion based on this study, (1) improving livestock productivity could reduce CH_4 emissions, however, this degree of reducing CH_4 emissions decreases with increasing BWG from 1.3 kg/day to 1.5 kg/day; (2) the positive effects of leguminous forage on BWG, CH_4 emissions, and NUE varied depending on legume species. For example, high proportion of CVH has greater potential to maintain a higher NUE than AH at the same target BWG (1.5 kg/day in Exp 3); (2) the optimal proportion of leguminous forage in oat-hay based diet differed with legume species, such as a low proportion of AH (16-20%) is recommended whereas it is around 30-40% for CVH. In addition, AH has a greater tolerance for drought and saline environment than CVH, and its DM yield was also higher than CVH. However, CVH is more commonly cultivated in highland areas, such as around 1000 - 3000 m (a.s.l.), which is not suitable for AH cultivation due to the unique ecological environment of the plateau. In this regard, AH and CVH have their own advantages and limitations, leguminous forage supplementation should promote in oat hay-based diet of smallholder farming systems based on local ecological environment and the characteristics of leguminous forage.

5.4 Importance of the study

This study provided significant guidance for smallholder beef cattle farming systems in the northwest of China, through determining the optimal proportion of basal forages (AH and CVH) which could be used to substitute the protein source from concentrate in the diet while maintaining maximum BWG. In addition, on one hand, the data obtained in this study regarding the NUE of crossbred Simmental calves could be used as the reference for local farmers and government to manage the protein resource for animal husbandry to reduce protein wastage and potential environmental pollution, such as leaching of NO_3^- and pathogens to the groundwater, and deterioration of sensitive ecosystems, degradation of soil production potential through accumulation of nutrients, salts, and metals. On the other hand, the data of enteric CH_4 emissions as well as the UN and FN outputs could be applied to establish equations to estimate CH_4 emissions and N output based on the GE intake or total N intake respectively, which are the main factors. In the end, this research give us a perspective to change the route from UN output to FN output considering UN is more violated to NH_3 emissions than FN, which not only accelerates global warming but also aggravates nitrification process of N. These aspects are useful to establish the scientific feeding standard which is attempted to minimize CH_4 emissions and improve NUE for beef cattle production systems.

5.5 Limitations of the study and recommendations for future research

This study is a traditional animal nutrition research that focused on the energy and nitrogen metabolism pathways. But, the rumen of a ruminant is a special anaerobic fermentation tank, which has an associative effects on the nutrient supply for ruminant after they fed on different mixed forages, especially for some leguminous forages which have secondary metabolites, such as saponin, tannins which may reduce the activity of methanogen and then reduce CH_4 production. In this case, common vetch, regarded as a new multi-purpose cereal leguminous forage for ruminant which has many anti-nutrition factors, needs further study to investigate the effects of common vetch mixture diets on the microbiome in the rumen and the nutrition absorption in the gastrointestinal tract.

In addition, it has been reported that there are more than 120 species of leguminous forages only in Gansu Province (Chen, 2007). Therefore, there is great potential to explore new leguminous forages for beef cattle production systems in the world, which could reduce CH_4 emissions and improve NUE. An appropriate level of leguminous forages in the diet, which could impact positive effects on nutrition digestive and metabolism for ruminant, appears to improve or maintain BWG (see section 2.3.1, section 3.3.1 and section 4.3.1). Therefore, the optimal ratios of grass and new leguminous forages deserves further study.

Acknowledgments

I would like to express my thanks to Prof. Tsunekawa Atsushi of the Arid Land Research Center, Tottori University (Japan), Prof. Ichinohe Toshiyoshi of the Faculty of Life and Environmental Science, Shimane University (Japan), especially Associate Prof. Peng Fei of International Platform for Dryland Research and Education, Tottori University (Japan) and Associate Prof. Kobayashi Nobuyuki of Arid Land Research Center, Tottori University (Japan) for their support and guidance on this study. My great thanks are extended to Prof. Hou Fujiang, Mr. Chang Shenghua, Mr. Zhang Cheng, Mr. Zhu Wanhe and their students at the College of Pastoral Agriculture Science and Technology, Lanzhou University in China for their support in conducting the feeding experiments with respiration chambers and for analyzing the feed, fecal, and urinary sample. I also wish to thank the Academician Nan Zhibiao of Lanzhou University in China for supporting common vetch seeds (*Vicia sativa* L.cv. Lanjian No. 3) for this study and thank to International Platform for Dryland Research and Education, Tottori University and United Graduated School of Agricultural Sciences, Tottori University for funding my participation in international conference during my PhD period.

This research was funded by the Marginal Region Agriculture Project of Tottori University, the Strategic Priority Research Program of Chinese Academy of Sciences of China (grant no. XDA20100102), the National Natural Science Foundation of China (no. 31672472), and the Program for Changjiang Scholars and Innovative Research Team at the University of China (IRT_17R50).

References

Abdoun, K., Stumpff, F., & Martens, H. (2006). Ammonia and urea transport across the rumen epithelium: a review. *Animal Health Research Reviews*, 7, 43–59. doi:10.1017/s1466252307001156

Aboagye, I. A., Oba, M., Castillo, A. R., Koenig, K. M., Iwaasa, A. D., & Beauchemin, K. A. (2018). Effects of hydrolyzable tannin with or without condensed tannin on methane emissions, nitrogen use, and performance of beef cattle fed a high-forage diet. *Journal of Animal Science,* 96, 5276–5286. doi:10.1093/jas/sky352

Abreu, A., Carulla, J. E., Lascano, C. E., Díaz, T. E., Kreuzer, M., & Hess, H. D. (2004). Effects of Sapindus saponaria fruits on ruminal fermentation and duodenal nitrogen flow of sheep fed a tropical grass diet with and without legume1. *Journal of Animal Science,* 82, 1392–1400. doi:10.2527/2004.8251392x

Agricultural and Food Research Council (AFRC). (1993). *Energy and Protein Requirements of Ruminants. An Advisory Manual Prepared by the AFRC Technical Committee on Responses to Nutrients;* Centre for Agriculture and Bioscience International: Wallingford. UK.

Agricultural Research Council (ARC). (1980). *The Nutrient Requirements of Ruminant Livestock*. Technical Review by an Agricultural Research Council Working Party, Common wealth Agricultural Bureau, Farnham Royal, UK.

Assefa, G., & Ledin, I. (2001). Effect of variety, soil type and fertiliser on the establishment, growth, forage yield, quality and voluntary intake by cattle of oats and vetches cultivated in pure stands and mixtures. *Animal Feed Science and Technology*, 92, 95–111. doi:10.1016/s0377-8401(01)00242-5

Association of Official Analytical Chemists (AOAC). (1990). *Official methods of analysis of the association of official analytical chemists*, 15th ed.. Arlington, VA: Association of Official Analytical Chemists.

Archimède, H., Eugène, M., Marie Magdeleine, C., Boval, M., Martin, C., Morgavi, D. P., Lecomte, P., & Doreau, M. (2011). Comparison of methane production between C3 and C4 grasses and legumes. *Animal Feed Science and Technology*, 166, 59–64. doi:10.1016/j.anifeedsci.2011.04.003

Bailey, C. B. (1989). Rate and efficiency of gain, body composition, nitrogen metabolism, and blood composition of growing Holstein steers given diets of roughage or concentrate. *Canadian Journal of Animal Science*, 69, 707–725. doi:10.4141/cjas89-084

Beauchemin, K. A., & McGinn, S. M. (2006). Methane emissions from beef cattle: Effects of fumaric acid, essential oil, and canola oil. *Journal of Animal Science*, 84, 1489–1496. doi:10.2527/2006.8461489x

Beauchemin, K. A., Kreuzer, M., O'Mara, F., & McAllister, T. A. (2008). Nutritional management for enteric methane abatement: a review. *Australian Journal of Experimental Agriculture*, 48, 21–27. doi:10.1071/ea07199.

Bhatta, R., Enishi, O., Yabumoto, Y., Nonaka, I., Takusari, N., Higuchi, K., Tajima, K., Takenaka, A., & Kurihara, M. (2013). Methane reduction and energy partitioning in goats fed two concentrations of tannin from *Mimosa* spp. *The Journal of Agricultural Science*, 151, 119–128. doi:10.1017/s0021859612000299

Bhatti, S. A., Bowman, J. G. P., Firkins, J. L., Grove, A. V., & Hunt, C. W. (2008). Effect of intake level and alfalfa substitution for grass hay on ruminal kinetics of fiber digestion and particle passage in beef cattle. *Journal of Animal Science*, 86, 134–145. doi:10.2527/jas.2006-693

Brouwer, E. (1965). Report of sub-committee on constants and factors. In *Proceedings of the 3rd symposium on energy metabolism of farm animals. European association for animal emission,* Blaxter, K.L., Ed.; Academic Press: Scotland, UK, p. 441-443.

Calabrò, S., Carone, F., Cutrignelli, M., D'Urso, S., Piccolo, G., Tudisco, R., Angelino, G., & Infascelli, F. (2006). The effect of haymaking on the neutral detergent soluble fraction of two intercropped forages cut at different growth stages. *Italian Journal of Animal Science,* 5, 327–339. doi:10.4081/ijas.2006.327

Chadwick, D. R. (2005). Emissions of ammonia, nitrous oxide and methane from cattle manure heaps: Effect of compaction and covering. *Atmospheric environment,* 39, 787–799. doi:10.1016/j.atmosenv.2004.10.012

Chaokaur, A., Nishida, T., Phaowphaisal, I., & Sommart, K. (2015). Effects of feeding level on methane emissions and energy utilization of Brahman cattle in the tropics. *Agriculture, Ecosystems & Environment*, 199, 225–230. doi:10.1016/j.agee.2014.09.014

Chen, B. (2007). Legume forage resources and floristic characteristics in Gansu Province.

Partacultural Science, 25, 42–45 (In Chinese).

Chen, L., Guo, G., Yu, C., Zhang, J., Shimojo, M., & Shao, T. (2014). The effects of replacement of whole-plant corn with oat and common vetch on the fermentation quality, chemical composition and aerobic stability of total mixed ration silage in Tibet. *Animal Science Journal*, 86, 69–76. doi:10.1111/asj.12245

Chinese Feeding Standard for Beef Cattle (CFSBC). 2004. Ministry of Agriculture of the People’s Republic of China; p. 1–27. Available from URL: https://wenku.baidu.com/view/154d59bdf121dd36a32d8269.html (Accessed on 4 October 2019 in Chinese).

Delgado, C., Rosegrant, M., Steinfeld, H., Ehui, S. & Courbios, C. (1999). *Livestock to 2020: The next food revolution.* Washington DC: International Food Policy Research Institute Food, Agriculture and the Environment Discussion Paper No. 28.

Dong, R. L., Zhao, G. Y., Chai, L. L., & Beauchemin, K. A. (2014). Prediction of urinary and fecal nitrogen excretion by beef cattle1. *Journal of Animal Science*, 92, 4669–4681. doi:10.2527/jas.2014-8000

Dong, L., Li, B., & Diao, Q. (2019). Effects of Dietary Forage Proportion on Feed Intake, Growth Performance, Nutrient Digestibility, and Enteric Methane Emissions of Holstein Heifers at Various Growth Stages. *Animals*, 9, 725. doi:10.3390/ani9100725

Doran, M. P., Laca, E. A., & Sainz, R. D. (2007). Total tract and rumen digestibility of mulberry foliage (*Morus alba*), alfalfa hay and oat hay in sheep. *Animal Feed Science and Technology*, 138, 239–253. doi:10.1016/j.anifeedsci.2006.11.016

Du, W., Hou, F., Tsunekawa, A., Kobayashi, N., Ichinohe, T., & Peng, F. (2019). Effects of the Diet Inclusion of Common Vetch Hay Versus Alfalfa Hay on the Body Weight Gain, Nitrogen Utilization Efficiency, Energy Balance, and Enteric Methane Emissions of Crossbred Simmental Cattle. *Animals*, 9, 983. doi:10.3390/ani9110983

Erol, A., Kaplan, M., & Kizilsimsek, M. (2009). Oats (*Avena sativa*)–Common vetch (*Vicia sativa*) mixtures grown on a low-input basis for a sustainable agriculture. *Tropical Grasslands*, 4, 191.

Estermann, B. L., Sutter, F., Schlegel, P. O., Erdin, D., Wettstein, H. R., & Kreuzer, M. (2002). Effect of calf age and dam breed on intake, energy expenditure, and excretion of nitrogen, phosphorus, and methane of beef cows with cattle. *Journal of Animal Science*, 80, 1124–1134. doi:10.2527/2002.8041124x

Evidente, A., Cimmino, A., Fernández-Aparicio, M., Rubiales, D., Andolfi, A., & Melck, D. (2011). Soyasapogenol B and *trans*-22-dehydrocam-pesterol from common vetch (*Vicia sativa* L.) root exudates stimulate broomrape seed germination. *Pest Management Science*, 67, 1015–1022. doi:10.1002/ps.2153

Ferrell, C. L., & Jenkins, T. G. (1998). Body composition and energy utilization by steers of diverse genotypes fed a high-concentrate diet during the finishing period: I. Angus, Belgian Blue, Hereford, and Piedmontese sires. *Journal of Animal Science,* 76, 637. doi:10.2527/1998.762637x

Fievez, V., Dohme, F., Danneels, M., Raes, K., & Demeyer, D. (2003). Fish oils as potent rumen methane inhibitors and associated effects on rumen fermentation *in vitro* and *in vivo*. *Animal Feed Science and Technology,* 104, 41–58. doi:10.1016/s0377-8401(02)00330-9

Food and Agriculture Organization of the United Nations (FAO). 2003. World Agriculture: Towards 2015/2030. An FAO Perspective. FAO, Rome, Italy; p. 97.

FAO. 2011a. *Mapping supply and demand for animal-source foods to 2030,* by T.P. Robinson & F. Pozzi. Animal Production and Health Working Paper. No. 2. Rome.

FAO, 2011b. *Successes and failures with animal nutrition practices and technologies in developing countries.* In H.P.S Makkar, ed. Proceedings of the FAO Electronic Conference, September 2010.

FAO. 2012. *Balanced feeding for improving livestock productivity – increase in milk production and nutrient use efficiency and decrease in methane emission*. By M.R. Garg. FAO Animal Production and Health Paper No. 173.

FAOSTAT. 2012. *FAO Statistical Database*. Food and Agriculture Organization of the United Nations, Rome, Italy, 352 pp.

FAOSTAT 2013. FAOSTAT database. *Food and Agriculture Organization of the United Nations*. Available at: http://faostat.fao.org/.

Garg, M. R., Sherasia, P. L., Bhanderi, B. M., Phondba, B. T., Shelke, S. K., & Makkar, H. P. S. (2013). Effects of feeding nutritionally balanced rations on animal productivity, feed conversion efficiency, feed nitrogen use efficiency, rumen microbial protein supply, parasitic load, immunity and enteric methane emissions of milking animals under field conditions. *Animal Feed Science and Technology,* 179, 24–35. doi:10.1016/j.anifeedsci.2012.11.005

Gerrits, W., Labussiere, E., Dijkstra, J., Reynolds, C., Metges, C., Kuhla, B., Lund P., & Weisbjerg, M. R. (2018). Letter to the Editors: Recovery test results as a prerequisite for publication of gaseous exchange measurements. *Animal*, 12, 4–4. doi:10.1017/s1751731117002397

Ghelichkhan, M., Eun, J. S., Christensen, R. G., Stott, R. D., & MacAdam, J. W. (2018). Urine volume and nitrogen excretion are altered by feeding birdsfoot trefoil compared with alfalfa in lactating dairy cows. *Journal of Animal Science*, 96, 3993–4001. doi:10.1093/jas/sky259

Givens, D. I., Owen, E., Omed, H. M., & Axford, R. F. E. (2000). Forage Evaluation in Ruminant Nutrition; CABI Publishing: Wallingford, UK; pp. 9–10.

Grainger, C., & Beauchemin, K. A. (2011). Can enteric methane emissions from ruminants be lowered without lowering their production? *Animal Feed Science and Technology*, 166, 308–320. doi:10.1016/j.anifeedsci.2011.04.021

Graham, P. H., & Vance, C. P. (2003). Legumes: Importance and constraints to greater use. *Plant Physiology*, 131, 872–877. doi:10.1104/pp.017004.

Guglielmelli, A., Calabrò, S., Primi, R., Carone, F., Cutrignelli, M. I., Tudisco, R., Piccolo, G., Ronchi, B., & Danieli, P. P. (2011). *In vitro* fermentation patterns and methane production of sainfoin (*Onobrychis viciifolia Scop.*) hay with different condensed tannin contents. *Grass and Forage Science*, 66, 488–500. doi:10.1111/j.1365-2494.2011.00805.x

Haddad, S. (2000). Associative effects of supplementing barley straw diets with alfalfa hay on rumen environment and nutrient intake and digestibility for ewes. *Animal Feed Science and Technology*, 87, 163–171. doi:10.1016/s0377-8401(00)00203-0

Haj-Ayed, M., González, J., Caballero, R., & Alvir, M. R. (2000, September). Nutritive value of on-farm common vetch-oat hays. II. Ruminal degradability of dry matter and crude protein. In *Annales de zootechnie* (Vol. 49, No. 5, pp. 391–398). EDP Sciences.

Hernández-Ortega, M., Heredia-Nava, D., Espinoza-Ortega, A., Sánchez-Vera, E., & Arriaga-Jordán, C. M. (2011). Effect of silage from ryegrass intercropped with winter or common vetch for grazing dairy cows in small-scale dairy systems in Mexico. *Tropical Animal Health and Production*, 43, 947–954. doi:10.1007/s11250-011-9788-2

Hess, H. D., Monsalve, L. M., Lascano, C. E., Carulla, J. E., Díaz, T. E., & Kreuzer, M.

(2003). Supplementation of a tropical grass diet with forage legumes and *Sapindus saponaria* fruits: effects on in vitro ruminal nitrogen turnover and methanogenesis. *Australian Journal of Agricultural Research*, 54, 703–713. doi:10.1071/ar02241

Hristov, A. N., Oh, J., Firkins, J. L., Dijkstra, J., Kebreab, E., Waghorn, G., Adesogan, A., Yang, W., Tricarico, J., Lee, C., Gerber, P. J., Henderson, B., & Tricarico, J. M. (2013). SPECIAL TOPICS — Mitigation of methane and nitrous oxide emissions from animal operations: I. A review of enteric methane mitigation options1. *Journal of Animal Science*, 91, 5045–5069. doi:10.2527/jas.2013-6583

Huang, Y. F., Gao, X. L., Nan, Z. B., & Zhang, Z. X. (2017). Potential value of the common vetch (*Vicia sativa* L.) as an animal feedstuff: A review. *Journal of Animal Physiology and Animal Nutrition*, 101, 807–823. doi:10.1111/jpn.12617

Huhtanen, P. (1991). *Associative effects of feeds in ruminants*. In Evaluation of the Energy Value of Feeds for Ruminants (pp. 37-57).

IPCC. 2006. *2006 IPCC Guidelines for National Greenhouse Gas Inventories prepared by the National Greenhause Gas Inventories Programme*. Eggleston, H.S., Buenida, L., Miwa, K., Nagara, T. & Tanabe, K. (eds). Published: IGES, Japan.

Ipharraguerre, I. R., & Clark, J. H. (2005). Varying Protein and Starch in the Diet of Dairy Cows. II. Effects on Performance and Nitrogen Utilization for Milk Production. *Journal of Dairy Science*, 88, 2556–2570. doi:10.3168/jds.s0022-0302(05)72932-5

Irie, H., Sudo, K., Akimoto, H., Richter, A., Burrows, J. P., Wagner, T., Wenig, M., Beirle, S., Kondo, Y., Sinyakov, V. P., & Goutail, F. (2005). *Evaluation of long-term tropospheric NO_2 data obtained by GOME over East Asia in 1996*–*2002*. Geophysical Research Letters, 32, L11810. doi:10.1029/2005GL022770, 2005.

Johnson, J. W., & Preston, R. L. (1995). Minimizing nitrogen waste by measuring plasma urea-N levels in steers fed different dietary crude protein levels. In: Texas Tech Univ. Res. Rep. T–5–355. Texas Tech Univ., Lubbock, TX. p. 62–63.

Johnson, K. A., & Johnson, D. E. (1995). Methane emissions from cattle. Journal of Animal Science, 73, 2483–2492. doi:10.2527/1995.7382483x

Kafilzadeh, F., & Heidary, N. (2013). Chemical composition,in vitrodigestibility and kinetics of fermentation of whole-crop forage from 18 different varieties of oat

(*Avena sativa* L.). *Journal of Applied Animal Research*, 41, 61–68. doi:10.1080/09712119.2012.739084

Karabulut, A., Canbolat, O., Kalkan, H., Gurbuzol, F., Sucu, E., & Filya, I. (2007). Comparison of In vitro Gas Production, Metabolizable Energy, Organic Matter Digestibility and Microbial Protein Production of Some Legume Hays. *Asian-Australasian Journal of Animal Sciences*, 20, 517–522. doi:10.5713/ajas.2007.517

Kebreab, E., Dijkstra, J., Bannink, A., & France, J. (2009). Recent advances in modeling nutrient utilization in ruminants1. *Journal of Animal Science*, 87, E111–E122. doi:10.2527/jas.2008-1313

Kirkpatrick, D. E., Steen, R. W. J., & Unsworth, E. F. (1997). The effect of differing forage:concentrate ratio and restricting feed intake on the energy and nitrogen utilization by beef cattle. *Livestock Production Science*, 51, 151–164. doi:10.1016/s0301-6226(97)00099-7

Kobayashi, N., Hou, F., Tsunekawa, A., Chen, X., Yan, T., & Ichinohe, T. (2017). Effects of substituting alfalfa hay for concentrate on energy utilization and feeding cost of crossbred Simmental male cattle in Gansu Province, China. *Grassland Science,* 63, 245–254. doi:10.1111/grs.12169

Kobayashi, N., Hou, F., Tsunekawa, A., Chen, X., Yan, T., & Ichinohe, T. (2018). Appropriate level of alfalfa hay in diets for rearing Simmental crossbred calves in dryland China. *Asian-Australasian Journal of Animal Sciences,* 31, 1881. doi:10.5713/ajas.18.0089

Koenig, K. M., & Beauchemin, K. A. (2018). Effect of feeding condensed tannins in high protein finishing diets containing corn distillers grains on ruminal fermentation, nutrient digestibility, and route of nitrogen excretion in beef cattle. *Journal of Animal Science*, 96, 4398–4413. doi:10.1093/jas/sky273

Kohn, R. A., Dinneen, M. M., & Russek-Cohen, E. (2005). Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. *Journal of Animal Science,* 83, 879–889. doi:10.2527/2005.834879x

Kossila, V.L. (1984) Location and potential feed use. In: Sundstøl, F. and Owen, E. (eds) Straw and Other Fibrous By-products as Feed. Elsevier, Amsterdam, pp. 4–24.

Larbi, A., El-Moneim, A. A., Nakkoul, H., Jammal, B., & Hassan, S. (2011). Intraspecies variations in yield and quality determinants in Vicia species: 3. Common vetch (*Vicia sativa ssp. sativa* L.). *Animal Feed Science and Technology,* 164, 241–251. doi:10.1016/j.anifeedsci.2011.01.004

Lee, J. M., Woodward, S. L., Waghorn, G. C., & Clark, D. A. (2004). Methane emissions by dairy cows fed increasing proportions of white clover (*Trifolium repens*) in pasture. In *Proceedings of the New Zealand Grassland Association* (Vol. 66, pp. 151-155).

Liu, C., Qu, Y., Guo, P., Xu, C., Ma, Y., & Luo, H. (2018). Effects of dietary supplementation with alfalfa (*Medicago sativa* L.) saponins on lamb growth performance, nutrient digestibility, and plasma parameters. *Animal Feed Science and Technology*, 236, 98–106. doi:10.1016/j.anifeedsci.2017.12.006

Livestock Research Group of the Global Research Alliance (2014). Technical Manual on Respiration Chamber Designs. Edited by Cesar Pinares and Garry Waghorn. Ministry of Agriculture and Forestry Pastoral House, Wellington 6140, New Zealand.

Luo, T., Mo, B. & Long, Z. (2000). A study on the comprehensive technique of cattle raising and planting a few kinds of high quality grasses on unoccupied cropland in winter. *Sichuan Grassland*, 4, 38–40.

Mackie, R. I., Gilchrist, F. M. C., & Heath, S. (1984). An in vivo study of ruminal micro-organisms influencing lactate turnover and its contribution to volatile fatty acid production. *The Journal of Agricultural Science*, 103, 37–51. doi:10.1017/s0021859600043306

Mandell, I. B., Gullett, E. A., Wilton, J. W., Allen, O. B., & Kemp, R. A. (1998). Effects of breed and dietary energy content within breed on growth performance, carcass and chemical composition and beef quality in Hereford and Simmental steers. *Canadian Journal of Animal Science,* 78, 533–541. doi:10.4141/a97-101

Mawuenyegah, P. O., Shen, M. N., Warly, L., & Fujihara, T. (1997). Effect of supplementary feeding with protein and energy on digestion and rumination behaviour of sheep consuming straw diets. *The Journal of Agricultural Science*, 129, 479–484. doi:10.1017/s0021859697004863

McCaughey, W. P., Wittenberg, K., & Corrigan, D. (1999). Impact of pasture type on methane production by lactating beef cows. *Canadian Journal of Animal Science*, 79, 221–226. doi:10.4141/a98-107

McDonald, P., Edwards, R. A., Geenhalgh, J. F. D., & Morgan, C. A. (2002). Animal nutrition, 6th ed. Pearson Education Limited, Essex, UK.

Mc Geough, E. J., O'Kiely, P., Hart, K. J., Moloney, A. P., Boland, T. M., & Kenny, D. A. (2010). Methane emissions, feed intake, performance, digestibility, and rumen fermentation of finishing beef cattle offered whole-crop wheat silages differing in grain content. *Journal of Animal Science*, 88, 2703–2716. doi:10.2527/jas.2009-2750

McMahon, L. R., McAllister, T. A., Berg, B. P., Majak, W., Acharya, S. N., Popp, J. D., Coulman, B. E., Wang, Y., Cheng, K. J. & Cheng, K. J. (2000). A review of the effects of forage condensed tannins on ruminal fermentation and bloat in grazing cattle. *Canadian Journal of Plant Science*, 80, 469–485. doi:10.4141/p99-050

Mc Parland, S., Kearney, J. F., Rath, M., & Berry, D. P. (2007). Inbreeding trends and pedigree analysis of Irish dairy and beef cattle populations. *Journal of Animal Science,* 85, 322–331. doi:10.2527/jas.2006-367

Minson, D. J. (1990). *Forage in Ruminant Nutrition*. Academic Press, San Diego, pp. 483

Montes, F., Meinen, R., Dell, C., Rotz, A., Hristov, A. N., Oh, J., Waghorn, G., Gerber, P. J., Henderson, B., Makkar, H. P. S., & Dijkstra, J. 2013. Special topics— Mitigation of methane and nitrous oxide emissions from animal operations: II. A review of manure management mitigation options. *Journal of animal science,* 91, 5070–5094. doi:10.2527/jas.2013-6584

Moss, A. R., Jouany, J. P., & Newbold, J. (2000). Methane production by ruminants: its contribution to global warming. *Annales de Zootechnie*, 49, 231–253. doi:10.1051/animres:2000119

Mould, F. L., Ørskov, E. R., & Mann, S. O. (1983). Associative effects of mixed feeds. I. effects of type and level of supplementation and the influence of the rumen fluid pH on cellulolysis *in vivo* and dry matter digestion of various roughages. *Animal Feed Science and Technology*, 10, 15–30. doi:10.1016/0377-8401(83)90003-2

Mtimuni, J. P. (1976). The nutritive evaluation of cereal silages using growing steers and chemical methods [M.Sc.Thesis]., SK, Canada: *Department of Animal and Poultry Science,* University of Saskatchewan.

National Research Council (NRC). (2003). *Air Emissions from Animal Feeding*

Operations: Current Knowledge, Future Needs; National Academies Press: Washington, DC, USA.

Newbold, C. J., Lassalas, B., & Jouany, J. P. (1995). The importance of methanogens associated with ciliate protozoa in ruminal methane production *in vitro*. *Letters in Applied Microbiology*, 21, 230–234. doi:10.1111/j.1472-765x.1995.tb01048.x

Niederecker, K. N., Larson, J. M., Kallenbach, R. L., & Meyer, A. M. (2018). Effects of feeding stockpiled tall fescue versus summer-baled tall fescue-based hay to late gestation beef cows: I. Cow performance, maternal metabolic status, and fetal growth. *Journal of Animal Science,* 96, 4618–4632. doi:10.1093/jas/sky341

Niderkorn, V., & Baumont, R. (2009). Associative effects between forages on feed intake and digestion in ruminants. *Animal*, 3, 951–960. doi:10.1017/s1751731109004261

Nkrumah, J. D., Okine, E. K., Mathison, G. W., Schmid, K., Li, C., Basarab, J. A., Price, M. A., Wang, Z., & Moore, S. S. (2006). Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle1. *Journal of Animal Science,* 84, 145–153. doi:10.2527/2006.841145x

O’Mara, F. P. (2011). The significance of livestock as a contributor to global greenhouse gas emissions today and in the near future. *Animal Feed Science and Technology*, 166, 7–15. doi:10.1016/j.anifeedsci.2011.04.074

Osuji, P. O., & Odenyo, A. A. (1997). The role of legume forages as supplements to low quality roughages—ILRI experience. *Animal Feed Science and Technology*, 69, 27–38. doi:10.1016/s0377-8401(97)81620-3

Owen, E. & Jayasuriya, M. C. N. (1989). Use of crop residues as animal feeds in developing countries. *Research and Development in Agriculture*, 6, 129–138.

Patra, A. K. (2010). Effects of supplementing low-quality roughages with tree foliages on digestibility, nitrogen utilization and rumen characteristics in sheep: a meta-analysis. *Journal of Animal Physiology and Animal Nutrition,* 94, 338–353. doi:10.1111/j.1439-0396.2008.00914.x

Pen, B., Sar, C., Mwenya, B., & Takahashi, J. (2008). Effects of *Quillaja saponaria* extract alone or in combination with Yucca schidigera extract on ruminal fermentation and methanogenesis *in vitro*. *Animal Science Journal,* 79, 193–199.

doi:10.1111/j.1740-0929.2008.00517.x

Phillips, C. J. (2018). *Principles of cattle production*. CABI Publishing.

Prakash, B., Saha, S. K., Khate, K., Agarwal, N., Katole, S., Haque, N., & Rajkhowa, C. (2013). Rumen microbial variation and nutrient utilisation in mithun (Bos frontalis) under different feeding regimes. *Journal of Animal Physiology and Animal Nutrition*, 97, 297–304. doi:10.1111/j.1439-0396.2011.01270.x

Reddy, M. S., Ilao, R. I., & Faylon, P. S. (Eds.). (2014). *Recent advances in biofertilizers and biofungicides (PGPR) for sustainable agriculture*. Cambridge Scholars Publishing. p. 381. ISBN 978-1-4438-7105-1.

Reynolds, C. K., & Kristensen, N. B. (2008). Nitrogen recycling through the gut and the nitrogen economy of ruminants: an asynchronous symbiosis. *Journal of animal science*, 86, E293–E305. doi:10.2527/jas.2007-0475

Rihawi, S., Iñiguez, L., Knaus, W. F., Zaklouta, M., Wurzinger, M., Soelkner, J., Larbi, A., & Bomfim, M. A. D. (2010). Fattening performance of lambs of different Awassi genotypes, fed under cost-reducing diets and contrasting housing conditions. *Small Ruminant Research,* 94, 38–44. doi:10.1016/j.smallrumres.2010.06.007

Sandoval-Castro, C. A., Capetillo-Leal, C., Cetina-Gongora, R., & Ramirez-Aviles, L. (2002). A mixture simplex design to study associative effects with an in vitro gas production technique. *Animal Feed Science and Technology,* 101, 191–200. doi:10.1016/s0377-8401(02)00137-2

Shibata, M., & Terada, F. (2010). Factors affecting methane production and mitigation in ruminants. *Animal Science Journal,* 81, 2–10. doi:10.1111/j.1740-0929.2009.00687.x

Smith P., Bustamante, M., Ahammad, H., Clark, H., Dong, H., Elsiddig, E. A., Haberl, H., Harper, R., House, J., Jafari, M., Masera, O., Mbow, C., Ravindranath, N. H., Rice, C. W., Robledo Abad, C., Romanovskaya, A., Sperling, F., &Tubiello. F. (2014): Agriculture, Forestry and Other Land Use (AFOLU). In: *Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change.* Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Steinfeld, H., Gerber, P., Wassenaar, T. D., Castel, V., Rosales, M., Rosales, M., & de Haan, C. (2006). *Livestock's long shadow: environmental issues and options*. Food &

Agriculture Origination.

Suttie, J. M., & Reynolds, S. G. (Eds.). (2004). Fodder oats: a world overview (No. 33). Food & Agriculture Origination.

Titgemeyer, E. C., Spivey, K. S., Parr, S. L., Brake, D. W., & Jones, M. L. (2012). Relationship of whole body nitrogen utilization to urea kinetics in growing steers. *Journal of Animal Science,* 90, 3515–3526. doi:10.2527/jas.2011-4621

United Nations. 2003. *World Population Prospects.* The 2002 Revision. New York.

United Nations. 2004. *World population to 2300*. New York.

United Nations. 2008. *World Population Prospects*. The 2007 Revision. New York

United Nations. 2009. *World Population Prospects. The 2008 Revision.* New York

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *Journal of Dairy Science,* 74, 3583–3597. doi:10.3168/jds.s0022-0302(91)78551-2

Waldrip, H. M., Todd, R. W., & Cole, N. A. (2013). Prediction of nitrogen excretion by beef cattle: A meta-analysis. *Journal of Animal Science,* 91, 4290–4302. doi:10.2527/jas.2012-5818

Wang, C., Zhang, C., Yan, T., Chang, S., Zhu, W., Wanapat, M., & Hou, F. (2019). Increasing roughage quality by using alfalfa hay as a substitute for concentrate mitigates CH4 emissions and urinary N and ammonia excretion from dry ewes. *Journal of Animal Physiology and Animal Nutrition,* 104, 22–31. doi:10.1111/jpn.13223

Weaver, J. E. (1926). *Root Habits of Alfalfa.* In: *Root Development of Field Crops*. McGraw-Hill Book Company, New York (USA).

Win, K. S., Ueda, K., & Kondo, S. (2015). Effects of grass hay proportion in a corn silage-based diet on rumen digesta kinetics and digestibility in dairy cows. *Animal Science Journal,* 86, 833–841. doi:10.1111/asj.12365

Yan, T., Agnew, R., Gordon, F., & Porter, M. (2000). Prediction of methane energy output in dairy and beef cattle offered grass silage-based diets. *Livestock Production Science,* 64, 253–263. doi:10.1016/s0301-6226(99)00145-1

Yan, T., Frost, J. P., Keady, T. W. J., Agnew, R. E., & Mayne, C. S. (2007). Prediction of nitrogen excretion in feces and urine of beef cattle offered diets containing grass silage. *Journal of Animal Science,* 85, 1982–1989. doi:10.2527/jas.2006-408

Yan, T., Porter, M. G., & Mayne, C. S. (2009). Prediction of methane emission from beef cattle using data measured in indirect open-circuit respiration calorimeters. *Animal,* 3, 1455–1462. doi:10.1017/s175173110900473x

Zhao, Y. G., Aubry, A., O'Connell, N. E., Annett, R., & Yan, T. (2015). Effects of breed, sex, and concentrate supplementation on digestibility, enteric methane emissions, and nitrogen utilization efficiency in growing lambs offered fresh grass. *Journal of Animal Science,* 93, 5764–5773. doi:10.2527/jas.2015-9515

Zhao, Y. G., Gordon, A. W., O'Connell, N. E., & Yan, T. (2016). Nitrogen utilization efficiency and prediction of nitrogen excretion in sheep offered fresh perennial ryegrass (*Lolium perenne*). *Journal of Animal Science,* 94, 5321–5331. doi:10.2527/jas.2016-0541

Zheng, K., Han, B., Yu, H., Hou, C., & Zhang, Q. 2002. Economic characters and usage of new variety of naked oats. *Journal of Inner Mongolia Agricultural University*, 23, 61–65.

Zou, C. X., Lively, F. O., Wylie, A. R. G., & Yan, T. (2015). Estimation of the maintenance energy requirements, methane emissions and nitrogen utilization efficiency of two suckler cow genotypes. *Animal*, 10, 616–622. doi:10.1017/s1751731115002268

Summary

The stock of beef cattle and beef production are increasing globally from 21 century, however, impacts of low nitrogen (N) utilization efficiency (NUE, ratio of retained N to N intake) and high enteric methane (CH_4) emissions of beef cattle production are the main concerns for the development of ruminant feeding system. For example, more than 70% of feed nitrogen (N) is excreted (such as in feces and urine) from livestock farming into the environment, and a low NUE could contribute more ammonia emissions to the air and more manure N outputs to the soil, which could damage air quality and lead to soil nitrification and acidification. In beef cattle feeding systems, approximately 60 to 80% of total N intake (NI) was excreted in urine, which has great potential to aggravate NH_3 emissions, and only 20 to 40% was excreted in feces. The enteric CH_4 emissions from ruminants not only represent a loss (2-12%) of diet energy but could also contribute to global warming. Globally, CH_4 emissions have increased nearly 40% globally from 1970 to 2004, and they are expected to increase 60% on the basis of proportional CH_4 emissions from expected livestock populations in 2030. Dietary manipulation, such as supplementing leguminous forage, was believed to have the potential to improve NUE and/or reduce CH_4 emissions from ruminants. Therefore, the development of a diet that can improve the NUE and reduce enteric CH_4 emissions is on demand and beneficial to both the animal husbandry and global environmental challenges.

In this research, we conducted 3 experiments to investigate how legume proportion (LP) and legume species (LS) to affect body weight gain (BWG), NUE and enteric CH_4 emissions of crossbred Simmental cattle. The forage-to-concentrate ratio was fixed at 60:40 (dry matter [DM] basis) for these 3 experiments.

In experiment (Exp) 1, 16 cattle were assigned to four diets with different oat hay (OH) to alfalfa hay (AH) ratios (60:0, AH0; 52:8, AH8; 44:16, AH16; and 36:24, AH24 on DM basis of total feed supplied) in a randomized block design. Forage dry matter intake (DMI) and total DMI increased from AH0 to AH24, and they significantly differed between AH24 and AH0 ($P<0.05$). Concentrate DMI did not differ among the four diets. The OM digestibility was significantly lower in AH24 than both AH0 and AH16 ($P<0.05$) and N digestibility tended to decrease linearly ($P<0.05$), and it was significantly lower in AH24 than AH0. No differences were found for dry matter (DM) and neutral detergent fiber

(NDF) digestibilities ($P>0.05$). The BWG gradually increased from the AH0 to AH24, and it was significantly higher in AH24 than in AH0 ($P<0.05$). Fecal N (FN) output and the ratio of FN to NI increased with increasing AH proportions, whereas urinary N (UN) output, the ratio of UN to NI. The blood urea N and ruminal ammonia N concentration decreased from AH0 to AH24 linearly, and they were significantly lower in AH24 than AH0. Although there were no differences in NUE, it still increased from AH0 to AH24, and FN and UN of calves significantly differed between AH0 and AH24. FN tended to increase linearly ($P<0.05$) with increasing AH proportions and it was significantly higher in the AH24 than in the AH0 group by 38% ($P<0.05$). However, UN tended to decrease with an increase in AH proportions and it was significantly lower in the AH24 than in AH0 by only 8.3% ($P<0.05$). The total volatile fatty acid (VFA) concentrations gradually increased with increasing AH proportions. CH_4 emissions and the ratio of CH_4 energy to gross energy intake did not differ from AH0 to AH16 ($P>0.05$), whereas it was significantly higher in AH24 than in AH16 ($P<0.05$).

In Exp 2, 16 cattle were assigned to 4 diets with different OH to common vetch hay (CVH) ratios (60:0, CVH0; 50:10, CVH10; 40:20, CVH20; and 30:30, CVH30 on DM basis of total feed supplied) in a randomized block design. There were no differences in forage DMI, concentrate DMI, BWG, and total VFA concentrations ($P>0.05$) among the four diets. The DM, OM, NDF and N digestibilities had a parabolic tendency from CVH0 to CVH30, and the highest values were observed in CVH20 ($P<0.05$). The fecal N (FN) output and the ratio of FN to NI increased with increasing CVH proportions, whereas urinary N (UN) output, the ratio of UN to NI, and the ruminal ammonia N concentration gradually decreased. The blood urea N and ruminal ammonia N concentrations showed a quadratic tendency from CVH0 to CVH30 and they were significantly greater in CVH10 than CVH30. NUE gradually increased from CVH0 to CVH30 although it was not significantly different. CH_4 emissions were significantly lower in CVH30 than in CVH0 and CVH20 ($P<0.05$) and the ratio of CH_4 energy to gross energy intake was significantly lower than in CVH10 ($P<0.05$).

In Exp 3, 16 cattle were allocated to four diets with 2×2 factorial arrangement of diets (2 kinds of leguminous forages (AH and CVH); 2 levels (20% and 40%) on DM basis of total feed supplied). Forage DMI and total DMI of cattle were significantly higher when fed on CVH40 than AH20 and AH40 ($P<0.05$). But no significant differences were found in concentrate DMI under LS ($P > 0.05$). The digestibilities of DM, OM and NDF of cattle when fed on AH40 were significantly lower than AH20 ($P < 0.05$). In the CVH diet groups, only NDF digestibility was significantly lower in the CVH40 than in the CVH20 ($P < 0.05$). Both LS and LP did not significantly influence the BWG of cattle ($P > 0.05$). Although the UN output of CVH20 and CVH40 was significantly lower than in the AH40 ($P < 0.05$), they had relatively higher FN output, especially between CVH40 and AH20 ($P < 0.05$). As a consequence, the RN of cattle in CVH20 and CVH40 were significantly higher than in AH40 ($P < 0.05$). CH_4 emissions were significantly lower in CVH40 than in AH20 ($P < 0.05$).

These findings suggested that, (1) appropriate proportions of leguminous forages in the diet could reduce CH_4 emissions and minimize the impact of volatile N excretion to the environment; (2) Too high proportion of leguminous forages in the diet could reduce nutrient digestibilities whereas the degree of reduction differs between CVH and AH; (3) CVH has greater potential to minimize the negative effects of CH_4 emissions and N excretion to the environment. Therefore, an opportunity for strategic feeding containing AH (16-20%) and CVH (30-40%) to reduce the direct impact of volatile N excretion and CH_4 emissions on the environment while maintaining BWG as well as nutrient digestibilities for crossbred Simmental cattle in dryland environments.

摘要

21 世紀以降、肉用牛頭数と牛肉消費量が世界的に急増する中、肉用牛飼養における低い窒素利用効率（NUE）と高いメタン産生量とが主な課題となっている。飼料窒素（N）の 70%以上が家畜飼養から環境に排出されます（糞便や尿など)。NUE の低下は、アンモニア発生量の増加による大気汚染と、窒素を含む排泄物の増加による土壌の窒素化および酸化を引き起こす。肉牛の給餌システムでは、総 N 摂取量（NI）の約 60～80%が尿中に排泄され、NH3 排出を悪化させる可能性が高く、糞便中に排泄されるのは 20～40%のみです。メタン発生量の増加は、飼料エネルギーの浪費と温室効果を増加させる。世界的に、メタン排出量は 1970 年から 2004 年にかけて世界的にほぼ 40%増加し、2030 年に予想される家畜個体群からの比例したメタン排出量に基づいて 60%増加すると予想されます。しかし、マメ科牧草の添加による飼料成分の調整により、NUE を高めてメタン排出量を抑制することができる。メタン排出量を抑制しつつも NUE が高い飼料を調整することで、畜産業と環境問題の双方への貢献が期待される。

本研究では３つの実験を通し、肉用牛飼料中のマメ科牧草の品種と比率が、シンメンタール種交雑育成子牛の増体効果、NUE、メタン排出量に与える影響を明らかにした。すべての実験で、粗飼料と濃厚飼料の比率は 60：40 とした。

実験 1 では、乱塊法によって 16 頭の供試牛を、対照区(エンバクと濃厚飼料のみ、AH0)、乾物(DM)給与量の 8%相当量をエンバクからアルファルファ乾草(AH)に代替した区(AH8)、16%相当量を AH で代替した区（AH16)、24%相当量を AH で代替した区（AH24）の４処理区に分けた。その結果、粗飼料摂取量は、AH0 から AH24 まで増加、AH24 においては AH0 より有意に高かった。濃厚飼料摂取量は４処理区で有意差がなかった。AH24 の有機物（OM）消化率は AH0 と AH16 より有意に高かった。N 消化率は直線的に減少する傾向があり、AH24 は AH0 よりも有意に低かった。DM 消化率と中性デタージェント繊維（NDF）の消化率は処理区間で差がなかった。増体効果は AH0 から AH24 まで増加し、AH24 は AH0 より有意に高かった。糞中窒素排泄量（FN）と、窒素摂取量（NI）に対する FN の比率は、飼料中の AH 比率の増加とともに増加したが、尿中窒素排泄量（UN)、NI に対する UN の比率、および第一胃液中のアンモニア N 濃度は徐々に減少した。AH0 から AH24 まで NUE は増加したが、増加量には有意差がなかった。FN と UN は、AH0 と AH24 の間で有意に異なっていました。FN は AH 比率の増加に伴って直線的に増加する傾向があり、AH0 グループよりも AH24 グループの方が 38%有意に高かった。しかし、国連は AH 比率の増加とともに減少する傾向があり、AH0 グループよりも AH24 グループの方が 8.3%だけ有意に低かった。第一胃液中の揮発性脂肪酸濃度（VFA）は、飼料中 AH 比率の増加とともに増加した。メタン産生量と、総エネルギー摂

取量に対するメタン中のエネルギーの比率は、AH0からAH16まで有意差がないが、AH24のメタン産生量はAH16より有意に高かった。

実験２では、乱塊法により16頭の供試牛を、対照区（エンバクと濃厚飼料のみ、CVH0）、DM給与量の10%相当量をエンバクからヤハズエンドウ（CVH）に代替した区（CVH10）、20%相当量をCVHで代替した区（CVH20）、30%相当量をCVHで代替した区（CVH30）の４処理区に分けた。その結果、粗飼料摂取量と濃厚飼料摂取量、増体量、VFAは処理区間で有意差がなかった。OM、DM、NDFの各消化率は、CVH0からCVH30 まで２次曲線的に変化し、CVH20で最高値となった。FNと、NIに対するFNの比率は、試料中CVH比率の増加とともに増加したが、UN、NIに対するUNの比率、および第一胃液中のアンモニアN濃度は徐々に減少した。血中尿素Nおよび第一胃アンモニアN濃度は、CVH0からCVH30への二次傾向を示し、CVH30よりもCVH10で有意に高かったCVH0からCVH30までNUEは増加したが、増加量には有意差がなかった。CVH30では、メタン産生量がCVH0とCVH20より有意に高く、総エネルギー摂取量に対するメタン中のエネルギーの比率はCVH10より有意に低かった。

実験３では、マメ科牧草品種（LS）及び同牧草の給与割合（LP）による2因子2水準の実験計画により、16頭の供試牛を、給与量の20%相当量をエンバクからCVHに代替した区（CVH20）、40%相当量をCVHで代替した区（CVH40）、20%相当量をAHで代替した区（AH20）、40%相当量をAHで代替した区（AH40）の４処理区に分けた。その結果、CVH40での総摂取量と粗飼料摂取量はAH20とAH40 より有意に高かったが、濃厚飼料摂取量は、LSによる違いがなかった。AH40 のDM消化率、OM消化率、NDF消化率はAH20 より有意に低かった。CVHの処理区内では、CVH40 でのNDF消化率がCVH20より有意に低かった。LSとLPはともに、供試牛の増体効果に影響しなかった。CVH処理区のUNはAH40より少なかったが、FNは高く、AH40 とCVH40 の間での差異がとくに顕著であった。CVH処理区の窒素蓄積量はAH40より高く、CVH40 のメタン産生量はAH20 より低かった。

本研究結果から、⑴ 飼料への適量のマメ科牧草の添加により、メタンと揮発性窒素の排出量を抑制し、環境への影響を軽減させられること、⑵ 高い比率での飼料へのマメ科牧草の添加は消化率を低下させるが、AHとCVHでは低下の程度が異なること、⑶ メタンと窒素の排出量を低減させる効果はAHよりもCVHが高いこと　が明らかになった。したがって、給与量の16%－20%をAHで、または30%－40%をCVHで代替することで、乾草地におけるシンメンタール牛の消化率と増体効果を維持しながら揮発性窒素とメタンの排出量を減らし、環境への負荷を軽減させることができると考えられた。

List of Publications

Du, W., Hou, F., Tsunekawa, A., Kobayashi, N., Ichinohe, T., & Peng, F. (2019). Effects of the Diet Inclusion of Common Vetch Hay Versus Alfalfa Hay on the Body Weight Gain, Nitrogen Utilization Efficiency, Energy Balance, and Enteric Methane Emissions of Crossbred Simmental Cattle. *Animals*, *9*(11), 983. (Published, this article covers Chapter 4)

Du, W., Hou, F., Tsunekawa, A., Kobayashi, N., Peng, F., & Ichinohe, T. (2019). Substitution of leguminous forage for oat hay improves nitrogen utilization efficiency of crossbred Simmental calves. *Journal of Animal Physiology and Animal Nutrition*. (Accepted, this article covers Chapter 3)