

**Utilization of alfalfa hay diets
for confined Simmental crossbred calf**

(舎飼いシンメンタール種交雑子牛に対する
アルファルファ乾草飼料の活用)

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2018

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Abbreviations

ADL	acid detergent lignin
AH	alfalfa hay
BHBA	β -hydroxybutylic acid
BUN	blood urea nitrogen
BW	body weight
C	concentrate feed (used for the trials in this study)
CH ₄	methane
CO ₂	carbon dioxide
CP	crude protein
CS	corn stover
CTRL	control feeding
DCP	digestible crude protein
DE	digestible energy
DG	daily body weight gain
DM	dry matter
DMI	dry matter intake
EBW	empty body weight
EE	dietary fat as ether extract
ERDP	effective rumen degradable protein
FCR	feed conversion ratio
FME	fermentable metabolizable energy
GE	gross energy
GHG	greenhouse gas
HA	high-level alfalfa hay feeding
HP	heat production
LA	low-level alfalfa hay feeding
MA	medium-level alfalfa hay feeding
MBS	metabolic body size
MT	metric ton
NDFom	ash-free neutral detergent fiber
MCP	microbial crude protein
ME	metabolizable energy
MEI	metabolizable energy intake
ME _m	metabolizable energy for maintenance

MP	metabolizable protein
N	nitrogen
NE	net energy
NEFA	non-esterified fatty acid
NE	net energy
NEI	net energy intake
NEmf	net energy for maintenance and fattening
OM	organic matter
RDP	rumen degradable protein
RE	retained energy
SEM	standard error of the mean
T1	trial 1
T2	trial 2
TDN	total digestible nutrients
UDP	digestible undegradable protein

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Chapter 1

General introduction

1.1. Background

1.1.1. Importance of effective feeding of beef cattle

Beef cattle production and consumption are increasing globally (FAO 2015) (Figure 1). Increased beef consumption within a country coincides with its economic growth (Tilman *et al.* 2011, Kastner *et al.* 2012, Delgado 2003) along with population expansion and urbanization (FAO 2009). More generally, increased consumption of animal products tends to reflect an increase in GDP per capita (FAO 2009). According to a projection by Rosegrant and Thornton (2008), under a business-as-usual scenario, the annual global per capita demand for meat will increase by an amount ranging between 6 kg and 23 kg in 2050 compared with the meat demand in 2000. Moreover, by 2050, this shift toward a greater demand for animal products is expected to encompass an estimated 40% of the world's population (Tilman *et al.* 2011, Cassidy *et al.* 2013).

The calories (energy) used until the time of consumption within the food system are much greater for animal products, including beef, than for plant-based diets (Rask and Rask 2011). The calorie-based conversion ratio of feed to animal products is about 10% (Rask and Rask 2011, Godfray *et al.* 2010), with the ratio for beef (3%) being considerably lower than that of other animal products such as pork (10%) and chicken (12%) (Smil 2000, Cassidy *et al.* 2013). Though utilization of waste products and crop residues is being promoted (Delgado 1999), the proportion of cereal consumption as animal feed has been steadily increasing (Naylor *et al.* 2005).

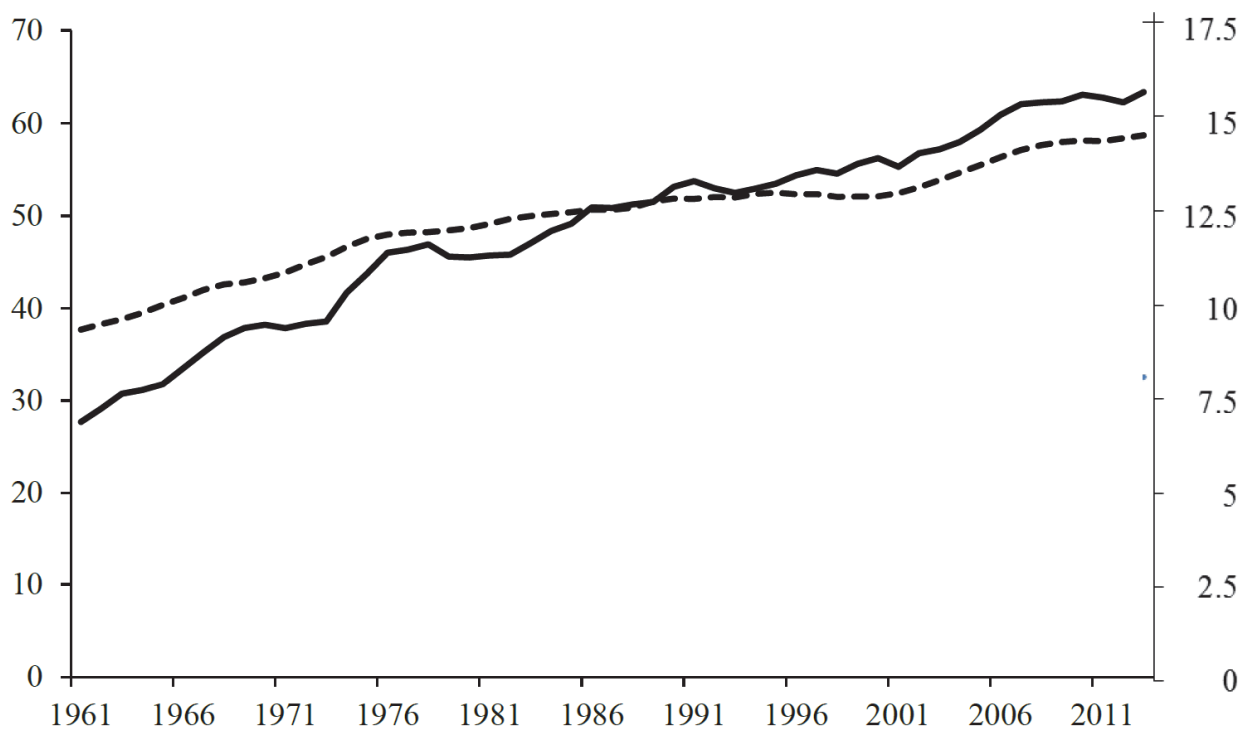


Figure 1 Beef production and number of cattle, worldwide, 1961–2013. The solid line depicts beef production (million MT), referring to the left scale; the dotted line depicts the number of cattle (100 million heads), referring to the right scale. Source: FAO (2015).

The increase in beef cattle’s feed consumption resulting from a shift in dietary preferences toward animal products may induce competition relating to the use of croplands for the cultivation of human food (FAO 2009). More efficient feeding of beef cattle is thus required in anticipation of a possible shortage of food and feed.

Studies have found that cattle are a major source of GHG emissions globally. Eighteen percent of the total GHG emissions worldwide reportedly originate from livestock production (Steinfeld *et al.* 2006). Most of the GHG emissions associated with livestock production comprise CH₄ released from enteric fermentation in livestock (Gerber *et al.* 2013). In fact, 77% of enteric CH₄ production is attributed to cattle (Steinfeld *et al.* 2006). The energy value of CH₄ is 55.5 kJ g⁻¹, and the CH₄ emission accounts for 5.5–7.5% of ingested energy in ruminants (IPCC 2006). Therefore a reduction in CH₄ emissions will

contribute to greater efficiency of the dietary energy utilization of cattle. Improvements in cattle feed that can alleviate the negative effects of GHG emissions are also required.

1.1.2. Global importance of Chinese beef production

Because of 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 (FAO 2015) (Figures 2 and 3). These increases in China, which are dramatic compared with increasing trends in other countries, is probably attributed to the country's vast population, which accounted for 19% of the world's population in 2015 (UN 2016). China is now the third largest beef producing country after the United States and Brazil (FAO 2015) (Figure 2). The annual increase in beef consumption in China is estimated to reach 3.1% by 2020 (Delgado 2003).

A consequent increase in the demand for animal feed has resulted in annual increases in quantities of imported concentrate feed ingredients and forages in China (MAFF 2013). The main feed ingredients used in China are corn (as an energy source) and soybean (as a protein source) (MAFF 2013). The corn accounts for approximately 90% of the domestic feed grain consumed in the country (Jiang 2013). In 2010, China imported more than one million MT of corn following a 15-year period of virtual self-sufficiency in corn production (Shimizu 2011) (Figure 4). It is estimated that corn consumption will increase to 193–226 million MT by 2020, mostly because of the increased demand for feed (MAFF 2013, Zhang 2012). An additional supply of more than 50 million MT of corn will be required (MAFF 2013). From the 1990s, China's soybean imports have also evidenced a rapidly increasing trend (Figure 5).

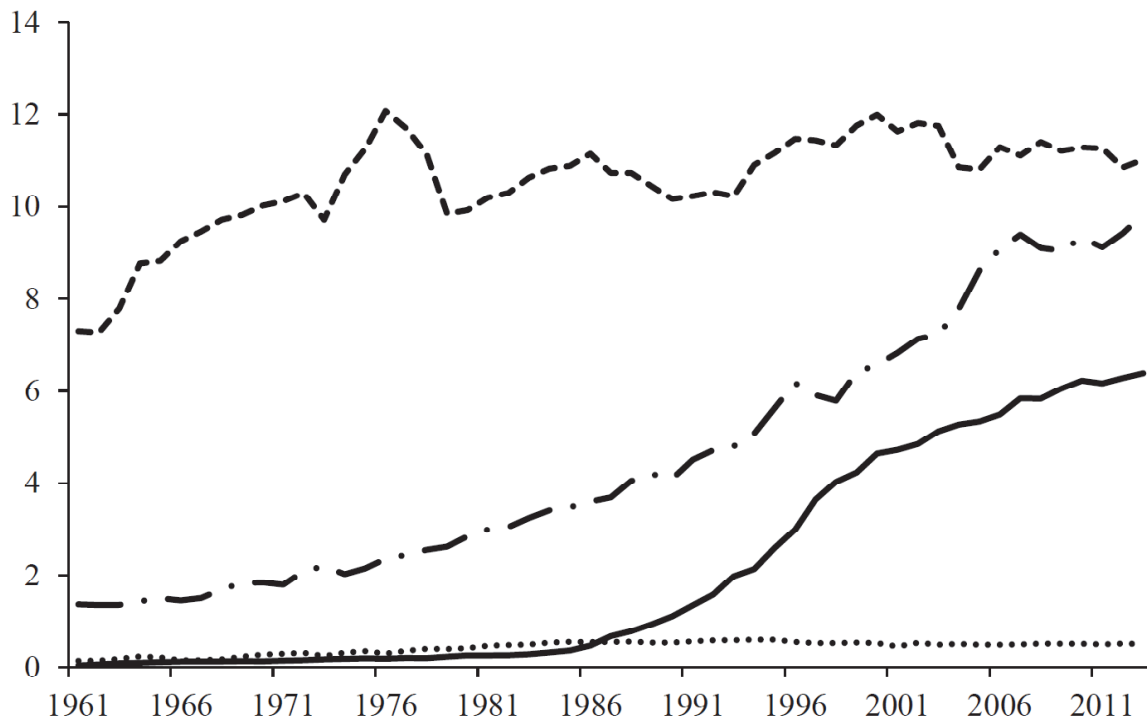


Figure 2 Beef production in major beef-producing countries, 1961–2013 (million MT). Beef production in China, the United States, Brazil, and Japan are depicted by a solid line, broken line, chained line, and dotted line, respectively. Source: FAO (2015).

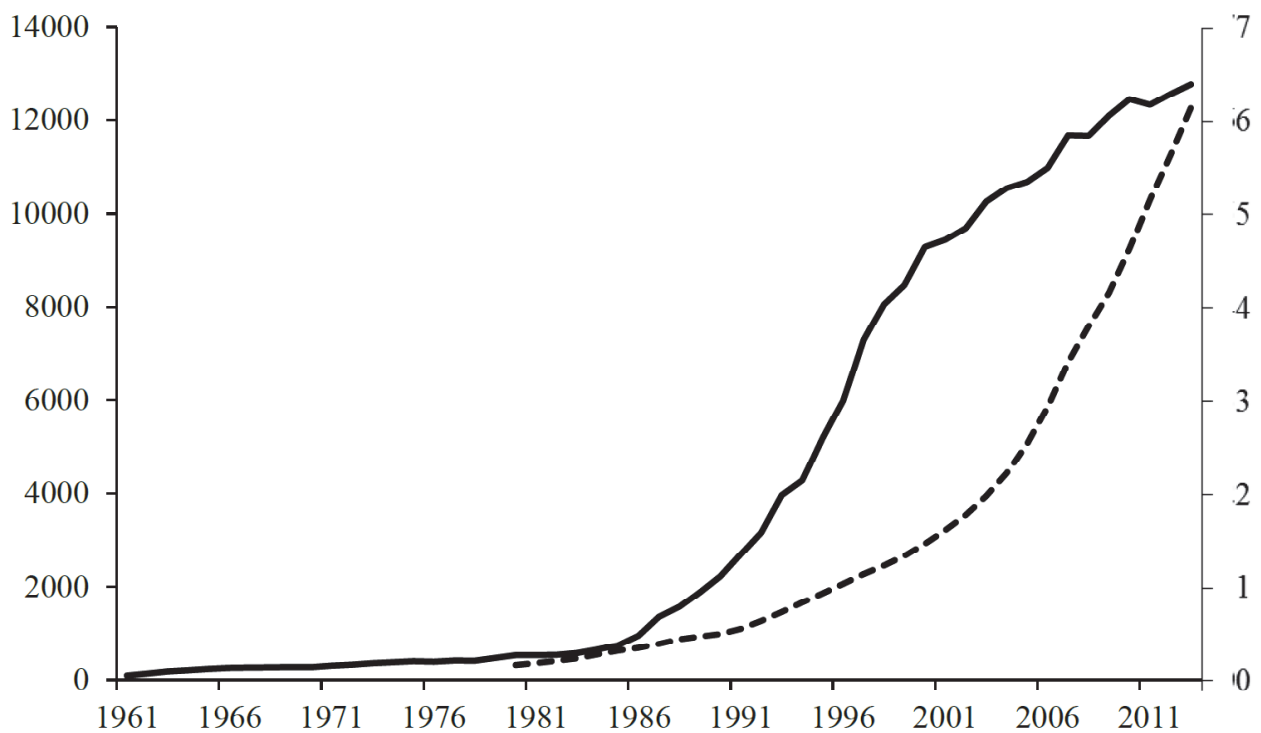


Figure 3 Beef production and GDP per capita in China, 1961–2013. The scale on the right refers to beef production (in million MT), depicted by a solid line; the scale on the left refers to GDP per capita (purchasing power parity in US\$), depicted by a dotted line. Sources: FAO (2015) and IMF World Economic Databases (2017).

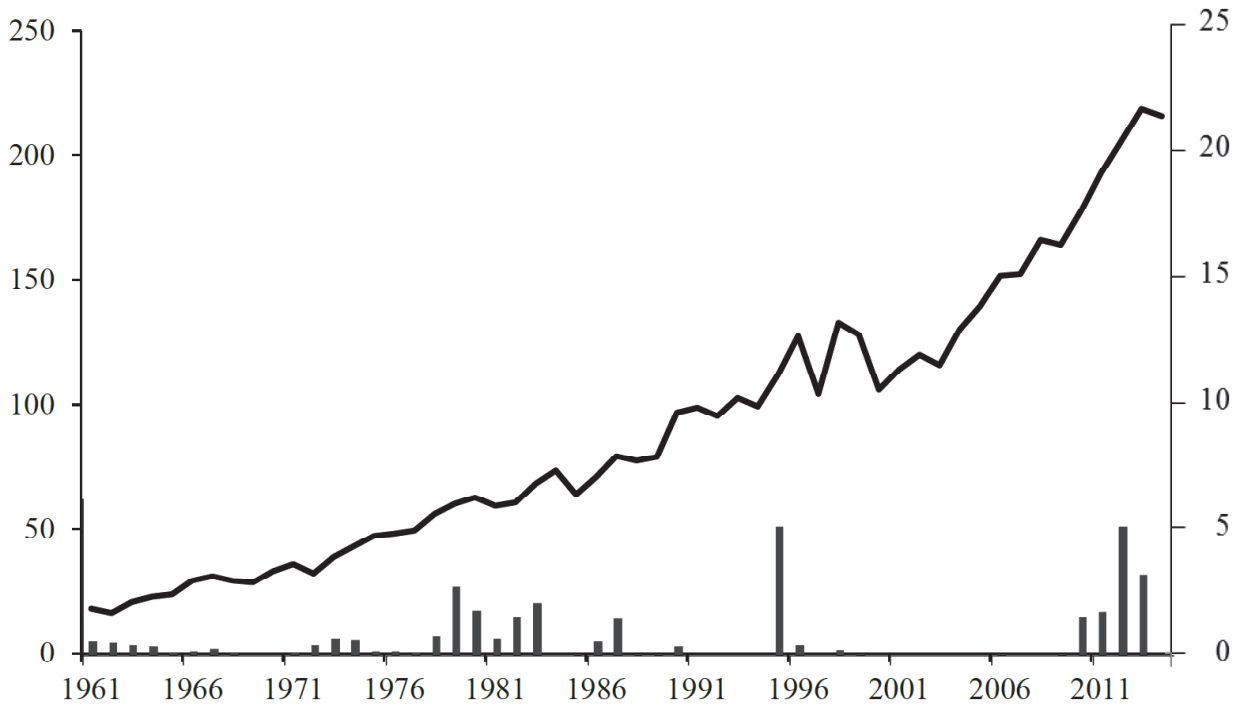


Figure 4 China's corn production and imports (million MT), 1961–2014. The scale on the left refers to production, depicted by a line; the scale on the right refers to imports, depicted by a bar. Source: FAO (2015).

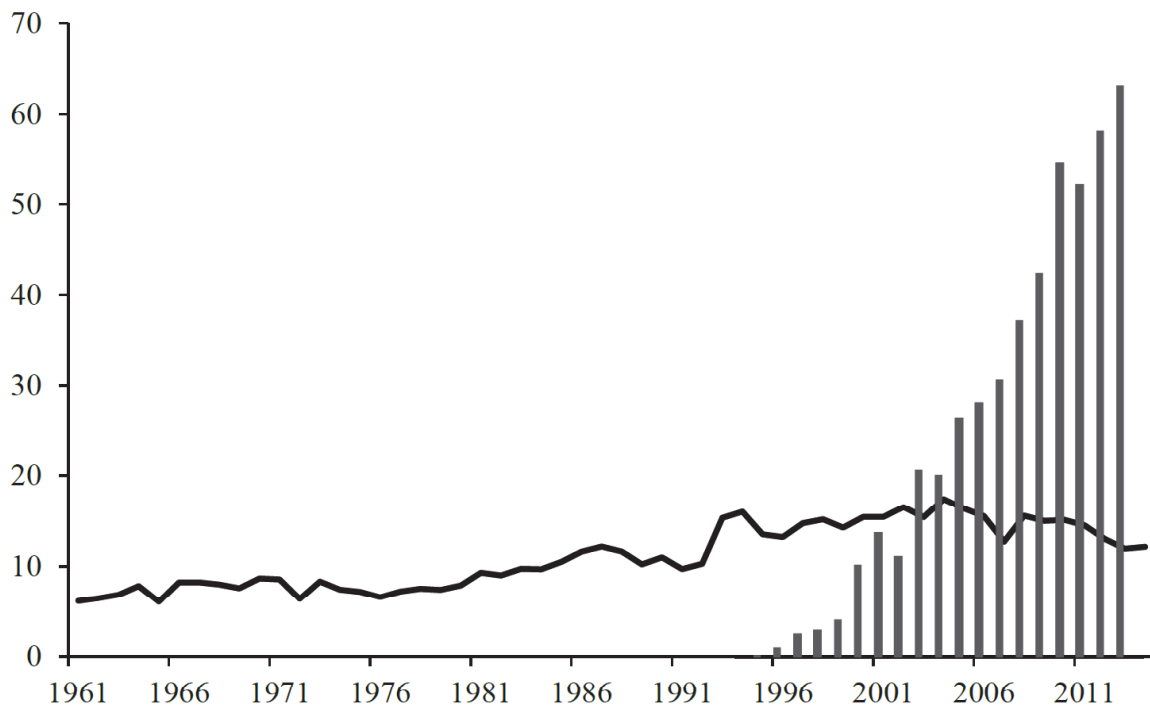


Figure 5 China's soybean production and imports, 1961–2014 (million MT). Production is depicted by a line, and imports are depicted by a bar. Source: FAO (2015).

In 2002, the quantity of imports exceeded that of domestic production, and in 2009, 50% of soybean exports in the global market were purchased by China (Shimizu 2011). In the latter year, China's sufficiency rate for soybean declined to 26%. Moreover, the Chinese government is promoting large-scale livestock management (Chinese State Committee for Development and Reform 2013) that requires more concentrate feed such as corn grain and soybean cakes. The consumption trend for these feed ingredients in China appears to be influencing their international prices.

1.1.3. Importance of foraging systems with locally produced roughage in Gansu Province

In 2013, the Chinese government prioritized eight western provinces along with three northeastern provinces and the provinces of Hebei, Henan, and Shandong as beef producing provinces in the country (Figure 7) (Chinese State Committee for Development and Reform 2013).



Figure 6 Map depicting the locations of Gansu Province and Linze Research Station in Lanzhou University along with the eight provinces in Western China that have been prioritized for beef production.

Within Western China, Gansu Province is one of the main beef producing provinces (National Bureau of Statistics of China 2014) (Table 1). Most of the land area of Gansu Province consists of typical drylands (Geng *et al.* 2014). Many of China’s dryland farmers traditionally graze livestock, utilizing limited resources available within expansive areas of dryland. Such extensive livestock grazing was concentrated in China’s western provinces, including Gansu Province (Hu and Zhang 2003). However, in Gansu Province, as in other provinces in China, livestock grazing has been restrained to prevent the desertification of natural pastures in accordance with the “restore agricultural land to forest and pasture” directive, which was issued in 2003 (Han *et al.* 2008). Raising beef cattle in pens or feedlots without grazing appears to be a common practice in the Province.

Table 1 Total meat and beef production in the prioritized provinces in China in 2012

Item	Total meat production, thousand MT	Beef production, thousand MT
Western region [†]		
Inner Mongolia 內蒙古	2458	512
Sichuan 四川	6702	293
Yunnan 雲南	3487	319
Tibet 西藏	252	151
Gansu 甘肅	878	167
Qinghai 青海	305	96
Ningxia 寧夏	265	79
Xinjiang 新疆	1342	362
North-eastern region		
Liaoning 遼寧	4187	432
Jilin 吉林	2600	450
Heilongjiang 黑龍江	2162	397
Others		
Hubei 河北	4429	553
Hunan 河南	6774	804
Shandong 山東	7642	670

[†] Provinces in western regions prioritized for beef production.

Source: National Bureau of Statistics of China (2014).

The average rural income within this province is the lowest compared with rural incomes in other Chinese provinces (National Bureau of Statistics of China 2014), and its human development index is also the lowest in the country, with the exceptions of those of Tibet and the provinces of Guizhou and Yunnan (UNDP China 2016) (Table 2).

Table 2 Average annual net incomes per capita in rural areas and a comparison of the human development index of Gansu Province with those of other provinces in 2013

Item	Annual net income per capita in 2013, yuan year ⁻¹	Human development index in 2014
National average	8895.9	0.754
Beijing 北京	18337.5	0.869
Shanghai 上海	19595.0	0.852
Western region [†]		
Inner Mongolia 内蒙古	8595.7	0.766
Sichuan 四川	7895.3	0.720
Yunnan 雲南	6141.3	0.668
Tibet 西藏	6578.2	0.600
Gansu 甘肅	5107.8	0.689
Qinghai 青海	6196.4	0.694
Ningxia 寧夏	6931.0	0.727
Xinjiang 新疆	7296.5	0.718

[†] Provinces in western regions prioritized for beef production.

Sources: National Bureau of Statistics of China (2014) and UNDP China (2016).

Agricultural by-products (such as CS) and concentrate feed are commonly used to feed beef cattle. Farmers in this province are not generally self-sufficient in their production of concentrate. According to farmers in Gansu Province who were interviewed prior to initiating this study in 2014, the expense associated with the purchase of concentrate feed was responsible for the increase in the feeding cost, which was one of the major issues reported by farmers. Less than half of the interviewed farm households ($n = 13$) used concentrate as a feed ingredient; the remaining households used only CS to feed their cattle.

It is therefore necessary to develop foraging systems for confined beef cattle in Gansu that simultaneously provide for maximum utilization of roughage produced on farm and high-level production performance. Such systems will contribute to the enhancement of farmers' livelihoods, and to the development of beef cattle production models based on diets of harvested roughage that could potentially be applied more broadly in dryland areas of Western China. Moreover, the data acquired in the course of developing the systems will be beneficial for improving actual feeding standards for beef cattle in China, some of which refer to the standards applied in other countries because of the limited number of available *in situ* studies.

1.2. A review of the related literature

1.2.1. Effects of using alfalfa as a feed for confined beef cattle

1.2.1.1. Compatibility of alfalfa with concentrate feed

Alfalfa (*Medicago sativa*) is a legume used for livestock forage and widely cultivated all over the world because of its resistance to drought and cold weather (Michaud *et al.* 1988, McKenzie *et al.* 1988). The area under alfalfa cultivation is estimated to be 320,000 km² worldwide (Michaud *et al.* 1988; Bouton 2001, 2012). Alfalfa's tolerance to drought is thought to be the result of the root's ability to extend straight down into the soil to a depth of 6 m or more (Weaver 1926). The amount of water consumed in the production of the alfalfa seems comparable with that for other major forage crops (such as corn). It has been reported that a volume of 628–678 m³ of water are required to produce 1 ton of alfalfa (on a DM basis) (Bauder *et al.* 1978, Mubako and Lant 2013), while the volume of water required to produce 1 ton of corn grain (on a DM basis) exceeds 500 m³ (Fader *et al.* 2010). Water consumption is an important factor to consider when assessing the feasibility of

producing alfalfa as feed for beef cattle in dryland areas, which are extensive in Gansu Province, China, as they are worldwide.

Alfalfa is a preferred forage species for feeding ruminants compared with other forage species, because of its rapid passage through the gastrointestinal tract and its provision of a large amount of soluble protein readily utilizable by ruminal microorganisms (ruminal degradable protein) for their protein synthesis (Waldo and Jorgensen 1981; Conrad and Klopfenstein 1988). The chemical composition and ME concentration of alfalfa compared with those of other feed ingredients based on standard values derived from published tables of feed composition, are shown in Table 3. Compared with other major crops used for forage, whether hay or green, alfalfa contains more protein and less fiber (as NDFom), whereas its energy concentration seems comparable. It has been used as a protein source for ruminants because of its higher N concentration compared with that of poaceous crops (Baumont *et al.* 2016; Delaby *et al.* 2016). However, alfalfa contains less protein and relatively more fiber than ingredients of concentrate feed such as oil seed meal. The energy concentration in alfalfa does not exceed that of ingredients of concentrate feed. When exploring the potential use of alfalfa as a substitute for concentrate feed, the economic feasibility as well as its nutrient concentration requires consideration.

Nutritious or high-quality alfalfa is highly palatable and appealing to animals. Heifers fed on AH with the inclusion of 8% of DM did not show any preference for the concentrate, whereas those fed on barley straw clearly showed such a preference (Madruga *et al.* 2017). It is worth conducting trials to improve the digestibility of alfalfa, which varies greatly according to harvesting times and parts used (leaves or stems) (Lacefield 2004). The rich nutritional qualities of alfalfa can be partly attributed to its ability to absorb soil nutrients through its deeply extended roots and to symbiosis with rhizobia that provides nitrogenous

nutrients through the roots (Vance *et al.* 1988). As the rhizobia remain in the soil after harvesting and continue to enable N fixation, alfalfa cultivation contributes to soil fertilization. The use of alfalfa cultivated as livestock feed may be appropriate and feasible.

Oba and Allen (2003) reported significant differences in DMI between groups of feed with high and low starch content (*i.e.*, feeds with high and low concentrate content) when fed to Holstein cows. In another study involving Holstein cows, McCarthy *et al.* (1989) reported differences in ruminal fermentation and in the passage of nutrients relating to various protein and carbohydrate sources. In a study of steers crossbred with Angus, Hereford, and Gelbvieh breeds, Hales *et al.* (2014) evaluated the effects of incorporating AH into a concentrate-based diet on energy metabolism, and reported possible improvements achieved by minimizing the amount of dietary roughage. An increase in the ratio of concentrate in the animals' diet reportedly caused the digestion coefficients of DM and GE to rise, leading to increased concentrations of propionic and butyric acids and decreased concentrations of acetic acid in their rumens (Cowsert and Montgomery 1969, Montgomery and Baumgardt 1965, Coppock *et al.* 1964, Brown *et al.* 1962, Elliot and Loosli 1959). By contrast, high-concentrate and low-roughage diets may cause ruminal acidosis, which is a risk factor for feedlot cattle because of the high rate of decomposition by microbes in their rumens (González *et al.* 2012). Low-level inclusion of roughage in high-concentrate diets helps to increase the DMI and energy intake (Galyean and Defoor 2003). The typical inclusion level of alfalfa (as forage) for feedlot finishing diets with corn silage is 8–10% (Samuelson *et al.* 2016; Vasconcelos and Galyean 2007), and the recommended amount of forage (bromegrass hay and corn silage) to be included in high-concentrate finishing diets is less than 15% (Swanson *et al.* 2017).

Table 3 Chemical compositions and metabolizable energy concentrations of alfalfa and other referenced feed ingredients

Category	Feed ingredients and condition	Chemical composition, % DM		ME, kJ g ⁻¹ DM
		CP	NDFom	
Forage fed green	Alfalfa, 1st-cut, late vegetative	26.2	33.3	10.52
Forage fed green	Red clover, 1st-cut, late vegetative	21.9	37.4	11.08
Forage fed green	Common vetch, bloom	18.3	–	9.74
Forage fed green	Orchardgrass, 1st-cut, late vegetative	18.3	–	9.74
Forage fed green	Oats, late vegetative	17.6	53.4	10.76
Hay	Orchardgrass, 1st-cut, late vegetative	16.5	49.6	10.51
Hay	Alfalfa, 1st-cut, late vegetative	21.8	36.0	9.04
Hay	Red clover, 1st-cut, bloom	15.4	45.9	8.88
Hay	Corn stover	5.8	65.5	9.03
Concentrate ingredient	Corn grain	8.8	12.5	15.16
Concentrate ingredient	Wheat bran	18.1	42.7	11.39
Concentrate ingredient	Soybean meal	51.1	15.5	14.00
Concentrate ingredient	Sunflower meal	35.6	–	7.26
Concentrate ingredient	Rape seed meal	42.3	27.2	11.80
Concentrate ingredient	Cotton seed meal	40.0	36.3	10.18

CP, crude protein; DM, dry matter; ME, metabolizable energy; NDFom, ash-free neutral detergent fiber; – signifies no data.

Source: NARO (2010).

The ratio of roughage (*i.e.*, alfalfa, CS, and others) to concentrate appears to be an important factor in designing a feed mixture for beef cattle and should be given due consideration.

1.2.1.2. Production of alfalfa and its substitutability for concentrates in Gansu Province

Rong *et al.* (2004) reported that alfalfa demonstrates a high degree of drought tolerance because of its long roots extending into the soil, and that there is strong potential for increasing the area under alfalfa cultivation because of its high growth levels in summer and its nutritional value. Studies have also pointed to its comparatively low requirement for water, strong resistance to pests/diseases, and the economic returns for farmers resulting from its cultivation (Guo *et al.* 2007). There has been an expansion of the area under alfalfa cultivation in Huanxian County in Gansu Province, which in 2006 accounted for 28% of the Province's entire area of irrigated farmland located in the Yellow River Basin (Hou *et al.* 2008). The volume of water consumed to produce 1 ton of alfalfa (on a DM basis) in the Province was reported to be 613–627 m³ (Gao *et al.* 2016, Ma and Xu 1998), which is a reasonable range compared to that for other crops (see section 1.2.1.1).

As revealed in interviews conducted with farmers in Gansu Province in 2014, concentrate cannot be utilized in the required quantities because of its high cost. Therefore commercial concentrate is mixed with other suitable ingredients such as cereal grains and husks. Because alfalfa is grown by farmers in Gansu province to feed their animals, it can be substituted for expensive commercial concentrates, and its utilization could consequently reduce the cost of beef cattle feeding in the Province. The utilization of alfalfa as a feed ingredient for confined beef cattle in Gansu Province is thus worth examining.

1.2.1.3. Risks of using alfalfa feed

Rumen gas formation (bloating), which is a life-threatening disease for ruminants, is caused by very high solubility of proteins that produce stable gas-trapping foam in the rumen (Pinchak *et al.* 2005). Referring to earlier studies conducted by Van Keuren and Matches (1988), Thompson and Paull (1990), and Howarth *et al.* (1991), Majak *et al.* (1995) discussed various hazards associated with bloat-inducing legumes such as alfalfa. Thompson *et al.* (2000) subsequently reported that incidences of frothy bloat in cattle were more frequent when the consumed alfalfa was harvested during the vegetative growth stage rather than during the budding and blooming stages of growth. By contrast, Veira *et al.* (2009) reported a reduction in incidences of frothy bloat when Jersey steers were grazed in pastures with alfalfa and orchardgrass. Supplementary feeding with alfalfa along with other roughage-based diets appeared to prevent the risk of bloat caused by exclusive alfalfa feeding.

The emission of enteric CH₄ is significantly associated with the constituents of animals' diets (Johnson and Johnson 1995, Boadi *et al.* 2004, Monteny *et al.* 2006, Banik *et al.* 2013). It has been reported that with increased levels of AH used in cattle feedlots, CH₄ and heat production associated with AH digestion correspondingly increase (Hales *et al.* 2014). Conversely, with improved digestibility of dietary intake, CH₄ production in the rumen decreases and is lower in animals that are fed grain-based diets than in those that are fed fibrous diets (Moss *et al.* 1995). Mixing fibrous diets such as those that include AH with grain-based diets appear to increase CH₄ emissions. However, CH₄ emissions from animals grazing pasture legumes (such as alfalfa) are lower than those from animals grazing pasture grasses (McCaughey *et al.* 1999; Waghorn *et al.* 2002; Chaves *et al.* 2006; Dorland *et al.* 2007; Archimede *et al.* 2011; Banik *et al.* 2013). The effects of a leguminous fibrous diet

that includes alfalfa on CH₄ production should be therefore carefully analyzed to determine whether substituting alfalfa for concentrate would have any negative impacts on GHG emissions.

Excessive use of alfalfa in feed could result in increased absorption of NH₃ through the rumen wall, inducing urinary excretion of N. Consequently, N retention and DG are reduced, especially when animals' diets are energy-deficient (Satter and Roffler 1975).

1.2.2. Studies on Simmental beef cattle in China

About 80% of beef cattle in China are the outcome of crossbreeding various foreign breeds with local breeds (such as Yellow Cattle), with the remaining 20% of these cattle comprising local breeds. Around half of the crossbred beef cattle are the progeny of Simmental mated with local breeds. Simmental has been promoted nationwide as an appropriate breed for milk as well as beef production, because of its tolerance of poor quality diets that are not well-formulated and the quantities of milk and beef that the breed provides (Kinoshita and Nishimura 2015). Simmental crossbreds are the main variety of beef cattle raised by farmers in Gansu Province. Several studies entailing feeding trials have been conducted with these cattle to examine their energy and N utilization. Li (2014) reported a significant increase in DG and a reduction in feeding costs per 1-kg DG for crossbred Simmental bulls that were fed alfalfa, CS, and concentrate compared with those that were fed only CS and concentrate, in Qinghai Province, China. In studies using Simmental steers, Liu *et al.* (2009) and Wang *et al.* (2009) reported on the effects of selenium methionine and malic acid on rumen fermentation. Wang *et al.* (2008) examined the effects of copper methionine on the physicochemical parameters of Simmental steers. These studies aimed to examine the effects of feeding regimens or additives on the status of

energy utilization and DG based on feeding trials, and provided a basic metabolic profile of Simmental crossbreds. However, as environmental and other conditions affecting livestock are difficult to control for within experiments, data on the energy utilization status of Simmental beef cattle in Gansu Province, obtained through *in situ* and *in vivo* studies, were needed to formulate an optimal feeding design for these cattle in the Province.

1.2.3. Studies for assessing energy utilization of cattle

Several feeding balance trials have been conducted to obtain a better understanding of the basic energy utilization of beef cattle. In a study involving Zebu (*Bos indicus*) and Brown Swiss (*Bos Taurus*) cows, Jose *et al.* (2010) reported differences in MEm and in energetic efficiency of BW gain. Tedeschi *et al.* (2002) obtained MEm values for Nellore (*Bos indicus*) bulls and steers. Based on feeding trials, Liu *et al.* (2013) calculated the MEm for Xiangzhong Black cattle using a linear multiple regression equations between DG, MBS (as explanatory variables), and MEI (as objective variable). Qin *et al.* (2011) evaluated the fasting metabolism of growing female water buffaloes. Douglas *et al.* (2005) estimated MEm values based on comparative data obtained from eight studies conducted on Nellore, Holstein, Gir, Guzerat, and Tabapuan breeds, and their crossbred progeny with Zebu, Angus, and Simmental breeds (all males, $n = 320$) after their slaughter. However, the application of the findings of these studies for feeding practices has not been sufficiently explored. There is therefore a need to conduct feeding trials in combination with studies on metabolism (*i.e.*, energy utilization status, etc.), which are unprecedented in Gansu Province and are now anticipated. Such feeding studies conducted with locally available feeds would facilitate the upgrading of existing Chinese feeding standards for beef cattle (MOA 2004).

It is possible to conduct a detailed analysis of the energy utilization status of cattle by measuring their basic respiration. MEI can be estimated by subtracting the energy released as exhaled gases (CO₂ and CH₄) measured through respiration trials and the energy excreted as urine from the value of DE intake, which is calculated as the GE intake from feed minus the energy excreted as feces. The MEm can be calculated by obtaining the estimated HP of several cattle and their MEI. The HP is calculated from the daily CO₂ and CH₄ production and the daily O₂ consumption, measured through respiration trials, using the following equation formulated by Brower (1965):

$$\text{HP (kJ day}^{-1}\text{)} = 16.18 \times \text{O}_2 \text{ consumption (L day}^{-1}\text{)} + 5.02 \times \text{CO}_2 \text{ production (L day}^{-1}\text{)} \\ - 2.17 \times \text{CH}_4 \text{ production (L day}^{-1}\text{)} - 5.99 \times \text{urinary N excreted (g day}^{-1}\text{)}$$

The MEm was estimated using a linear regression equation between NEI (obtained by subtracting HP from MEI) and MEI, both of which were based on MBS (kg^{0.75} BW), as presented by Freetly *et al.* (2006).

Various devices can be used to measure respiration for obtaining HP. In a confined system, the composition of gases and their differences are measured in a closed animal respiration chamber before and after conducting the trial. In this closed-circuit system, the chamber is continuously supplied with O₂ while these measurements are being taken. The O₂ consumption is calculated from the volume of supplied O₂ and the difference between the amount of O₂ in the chamber before and after the trial. Exhaled CO₂ is absorbed by the collecting equipment. Production of CO₂ is calculated as the difference between the CO₂ concentrations before and after the trial, and the volume absorbed by the equipment. In an open-circuit system, air from outside always circulates in the chamber. The outflow of air from the chamber and the composition of gases (O₂, CO₂ and CH₄) included in the chamber's inflow and outflow air are measured. Then O₂ consumption as well as CO₂ and

CH₄ production is calculated. Derno *et al.* (2005) estimated the MEM of Hereford steers using open-circuit chambers. Nonaka *et al.* (2012a, 2012b) used open-circuit respiration chambers when examining the effects of high environmental temperatures on the status of nutrition, energy, and N utilization of Holstein heifers. The gases can also be collected using a mask or hood attached to the mouth of the animal. This method does not require a respiration chamber and is therefore less costly. Qin *et al.* (2011) evaluated the fasting metabolism of growing female buffaloes using open-circuit respiratory hoods. Hales *et al.* (2014) reported on the effects of dietary roughage concentration on energy metabolism using open-circuit respiration head-boxes. Morooka *et al.* (1983) evaluated seasonal changes in the HP of calves using open-circuit hoods. These respiration trials, which were mostly aimed at elucidating the effects of feeding and environmental conditions on the basic metabolism of animals, do not appear to have been well integrated with the feeding trials.

1.2.4. Studies for assessing nitrogen utilization of cattle

Studies indicate that the N utilization efficiency expressed as the ratio of N in the beef product to N intake, only improved marginally from 23.7% in 1960 to 24.0% in 2008 (Stone *et al.* 1960, Hristov and Huhtanen 2008, Calsamiglia *et al.* 2010). Rumen metabolism is thought to be the most important factor contributing to efficient N utilization (Tamminga 1992). Efficient utilization of dietary carbohydrates as energy sources, and of N (protein) for optimizing ruminal microbial protein synthesis and reducing N outputs, should be considered as factors for improving ruminal functions (Ma *et al.* 2014, Nejad *et al.* 2017). Reducing amino acid deamination without limiting the supply of peptides and amino acids for ruminal bacteria through the control of specific ruminal bacteria is also important

for preventing excessive NH₃ production in the rumen, which negatively affects N utilization efficiency (Cotta and Russell 1982, Argyle and Baldwin 1989, Griswold *et al.* 1996).

Nitrogen utilization efficiency varies considerably according to dietary treatments or feed components (Calsamiglia *et al.* 2010). Steers fed on dry-rolled corn-based diets with increased proportions of AH showed increasing N retention (Farran *et al.* 2006). Conversely, decreased N retention was observed when steers were fed on diets in which wet distillers grain was the basal component (Hales *et al.* 2014). AFRC (1993) estimated the N requirements of ruminants, using the MP comprising RDP for the synthesis of microbial protein in the rumen and UDP.

A number of trials have been conducted in China with the aim of improving N utilization efficiency for cattle. Studies have reported on the effects of feed additives in CS-based diets with corn grain on rumen fermentation and N utilization of Simmental cattle (Liu *et al.* 2009, Wang *et al.* 2009; see section 1.2.2). However, the N utilization status of Simmental or crossbred cattle fed on concentrate-based diets with substitution of AH in drylands in China (Gansu Province) has not been studied. Therefore, the results of feeding trials conducted to assess the N utilization of these cattle, and the application of these findings for formulating feeding regimens, are expected to make a significant contribution to the literature.

1.3. Objectives of the study

The discussion presented in sections 1.1 and 1.2 can be summarized as follows.

- 1) Effective feeding practices for beef cattle are required in anticipation of a possible shortage of food and feed. Considering the influence of feed consumption in China

on global feed prices, studies conducted on foraging systems that include locally produced roughage are important in China.

- 2) The alfalfa cultivated in Gansu Province can be considered a locally available substitute for expensive commercial concentrates.
- 3) An optimal feeding design for Simmental beef cattle should be established based on the findings of studies, entailing *in situ* and *in vivo* trials, on the metabolism of these cattle (particularly their energy and N utilization status).
- 4) Therefore, a study aimed at improving feeding regimens, entailing both feeding and respiration trials, is pertinent.

Based on the above considerations, this study aimed at proposing measures for improved feeding regimens of Simmental beef cattle through the substitution of alfalfa for concentrate feed. The findings of the study would provide useful guidelines for revising actual feeding standards and would contribute to enhancing the productivity of beef cattle.

More specific aims of the study were:

- 1) To assess the effects of substituting alfalfa for concentrate on BW gain, efficiency of dietary energy and N utilization, GHG emissions, and feeding costs by performing two seasonal feeding and respiration trials (in the warm and cool seasons); and
- 2) To determine the appropriate alfalfa inclusion level in relation to the total DM allowance using the outputs of dietary energy and N utilization status obtained from two further feeding trials and from the trials mentioned in 1).

1.4. Approaches taken in the study

To achieve the aim of this study, the following approaches were taken.

1) Substitution of feed concentrates by alfalfa hay

Hay processing from fodders is commonly practiced in dryland areas. The hay can be easily stored and provides the most cost-effective feed in Gansu Province. Consequently, I selected AH as a possible substitute for concentrate feed for my study.

2) Use of Simmental male calves in this study

In Gansu Province, farmers prefer male calves to female ones because of their relatively fast growth, thereby saving on feeding costs. According to the findings of interviews conducted with farmers in the Province in 2014, small farmers who keep cows generally sell their calves aged around 12 months in local markets to other farmers or companies that further fatten them. Feeding improvements implemented up to the time of selling the calves would enable small farmers to enhance their incomes. Therefore calves aged about 6 months were selected in this study to improve their growth before reaching 12 months of age when they are sold.

During the growing stage of calves from 6 to 12 months, the development of ruminal functions, the skeleton, and inner muscular fat is intensive. Roughage at the right feeding levels and concentrate are required for proper growth of the rumen entailing expansion of ruminal volume, bacterium propagation, and formation of the rumen wall (NARO 2008). Fibrous ingredients cause an expansion in the ruminal volume and facilitate an increase in the number of bacteria and the concentration of propionate acid in the rumen, which promote a well-functioning rumen (Harrison *et al.* 1960). Feeding management during this stage before the calves reach the age of 12 months is important for achieving proper fattening. However, there seems to be a lack of studies in China that focus on this stage (6–

12 months of age) for beef cattle fed under varying geographical, genetic, and environmental conditions. Consequently, calves that were at the growing stage were selected for this study.

1.5. Structure of the thesis

The structure of this thesis is presented in Figure 6. Following this introductory chapter, the effects of substituting AH for C on energy utilization and feeding costs, and on N utilization, respectively, are discussed in Chapters 2 and 3. In Chapter 4, the appropriate AH inclusion level to the total DM allowance is elaborated, based on the follow-up trials and the discussion in Chapters 2 and 3. Chapter 5 presents the conclusions of the study.

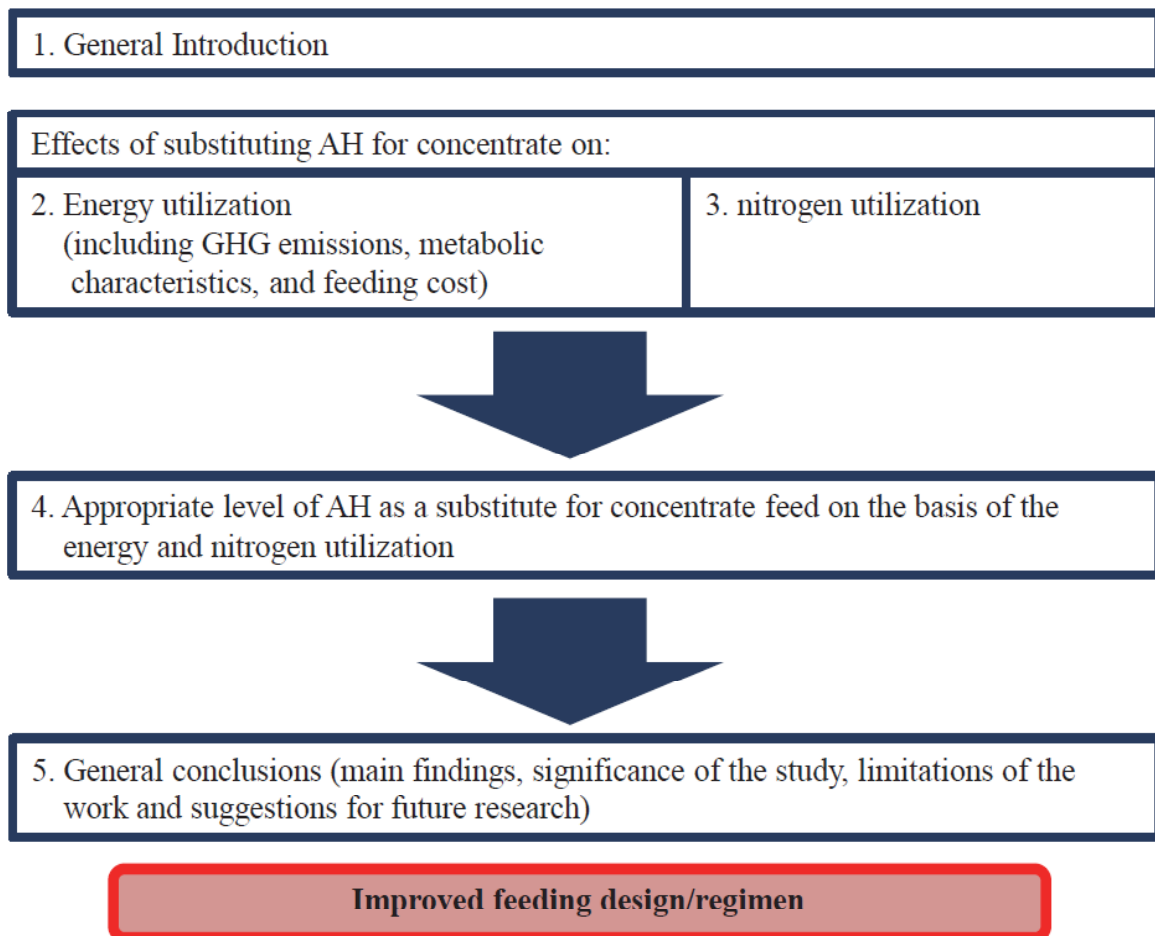


Figure 7 Structure of the thesis.

Chapter 2

Effects of substituting alfalfa hay for concentrate on energy utilization and feeding cost

2.1. Introduction

As the first step of this study, I evaluated the effects of substituting AH for C on energy utilization and feeding costs for two feeding trials (T1 and T2) conducted in 2015. In this chapter, unless otherwise specifically indicated, T1 and T2 denote the trials conducted in 2015.

2.2. Materials and methods

The animals used for this study were cared for according to the provisions of the *Guide for the Care and Use of Laboratory Animals* (Gansu Province Animal Care Committee) throughout the experimental periods.

2.2.1. Study site

The trials were conducted at the Linze Research Station of the College of Pastoral Agriculture Science and Technology of Lanzhou University, which is located in Linze County of Gansu Province (Figure 7). The research station is situated at a latitude of 39.24° N and a longitude of 100.06° E and at an elevation of 1390 m above sea level (Zhu *et al.* 1997). The annual mean precipitation is about 130 mm in this region, and the total precipitation was 85 mm in 2015, all of which occurred between April and October. The annual average temperature during that year was 7.8 °C. In 2015, the average temperatures during the warm season (August–September) and the cool season (September–November)

were 17.5 °C and 10.7 °C, respectively (data supplied by the Linze Research Station). The study site is categorized as a typical arid zone (United Nations Environment Programme 1997).

2.2.2. Cattle, applied diets, and feeding management

Two feeding trials were conducted, from August 6 to September 16, 2015 (T1) in the warm season and from September 24 to October 27, 2015 (T2) in the cool season, with the aim of achieving 1-kg DG. Eighteen male crossbred Simmental calves with a mean BW of 175.8 kg \pm 23.8 kg (at 7 months of age) and with a mean BW of 218.8 kg \pm 27.4 kg (at 8 months of age) were purchased from a local market and used for T1 and T2, respectively.

The 18 calves in each trial were divided into three groups, according to their initial BW, each associated with one of the following dietary treatments: a conventional CTRL group ($n = 6$), a LA group ($n = 6$), and a HA group ($n = 6$). Calves allocated to the CTRL groups of T1 and T2 (T1-CTRL and T2-CTRL), were offered CS as a sole forage diet together with C. The diets for the control groups in T1 and T2 were designed in accordance with conventional diets used by beef cattle farmers in Gansu Province. In light of feedback obtained from farmers interviewed in Gansu Province, I prepared the diets to contain a greater proportion of C during the cool season (*i.e.*, T2) compared with the proportion of C in diets formulated for the warm season (*i.e.*, T1). The calves in the LA group were given CS along with a low level of AH and with C, whereas those in the HA group were given CS along with a high level of AH and with C. The experimental diet for each treatment was designed to provide sufficient ME and MP requirements for 1-kg DG for a bull calf based on the estimation equation formulated by the AFRC (1993), the values listed in the MOA (2004), and the calves' BW weekly measured. The DMI for each group calculated by the

equation of AFRC (1993) was used for designing the diet. Tabular values listed in the *Standard Tables of Feed Composition in Japan (2009)* (NARO 2010) were used for converting the DE values of CS, AH, and the feed ingredients of C listed in MOA (2004) into ME concentrations. Confirmed nutritional values of the commercial concentrate component of C were also used to calculate the ME concentration of C. Ultimately, in the T1-LA group, 22% of the daily allowance of C (on a DM weight basis) used for the T1-CTRL group was replaced with AH. In the T1-HA group, 44% of the daily C allowance used for the T1-CTRL group was replaced with AH. For the T2-LA and T2-HA groups, 13% and 25%, respectively, of the daily C allowances used for the T2-CTRL group were replaced with AH.

The CS and AH used in T1 and T2 were cultivated at the Linze Research Station. The CS used for both trials was harvested in September 2014. The AH used for these trials was harvested in May 2015 (first-cut hay harvested before the blooming stage of growth in 2015) for T1, and later the same year in July (second-cut hay) and September (third-cut hay) for T2. The AH used for T1 and T2 was mixed and stored for use as the experimental feed. The C comprised commercial concentrate (31%), wheat bran (10%), and corn grain (59%). The commercial concentrate was composed of soybean meal, sunflower meal, rape seed meal, cotton seed meal, urea, sodium chloride, and a vitamin and mineral premix (precise composition unavailable). The corn grain was produced at the Linze Research Station in September 2014. Throughout the feeding trials, the calves were housed in individual pens and had free access to fresh water and mineral blocks. They were fed daily on a mix of coarsely chopped CS and AH (5–10 cm in length) and a separate meal of C, and the feed was accessed via separate troughs provided for each animal. The chopped roughage was provided twice a day (07.30 and 19.30 hours), and C was provided once a

day at 14.30 hour.

2.2.3. Measurements and sample collection

Throughout the experimental period, and for both trials, the amount of feed offered to the calves and the refusals were weighed and recorded daily to calculate the daily feed intake. The calves were weighed weekly as well as at the beginning and end of each trial. Based on the BW of the animals, the individual feed levels of the animals allocated to the CTRL, LA, and HA groups were calculated weekly, keeping the designed dietary ingredient composition. The roughage (CS and AH) was fed *ad libitum* at 20% more than the calculated allowance; whereas C was fed as calculated. Representative samples of CS, AH, and C were collected several times during the feeding trials and composited for analyses of their chemical composition.

At the ends of the feeding trials, respiratory measurements were conducted at the Linze Research Station for 15 calves in T1 and 12 calves in T2 selected from the three dietary groups (*i.e.*, five calves in T1 and four calves in T2 per group) using ventilated open-circuit respiration chambers (the interior volume of each of the four chambers was 17810 L). During a 5-day period of adaptation of the animals to the respiration chamber, 24-hour urine samples were collected for a day, and representative fecal samples were collected every morning for 3 days to determine the daily urinary and fecal GE excretion of calves allocated to the CTRL, LA, and HA groups. The total feces excreted 24 hours was not able to be collected because of the structure of the floor of pens. Excreted urine was collected by using a plastic funnel, weighed, and treated with 10% (v/v) H₂SO₄ to keep urine pH below 3. Urine samples (about 100 mL) taken from each animal were stored at -15 °C for further analysis. Daily fecal excretion was estimated using ADL as an internal marker to determine

digestibility. After the 5-day adaptation period, O₂ consumption and CO₂ and CH₄ production were measured for 48 hours using both an infrared-absorption based gas analyzer (CO₂ and CH₄) and a paramagnetic-based O₂ gas analyzer (VA-3000, Horiba Ltd., Kyoto, Japan). The average BW values of the calves at the start of the respiratory measurements were 209.5 ± 25.2 kg and 257.0 ± 27.2 kg in T1 and T2, respectively. During the respiration trials, the same schedule of experimental diets offered to the animals in the feeding trials was continued. Daily HP of each animal was calculated using Brower's (1965) equation. The MEm of male calves was estimated using a linear regression equation between NEI and MEI, as presented by Freetly *et al.* (2006) (see section 1.2.3).

2.2.4. Chemical analysis

Collected feed and fecal samples were dried in a forced air oven at 60 °C. The dried samples were ground and sieved so that they could be passed through a 1 mm screen. Concentrations of GE, DM, CP, NDFom, and ADL in the dried feed samples and fecal samples were determined. Concentrations of DM and CP were determined using the method outlined by the Association of Official Analytical Chemists (AOAC 1984). Concentrations of NDFom and ADL were determined using the procedure developed by Van Soest *et al.* (1991). Concentrations of GE were determined using a bomb calorimeter (CA-4AJ, Shimadzu Corp., Kyoto, Japan). Urine samples were placed in a freeze dryer (VD550R, Taitec Corp., Koshigaya, Japan) for 48 hours, and the GE concentration was determined using the above described bomb calorimeter. The urinary N concentration was determined as described previously (AOAC 1984).

The collected feed samples were used in an analysis of *in vitro* gas production to verify MEI values calculated using the results of feeding trials in the current study. Feed

ingredients (CS, AH, and C) in the diets were mixed according to the actual feed intake ratio. The mixed feed samples (2 g DM) were then mixed with ruminal fluid obtained from sheep kept in the Faculty of Life and Environmental Science at Shimane University to provide solutions that were each made up to 200 mL. The solutions were then kept at 39 ± 0.5 °C for 24 hours, and the volume (mL) of gas generated from the solution was measured. The two ME concentrations for all of the groups in T1 and T2 were calculated using the following formulas (Menke and Steingass 1988).

$$\text{ME (MJ kg}^{-1}\text{ DM)} = 0.01 + 0.1607 \times \text{OM digestibility (\%)} \\ - 0.0133 \times \text{Ash concentration (g kg}^{-1}\text{ DM)}$$

$$\text{OM digestibility (\%)} = 0.0448 \times \text{CP concentration (g kg}^{-1}\text{ DM)} \\ + 0.0651 \times \text{Ash concentration (g kg}^{-1}\text{ DM)} \\ + 0.8893 \times \text{In vitro gas production at 24 hours (mL per 200 mL solution)} \\ + 14.88$$

$$\text{ME (MJ kg}^{-1}\text{ DM)} = 14.78 - 0.0147 \times \text{ADF concentration (g kg}^{-1}\text{ DM)}$$

2.2.5. Economic analysis

To examine the economic feasibility of substituting AH for C, differences in feeding costs for each of the dietary treatment groups in T1 and T2 were calculated. Feed costs were calculated by using the sum of the expenses incurred for the purchase of AH and commercial concentrate at their market prices (1.7 yuan kg⁻¹ for AH and 2.9 yuan kg⁻¹ for C) and the daily intake of AH and C for each calf in T1 and T2. The CS feed cost was not considered, because CS was generally prepared by each farmer as a forage source for feeding their cattle. In addition, the economic benefit of the calves' DG was estimated by

subtracting the feed cost from the expected income (profit) from the DG values calculated according to the market price of the calves (22 yuan kg⁻¹ BW). The estimates were subsequently converted at the rate of US\$1 = 6.36 yuan (based on the average values for the period of August 6 – November 1, 2015).

2.2.6. Statistical analysis

Differences in means between the three groups obtained for each trial were evaluated using one-way analysis of variance (ANOVA) and Tukey's test after the normality and homoscedasticity of data distribution were evaluated (Kolmogorov–Smirnov and Barlett tests). Possible seasonal differences in the efficiency of energy utilization or GHG production were not considered because of differences in the feeding regimens applied in T1 and T2. All statistical analyses were performed with R statistical software (Version 3.1.1, the R Foundation for Statistical Computing, Vienna, Austria). Significance was declared at $P \leq 0.05$ and trends in the results when $0.05 < P \leq 0.10$ were identified.

2.3. Results and discussion

2.3.1. Chemical composition of diet

The GE concentration and chemical composition of CS did not differ between T1 and T2 (Table 4). Minson (1980) reported that a CP concentration of at least 8% (on a DM basis) is needed if the diet of ruminants is solely composed of forage. The concentration of CP in CS ranged between 4% and 5% (on a DM basis) for both years and that of NDFom was about 70% of DM, indicating that high production levels cannot be achieved when CS is used as the basal diet for beef cattle.

Table 4 Gross energy concentrations and chemical compositions of feed ingredients in experimental diets designed for Simmental beef calves

Item	Diets used in 2015			Diets used in 2016					
	GE, kJ g ⁻¹ DM	CP	NDFom	ADL	Chemical composition, % DM	GE, kJ g ⁻¹ DM	CP	NDFom	ADL
Trial 1									
Corn stover	16.3	5.2	70.3	5.7	16.2	5.1	72.6	5.3	5.3
Alfalfa hay [†]	16.7	15.8	52.2	7.5	17.4	13.5	46.9	7.6	7.6
Concentrate [‡]	17.6	13.1	17.1	2.2	18.1	12.7	15.0	1.0	1.0
Trial 2									
Corn stover	16.2	4.3	72.0	5.1	16.4	4.4	77.6	5.2	5.2
Alfalfa hay [†]	16.5	12.0	52.8	8.7	17.1	12.3	52.4	8.3	8.3
Concentrate [‡]	17.7	12.8	23.4	2.4	17.5	18.0	18.1	1.4	1.4

In 2015, Trials 1 and 2 were conducted from August 6 to September 23 (samples were collected on September 14 and 16) and from September 24 to November 3 (samples were collected on October 23 and 28).

In 2016, Trial 1 and Trial 2 were conducted from July 3 to August 15 (samples were collected on July 3), and from August 16 to October 25 (samples were collected on August 15).

ADL, acid detergent lignin; CP, crude protein; DM, dry matter; GE, gross energy; NDFom, ash-free neutral detergent fiber.

[†] In Trial 2 of 2015, and for both of the trials conducted in 2016, a mixture of the alfalfa hay harvested in July and September 2015 (mixed ratio = 50:50) used as feed was analyzed.

[‡] The concentrate used as feed in 2015 and 2016, respectively, comprised 59% and 60% of corn grain, 31% and 30% of commercial concentrate, and 10% and 10% of wheat bran.

Thus, when CS is chosen as the basal diet for crossbred Simmental male calves, concentrate feed or a leguminous forage supplement is required to achieve 1-kg DG. Reflecting differences in the harvesting stage of alfalfa, the concentrations of CP, NDFom, and ADL of AH differed slightly between T1 and T2. The CP concentrations of AH in T1 and T2 (15.8% and 12.0% on a DM basis, respectively) were lower than the tabular values listed by NARO (2010) (ranging from 19.1% to 21.8% on a DM basis) but were close to the value listed in MOA (2004) (13.1% on a DM basis). The finding of this study that CP concentrations of AH in both trials were close to that of C suggests that it may be possible to provide CP in CS-based feeding regimens for beef calves by replacing concentrate feed with AH. The NDFom and ADL concentrations of the second- and third-cut AH in T2 were slightly higher than those of the first-cut AH in T1. The NDFom concentrations in AH harvested before the bloom stage vary between 36.0% and 39.3% on a DM basis (NARO 2010). The higher NDFom concentrations of AH obtained in this study may have been caused by unavoidable contamination at the time of harvesting with other poaceous forage crops grown at the research station. The difference in the NDFom concentrations of C between T1 and T2 was probably the result of variations in nutrient concentrations in the wheat bran and corn grain that were added to the commercial concentrate. The higher ADL concentrations of AH in T2 compared with those in T1 could be attributed to the growing stage of first-cut AH used in T1, which was earlier than that of second- and third-cut AH in T2. This explanation is in agreement with the reported finding that the ADL concentration of alfalfa increases during its successive growth stages (Orloff and Putnam 2004, Reddy *et al.* 2005, Burns 2011).

The daily feed allowances for calves at the start of T1 and T2 are shown in Table 5.

Table 5 Feed allowances and their chemical compositions within experimental diets designed for Simmental beef calves in 2015

Item	Trial 1			Trial 2		
	T1-CTRL	T1-LA	T1-HA	T2-CTRL	T2-LA	T2-HA
Feed ingredient [†] , kg DM day ⁻¹						
Corn stover	3.0	3.0	3.0	0.6	0.6	0.6
Alfalfa hay	0.0	0.7	1.4	0.0	1.0	1.9
Concentrate	2.7	2.1	1.5	4.8	4.2	3.6
Chemical composition [‡] , % DM						
Dry matter, %	83.6	83.5	83.5	81.9	83.0	83.9
CP	8.9	9.3	9.7	11.9	11.8	11.7
NDFom	45.1	48.9	52.5	29.1	33.7	37.5
ADL	3.4	4.0	4.7	2.9	4.3	5.2

See the footnote in Table 4 for details on Trials 1 and 2.

T1-CTRL and T2-CTRL were controls with no alfalfa hay feeding; T1-LA and T2-LA denoted low levels of alfalfa hay feeding; and T1-HA and T2-HA denoted high levels of alfalfa hay feeding.

ADL, acid detergent lignin; CP, crude protein; DM, dry matter; NDFom, ash-free neutral detergent fiber.

[†] Calculated using the equation provided by AFRC (1993) based on the initial average body weights of male calves in Trial 1 (175.8 kg) and Trial 2 (218.8 kg) to meet the metabolizable energy requirement for a daily weight gain of 1 kg.

[‡] Values were estimated based on the chemical composition of feed ingredients (Table 4) and the composition of ingredients in the experimental diets.

As described above, the feed allowance for each animal was calculated weekly on the basis of its BW, maintaining the feed proportion of the feeding treatments in each trial. The values shown in Table 5 were for diets fed to calves with BWs of 175.8 kg and 218.8 kg in T1 and T2, respectively. The estimated CP concentration in the calves' diets (on a DM basis) ranged between 8.9% and 9.7% in T1 and between 11.7% and 11.9% in T2 (Table 5). The estimated NDFom concentration in the diets (on a DM basis) ranged between 45.1% and 52.5% in T1 and between 29.1% and 37.5% in T2. The estimated NDFom concentration tended to increase with an increase in the level of AH feed. By contrast, the variation in estimated CP concentrations among the feeding groups in T1 and T2 (8.9–9.7% in T1, 11.7–11.9% in T2) was less than that of the estimated NDFom concentration. The ADL concentrations exceeded 2% on a DM basis for all of the diets, and were sufficiently high to justify their use as an internal marker to estimate fecal DM excretion (Munitifering 1982).

2.3.2. Feed intake, feed efficiency, and digestibility

A summary of average feed intakes of the calves over the one-month period of each feeding trial is shown in Table 6. For both trials, refusal of C was observed for calves in the CTRL groups. The quantity of refused forage (CS and AH) in the T2-HA group (0.13 kg DM day⁻¹) exceeded that in the T2-CTRL (0.03 kg DM day⁻¹) and T2-LA (0.07 kg DM day⁻¹) groups. There were no significant differences in the total DMI (in kg day⁻¹ and % BW) among the calves in the T1 feeding groups. The NDFom concentrations of daily ingested feed (on a DM basis) by calves in the T1-CTRL, T1-LA, and T1-HA groups were 39.9%, 44.3%, and 49.5%, showing a corresponding increase with increasing amounts of AH feed.

Table 6 Feed and nutrient intake, and digestibility in Simmental crossbred beef calves in trials conducted in 2015

Item	Trial 1				Trial 2				SEM	P
	T1-CTRL	T1-LA	T1-HA	P	T2-CTRL	T2-LA	T2-HA	P		
Feed intake										
Corn stover, kg DM day ⁻¹	1.5 ^C	1.8 ^B	2.0 ^A	0.000 [†]	0.8	0.8	0.8	0.78	0.00	0.78
Alfalfa hay, kg DM day ⁻¹	0.0 ^C	0.4 ^B	0.9 ^A	0.000 [†]	0.0 ^C	1.2 ^B	2.4 ^A	0.000 [†]	0.01	0.000 [†]
Concentrate, kg DM day ⁻¹	3.1 ^A	2.7 ^B	1.8 ^C	0.000 [†]	3.1	3.3	3.1	0.39	0.12	0.39
Total DMI, kg day ⁻¹	4.6	4.9	4.7	0.25	3.8 ^C	5.3 ^B	6.3 ^A	0.000 [†]	0.12	0.000 [†]
Total DMI, % BW	2.4	2.5	2.6	0.25	1.7 ^C	2.2 ^B	2.6 ^A	0.000 [†]	0.08	0.000 [†]
Nutrient intake, kg DM day⁻¹										
Crude protein	0.5	0.5	0.5	0.47	0.4 ^C	0.6 ^B	0.7 ^A	0.000 [†]	0.02	0.000 [†]
NDFom	1.8 ^C	2.2 ^B	2.3 ^A	0.000 [†]	1.3 ^C	2.0 ^B	2.6 ^A	0.000 [†]	0.03	0.000 [†]
Roughage-intake total-DMI ⁻¹ , %	33.4 ^C	45.9 ^B	61.4 ^A	0.000 [†]	19.7 ^C	37.8 ^B	50.6 ^A	0.000 [†]	2.87	0.000 [†]
Digestibility, %										
Dry matter	64.6	60.8	59.3	0.78	76.7 ^A	66.0 ^B	62.4 ^B	0.000 [†]	1.53	0.000 [†]
Crude protein	58.6	55.5	59.7	0.89	72.9 ^A	62.9 ^B	61.3 ^B	0.01	2.36	0.01
NDFom	52.6	50.7	49.2	0.93	60.1 ^A	49.4 ^B	48.2 ^B	0.01	2.40	0.01

Trials 1 and 2 comprised the following groups: T1-CTRL, T1-LA, T1-HA, T2-CTRL, T2-LA, and T2-HA; for details, see Tables 4 and 5 or the section on materials and methods in Chapter 2.

BW, body weight; DM, dry matter; DMI, dry matter intake; NDFom, ash-free neutral detergent fiber; SEM, standard error of the mean.

^{A, B, C} Means with different superscripts within a row for each of Trial 1 and Trial 2 differed significantly ($P \leq 0.05$).

[†] $P < 0.0005$.

Forage fiber concentration evidently affects voluntary feed intake through the physical regulation caused by the filling of the rumen (Ichinohe *et al.* 1994). By contrast, Hales *et al.* (2014) found no difference in DMI when concentrate-based diets were substituted for those entailing various ratios of AH. The results in T1 also revealed that dietary substitution with AH, regardless of the consequent increase in NDFom intake, did not reduce the total DMI. However, the results for T2 revealed significant differences ($P < 0.05$) in the total DMI (kg day⁻¹ and % BW) between the three feeding groups. The total DMI for the T2-CTRL group was the lowest, while the total DMI for the T2-HA group was significantly higher ($P < 0.05$) than that for the T2-LA group. The NDFom concentrations in the ingested feed (on a DM basis) for the T2-CTRL, T2-LA, and T2-HA groups were 33.3%, 37.5%, and 40.7%, respectively. As in T1, they increased with a corresponding increase in the amount of AH feed. However, contrasting with the results obtained in T1, the increased NDFom intake in T2 with an increasing amount of AH feed, resulted in a significant increase in the total DMI. The results for the total DMI in T2 supported the findings of a study of growing ruminants conducted by Defoor *et al.* (2002), in which the authors suggested that growing heifers ate more with increasing concentrations of NDFom in the diet to compensate for the reduction in dietary energy intake. Furthermore, the results of feed intake in T2 indicated that differences in the dietary forage to concentrate ratio affected feed intake, as suggested by McCarthy *et al.* (1989), Krause *et al.* (2002), and Oba and Allen (2003). Kennedy and Murphy (1988) reviewed digesta particle outflow from the rumen and noted that in addition to the physiological rumen filling caused by forage feeding, cold temperatures also led to an increased rate of rumen digesta outflow. Hence, both the cold temperature evident in October 2015 at Linze Research Station and differences in the ruminal NDFom content caused by variations in NDFom intake could conceivably have led to the significant

differences in total DMI among the dietary treatments implemented in T2. The results obtained in this study suggested that a dietary AH allowance of up to 50% of C in the warm season and an allowance of up to 80% of C in the cool season (Table 6) did not have any detrimental effects on feed intake in both trials.

The average values for daily MEI and DG of the calves were also calculated (Figure 8, Table 7). The MEI values did not differ significantly among the feeding groups in T1, although the T1-HA group showed a slightly lower value than the other feeding groups ($P > 0.05$). By contrast, the MEI value was significantly higher ($P < 0.05$) for the T2-HA group than for the T2-CTRL group, and there was no difference between the T2-LA and T2-HA groups. Over the one-month feeding period, the following MEI values, expressed as percentages of the ME required for 1-kg DG in male calves, were calculated for each group: T1-CTRL: 97, T1-LA: 90, T1-HA: 78, T2-CTRL: 98, T2-LA: 108, and T2-HA: 119. There were no significant differences in the MEI values calculated for the T2-CTRL and T2-LA groups or for the T2-LA and T2-HA groups ($P > 0.05$; Figure 8). The lower average DG obtained for the T1-HA group compared with the average DG obtained for other groups in T1 ($P < 0.05$; Table 7) may have been caused by the numerically lower MEI value for the T1-HA group. The average DG of calves in the T2-CTRL group was significantly lower ($P < 0.05$) than those for the other groups in T2, whereas those for the T2-LA and T2-HA groups did not differ significantly ($P > 0.05$). The target growth performance (1-kg DG) was achieved for T2-LA and T2-HA calves. Hales *et al.* (2014) reported that the RE for BW gain in finishing beef cattle decreased as the dietary concentration of AH increased.

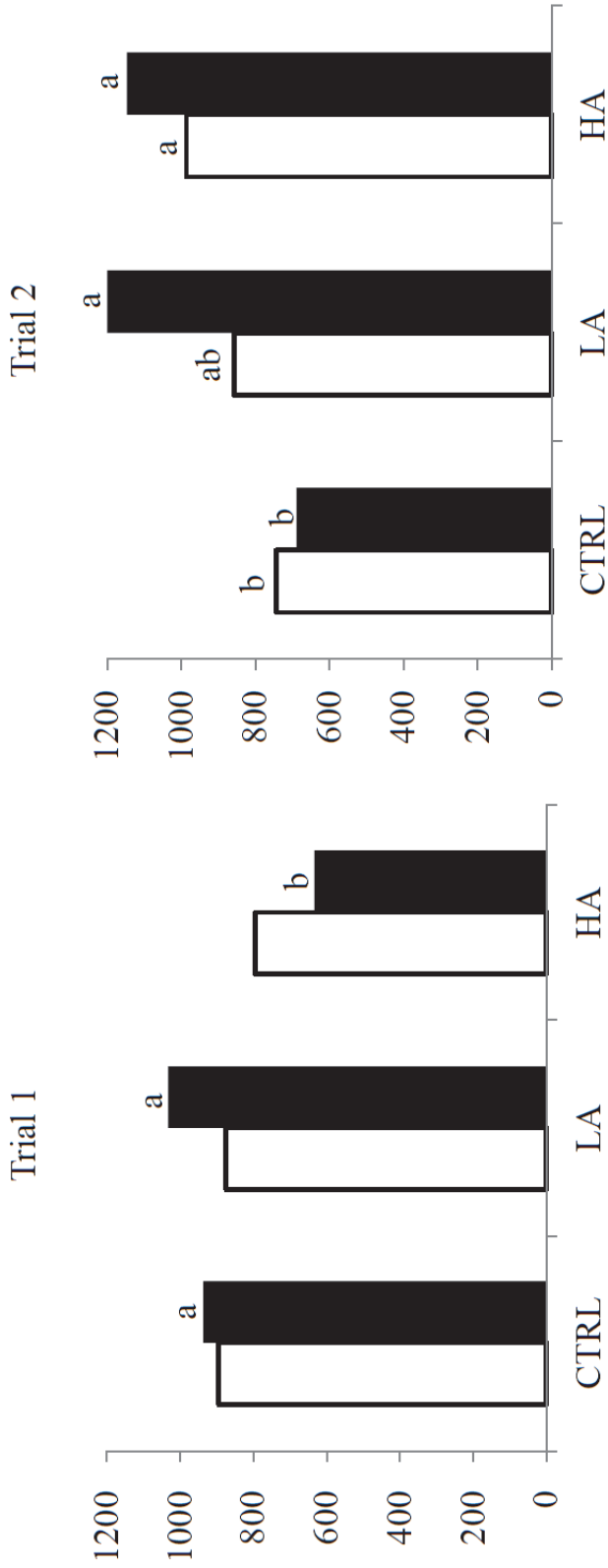


Figure 8 Metabolizable energy intake and daily gain of calves in 2015. CTRL, control group; LA, low-level alfalfa hay feeding group; HA, high-level alfalfa hay feeding group. ^{a, b} Mean values with different superscripts within each of Trial 1 and Trial 2 differed significantly among the three feeding groups ($P \leq 0.05$). Unshaded bars depict metabolizable energy intake (kJ kg^{-0.75} BW day⁻¹); solid bars depict daily gain (g day⁻¹).

Table 7 Growth performance and energy utilization of Simmental crossbred beef calves during trials conducted in 2015

Item	Trial 1				Trial 2					
	T1-CTRL	T1-LA	T1-HA	SEM	P	T2-CTRL	T2-LA	T2-HA	SEM	P
Growth performance										
Daily gain, kg day ⁻¹	0.94 ^A	1.03 ^A	0.63 ^B	0.08	0.01	0.69 ^B	1.20 ^A	1.15 ^A	0.12	0.02
Feed conversion ratio	5.01 ^B	4.89 ^B	8.42 ^A	0.95	0.005	5.83	4.58	6.09	0.71	0.31
Energy utilization										
GE intake, kJ kg ^{-0.75} BW day ⁻¹	1543.0	1612.4	1592.7	42.1	0.50	1157.7 ^C	1482.6 ^B	1767.1 ^A	40.4	0.000 [†]
Energy digestibility (DE GE ⁻¹), %	65.2	60.8	59.2	5.4	0.73	77.1 ^A	66.7 ^B	63.5 ^B	1.7	0.001
Energy metabolizability (ME GE ⁻¹), %	58.6	54.4	51.7	5.5	0.67	67.2 ^A	57.9 ^B	55.6 ^B	1.6	0.001
ME intake, kJ kg ^{-0.75} BW day ⁻¹	895.2	875.1	795.3	81.2	0.70	744.3 ^A	857.3 ^{AB}	986.8 ^B	39.5	0.01

Trials 1 and 2 comprised T1-CTRL, T1-LA, T1-HA, T2-CTRL, T2-LA, and T2-HA; for details, see Tables 4 and 5 or the section on materials and methods in Chapter 2.

BW, body weight; DG, daily gain; DMI, dry matter intake; DE, digestible energy; GE, gross energy; ME, metabolizable energy; SEM, standard error of the mean.

^{A, B, C} Means with different superscripts within a row for each of Trial 1 and Trial 2 differed significantly ($P \leq 0.05$).

[†] $P < 0.0005$.

The numerically lower MEI value for the T1-HA group compared with the values obtained for the T1-CTRL and T1-LA groups may have resulted in a reduction of the RE in the T1-HA group compared with the RE values in the other two groups, leading to the significantly lower DG value ($P < 0.05$). However, the absence of any difference in the DG of calves in the T1-CTRL and T1-LA groups indicated that the amount of AH feed in the T1-LA treatment did not cause any reduction in RE that might have occurred in the T1-HA group. The lower DG value observed in the T2-CTRL group compared with the values observed in the other two groups in T2 may have been attributable to the T2-CTRL treatment, which did not satisfy the ME requirement for achieving the DG target of 1 kg. Considering the daily consumption of C in the T2-CTRL group, which accounted for 65% of the dietary allowance (4.8 kg DM day⁻¹) (Tables 5 and 6), the lack of ME supply in this group appeared to be attributable to the excessive allowance of C rather than to the lower intake of C when growing calves were fed a CS-based diet in the cool season in Gansu Province. The results for average MEI and DG suggested that the provision of AH to the LA groups in both T1 and T2 had no negative effect on energy retention for BW gain of growing male calves.

The average FCR trends among the three feeding groups differed between T1 and T2 (Table 7). Though a difference between the FCR values obtained for the T1-CTRL and T1-LA groups was negligible, they were significantly lower (more preferable) than the value obtained for the T1-HA group ($P < 0.05$). The trend for FCR values obtained in T1 was the reverse of that for DG, indicating that the T1-LA diet was comparable to a conventional concentrate-based diet for cattle in Gansu Province in the warm season. The FCR values for the feeding groups in T2 did not differ significantly, but the value for the T2-LA group was numerically lower than the values obtained for the other feeding groups.

Although no clear relationship was evident between DG and FCR in T2, the advantage of low-level substitution of AH for C compared with high-level AH feeding was apparent in both T1 and T2.

The digestibility of DM, CP and NDFom in the three feeding groups did not differ significantly in T1 ($P > 0.05$, Table 6). Conversely, the digestibility of DM, CP, and NDFom in the T2-CTRL group was significantly higher than their digestibility in the T2-LA and T2-HA groups ($P < 0.05$). As previously noted, the reduced NDFom digestibility for AH feeding groups in T2 may have been partially explained by a higher outflow rate of rumen digesta in the cool season (Kennedy and Murphy 1988). The difference in amounts of AH feed in the LA and HA groups in both trials did not affect nutrient digestibility.

2.3.3. Energy utilization efficiency

Gross energy intake did not differ significantly ($P > 0.05$) among the feeding groups in T1, whereas it differed significantly ($P < 0.05$) among the feeding groups in T2 with the highest value obtained for T2-HA, followed by values for T2-LA and T2-CTRL (Table 7). In T1, the energy digestibility of the feeding groups did not differ significantly ($P > 0.05$), although the increase in fecal energy excretion (from 528.2 kJ kg^{-0.75} BW day⁻¹ for the T1-CTRL group to 633.5 kJ kg^{-0.75} BW day⁻¹ for the T1-HA group), associated with an increasing amount of AH feed, resulted in a slight decrease in energy digestibility. By contrast, energy digestibility was significantly ($P < 0.05$) greater for the T2-CTRL group than for the other two groups; in T2, the values were ranked in the reverse order for total DMI (Table 7). As was the trends observed for digestion coefficients of GE in the two trials, energy metabolizability did not differ among the T1 groups ($P > 0.05$), but it was

significantly greater for the T2-CTRL group ($P < 0.05$) than for other T2 groups. Plausibly, the lack of significant differences in MEI and energy metabolizability ($P > 0.05$) between the T2-LA and T2-HA groups could explain the lack of any significant difference in DG between these two groups (Figure 8). Increasing the dietary substitution rate of AH did not result in any marked improvement in energy metabolizability in either trial. Hales *et al.* (2014), who adopted dry-rolled corn-based diets supplemented with AH, reported that fecal energy loss increased with decreasing energy digestibility, as the amount of AH feed increased, owing to an increase in the NDF intake and a coinciding decrease in NDF digestibility. Numerical differences and significant differences in NDFom digestibility were observed in T1 and T2, respectively, and the trends of NDFom digestibility and NDFom intake observed in each trial appeared consistent with those reported by Hales *et al.* (2014). These values for energy digestibility and metabolizability (Table 7) are in agreement with the results of Liu *et al.* (2013), who calculated 66% energy digestibility and 58% energy metabolizability in Xiangzhong Black bulls. There was no marked difference in energy metabolizability between crossbred Simmental calves and other breeds.

The DG values obtained for the T1-LA, T2-LA, and T2-HA groups exceeded the DG target of 1 kg, and the ratios of DG to MEI (DG ME^{-1} , in $\text{g MJ}^{-1} \text{day}^{-1}$) calculated for the T1-LA, T2-LA, and T2-HA groups were 23.2, 20.1, and 19.3, respectively. These values were higher than previously reported results: $19.2 \text{ g MJ}^{-1} \text{day}^{-1}$ for Zebu cows and $15.7 \text{ g MJ}^{-1} \text{day}^{-1}$ for Brown Swiss cows (Jose *et al.* 2010), and were lower than the value of $31.1 \text{ g MJ}^{-1} \text{day}^{-1}$ reported for Xiangzhong Black bulls (Liu *et al.* 2013). The ME utilization efficiency for BW gain in crossbred Simmental male calves appeared lower than that for Chinese indigenous Xiangzhong Black cattle. The lower ME utilization efficiency of crossbred Simmental male calves indicates the need for a greater energy allowance to

ensure production performances that are comparable to those of Xiangzhong Black cattle.

The MEm (in $\text{kJ kg}^{-0.75} \text{ BW day}^{-1}$), which was calculated as 652 in T1 and 600 in T2 (Figure 9), was higher than the previously reported values of 469 (Henrique *et al.* 2005), 460 for Nellore bulls and 485 for Nellore steers (Tedeschi *et al.* 2002), 506 for Chinese water buffaloes (Qin *et al.* 2011), 506 for Xiangzhong Black bulls (Liu *et al.* 2013), and 585 for Holstein cows (Hayasaka *et al.* 1995). This finding suggests that the relatively higher MEm requirement of crossbred Simmental bull calves may have resulted in a greater ME requirement for BW gain than that required for other species.

Differences between the MEI values estimated from ADF concentrations (Menke and Steingass 1988) and those obtained from the feeding trials conducted in the current study were compared with differences between the MEI values estimated by conducting *in vitro* gas production analysis and those obtained from the feeding trials mentioned above (Table 8). The ratios of the MEI values estimated with ADF concentrations to the MEI values obtained through the feeding trials ((3) $(1)^{-1}$ in Table 8) were lower than those of the MEI values estimated as a result of the *in vitro* gas production analysis to the MEI values obtained through the feeding trials ((2) $(1)^{-1}$ in Table 8) in all of the feeding groups in 2015. Variations in the former ratios among the three groups in each of T1 and T2 were relatively small compared with variations in the latter ratios, which increased as the AH inclusion level increased in both the T1 and T2 conducted in 2015. The MEI estimated from the ADF concentrations appeared to be linearly proportional to the MEI obtained through the feeding trials, and this method could be utilized as an alternative *in vitro* method for estimating MEI values using certain parameters for calibration.

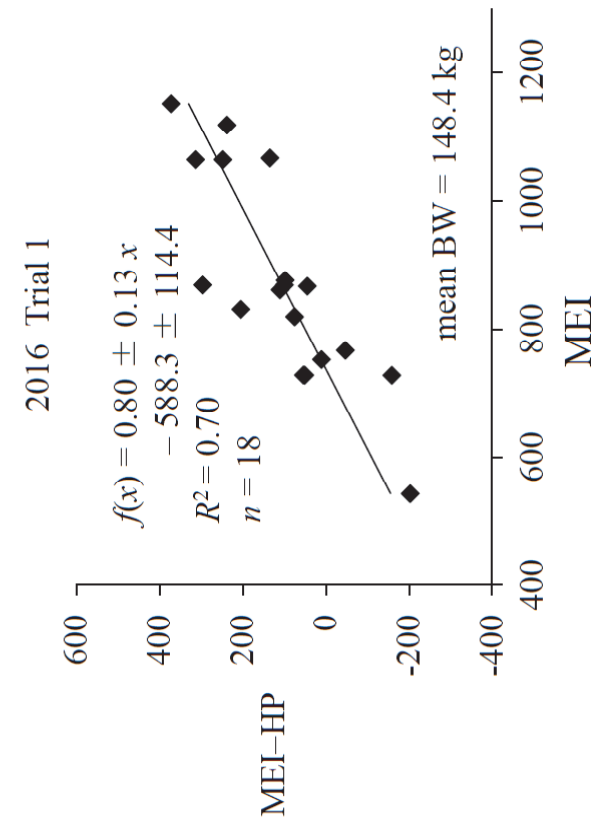
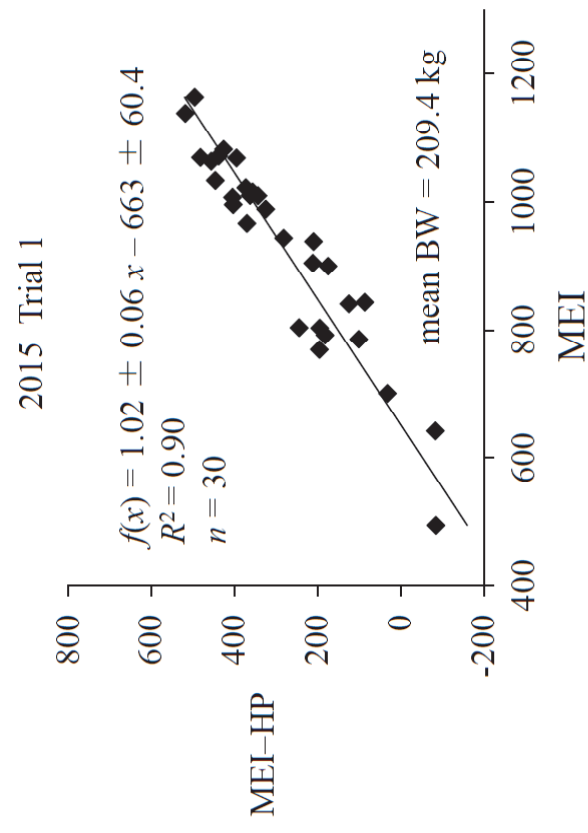
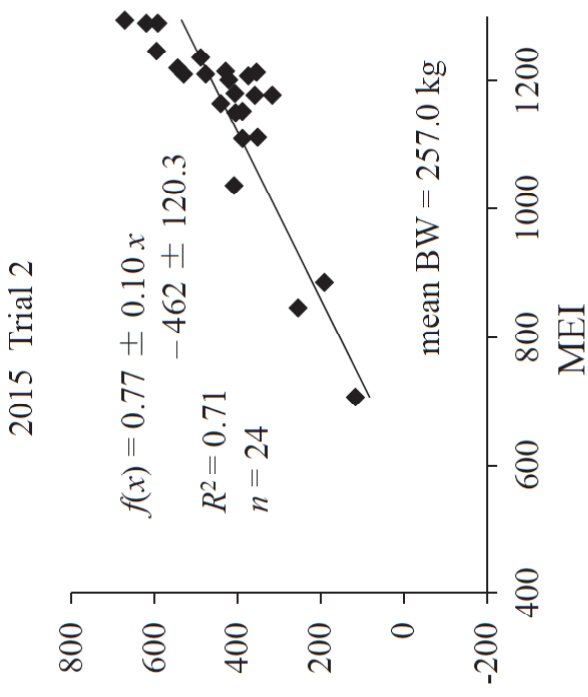


Figure 9 Linear regression of net energy intake (NEI, $f(x)$) and metabolizable energy intake (MEI, x) of calves in Trials 1 and 2 conducted in 2015 and Trial 1 conducted in 2016. The NEI was estimated as MEI – HP (heat production). Units referred to metabolic body size ($\text{kJ kg}^{-0.75} \text{BW day}^{-1}$). Metabolizable energy for maintenance (ME_m) was the interpolant of x at the point where $f(x)$ is zero.

Table 8 Metabolizable energy intake of Simmental crossbred beef calves during trials conducted in 2015 and 2016

Item	2015					
	Trial 1			Trial 2		
	T1-CTRL	T1-LA	T1-HA	T2-CTRL	T2-LA	T2-HA
Metabolizable energy intake [†] , MJ day ⁻¹ (1)	46.4	45.3	42.0	45.4	52.2	60.3
Metabolizable energy intake [‡] , MJ day ⁻¹ (2)	60.1	68.2	71.9	57.0	68.4	80.8
Metabolizable energy intake [§] , MJ day ⁻¹ (3)	55.5	55.7	50.3	47.4	61.3	69.3
Ratio for comparison as (2) (1) ⁻¹ , %	129.5	150.5	171.2	125.5	131.0	134.1
Ratio for comparison as (3) (1) ⁻¹ , %	119.5	122.8	119.9	104.4	117.5	115.0

Item	2016					
	Trial 1			Trial 2		
	T1-LA	T1-MA	T1-HA	T2-LA	T2-MA	T2-HA
Metabolizable energy intake [†] , MJ day ⁻¹ (1)	32.1	36.0	34.0	54.0	50.7	52.9
Metabolizable energy intake [‡] , MJ day ⁻¹ (2)	48.2	42.9	42.7	77.0	63.8	69.6
Metabolizable energy intake [§] , MJ day ⁻¹ (3)	40.5	42.8	38.4	60.9	58.9	58.2
Ratio for comparison as (2) (1) ⁻¹ , %	150.0	119.2	125.6	142.5	125.7	131.6
Ratio for comparison as (3) (1) ⁻¹ , %	125.9	119.1	113.0	112.8	116.1	109.9

Trials 1 and 2 comprised T1-CTRL, T1-LA, T1-MA, T1-HA, T2-CTRL, T2-LA, T2-MA, and T2-HA; for details, see Table 4, 5, 11 or the section on materials and methods in Chapter 2 and 4.

[†] Calculated by the feeding trials conducted in 2015 and 2016.

[‡] Calculated using the *in vitro* gas production analysis (Menke and Steingass 1988).

[§] Calculated using the concentration of acid detergent fiber (Menke and Steingass 1988).

2.3.4. Greenhouse gas emissions as a loss of the energy intake

In T1, there were no differences in CO₂ production among the three groups (Table 9). CH₄ production (L day⁻¹) tended to be greater for the T1-HA group than for the T1-CTRL and T1-LA groups ($P < 0.10$). This also applied to the ratio of the energy of emitted CH₄ to the total GE intake ($P < 0.10$). In T2, as in T1, there were no differences in CO₂ production among the three groups, and CH₄ production (L day⁻¹, L kg^{-0.75} BW day⁻¹) was greater for the T2-HA group than for the T2-CTRL and T2-LA groups ($P < 0.10$). The ratio of the energy of emitted CH₄ was numerically the lowest in the T2-LA group. Increased CH₄ production correlating to an increase in DMI has been reported in previous studies (Moe and Tyrrell 1979, Shibata *et al.* 1993). In this study, an increase in CH₄ production (L day⁻¹, $P < 0.10$) along with a significant increase in DMI (kg day⁻¹, $P < 0.05$) and in the proportion of the AH mixture, was observed in T2; whereas in T1, an increase in CH₄ production ($P < 0.10$) with no significant difference in DMI between the three groups was observed. A negative relationship has been reported between CH₄ production and inclusion levels of concentrate feed (Johnson and Johnson 1995, Ferris *et al.* 1999, Yan *et al.* 2000, McGeough *et al.* 2010, Aguerre *et al.* 2011). Hales *et al.* (2014), in a study using corn-based diets with AH, reported that CH₄ production increased with decreasing energy digestibility as the AH feeding level increased owing to an increase in NDF intake and a coinciding decrease in NDF digestibility. The relationship between inclusion levels of C, CH₄ production, NDFom digestibility, and NDFom intake observed in T1 and T2 was consistent with the findings of these earlier studies. The ratios of the energy of emitted CH₄, which ranged from 4.2% to 6.1 %, were regarded as consistent with reported ratios of 4.1% (Dämmgen *et al.* 2013), 2–15% (Flachowsky and Lebzien 2012), 6.1–7.9% (Jordan *et al.* 2006a), 6.1–8.3% (Jordan *et al.* 2006b), and 5.2–7.7% (Zhu *et al.* 2014).

Table 9 Methane and carbon dioxide emission, metabolizable energy intake, and body weight of Simmental crossbred beef calves during trials conducted in 2015

Item	Trial 1					Trial 2				
	T1-CTRL	T1-LA	T1-HA	SEM	P	T2-CTRL	T2-LA	T2-HA	SEM	P
Methane production, L day ⁻¹	85.36 ^b	97.93 ^b	121.67 ^a	10.23	0.07	102.82 ^B	128.22 ^B	161.25 ^A	12.30	0.03
Methane production, L day ⁻¹ kg ⁻¹ DG	93.81 ^B	97.59 ^B	215.07 ^A	26.34	0.01	154.67	123.67	166.46	30.55	0.61
Methane production, L kg ^{-0.75} BW day ⁻¹	1.60 ^B	1.89 ^B	2.31 ^A	0.13	0.01	1.66 ^{Bb}	2.09 ^{ABb}	2.63 ^{Aa}	0.15	0.004
Methane production, g kg ⁻¹ DMI	13.1	14.2	18.0	1.21	0.04	19.1	17.3	18.1	1.85	0.80
Methane-energy GE-intake [†] , %	4.23 ^{Bb}	4.64 ^{ABb}	5.93 ^{Aa}	0.48	0.03	6.08	5.58	5.88	0.59	0.84
Carbon dioxide production, L day ⁻¹	1790.46	1728.87	1732.15	122.27	0.94	2124.78	2398.82	2421.66	217.29	0.58
ME intake [†] , MJ day ⁻¹	54.54	54.41	46.17	3.11	0.14	72.01	74.95	73.85	6.63	0.95
Body weight [‡] , kg	212.63	211.86	208.65	12.39	0.97	253.39	256.95	256.93	14.94	0.98

Trials 1 and 2 comprised T1-CTRL, T1-LA, T1-HA, T2-CTRL, T2-LA, and T2-HA: for details, see Tables 4 and 5 or the section on materials and methods in Chapter 2.

DG, daily gain; BW, body weight; DMI, dry matter intake; GE, gross energy; ME, metabolizable energy; SEM, standard error of the mean.

^{A, B, a, b} Means with different superscripts within a row for each of Trial 1 and Trial 2 differ significantly (^{A, B} for $P \leq 0.05$, ^{a, b} for $P \leq 0.10$).

[†] Calculated using values from MOA (2004) and NARO (2010) on the basis of actual intakes of feed ingredients.

[‡] Obtained at the start of measurements conducted in respiration chambers.

The increasing trends in CH₄ production with an increasing proportion of the AH mixture showed that incorporating the AH mixture into concentrate-based diets did not appear to be an optimal strategy in terms of reducing CH₄ emissions.

Nevertheless, neither differences in CH₄ production (L day⁻¹) nor those in CH₄ production per 1-kg DG between the CTRL and LA groups were significant in either trial ($P > 0.10$, Table 9). The ratio of energy loss as CH₄ emission to total GE intake was lower in the T2-LA group than in the T2-CTRL group. Zhao *et al.* (2015) suggested the possible substitution of high-quality forages for concentrate to mitigate enteric CH₄ emissions. A certain amount of AH inclusion could be incorporated as a substitute for concentrate feed, when factors other than GHG emissions, such as feed cost, are considered.

2.3.5. Economic evaluation of feeding alfalfa hay to calves

The feed cost (US\$ day⁻¹ head⁻¹) decreased significantly from T1-LA to T1-HA ($P < 0.05$, Table 10), because the influence of a decrease in the DMI of C on the feeding cost from T1-LA to T1-HA was greater than that of an increase in the DMI of AH (Table 6). On the other hand, there was a significant increase in feed cost from T2-CTRL to T2-HA ($P < 0.05$) because of an increase in the DMI of AH without a decrease in the DMI of C from T2-CTRL to T2-LA and T2-HA. The feed costs for the LA groups in both trials were numerically lower than the cost for the T1-CTRL group in T1 and significantly less than that for the T2-HA group in T2, respectively. The T1-LA, T2-LA, and T1-HA groups evidenced 1-kg DG (Figure 8). The feed cost was lower ($P < 0.05$) and the economic benefit was numerically higher for the T2-LA group than for the T2-HA group. To achieve 1-kg DG, AH feeding at the LA level would appear to be an appropriate feeding strategy in terms of economic feasibility in Gansu Province.

Table 10 Economic assessment of incorporating alfalfa into the feeding regimen of growing Simmental male calves for trials conducted in 2015

Item	Trial 1				Trial 2			
	T1-CTRL	T1-LA	T1-HA	SEM P	T2-CTRL	T2-LA	T2-HA	SEM P
Feed cost, US\$ day ⁻¹ head ⁻¹	1.64 ^A	1.55 ^A	1.28 ^B	0.06 0.001	1.70 ^C	2.20 ^B	2.47 ^A	0.07 0.000 [§]
Profit [†] , US\$ day ⁻¹ head ⁻¹	3.24 ^A	3.57 ^A	2.19 ^B	0.26 0.01	2.38 ^B	4.16 ^A	3.97 ^A	0.42 0.02
Economic benefit [‡] , US\$ day ⁻¹ head ⁻¹	1.60 ^{AB}	2.03 ^A	0.91 ^B	0.27 0.03	0.68	1.96	1.50	0.44 0.15
Feed cost, US\$ day ⁻¹ kg ⁻¹ DG head ⁻¹	1.78	1.54	2.29	0.29 0.21	2.60	1.91	2.40	0.30 0.28

Trials 1 and 2 comprised T1-CTRL, T1-LA, T1-HA, T2-CTRL, T2-LA, and T2-HA: for details, see Tables 4 and 5 or the section on materials and methods in Chapter 2.

SEM, standard error of the mean.

Values for feed costs were calculated based on the results for feed intakes (Table 6) obtained in the feeding trials.

[†] Calculated by multiplying DG (Table 7) by the market price of a growing calf.

[‡] Calculated by subtracting the feed cost from the profit.

[§] $P < 0.0005$.

^{A, B, C} Means with different superscripts within a row for each of Trial 1 and Trial 2 differed significantly ($P \leq 0.05$).

The additional income for farmers was calculated for each trial, based on the difference in economic benefits obtained per calf for a 100-day period between the LA and CTRL groups. Estimated incomes were US\$43 and US\$128 for the T1-LA and T2-LA groups, respectively. These amounts accounted for 17–50% of the average annual livestock-derived income of farming households in Gansu Province (US\$254 year⁻¹) (Editorial committee for Gansu statistics yearbook 2014).

Shi *et al.* (2014) reported that partial replacement of maize with an alternative feed source (a compound of Chinese jujube, soybean hulls, and molasses) did not have an adverse effect on the DG of Limousin crossbreeds. Though the conventional diet comprising maize, tofu residue, and brewer's grain was associated with higher growth rates, the alternative feed source cost less. Sithyphone *et al.* (2011) showed that the cost of hay feed required to achieve 1-kg DG in Japanese Black cattle was lower than that of concentrate, though the growth rate of the hay-fed animals was slower than that of the concentrate-fed animals. This finding indicates that the efficient production of hay using farmers' own resources would considerably reduce feeding costs. The findings of the current study similarly showed that the incorporation of a low level of AH into concentrate-based diets was economically advantageous.

2.4. Conclusions

Replacement of C with AH at differing levels did not cause a reduction in DMI associated with increased NDFom intake, but a high level of AH feed did cause an increase in CH₄ emission. Low-level AH supplementation increased the DG of growing beef calves. There were no significant differences in energy metabolizability between the low-level and high-level AH diets. Considering the importance of economic feasibility, the low-level AH

diet provided relatively high economic benefits. The inclusion of low levels of AH in the diets of growing beef cattle would be an appropriate practice in the drylands of Gansu Province, China. Because DMI and DG values were lower for the T2-CTRL group than for other T2 groups, a careful evaluation may be necessary when considering the introduction of feeding practices with relatively high proportions of concentrate feed in the cool season. The MEm requirements of crossbred Simmental calves were calculated to be higher than those for other breeds, suggesting that the energy allowance should be carefully calculated to ensure the energy requirement of crossbred Simmental. In conjunction with the energy metabolism study discussed in Chapter 2, further evaluation of the N utilization status of Simmental beef calves was proposed to establish an appropriate feeding regimen.

Chapter 3

Effects of substituting alfalfa hay for concentrate on nitrogen utilization

3.1. Introduction

The discussion in the previous chapter of dietary energy utilization in growing Simmental crossbred calves demonstrated that the DG was higher ($P < 0.05$) in groups with high ME intake levels (the CTRL and LA groups) in the warm season. By contrast, in the cool season, increased ME intake moving from the LA to the HA group was not associated with an increase in DG. The effects of AH inclusion levels on the dietary N utilization status and DG requires evaluation. Therefore, using the data obtained from two feeding trials conducted in 2015, I evaluated the effects of the dietary AH level on the DG and efficiency of dietary N utilization. In this chapter, unless otherwise specified, T1 and T2 denote the trials conducted in 2015 (as mentioned in Chapter 2).

3.2. Materials and methods

The data collected in the feeding trials conducted in 2015 (see Chapter 2) were used to assess the effects of the dietary AH level on the DG and efficiency of dietary N utilization in Simmental calves. Additionally, the MCP content synthesized in the rumen with the FME provided to each group was calculated. The FME concentrations in feed ingredients (CS, AH, and C) were referenced from the values listed in AFRC (1993), and yields of MCP per FME unit ($\text{g MJ}^{-1} \text{FME}$) were calculated based on feeding levels for each calf using the AFRC estimation equation (1993). In addition, the amount of MCP synthesized from ERDP was estimated (AFRC 1993). The estimation equation (AFRC 1993) was used to calculate the rumen outflow rate per hour based on the level of feeding. Other parameters

related to feed ingredients required to establish ruminal N degradability were referenced from the tabular values listed in AFRC (1993). The same statistical analysis described in Chapter 2 was applied to evaluate differences in means between the three groups in each trial.

3.3. Results

Feed and nutrients intake, and digestibility are presented in Table 6. In T1, CP intake and digestibility did not differ between the groups, but NDFom intake did increase with a corresponding increase in the amount of AH feed. Conversely, in T2, an increase in the amount of AH feed corresponded to increased intakes of CP and NDFom ($P < 0.05$). The digestibility of CP and NDFom was lower in the T2-LA and T2-HA groups than in the T2-CTRL group ($P < 0.05$). In both trials, ratios of roughage intake to total DMI increased as the AH feeding level increased ($P < 0.05$).

The N balance and N utilization efficiency in the feeding trials are shown in Table 11. In T1, N intake from AH increased linearly and that from C decreased as the AH feeding level increased, although total N intake did not differ among the three feeding groups. Neither the total N intake nor fecal N excretion differed significantly as the amount of AH inclusion increased. Urinary N excretion, however, tended to decrease as AH inclusion increased ($P = 0.09$). The N retention numerically increased ($P = 0.37$) with increasing AH feeding level. Nitrogen utilization efficiency expressed as the ratio of urinary N excretion to N intake tended to decrease ($P = 0.08$), and the efficiency expressed as the ratio of N retention to N absorption (56.0–75.9%) showed a numerical increase ($P = 0.14$) with an increase in the proportion of AH in the feed. In T2, N intake from AH increased as the AH feeding level increased ($P < 0.05$), whereas N intake from C did not differ among the three

groups. Total N intake and fecal N excretion increased significantly ($P < 0.05$) as the AH allowance increased, but urinary N excretion did not differ between the treatments ($P = 0.62$). Nitrogen retention in the T2-HA group was significantly greater than in the T2-LA and T2-CTRL groups ($P < 0.05$). The ratio of urinary N excretion to N intake showed a numerical decrease ($P = 0.12$), while the N utilization efficiency, expressed as the ratio of N retention to N absorption (31.6–56.4%), showed a numerical increase ($P = 0.15$) with an increasing amount of AH in the feed.

The following values of MCP (g day^{-1}) synthesized from the FME provided for each group were calculated: 422.0 (T1-CTRL), 412.0 (T1-LA), 360.6 (T1-HA), 391.3 (T2-CTRL), 482.6 (T2-LA), and 565.8 (T2-HA). Estimated values of MCP (g day^{-1}) synthesized from ERDP intake were: 288.5 (T1-CTRL), 313.4 (T1-LA), 333.0 (T1-HA), 282.2 (T2-CTRL), 384.6 (T2-LA), and 473.0 (T2-HA). Hence the amounts of MCP synthesized from ERDP intakes expressed as percentages of the MCP synthesized from the FME provided were calculated as follows: 68.4 (T1-CTRL), 76.1 (T1-LA), 92.3 (T1-HA), 72.1 (T2-CTRL), 79.7 (T2-LA), and 83.6 (T2-HA). All of these values were below 100%.

3.4. Discussion

3.4.1. Nitrogen utilization efficiency

Lack of significant difference in the total N intake among the groups in T1 (Table 11) indicates that substituting AH for C in the diets does not affect the N intake. Simple regression analysis of AH intake ($f(x)$, $\text{g kg}^{-0.75} \text{ BW day}^{-1}$) with C intake (x , $\text{g kg}^{-0.75} \text{ BW day}^{-1}$) in T1 reveals that an AH intake of 1 kg DM can effectively replace a C intake of 1.36 kg DM ($R^2 = 0.90$).

Table 11 Nitrogen balance and nitrogen utilization efficiency in Simmental crossbred beef calves in trials conducted in 2015

Item	Trial 1				Trial 2					
	T1-CTRL	T1-LA	T1-HA	SEM P	T2-CTRL	T2-LA	T2-HA	SEM P		
N balance										
N intake, g kg ^{-0.75} BW day ⁻¹	1.46	1.52	1.50	0.04	0.62	1.11 ^C	1.49 ^B	1.89 ^A	0.05	0.000 [‡]
N intake from alfalfa hay	0.00 ^C	0.20 ^B	0.44 ^A	0.01	0.000 [‡]	0.00 ^C	0.36 ^B	0.75 ^A	0.02	0.000 [‡]
N intake from concentrate	1.22 ^A	1.04 ^B	0.76 ^C	0.04	0.000 [‡]	1.03	1.05	1.05	0.05	0.94
Fecal N excretion, g kg ^{-0.75} BW day ⁻¹	0.53	0.54	0.53	0.09	0.99	0.33 ^C	0.54 ^B	0.73 ^A	0.04	0.000 [‡]
Urinary N excretion, g kg ^{-0.75} BW day ⁻¹	0.40	0.34	0.23	0.05	0.09	0.54	0.66	0.51	0.10	0.62
N retention, g kg ^{-0.75} BW day ⁻¹	0.54	0.64	0.74	0.10	0.37	0.24 ^B	0.30 ^B	0.65 ^A	0.07	0.01
N utilization efficiency										
Urinary-N-excretion N-intake ⁻¹ , %	27.3 ^a	22.3 ^{ab}	15.5 ^b	3.17	0.08	48.4	43.8	26.7	6.76	0.12
N-retention N-intake ⁻¹ , %	36.9	41.9	49.4	6.17	0.39	22.0	20.3	34.6	5.81	0.24
N-retention N-absorbed ⁻¹ , % [†]	56.0	64.6	75.9	6.35	0.14	31.6	32.3	56.4	8.92	0.15

Trials 1 and 2 comprised T1-CTRL, T1-LA, T1-HA, T2-CTRL, T2-LA, and T2-HA; for details, see Tables 4 and 5 or the section on materials and methods in Chapter 2.

BW, body weight; SEM, standard error of the mean.

^{A, B, C, a, b, c} Means with different superscripts within a row for each of Trial 1 and Trial 2 differed significantly (^{A, B, C} for $P \leq 0.05$, ^{a, b, c} for $P \leq 0.10$).

[†] Absorbed N was calculated as N intake – N excreted in feces.

[‡] $P < 0.0005$.

The result in T1 and the increase in total N intake in T2 as the increase in AH feeding level ($P < 0.05$) suggest no particularly detrimental effect of the increase in AH feeding level on the N intake.

The N retention tended to increase as the amount of AH increased due to the reduction in urinary N excretion in T1 and to a greater increase in N intake than in fecal N excretion in T2 (Table 11). Regression analysis of N retention ($f(x)$, $\text{g kg}^{-0.75} \text{ BW day}^{-1}$) with N intake from AH (x , $\text{g kg}^{-0.75} \text{ BW day}^{-1}$) revealed that an increase of 100 g in N intake from AH corresponded to increases in N retention of 1.15 g ($R^2 = 0.20$) in T1 and 1.03 g in T2 ($R^2 = 0.63$), respectively. This finding suggests an increasing trend relating to N retention with increasing AH feed levels. Agarwal *et al.* (2015) reported that the proportion of urea recycled into the gastrointestinal tract of growing sheep was greater with forage-type diet than with concentrate-type diet. Increases in the recycled urea in AH feeding groups (T1-LA and T1-HA) might have led to a reduction in the excreted urinary N, and to an increase in N retention. The increase in N retention with increasing AH feeding levels in T1 and T2 may have been partly caused by the difference in the amount of recycled urea attributable to the forage (*i.e.*, AH in this study) amount in the diets (Table 5).

Nitrogen utilization efficiency, calculated as the ratio of N retention to N absorbed, of N retention to N intake, or of urinary N to N intake (Table 11), are in agreement with those of Dong *et al.* (2014), Lobley (1992), Yan *et al.* (2007), Zhao *et al.* (2015), and Niderkorn *et al.* (2015). Stergiadis *et al.* (2015) reported that N utilization efficiency increased as NDF intake increased with constant N intake in dairy cows fed fresh fodder, and suggested that the increase of N utilization efficiency was due to lengthened retention times of rumen digesta. The increase in NDF intake also enables microbes in the hindgut to utilize the available fermentable energy source to capture N from recycled blood urea, leading to a

reduction of urinary N (Hoekstra *et al.* 2007). The results of this study showed similar trends to those reported in the above studies. In both trials, the N utilization efficiency numerically increased (Table 11) with increased dietary NDFom concentrations (Table 5) and increased NDFom intakes (Table 6, $P < 0.05$), whereas the results for N intake were either comparable (T1) or they showed an increase (T2, $P < 0.05$) that corresponded to higher levels of dietary AH. These results suggest an effect of NDFom intake, accompanied by the AH feeding level, on N utilization efficiency.

3.4.2. Relation between nitrogen retention and daily body-weight gain

In the two trials, the 1-kg DG was achieved for the calves in the T1-LA, T2-LA, and T2-HA groups, but not for the calves in the T1-CTRL, T1-HA, and T2-CTRL groups (Figure 10). The DG goal of 1 kg was set as an appropriate goal for the trials in light of farmers' responses during previous interviews conducted in Gansu Province. Although T1-LA achieved 1-kg DG, T1-HA did not (Figure 10). Because both N intake and N retention did not differ significantly between T1-LA and T1-HA (Table 11), the DG reduction in T1-HA (demonstrating the altered relationship between N retention and DG in T1-HA) might be due to a decrease in ME intake from T1-LA to T1-HA with an increase in NDFom intake (see Chapter 2). Although T2-CTRL did not achieve 1-kg DG, T2-LA did. The N intake increased ($P < 0.05$), and there was a numerical increase in N retention from T2-CTRL to T2-LA (Table 11, Figure 10). The MEI of calves in the T2-CTRL group may have been less than that required for 1-kg DG (see Chapter 2). In the current study, either the N intake or the MEI (or both) in the T2-CTRL group may have been insufficient to yield 1-kg DG.

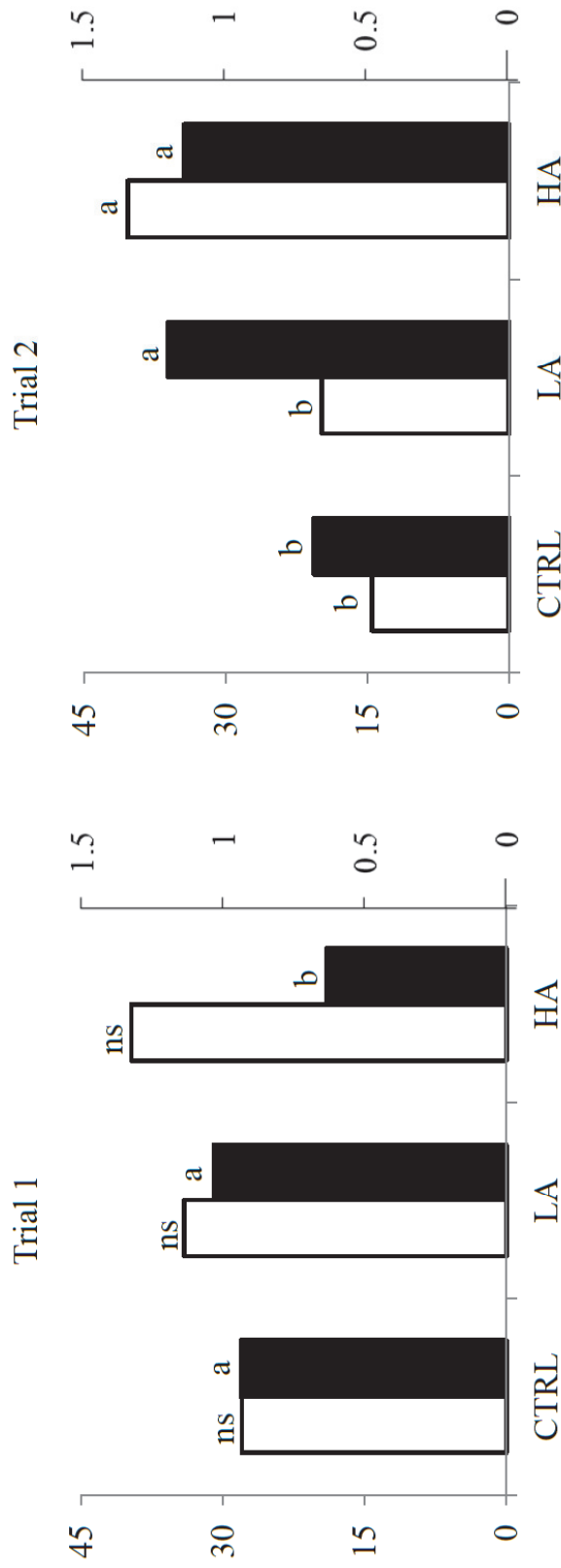


Figure 10 Nitrogen retention and daily weight gain of calves in 2015. CTRL, control group; LA, low-level alfalfa hay feeding group; HA, high-level alfalfa hay feeding group; ns, no significance. ^{a, b} Mean values with different superscripts within each of Trial 1 and Trial 2 differed significantly among the three feeding groups ($P \leq 0.05$). Unshaded bars depict nitrogen retention (g day^{-1} , left scale); solid bars depict daily weight gain (kg day^{-1} , right scale).

By contrast, both the T2-LA and T2-HA feeding regimens provided sufficient amounts of N and ME required for 1-kg DG. The N intake and N retention were higher in T2-HA than in T2-LA ($P < 0.05$; Table 11, Figure 10). However, there was no difference in the DG of calves in the T2-LA and T2-HA groups. A significant increase ($P < 0.05$) in N retention from T2-LA to T2-HA (Figure 10) did not correspond to an increase in the DG. Malekjahani *et al.* (2017) suggested a decrease in synchronization index (for balance of N and energy supplies for ruminal microbes) when the inclusion level of AH in the diet (on a DM basis) increased from 34% to 43% and the inclusion level of corn grain (on a DM basis) decreased from 15% to 5%. Unbalanced N and energy supplies for ruminal microbes leads to increases in ruminal NH_3 concentration, BUN, and urinary N excretion, which in turn reduce N utilization efficiency (Hoekstra *et al.* 2007). The lack of DG increase from T2-LA to T2-HA with the increase in the inclusion level of AH from 22.6% to 38.1%, indicates that the energy and N supplies for ruminal microbes in T2-HA were less balanced than those in T2-LA. However, in both trials, the estimated ratio of MCP synthesized from the ERDP to the MCP synthesized from the FME approached 100% as the AH inclusion level increased. Because this ratio shows the balance between the ERDP and FME supply, the finding indicates that ERDP and FME supplies required for the effective functioning of ruminal microbes were more balanced for the HA groups compared with these supplies for other groups (see section 3.3).

The feeding regimens prepared in T1 were for a season requiring minimal feeding of concentrate, and those in T2 were for a season requiring increased concentrate. In T1, insufficient ME intake for 1 kg DG caused a DG reduction in T1-HA because the increase in AH intake did not compensate for the reduced ME intake from C (see Chapter 2). In T2, the higher CP concentrations due to higher C inclusion levels than in T1 groups (Table 5)

may have provided abundant degradable protein for ruminal microbes, thereby exceeding the amount of microbial protein synthesized by using available fermentable energy in the rumen. Protein assimilation requires more energy than does assimilation of carbohydrates (Sato 2014). Excess dietary protein increases maintenance energy requirements of steers because of the energy costs for removing excess N as urea in urine (Jennings *et al.* 2018). ATP or energy is required to detoxify or catabolize the ammonia, which could be produced by excess of N supply in the rumen and absorbed through rumen wall, into urea mainly in the liver. The protein supply, which exceeded the required amount in the current study, would lead to wastage of energy needed for BW gain. Moreover, a high-protein diet may induce fat mobilization in dairy cows and steers to use the additional protein for net tissue accretion during exogenous energy undernutrition (Ørskov *et al.*, 1983). It is plausible that the mobilization of body fat as an energy source to assimilate the amino acids absorbed through the digestive tract negatively affects BW gain. The lack of a significant DG increase in T2-HA compared with the DG increase in T2-LA (Table 7), despite greater N retention in T2-HA than T2-LA (Table 11, Figure 10), could be attributed to this fat mobilization.

The need to control the release of excreted N from livestock into the environment has become a matter of significant concern. This issue is partly caused by the asynchrony between the degradation of carbohydrates and proteins in the rumen, which contributes to the loss of N in urine (Stern *et al.* 1994, Ma *et al.* 2014). In this context, it is important to achieve efficient N utilization through the appropriate supply of dietary carbohydrates and protein for optimal microbial protein synthesis and a reduction in the N output. Further study is needed to elucidate a ration formula including AH for growing beef calves that provides better balanced N (*i.e.*, MP) and energy (*i.e.*, ME) to yield DG above 1 kg.

3.5. Conclusions

Alfalfa-hay inclusion in the concentrate-based diets did not cause any detrimental effect on N utilization efficiency and N retention in both trials. However, high-level AH inclusion did not markedly contribute to improving the DG, due to the reduction in ME intake and/or an imbalance between dietary energy and N supply; although the N utilization efficiency seemed preferable. To achieve the 1-kg DG with sufficient N utilization and no detrimental effect on dietary energy utilization, low-level AH feeding (*i.e.*, AH allowance as a percentage of DM at 8.4% in T1 and 23.6% in T2) would be a beneficial practice when a concentrate-based diet is provided for crossbred Simmental male calves in the drylands of China. Further evaluation is expected to elucidate appropriate inclusion levels of AH in the drylands of China.

Chapter 4

Appropriate inclusion level of alfalfa hay to total dry-matter allowance

4.1. Introduction

The dietary energy and N utilization of growing Simmental crossbred calves have been discussed in previous chapters. The key finding reported thus far is that the dietary inclusion of AH at low levels did not reduce energy digestibility and metabolizability, while entailing high economic benefits, increased N intake, and no significant increase in CH₄ production, as compared with a conventional concentrate-based diet. Substitution of AH for C in an appropriate ratio would thus lead to China's increased self-sufficiency in animal feed and would yield economic benefits and high production performance for farmers.

The effects of low-level AH inclusion in the diets of beef cattle were assessed in terms of the animals' performances and economic benefit. However, a broad range of AH inclusion levels should be evaluated to optimize the ratio to be used in Gansu Province. Therefore, I evaluated the effect of the dietary AH inclusion level on the DG, energy utilization status, and economic profitability through two additional feeding trials and a respiration trial conducted in 2016, in which the diets of the animals were designed on the basis of the results presented in previous chapters. Subsequently, I used the outputs obtained from the trials conducted in 2015 and 2016 to determine the optimal ratio of AH to the total DM allowance when substituting AH for C. In this chapter, unless otherwise specifically indicated, T1 and T2 denote the trials conducted in 2016.

4.2. Materials and methods

4.2.1. Cattle, applied diets, and feeding management

Two feeding trials were conducted in the Linze Research Station of Lanzhou University (see section 2.2.1) from July 3 to 17, 2016 (T1) and from August 15 to September 23, 2016 (T2), with the aim of achieving 1-kg DG. The average temperatures during the periods of T1 and T2 were 19.8 °C and 16.1 °C, respectively. The annual temperature in 2016 was 6.3 °C. The total precipitation in the Station during that year was 50 mm, all of which occurred between March and October (data supplied by the Linze Research Station).

Twelve male crossbred Simmental calves with a mean BW of 126.2 ± 8.0 kg (at 6 months of age) and with a mean BW of 159.4 ± 9.9 kg (at 7 months of age) were purchased from a local market for the T1 and T2, respectively. Conventional feeding practices of beef cattle farmers in Gansu Province, which entail a greater amount of C in the animals' diets during the cool season compared with the C component of their diets in the warm season, were incorporated into the design of the experimental feeding regimens. The 12 calves in each trial were assigned to one of three groups (LA, MA, and HA; $n = 4$ per group) so that initial BW did not differ significantly among groups. These three groups differed in the amount of AH fed. All calves were fed forage diets comprising CS and AH, supplemented with C. The diets of the T1-LA and T2-MA groups were designed based on the low-level AH mixtures used for the feeding trials conducted in 2015 (see section 2.2.1); these dietary allowances (T1-CTRL and T2-LA in 2015) were regarded as being practically appropriate for the drylands of Gansu Province, China. In T1, to assess the effect of adding more of the AH mixture than the proportion used in the LA group in 2015, the calves in the MA group (T1-MA) were offered an increased amount of AH with CS and C, whereas those in the HA group (T1-HA) received a proportionately greater amount of AH with CS and C. In T2, to assess the effect of changing the proportion of the AH mixture from the quantity in the LA group in 2015, the calves were offered CS and C with a decreased amount of AH in the LA

group (T2-LA) but an increased amount of AH in the HA group (T2-HA). The experimental diet for each treatment was designed in the same way as the treatments applied in 2015 (see section 2.2.1). In a one-way-layout design, calves were fed the diets comprising AH (as a percentage of DM) at 15% (T1-LA), 23% (T1-MA), 31% (T1-HA), 9% (T2-LA), 24% (T2-MA), or 34% (T2-HA); a constant ratio (as a percentage of DM) of CS; and decreasing quantities of C in proportion to the increase in AH.

The CS and AH used in both trials were cultivated at the Linze Research Station. The CS used in both trials was harvested in September 2015. The AH used in both trials was harvested in July (second-cut hay) and in September (third-cut hay) of 2015. The C comprised commercial concentrate (30%), wheat bran (10%), and corn grain (60%). The corn grain was produced at the Linze Research Station in September 2015. Other dietary conditions in the trials remained the same as those in 2015 (as described in Chapters 2 and 3).

4.2.2. Measurements and sample collection

As in the trials in 2015, the amount of feed offered and the refusals were weighed, the calves were weighed, and the feed and fecal samples were collected, weighed, and used for further chemical analysis (see section 2.2.2). At the beginning and end of both trials, blood samples (10 ml per calf) were collected from their jugular veins after the morning feeding of roughage. The samples were immediately centrifuged at 3000 RPM ($600 \times g$) for 10 minutes at room temperature, and the plasma was stored at -20°C . After completion of T1, respiratory measurements for 12 calves (all of the calves in T1) were conducted for 48 hours after a 5-day adaptation period, as in 2015. The average BW of the calves in T1 at the start of the respiratory measurements was 144.4 ± 14.2 kg. Metabolizable energy intake for

each calf was calculated using a ratio of 0.88 for the conversion of DE into ME obtained from the results of the trials conducted in 2015 (Table 7). The estimates of MEI were compared with those calculated on the basis of dietary ADF concentrations and *in vitro* gas production analysis (see section 2.2.4). Then HP was calculated and MEm was estimated (see section 2.2.3). The respiratory measurements were not performed at the end of T2 due to technical problems with the measuring apparatus. The same statistical analysis, as described in Chapter 2, was applied for the three groups in each trial as well as for all of the trials conducted in 2015 and 2016.

4.2.3. Analysis for blood metabolites

Plasma concentrations of the following metabolites were determined by using commercial kits: glucose (Glucose C-test; Wako Pure Chemical Industries, Osaka, Japan), NEFA (NEFA C-test; Wako Pure Chemical Industries), BUN (C013-2; Nanjing Jiancheng Bioengineering Institute, Nanjing, China), and BHBA (3-HB auto; Wako Pure Chemical Industries). A spectrophotometer (Cary 60; Agilent Technologies Corp., United States) was used for performing the analyses.

4.2.4. Economic analysis

Differences in feeding costs for the dietary treatment groups were calculated as described in Chapter 2 (see section 2.5). The market prices applied for the calculation were 1.70 yuan kg⁻¹ for AH, 2.54 yuan kg⁻¹ for commercial concentrate, and 22 yuan kg⁻¹ BW for calves. The estimates were converted at the rate of US\$1 = 6.68 yuan (based on the average of values for the periods of July 3 – 18 and of August 15 – September 23, 2016).

4.2.5. Estimation of net energy intake and requirement

The *Feeding Standard for Beef Cattle* (MOA 2004) provides instructions on the preparation of feeding regimens by estimating the NEmf intake and the NEmf requirement. Accordingly, the applicability of tabular NEmf values and functions for estimating the NEmf intake (MOA 2004) was verified, and the energy utilization efficiency for the calves in the current study was assessed. The NEmf requirements of all of the calves used for the trials conducted in 2015 and 2016 were calculated using BW (kg) and DG (kg day⁻¹) by applying the following formulas (MOA 2004):

$$\text{NEm (kJ day}^{-1}\text{)} = 322 \times \text{BW}^{0.75}$$

$$\text{NEg (kJ day}^{-1}\text{)} = (2092 + 25.1 \times \text{BW}) \times \text{DG} / (1 - 0.3 \times \text{DG})$$

$$\text{NEmf (kJ day}^{-1}\text{)} = (\text{NEm} + \text{NEg}) \times (\text{F, calibration factor according to BW and DG})$$

Average BW values obtained by weighing the calves at the beginning and end of each of the trials and the actual DG were used for the calculation. F values of 1.020, 1.034, 1.047, and 1.061 were used for the calves with BWs (kg) of <200, 200–225, 225–250, and 250–275, respectively (MOA 2004).

Next, the NEmf intakes for all of the calves were calculated using the actual intakes of dietary ingredients (AH, CS, and C) and the tabular values listed in MOA (2004) which were calculated on the basis of GE and DE as follows:

$$\text{NEm} = \text{DE} \times \text{K}_m \quad \text{K}_m = 0.1875 \times (\text{DE}/\text{GE}) + 0.4579$$

$$\text{NEg} = \text{DE} \times \text{K}_f \quad \text{K}_f = 0.523 \times (\text{DE}/\text{GE}) + 0.00589$$

$$\text{NEmf} = \text{DE} \times \text{K}_{mf} \quad \text{K}_{mf} = \text{K}_m \times \text{K}_f \times 1.5 / (\text{K}_f + 0.5 \times \text{K}_m).$$

The NEmf values (MJ kg⁻¹ DM) for estimating the above NEmf intake were 2.81 for CS, 3.53 for AH, and 8.34 for C. Simple regression analysis of the required NEmf with the NEmf intake was performed to validate the applicability.

Last, the other values of NEmf intake were calculated using the actual DE GE⁻¹ for calculating K_f, K_m, K_{mf}, and NEmf. The NEmf intake calculated using this method was compared with the required NEmf by conducting a simple regression analysis. The NEmf intake and the energy utilization efficiency (DE GE⁻¹) of the calves used in this study were then evaluated on the basis of the results of two simple regression analyses.

4.3. Results and discussion

4.3.1. Chemical composition of diets

The GE concentration and chemical composition of feed ingredients are shown in Table 4. Despite the use of alfalfa harvested at the same stage for both feeding trials, there were slight differences in the concentrations of CP, NDFom, and ADL in the AH feeds used in T1 and T2. The CP concentration of C was higher in T2 than in T1, because commercial concentrate used in T1 was not available, and replacement with a new concentrate was necessary at the commencement of T2. The NDFom concentrations of AH on a DM basis (46.9% for T1 and 52.4% for T2) were higher than those reported by NARO (2010) (36.0–39.3%) and were similar to those of the samples obtained in 2015 (52.2–52.8%).

The daily feed allowance and estimated chemical compositions of the experimental diets of the calves at the start of each trial are shown in Table 12. The feed allowance for each animal was calculated in the same way as in 2015 (see section 2.3.1). The values shown in Table 12 were for diets provided to calves with BWs of 126.2 kg and 159.4 kg in T1 and T2, respectively. In both trials, the estimated dietary NDFom concentration tended to increase as the proportion of AH included in the feed increased because of the higher concentrations of NDFom in AH compared with NDFom concentrations in C (Table 4).

Table 12 Feed allowances and the chemical composition of experimental diets formulated for Simmental beef calves in 2016

Item	Trial 1			Trial 2		
	T1-LA	T1-MA	T1-HA	T2-LA	T2-MA	T2-HA
Feed ingredient [†] , kg DM day ⁻¹						
Corn stover	1.2	1.2	1.2	1.3	1.3	1.3
Alfalfa hay	0.6	1.0	1.4	0.4	1.2	1.8
Concentrate	2.3	2.1	1.9	3.0	2.5	2.2
Estimated chemical composition [‡] , % DM						
CP	10.6	10.8	10.9	13.8	13.1	12.7
NDFom	36.6	38.4	40.4	37.1	42.1	44.4
ADL	3.2	3.7	4.2	3.0	4.1	4.7

See the footnote in Table 4 for details on Trials 1 and 2.

Feeding regimens for T1-LA and T2-MA were designed with reference to the low level of alfalfa hay used in feeding trials in 2015 (see Chapter 4). T1-LA and T2-LA denoted low-levels of alfalfa hay feeding; T1-MA and T2-MA denoted medium levels of alfalfa hay feeding; and T1-HA and T2-HA denoted high levels of alfalfa hay feeding.

ADL, acid detergent lignin; CP, crude protein; DM, dry matter; NDFom, ash-free neutral detergent fiber.

[†] Calculated using the equation provided by AFRC (1993) based on the initial average body weight of male calves in Trial 1 (126.2 kg) and Trial 2 (159.4 kg) to meet the metabolizable energy requirement for a daily gain of 1 kg.

[‡] Values were estimated based on the chemical composition of feed ingredients (Table 4) and the composition of ingredients in the experimental diets.

The ADL concentrations were above 3% in all of the groups (Table 12), and were sufficiently high for their use as an internal marker to estimate fecal DM excretion (see section 2.3.1). The concentrations of CP in CS and those of NDFom (Table 4) further confirmed that inclusion of a concentrate or leguminous forage supplement is required for crossbred Simmental male calves when they are fed CS as the basal forage. Differences between the MEI value estimated on the basis of ADF concentrations and those obtained through the feeding trials ((1) (3)⁻¹ in Table 8) were less than those between the MEI estimated on the basis of *in vitro* gas production analysis and those obtained through the feeding trials ((1) (2)⁻¹ in Table 8) for all of the feeding groups in 2016. This finding suggests the possible use of the MEI estimated from the ADF concentration as an alternative *in vitro* method to estimate the MEI (see section 2.3.3).

The results of all of the trials in 2015 and 2016 led to the following conclusion: CP concentrations of AH approximate or exceed those of C, suggesting the possible replacement of C with AH to provide CP in CS-based diets.

4.3.2. Feed intake, feed and energy utilization efficiency, and growth performance

Feed and nutrient intake and digestibility for each group are shown in Table 13. Dry-matter intake (in kg day⁻¹ and % BW) did not differ between groups in either T1 or T2 and was consistent with the results obtained from both T1 and T2 in 2015, which indicated that dietary substitution with AH did not reduce total DMI. In T1, the C intake did not decrease from T1-LA to T1-HA in proportion to the increase in the AH intake. Consequently the ratio of C intake (on a DM basis) to total DMI did not gradually decrease as AH intake increased, and the ratio of roughage (CS and AH) intake to total DMI did not differ among the three groups in T1 ($P = 0.51$).

Table 13 Feed and nutrient intake, and digestibility in Simmental crossbred beef calves in trials conducted in 2016

Item	Trial 1				Trial 2					
	T1-LA	T1-MA	T1-HA	SEM	P	T2-LA	T2-MA	T2-HA	SEM	P
Feed intake										
Corn stover, kg DM day ⁻¹	1.0 ^b	0.9 ^{ab}	0.7 ^a	0.08	0.06	1.3 ^a	1.3 ^a	1.2 ^b	0.03	0.01
Alfalfa hay, kg DM day ⁻¹	0.5 ^B	0.7 ^{AB}	0.8 ^A	0.06	0.02	0.4 ^C	1.1 ^B	1.6 ^A	0.04	0.000 [†]
Concentrate, kg DM day ⁻¹	1.9	2.0	1.7	0.14	0.34	3.4 ^a	2.8 ^b	2.5 ^c	0.08	0.000 [†]
Total DMI, kg day ⁻¹	3.5	3.6	3.3	0.21	0.63	5.1	5.2	5.2	0.13	0.76
Total DMI, % BW	2.63	2.57	2.47	0.17	0.58	2.73	2.73	2.84	0.04	0.09
Nutrient intake, kg DM day⁻¹										
Crude protein	0.36	0.39	0.37	0.03	0.63	0.71	0.69	0.69	0.02	0.66
NDFom	1.28	1.26	1.18	0.09	0.74	1.85 ^B	2.09 ^A	2.18 ^A	0.05	0.004
Roughage-intake total-DMI ⁻¹ , %	45.3	43.1	47.8	2.75	0.51	33.9 ^C	46.8 ^B	52.4 ^A	4.75	0.000 [†]
Digestibility, %										
Dry matter	58.7	63.2	66.2	4.35	0.45	69.6	64.0	66.1	2.37	0.31
Crude protein	49.5	51.2	60.5	5.72	0.39	71.6	66.8	68.1	2.89	0.51
NDFom	39.6	50.3	48.0	3.95	0.19	58.2	51.1	57.6	2.62	0.16

Trials 1 and 2 comprised T1-LA, T1-MA, T1-HA, T2-LA, T2-MA, and T2-HA; for details, see Tables 4 and 12 or the section on materials and methods in Chapter 4.

BW, body weight (kg); DM, dry matter; DMI, dry matter intake; NDFom, ash-free neutral detergent fiber; SEM, standard error of the mean.

A, B, C, a, b, c Means with different superscripts within a row for each of Trial 1 and Trial 2 differ significantly (^{A, B, C} for $P \leq 0.05$, ^{a, b, c} for $P \leq 0.10$).

† $P < 0.0005$.

I attributed the lack of proportional decrease in the C intakes in T1-MA and T1-HA to the calves' preference for C rather than AH, which was facilitated by separately feeding C and AH at different times. Sorting behavior in relation to forage and concentrate feed has been observed in growing calves (Miller-Cushon *et al.* 2013) and growing heifers (DeVries *et al.* 2014) fed on high-concentrate diets. The ratio of roughage intake to total DM allowance was reportedly 12% when the rice straw (as roughage) and concentrate (ingredients not specified) were fed separately and *ad libitum* to fattening steers (Okushima and Wada 1976). The other studies reported ~10% as a typical ratio of forage intake to total DMI when forage and concentrate feeds are offered separately (González *et al.* 2008; Faleiro *et al.* 2011). In this study, the designed ratios of roughage (CS and AH) to total DM allowance in T1 (44–58%, Table 12) were much greater and might have caused the preferred intake of C by calves. By contrast in T2, according to the increase in the ratios of AH intake to total DMI ($P < 0.05$), the C intakes tended to decrease proportionally ($P < 0.10$) (Table 13).

The growth performance and energy and N utilization of each group are shown in Table 14. The DG in T1-LA and T1-HA tended to be higher than in T1-MA ($P < 0.10$) (Figure 11). The DG in T1-MA did not meet the target, which was achieved in the other groups in T1. In T2, the DG was slightly greater in the T2-LA and T2-MA groups than in the T2-HA group ($P = 0.12$). In both trials, the LA groups, which received AH at low levels (*i.e.*, 14.2% and 7.8% of total DMI), achieved the desired DG (1 kg day^{-1}) and the highest DG in each trial. The digestion coefficients of DM, CP, and NDFom did not differ among the three groups in either T1 or T2 (Table 13). Decreased NDFom digestibility with concurrent increased NDFom intake is the suspected source of an AH-associated decrease in energy retention for DG (Hales *et al.* 2014).

Table 14 Growth performance, and energy and nitrogen utilization of Simmental crossbred beef calves during trials conducted in 2016

Item	Trial 1				Trial 2					
	T1-LA	T1-MA	T1-HA	SEM	P	T2-LA	T2-MA	T2-HA	SEM	P
Growth performance										
Daily gain, kg day ⁻¹	1.09	0.92	1.06	0.07	0.27	1.46	1.40	1.23	0.10	0.30
Feed conversion ratio	3.16 ^{ab}	3.98 ^a	3.12 ^b	0.27	0.09	3.55	3.71	4.31	0.31	0.29
Energy utilization										
GE intake, kJ kg ^{-0.75} BW day ⁻¹	1555.1	1547.8	1470.5	70.1	0.65	1736.1	1739.1	1793.0	21.9	0.17
Energy digestibility (DE GE ⁻¹), %	61.0	64.2	67.5	4.1	0.56	70.6	65.4	67.4	2.43	0.35
DE intake, kJ kg ^{-0.75} BW day ⁻¹	946.6	1000.8	992.2	84.8	0.89	1226.8	1135.9	1208.6	45.0	0.30
Nitrogen utilization, g kg^{-0.75} BW day⁻¹										
Nitrogen intake	1.50	1.55	1.50	0.07	0.84	2.26 ^a	2.17 ^b	2.22 ^{ab}	0.02	0.08
Nitrogen absorbed [†]	0.74	0.81	0.91	0.10	0.57	1.62	1.45	1.51	0.06	0.21

Trials 1 and 2 comprised T1-LA, T1-MA, T1-HA, T2-LA, T2-MA, and T2-HA; for details, see Tables 4 and 12 or the section on materials and methods in Chapter 4.

BW, body weight; DG, daily gain; DMI, dry matter intake; DE, digestible energy; GE, gross energy; SEM, standard error of the mean.

^{a, b} Means with different superscripts within a row for each of Trial 1 and Trial 2 differ significantly ($P \leq 0.10$).

[†] Absorbed nitrogen was calculated as nitrogen intake – nitrogen excreted in feces.

[‡] $P < 0.0005$.

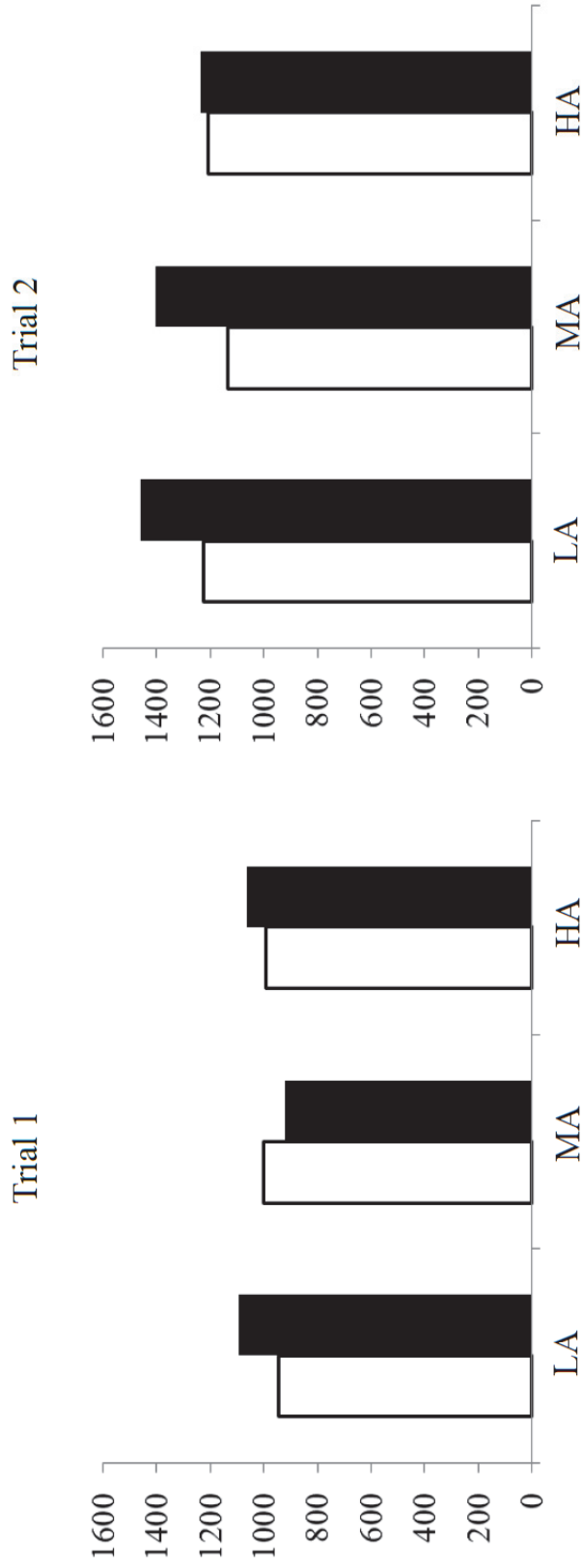


Figure 11 Digestible energy intake and daily weight gain of calves in 2016. There were no significant differences between the three feeding groups in either trial ($P > 0.10$). Unshaded bars depict digestible energy intake (MJ day^{-1} , left scale); solid bars depict daily weight gain (kg day^{-1} , right scale).

The increase in NDFom intake from T2-LA to T2-MA might have induced the observed decline in DG (Tables 13 and 14). In both trials, energy digestibility did not differ among the three groups ($P > 0.10$) (Table 14).

Associative effects of forages and concentrate supplements have been reported (Berge and Dulphy 1985, Huhtanen 1991, Doyle *et al.* 2005, Niderkorn and Baumont 2009). Interactions of mixed forages may modify intake and/or digestibility of the fibrous components of forage (Dixon and Stockdale 1999), and iso-energetic supplies of various forages can lead to differential nutrient distribution in muscle and adipose tissues (Kim *et al.* 2015). The dietary incorporation of easily fermentable carbohydrates provided by grains or concentrate feed could cause a decrease in ruminal pH and digestion coefficients (Mould *et al.* 1983). The slight increase (without significance) in the digestion coefficients along with a decrease in the ratio of C intake to total DMI observed in T1 (from T1-LA to T1-HA) and from T2-MA to T2-HA (Table 13) may have been caused by such associative effects. In other words, the decrease in the provision of carbohydrates associated with the AH mixture positively affected the digestion coefficients of DM, CP, and NDFom, and these positive effects exceeded the negative ones that were possibly caused by an increase in the NDFom intake and dietary NDFom concentration.

The DG of the calves in the T1-LA, T1-HA, T2-LA, T2-MA and T2-HA groups exceeded the target (1 kg day⁻¹). The ratio of DG to MEI (DG ME⁻¹, g MJ⁻¹ day⁻¹) was calculated as 34.2, 32.0, 27.2, 27.9 and 23.5 for T1-LA, T1-HA, T2-LA, T2-MA and T2-HA, respectively. These values were higher than those calculated in the trials in 2015 (19.3–23.2 g MJ⁻¹ day⁻¹), and exceeded or approximated that reported by Liu *et al.* (2013) in Xiangzhong Black bulls (31.1 g MJ⁻¹ day⁻¹). The ME utilization efficiency for DG in appropriately fed crossbred Simmental male calves yields similar growth performance to

that of indigenous Xiangzhong Black cattle; whereas a lower level of ME utilization efficiency was reported in Chapter 2 for Simmental cattle compared with Xiangzhong Black cattle (see section 2.3.3). Further investigation of ME utilization efficiency would be expected to elaborate the ME requirement to ensure target production performance of Simmental crossbred calves.

The MEm of $737 \text{ kJ kg}^{-0.75} \text{ BW day}^{-1}$ calculated in T1 (Figure 9) was higher than the values reported in Chapter 2. These estimated values of MEm in T1-2015, T2-2015, and T1-2016 also suggested the relatively greater MEm requirement of crossbred Simmental bull calves for BW gain than the MEm requirement of other species (see section 2.3.3), though it is not known whether this characteristic of a relatively high MEm requirement is hereditary or acquired. An inverse relationship in the values of the calculated MEm (737, 652, and $600 \text{ kJ kg}^{-0.75} \text{ BW day}^{-1}$ in T1-2016, T1-2015, and T2-2015, respectively) to those of the average BW and age (*i.e.*, growth stage of the calves) in the study (144 kg and 6 months in T1-2016, 209 kg and 7 months in T1-2015, and 257 kg and 8 months in T2-2015) was found. The average BW (in kg) and the estimated MEm (in $\text{kJ kg}^{-0.75} \text{ BW day}^{-1}$) of cattle in the previous reports were: 300–327 (21–24 months) and 460–485 for Nellore bulls and steers (Tedeschi *et al.* 2002), 254–388 (12–24 months) and 506 for Chinese water buffaloes (Qin *et al.* 2011), and 276 and 585 for Xiangzhong Black bulls (Liu *et al.* 2013). The MEm values in these reports, which were lower than those in the current study, were estimated using cattle with a greater average BW. Van Es (1980) reported that the MEm for growing calves exceeded that of beef cattle finishing fattening because of the high turnover rate of protein for growth (*i.e.*, the costs of protein deposition were underestimated) and a higher level of physical activity of growing calves. Some protein deposition or “growth work” was recommended for cattle in the BW range of 70–

250 kg, and it was tentatively estimated that the utilization of 500 MJ as ME by older cattle was equivalent to only 400 MJ ME for calves (Van Es 1980). The higher MEm estimated in this study appeared to be related to the growth stage of the animals, suggesting a possible increase in the efficiency of ME for BW gain (referred to as K_f in AFRC (1993)) according to the growth stage of cattle.

The increase in the calculated MEm values in T2-2015, T1-2015, and T1-2016 may be correlated also with the average interior temperatures of the respiration chambers (8.2, 15.5, and 23.2 °C in T2-2015, T1-2015, T1-2016, respectively). Morooka *et al.* (1983) reported that the HP tended to be higher in winter than in summer, based on the results of experiments using Holstein male calves (7–25 weeks in age). An increase in HP corresponding to a decrease in environmental temperature was also reported by Holmes and McLean (1975) and by Berman (1968). However, Fujita *et al.* (1982) did not report any increase in HP for Holstein dairy cows under cold conditions. They found a decrease in the number of breaths as a quick reaction to cold conditions, indicating a delayed response as the increase in HP under conditions of cold-related stress. Webster *et al.* (1976) found no variation in HP within a temperature range of 5–20 °C. Another study also reported no significant effect of temperature on the HP of growing cattle over 150 kg in the absence of severely cold conditions (*e.g.*, –15 °C or below) (Webster 1978). By contrast, Kurihara *et al.* (1990, 1991) reported higher MEm values for Holstein cows at 32 °C than at 18 °C and 26 °C because of an increase in heat increment resulting from feed ingestion at 32 °C. The increase in heat increment was possibly caused by accelerated heat radiation with increased breathing (Kurihara *et al.* 1990). The results of the current study were similar to those of Kurihara *et al.* (1990, 1991). Specifically, the estimated MEm values for the warm periods (T1-2015 and T1-2016) were higher than those for the cool period (T2-2015). It is possible

that the finding of no increase in HP or MEm under cold conditions reflects a characteristic of Simmental crossbred calves. A greater amount of C is fed to Simmental calves in Gansu Province during the cold season than during the warm season (see section 2.2.2). A possible reason for this is that farmers in Gansu Province understand that energy and nutrients are more concentrated in C than in forage, and calves' energy requirements are higher in winter. However, the characteristics of Simmental calves indicated by the results of this study (*i.e.*, no increase in HP under cold conditions) imply the need to reconsider the increased proportion of C in the feed of calves during the cold season and the extension of the period of minimal use of C in their feed during the year, which would benefit farmers (see section 1.1.3) and contribute to lower the quantities of imported concentrate feed ingredients (see section 1.1.2). It is still necessary to further investigate whether the temperatures applied in this study were below the range affecting energy metabolism and whether the age or BW of the calves had a greater effect on their energy metabolism.

Kleiber (1988) reported that basic metabolism can be expressed as a constant when it is indicated as MBS ($\text{kg}^{0.75} \text{ BW}$). However, the basic metabolism was reportedly proportional to the surface area of the animals, *i.e.*, to the two-thirds power of BW (Bergmann 1847, Heusner 1982). AFRC (1993) estimated that the fasting metabolism (MJ day^{-1}) was proportional to the two-thirds power of BW. By contrast, Feldman *et al.* (1983) reported that both exponents (0.75 and two-thirds) were valid for describing variations of basal energy metabolism among animals of different species. Suppose the MEm is calculated according to the assumption that the MEm is proportional to the MBS, even though the MEm is in reality proportional to the two-thirds power of BW, the rates of change in the calculated MEm with the BW increase will be higher than expected. That is, the values of MEm (per MBS) decrease along with increasing BW as evidenced in the current study,

contrary to the assumption that the MEm per MBS is constant (no change in the rates regardless of different BWs). This trend for MEm (per MBS) with BW indicated that the MEm or basic metabolism of cattle used in this current study could be explained as being proportional to the two-third power of BW reported by Bergmann (1847) and Heusner (1982).

The following conclusions emerged from the results of all of the trials conducted in 2015 and 2016.

- 1) Replacement of C with AH did not cause DMI depression associated with increased NDFom intake.
- 2) A decrease or increase in digestion coefficients may be caused by an increase in NDFom intake and/or by the associative effects of different mixtures of feed ingredients.
- 3) There were no significant differences between low- and high-level AH diets in relation to either energy digestibility or metabolizability.
- 4) The calculated MEm values were higher than the values reported in previous studies, suggesting that crossbred Simmental bull calves have a relatively higher MEm requirement for achieving BW gain than do other species.
- 5) The higher MEm values estimated in this study appeared to be related to the growth stage of the calves, suggesting a possible increase in the efficiency of ME for BW gain (K_f) according to the animals' growth stage.
- 6) The finding of no increase in HP or in MEm under cold conditions may be also a characteristic of Simmental crossbred calves. The question of whether the temperatures applied in this study were under the range affecting energy metabolism requires further investigation.

4.3.3. Daily gain and the roughage-intake ratio to total dry-matter intake

The DG showed a slight decline moving from T1-LA to T1-MA (Table 14), increasing again in T1-HA. This trend for DG in T1 was the same as that for the ratio of roughage intake to total DMI (Table 13). The reduction in DG from T1-LA to T1-MA might plausibly be related to the reduction in blood glucose values (Table 15), indicating an insufficient energy supply in T1-MA. The increase of DG from T1-MA to T1-HA may mirror the increase in digestion coefficients, probably caused by the associative effects (see section 4.3.2). In T2, whereas all of the groups achieved the designed growth target (1-kg DG), there was a numerical decrease in DG with increasing amount of the AH mixture. No clear relation was evident between DE intake (Table 14, Figure 11) and DG in either trial. The trend relating to DG in T2 was the reverse of that for the ratio of roughage intake to the total DMI (Figure 13). It has been suggested that differences in the dietary forage to concentrate ratio affect the feed intake (McCarthy *et al.* 1989, Krause *et al.* 2002, Oba and Allen 2003). Here, an assumption was held that a certain relation exists between the above ratio and the DG, namely that the DG would increase up to a certain level of AH mixture in the diet and would decline above that level. Regression of the ratio of roughage intake to total dry-matter intake with the DG using the results of all the trials for 2 years showed a quadratic correlation ($R^2 = 0.29$) (Figure 14). The correlation suggested that the optimal DG would be achievable with the ratio of roughage intake close to 41%. Accordingly, the results showed a decline in DG from T1-LA to T1-HA (from 1.09 kg to 1.06 kg day⁻¹) with an increase in the ratio (of roughage intake to total DMI) from 45.3% to 47.8% (Figure 13). The DG also declined from T2-MA to T2-HA (from 1.40 kg to 1.23 kg day⁻¹) with an increase in the ratio from 46.8% to 52.4%.

Table 15 Blood metabolites in Simmental beef calves in trials conducted in 2016

Item	Before Trial 1				After Trial 1			
	T1-LA	T1-MA	T1-HA	SEM	T1-LA	T1-MA	T1-HA	SEM
Glucose, mmol L ⁻¹	2.92	3.24	3.54	0.27	3.44	3.15	2.61	0.27
Blood urea nitrogen, mmol L ⁻¹	5.37	6.28	6.07	0.37	3.63	4.48	4.66	0.64
Non-esterified fatty acid, $\mu\text{Eq L}^{-1}$	737.64 ^a	449.95 ^b	496.69 ^{ab}	80.15	497.28	426.93	473.02	85.88
β -hydroxybutylic acid, $\mu\text{mol L}^{-1}$	66.87	98.76	115.83	21.15	233.58	81.44	116.15	54.81

Item	Before Trial 2				After Trial 2			
	T2-LA	T2-MA	T2-HA	SEM	T2-LA	T2-MA	T2-HA	SEM
Glucose, mmol L ⁻¹	4.35	3.87	3.67	0.30	4.47	4.37	4.53	0.10
Blood urea nitrogen, mmol L ⁻¹	4.88	3.00	4.32	0.67	4.97 ^{AB}	3.25 ^B	5.35 ^A	0.49
Non-esterified fatty acid, $\mu\text{Eq L}^{-1}$	402.54	323.88	355.74	88.55	214.33	221.24	211.30	7.91
β -hydroxybutylic acid, $\mu\text{mol L}^{-1}$	204.88	415.90	160.59	81.59	195.81 ^b	261.67 ^{ab}	367.08 ^a	47.41

Trials 1 and 2 comprised T1-LA, T1-MA, T1-HA, T2-LA, T2-MA, and T2-HA; for details, see Tables 4 and 12 or the section on materials and methods in Chapter 4.

SEM, standard error of the mean.

^{A, B, a, b} Means with different superscripts within a row before and after each trial differ significantly (^{A, B} for $P \leq 0.05$, ^{a, b} for $P \leq 0.10$).

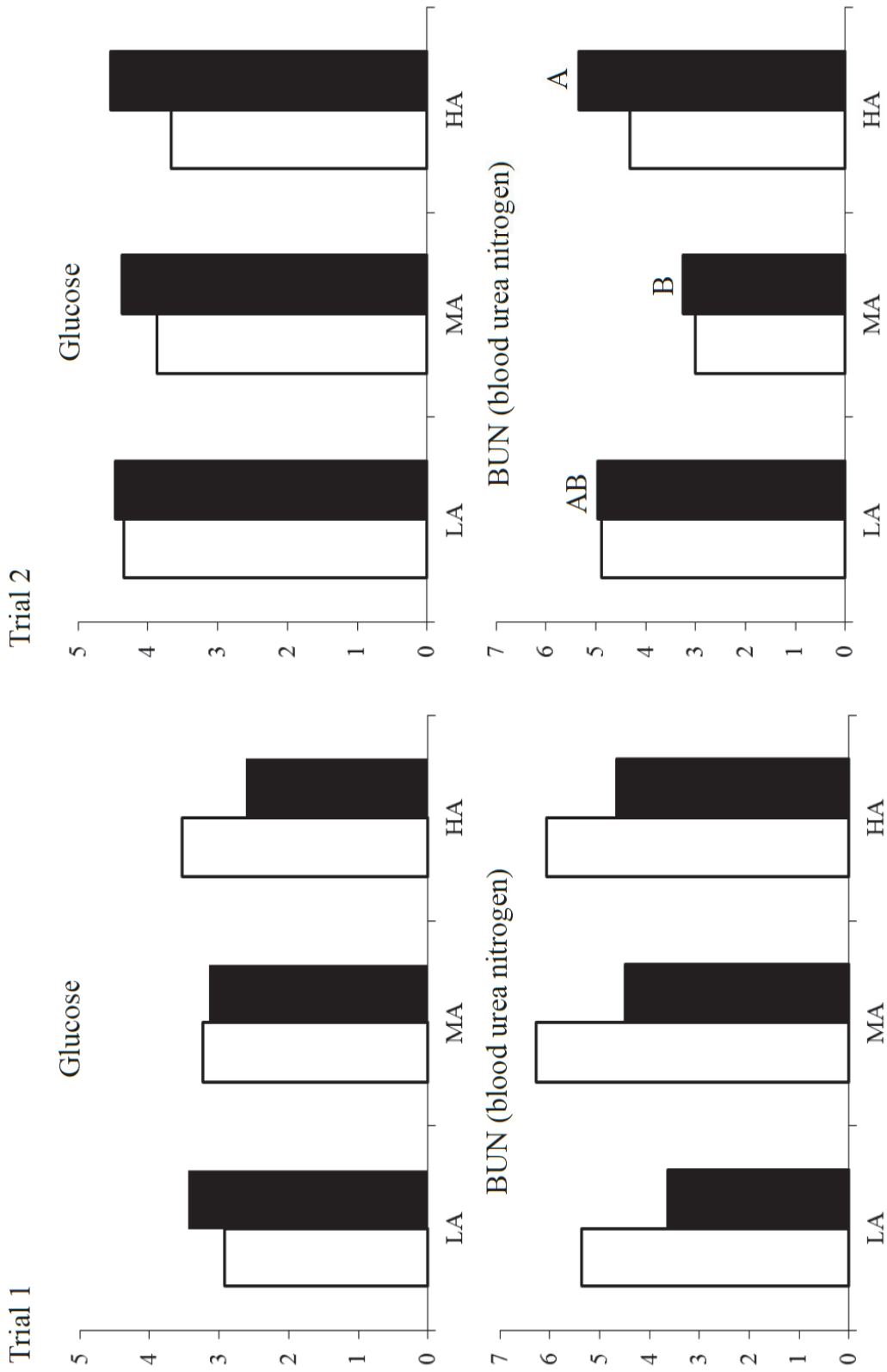


Figure 12 Values of glucose and blood urea nitrogen in the plasma collected before and after Trials 1 and 2 in 2016. ^{A, B, a, b} Mean values with different subscripts within each of Trial 1 and Trial 2 differed significantly among the three feeding groups (^{A, B} for $P \leq 0.05$; ^{a, b} for $P \leq 0.10$). Unshaded bars depict pre-trial values (mmol L⁻¹); solid bars depict post-trial values (mmol L⁻¹).

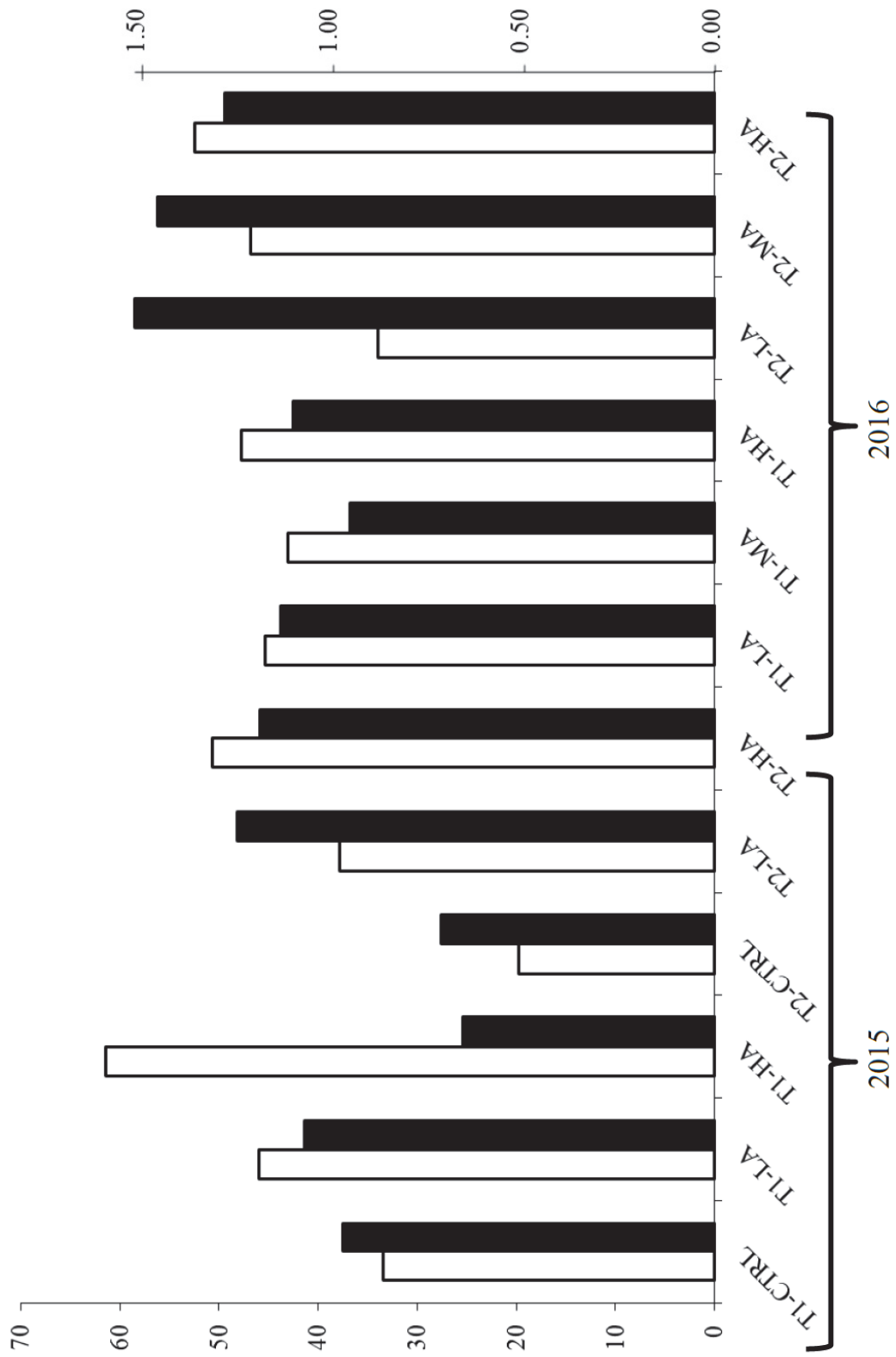


Figure 13 Ratio of roughage intake to total feed intake (based on dry matter) and daily weight gain of calves during feeding trials conducted in 2015 and 2016. Along the scale on the left, unshaded bars depict the ratio of roughage intake to total feed intake (% based on dry matter); along the scale on the right, solid bars depict daily weight gain (kg day⁻¹).

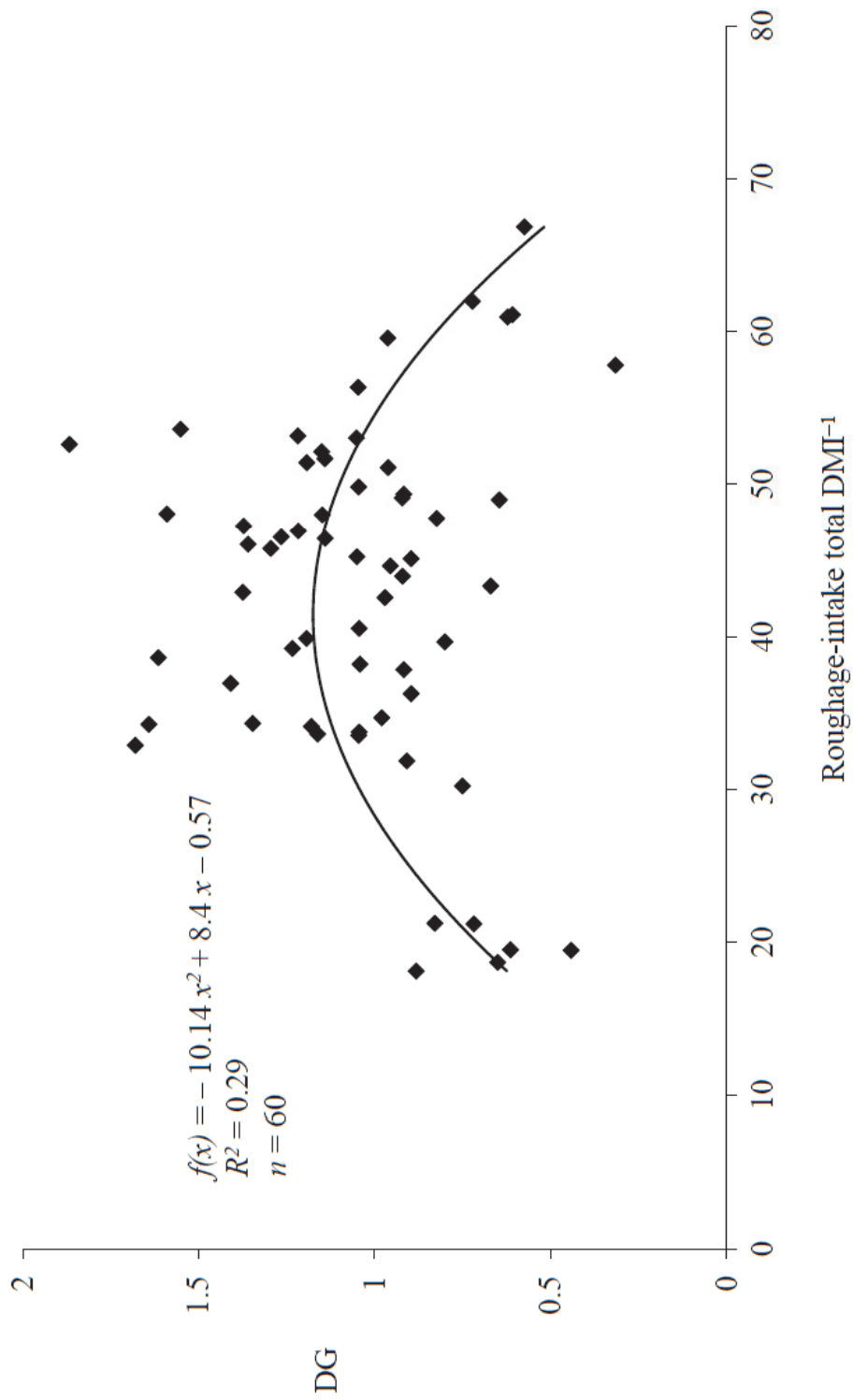


Figure 14 Quadratic regression of daily weight gain (DG, kg day⁻¹, $f(x)$) and ratio of roughage intake (on a DM basis) to total DMI (% x) of calves during feeding trials conducted in 2015 and 2016.

In 2015, a similar decline in DG was observed from T1-LA to T1-HA (from 1.03 kg to 0.63 kg day⁻¹) when the ratio increased from 45.9 % to 61.4%. The MEI was lower when calves were fed diets entailing a high level of AH mixture, leading to lower DG (as in T1-HA in 2015). Hales *et al.* (2014) reported a decrease in energy retention for DG with the addition of more AH as a result of increased NDFom intake and decreased NDFom digestibility. The increase in NDFom intake from T2-MA to T2-HA in 2016 (with the roughage-intake ratio, 46.8% and 52.4%, respectively) may have led to the decline in DG (Tables 13 and 14). A decline in the DG with the ratio of roughage intake to total DMI that exceeded 41% was thus explained. Furthermore, the results of the trials conducted in 2015 showed an increase in DG from 0.69 kg to 1.20 kg day⁻¹ (from T2-CTRL to T2-LA) when the ratio increased from 19.7% to 37.8%.

However, in T1-2016, an increase in the ratio from 43.1% to 45.3% (from T1-MA to T1-LA) was associated with an increase in the DG. The results of the trials in 2015 showed a similar increase in DG from 0.94 kg to 1.03 kg day⁻¹ (from T1-CTRL to T1-LA) when the ratio increased from 33.4% to 45.9%. Guided by the quadratic correlation that showed the DG decrease at the ratio above 41%, the ratio for achieving the optimal DG might have a certain margin (*i.e.*, ~41%).

4.3.4. Nitrogen intake and efficiency

In T1, there were no significant differences in the N intake values among all of the groups (Table 14). The absorbed N (g kg^{-0.75} BW day⁻¹) and N utilization efficiency (as N-absorbed N-intake⁻¹) showed a numerical increase with increasing amount of the AH mixture, and the post-T1 BUN values appeared to increase accordingly (Table 15). The calves in T1-LA achieved the 1-kg DG target. The N supply appeared sufficient for 1-kg

DG in all of the T1 groups, though it was difficult to explain the slight shortfall in meeting the DG target in T1-MA. In T2, there did not appear to be a clear trend relating to N intake with increasing amount of the AH mixture (Table 14). The N intake tended to decrease from T2-LA to T2-MA ($P < 0.10$) and showed a numerical increase from T2-MA to T2-HA. This trend corresponded with trends for the absorbed N and BUN values (Tables 14 and 15). The N intake, N absorption, and BUN values were lowest in the T2-MA group in which 1-kg DG was achieved. Therefore N supplies for all of the T2 groups appeared to be sufficient for meeting the DG goal of 1 kg, although the post-trial BUN value for T2-MA was slightly below the standard range of values (3.57–8.93 mmol L⁻¹) (National Agricultural Insurance Association 1997). The results obtained for both trials in 2016, indicated that N supplies were sufficient for a 1-kg DG in all of the groups. This supports the finding of effective N utilization for DG using low-level AH feeding (T1- and T2-LA groups in 2015) without any detrimental effects on dietary energy utilization, as suggested in Chapter 3.

The following conclusions emerged from the results of all of the trials conducted in 2015 and 2016.

- 1) Low-level AH feeding evidenced effective N utilization for DG without any detrimental effects on dietary energy utilization by calves.
- 2) Both N retention and its efficiency were higher among the groups with higher levels of AH in their diets (see section 3.4).

4.3.5. Greenhouse gas emissions as a loss of the energy intake

No significant difference in CH₄ production was observed among the groups (Table 16).

Table 16 Methane and carbon dioxide emission, metabolizable energy intake, and body weight of Simmental crossbred beef calves for Trial 1 in 2016

Item	Trial 1				SEM	P
	T1-LA	T1-MA	T1-HA			
Methane production, L day ⁻¹	75.79	88.29	82.26	7.96	0.56	
Methane production, L day ⁻¹ kg ⁻¹ DG	70.02	98.42	78.10	10.15	0.18	
Methane production, L kg ^{-0.75} BW day ⁻¹	1.97	2.17	2.11	0.18	0.73	
Methane production, g kg ⁻¹ DMI	15.9	17.6	17.8	1.42	0.61	
Methane-energy GE-intake ⁻¹ , %	4.51	3.97	4.90	0.73	0.67	
Carbon dioxide production, L day ⁻¹	1470.38	1695.51	1546.83	111.64	0.39	
ME intake [†] , MJ day ⁻¹	35.13	37.58	36.72	2.83	0.83	
Body weight [‡] , kg	142.64	148.96	151.41	7.92	0.73	

Trial 1 comprised T1-LA, T1-MA, and T1-HA: for details, see Tables 4 and 12 or the section on materials and methods in Chapter 4.

DG, daily gain; BW, body weight; DMI, dry matter intake; GE, gross energy; ME, metabolizable energy; SEM, standard error of the mean.

There were no significant differences between the three feeding groups ($P > 0.10$).

[†] Calculated using values from MOA (2004) and NARO (2010) on the basis of actual intakes of feed ingredients.

[‡] Obtained at the start of measurements conducted in respiration chambers.

This lack of any difference in CH₄ production can be explained by the lack of any difference in the NDFom intake ($P = 0.74$) and NDF digestibility ($P = 0.19$) among the groups (Table 13). The relationship between CH₄ production, inclusion levels of concentrate, and NDFom digestibility and intake as evidenced in the 2015 trials (see section 2.3.4) was not clearly observed in T1-2016. The ratio of the energy of emitted CH₄ to total GE intake (4.0–4.9% in T1-2016) did not differ among the three groups ($P = 0.67$), and was less than the reported ratio of 5.5–7.5% (IPCC 2006). Inclusion of a certain amount of AH appeared not to affect CH₄ production. In other words, a certain proportion of concentrate could effectively be replaced by AH, as reported in Chapter 2.

A strong relationship between DMI and CH₄ production and equations to estimate CH₄ emissions associated with DMI have been reported (Shibata *et al.* 1992, 1993; Cottle *et al.* 2011; Kennedy and Charmley 2012). Simple regression of CH₄ production ($f(x)$, L day⁻¹) conducted with each of the DMI values (x_1 , MJ day⁻¹), the BW (x_2 , kg), the ratio of roughage intake (on a DM basis) to the total DMI (x_3 , %), and the MEI (x_4 , MJ day⁻¹) in the trials in 2015 also revealed that the correlation coefficient with the DMI was relatively high ($R^2 = 0.50$) (Figure 15). The regressed function of DMI for CH₄ production, $f(x) = 30.2 x_1 - 35.6$, indicates that 21.6 g of CH₄ was produced per 1 kg of DMI, which was similar to the finding of Kennedy and Charmley (2012) (19.6 g kg⁻¹ DMI) and much lower than that of Purnomoadi (2013) (34 g kg⁻¹ DMI). Hristov *et al.* (2013) proposed an equation for estimating the CH₄ emissions of non-lactating animals that incorporated factors relating to the composition of diet nutrients (NDF, EE), BW, and GE intake. However, application of this equation in the current study, using the values of EE concentrations listed by MOA (2004) (1.0%, 1.4%, 5.0% for CS, AH, and C, respectively) and of actual GE and NDFom intakes, and of BW, did not yield comparable figures.

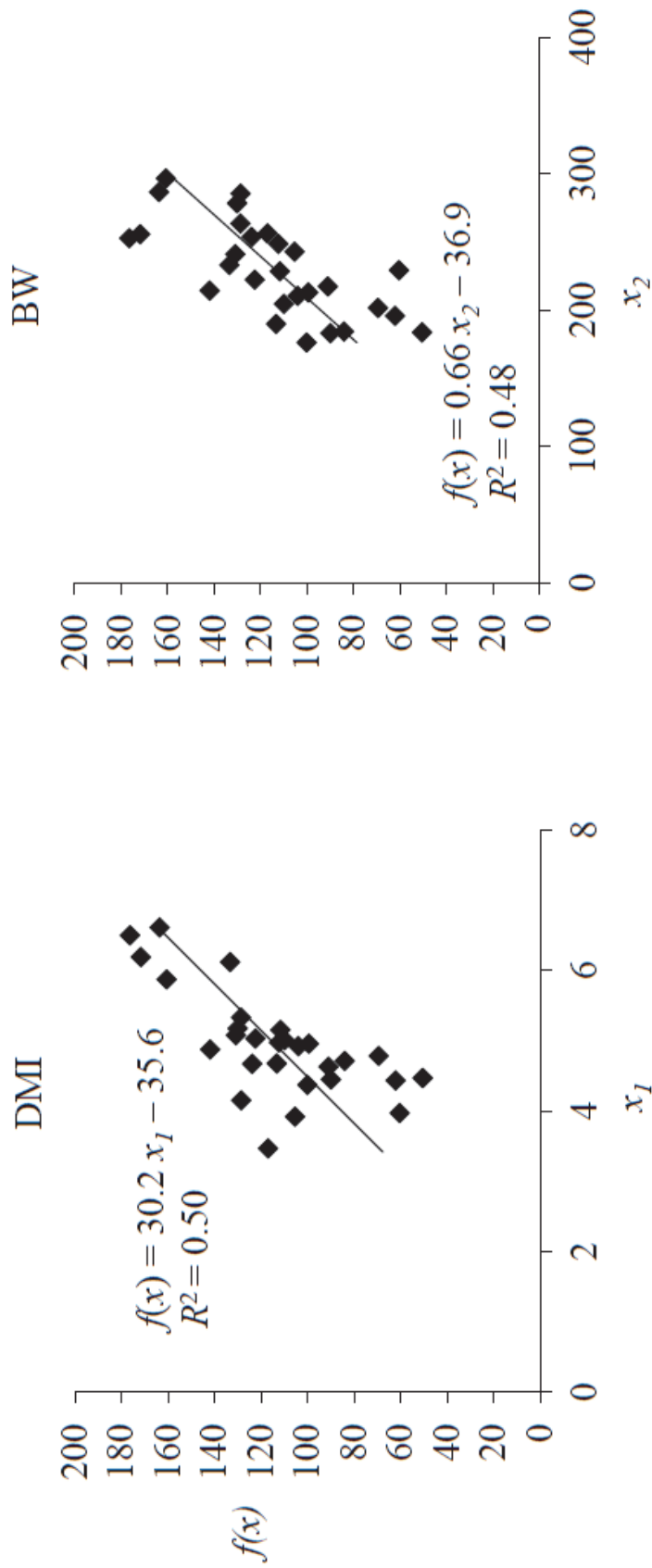


Figure 15a Simple regression of methane production ($f(x)$, L day⁻¹) with dry matter intake (DMI) (kg day⁻¹, x_1) and body weight (kg, x_2) of calves in 2015 ($n = 28$).

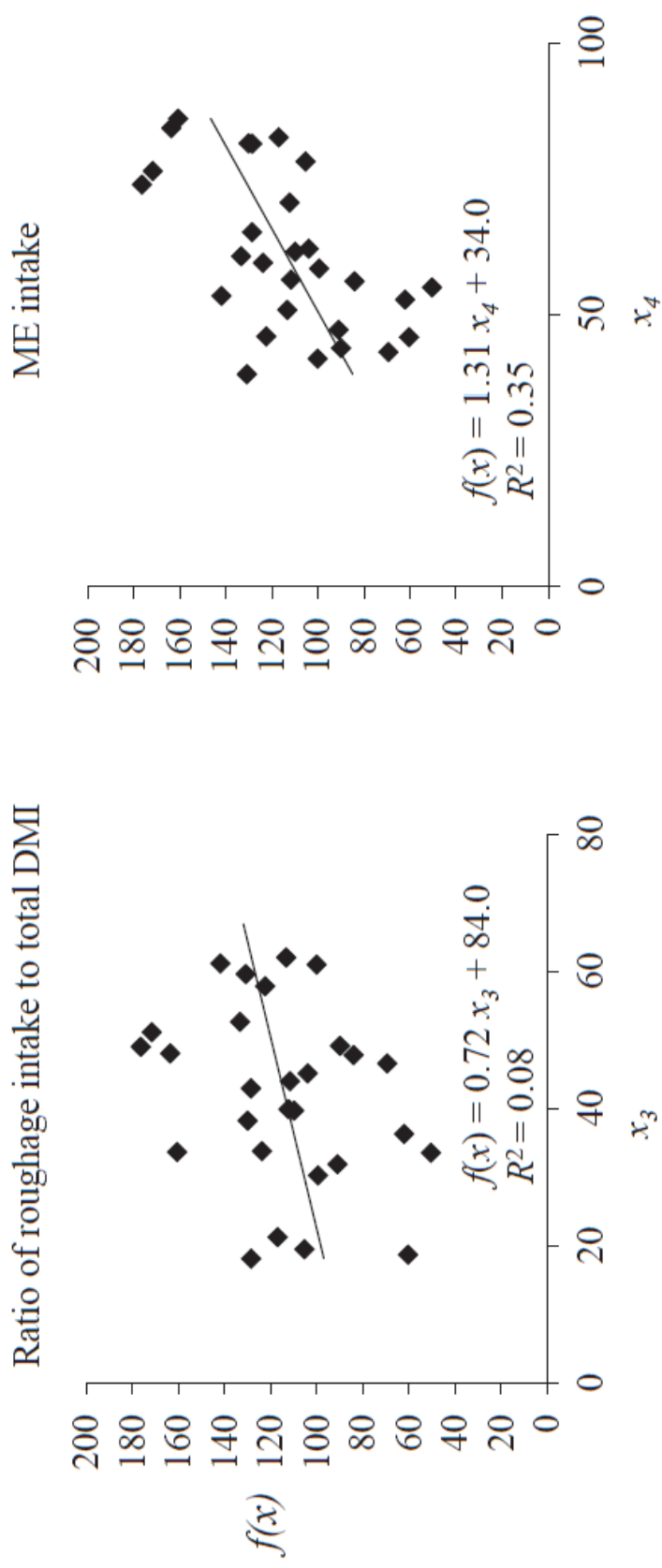


Figure 15b Simple regression of methane production ($f(x)$, L day⁻¹) with the ratio of roughage intake (on a DM basis) to total DMI (x_3 , %) and metabolizable energy (ME) intake (MJ day⁻¹, x_4) of calves in 2015 ($n = 28$).

Therefore, in this study, a multiple regression of CH₄ production ($f(x)$) was performed with DMI (x_1), BW (x_2), and the ratio of roughage intake to total DMI (x_3), all of which are common in farming practice. The regression revealed the following equation: $f(x) = 7.358 x_1 + 0.749 x_2 + 1.195 x_3 - 144.95$ ($R^2 = 0.82$), which indicates that there is a possibility of estimating CH₄ emissions with a certain degree of reliability using commonly available information related to actual feeding practices. No parameters per MBS ($\text{kg}^{0.75}$ BW) expressed clear correlations with the other parameters used in this study. It may be important to compare parameters related to CH₄ production using the several units (such as kg^{-1} BW, kg^{-1} DG, and kg^{-1} DMI) including the MBS unit ($\text{kg}^{0.75}$ BW).

The CO₂ production did not differ between the three groups in all of the trials conducted in 2015 and 2016 (Tables 9 and 16). However, the average values of CO₂ production (L day^{-1}) for all three groups in each trial (1570.9, 1750.5, and 2315.5 for T1-2016, T1-2015, and T2-2015, respectively) seemed to increase with an increase in the average BW (kg) in each trial (147.67, 210.05, and 255.96 for T1-2016, T1-2015, and T2-2015, respectively). The average CO₂ production was significantly higher in T2-2015 than in T1-2015 ($P < 0.05$), as was the CO₂ production in T1-2015 compared with T1-2016. The CO₂ production was affected by the BW of the calves and the consequent volume of respiration rather than by the applied feeding regimens. Livestock respiration is not considered a net source of the effects of GHGs (global warming) in terms of equivalent quantities of emitted and absorbed gases, though more than half of the total CO₂ generated through animals' respiration is emitted from cattle (Steinfeld *et al.* 2006). The results for CO₂ emission in this study were not considered a major factor affecting AH substitution for concentrate as a preferred feeding option.

The following conclusions emerged from the results of all of the trials in 2015 and

2016.

- 1) Carbon-dioxide production did not differ between treatments.
- 2) The ratio of the energy of emitted CH₄ to total GE intake was lower in the groups with no AH or low levels of AH than in those with high levels of dietary AH.
- 3) The increasing trend in CH₄ production with increasing amount of the AH mixture showed that the addition of the AH mixture to concentrate-based diets did not appear to be a preferred option in terms of reducing CH₄ emissions. However, low-level addition of AH did not cause a significant increase in CH₄ production.
- 4) A certain proportion of AH could be substituted for concentrate feed if factors other than GHG emissions (such as DG, FCR and feed cost) are considered.

4.3.6. Economic evaluation of feeding alfalfa hay to calves

In T1, economic benefit was numerically highest in the LA group (Table 17). Economic benefit declined slightly from T1-LA to T1-MA, reflecting an increase in feed cost (for T1-LA vs. T1-MA) due to the increased AH and C intakes. In T2, economic benefit was numerically highest in the LA group because the significant increase in AH intake ($P < 0.05$) was accompanied by a relatively small decrease in C intake ($P < 0.10$) from T2-LA to T2-HA (Table 13). Both LA groups achieved the 1-kg DG target by consuming a diet that included a low level of AH (*i.e.*, 7.8%–14.2% on a DM basis) added to C-based ration. As reported in Chapter 2, incorporating small proportions of AH into a concentrate-based regimen (T1-MA and T2-MA in 2016) appears to be acceptable in terms of economic feasibility for feeding Simmental beef calves.

Table 17 Economic assessment of incorporating alfalfa into the feeding regimen of growing Simmental male calves for trials conducted in 2016

Item	Trial 1			Trial 2			SEM	P
	T1-LA	T1-MA	T1-HA	T2-LA	T2-MA	T2-HA		
Feed cost, US\$ day ⁻¹ head ⁻¹	0.95	1.06	0.96	1.53	1.48	1.49	0.04	0.64
Profit, US\$ day ⁻¹ head ⁻¹	3.60	3.03	3.50	4.82	4.63	4.07	0.33	0.30
Economic benefit, US\$ day ⁻¹ head ⁻¹	2.65	1.97	2.54	3.28	3.14	2.58	0.34	0.35
Feed cost, US\$ day ⁻¹ kg ⁻¹ DG head ⁻¹	0.87 ^b	1.18 ^a	0.91 ^{ab}	1.07	1.07	1.23	0.09	0.39

Trials 1 and 2 comprised T1-LA, T1-MA, T1-HA, T2-LA, T2-MA, and T2-HA; for details, see Tables 4 and 12 or the section on materials and methods in Chapter 4.

SEM, standard error of the mean.

Values for feed costs were calculated based on the results for feed intakes (Table 13) obtained in the feeding trials.

^{a, b} Means with different superscripts within a row for each of Trial 1 and Trial 2 differed significantly ($P \leq 0.10$).

The following conclusion emerged from the results of all of the trials conducted in 2015 and 2016.

- 1) A low level of AH added to concentrate-based feed was demonstrated to be economically advantageous for achieving 1-kg DG and appeared to be a preferred feeding option in Gansu Province.
- 2) Economic benefits for farmers would be considerable compared with actual farmers' incomes derived from livestock within the Province (see section 2.3.5).

4.3.7. Appropriate ratio of alfalfa hay to total dry-matter allowance

I analyzed the data obtained in the trials in 2016 regarding the optimal ratio of AH intake to total DMI in the context of results of feeding trials in 2015. Because of the differences between the feeding regimens for the 2 trials in both years, I compared T1 in 2016 with the trial performed during the 2015 warm season (*i.e.*, T1 in 2015) and compared T2 in 2016 with the trial completed during the 2015 cool season (*i.e.*, T2 in 2015).

In T1 (Table 18), the DG at the AH-intake ratio of 19.1% (T1-HA in 2015) was lower than the DG in the other groups ($P < 0.05$). The DG gradually increased when the AH-intake ratio was $\leq 14.2\%$. The trend for FCR values appeared to be opposite to that for DG values. The FCR was lower for the AH-intake ratio of 14.2% and 24.2%, supporting the difference from that for a ratio of 19.1% ($P < 0.05$). At the AH-intake ratio of 19.1%, the roughage to total DMI ratio (61.4%) was significantly higher than in the other five T1 groups (33.4–47.8%) ($P < 0.01$).

Table 18 Ratio of alfalfa-hay intake to total dry-matter intake for Trial 1 in 2015 and 2016, and parameters for the preferred ratio for Simmental crossbred male calves

Item	Year						SEM	P
	2015		2016		2016			
Trial	T1-CTRL	T1-LA	T1-LA	T1-LA	T1-HA	T1-MA	T1-HA	
AH-intake [§] total-DMI ⁻¹ , %	0	8.1	45.9	14.2	19.1	19.4	24.2	
Roughage-intake [§] total-DMI ⁻¹ , %	33.4	45.9	45.3	45.3	61.4	43.1	47.8	
Dairy gain, kg day ⁻¹	0.94 ^{AB}	1.03 ^A	1.09 ^A	1.09 ^A	0.63 ^B	0.92 ^{AB}	1.06 ^A	
Feed conversion ratio	5.01 ^{AB}	4.89 ^{BC}	3.16 ^D	3.16 ^D	8.42 ^A	3.98 ^{BCD}	3.12 ^D	
ME intake, kJ kg ^{-0.75} BW day ⁻¹	895.2	875.1	831.0 [‡]	831.0 [‡]	795.3	878.6 [‡]	871.0 [‡]	
Methane production, L kg ^{-0.75} BW day ⁻¹	1.60 ^B	1.89 ^{AB}	1.97 ^{AB}	1.97 ^{AB}	2.31 ^A	2.17 ^{AB}	2.11 ^{AB}	
Methane production, g kg ⁻¹ DMI	13.1	14.2	15.9	15.9	18.0	17.6	17.8	
Economic benefit, US\$ day ⁻¹ head ⁻¹	1.60 ^{AB}	2.03 ^A	2.65 ^A	2.65 ^A	0.91 ^B	1.97 ^{AB}	2.54 ^A	

Trial 1, T1-CTRL, T1-LA, T1-MA, and T1-HA: for details, see Table 4, 5, 11 or the section on materials and methods in Chapters 2 and 4.

AH, alfalfa hay; DM, dry matter; DMI, dry matter intake; DG, daily gain; ME, metabolizable energy; BW, body weight; SEM, standard error of the mean.

^{A, B, C, D, E} Means with different superscripts within a row differ significantly ($P \leq 0.05$).

[†] $P < 0.0005$.

[‡] Calculated by using the ratio for converting DE into ME obtained in the trials in 2015.

[§] On a dry-matter basis.

The roughage:intake ratio affects the DG and FCR (Nakatsuji 1999, NARO 2008, McCroskey *et al.* 1961); the roughage:intake ratio at the AH-intake ratio of 19.1% was much higher than that reported as appropriate for Holstein steers fed commercial concentrate and rice straw (12%) (Okushima and Wada 1976) and that for Japanese Black steers fed commercial concentrate, timothy grass (*Phleum pretense*) and corn silage (50%) (Kurohiji *et al.* 1970). The increase in FCR from the AH-intake ratio of 14.2% (roughage:intake ratio, 45.3%) (T1-LA in 2016) to the AH-intake ratio of 19.1% (roughage:intake ratio, 61.4%) indicates that excessive roughage intake increased the FCR and reduced the DG. The DG and FCR did not differ between the groups with AH-intake ratios of 24.2% and 14.2% ($P = 0.99$ for both DG and FCR), likely because the roughage:intake ratio for the former (47.8%) did not markedly differ from that for the latter (45.3%). When AH is substituted for C in diets with a constant ratio of CS to total DMI, adding more AH than that used for the group with the AH-intake ratio of 14.2% risks reducing DG. The FCR of feedlot cattle is typically less than 6 (Shike 2013), and FCR values of 8.3 in Chongqing (Western China) and of 6.4 to 7.1 in Inner Mongolia (feeding style not specified) are reported (USDA 2016). Even though FCR values are typically lower for younger growing calves than for older animals (AFRC 1993, NARO 2008), the FCR value (3.16) achieved by using an AH-intake ratio of 14.2% indicates a particularly high level of feeding efficiency in Simmental crossbred beef cattle. The inclusion of AH at $\leq 14.2\%$ and 14.2% of total DMI seemed appropriate in terms of DG and FCR, respectively. The MEI ($\text{kJ kg}^{-0.75} \text{ BW day}^{-1}$) did not differ between groups at AH-intake ratios of $\leq 14.2\%$. The concentrations of blood metabolites (blood glucose, NEFA, and BHBA) at the AH-intake ratio of 14.2% (T1-LA in 2016) were within physiologically normal ranges (2.50–3.89 mmol L^{-1} , 200–800 $\mu\text{Eq L}^{-1}$, $<1200 \mu\text{mol L}^{-1}$, respectively) (National

Agricultural Insurance Association 1997, Oikawa 2015), and indicate sufficient energy supply. A reduction in MEI due to the mixture of AH into C-based diets decreased DG or energy utilization efficiency when the ratio of AH incorporated was 19.1% (see section 2.3.2). The energy supply in the groups with the AH-intake ratio of $\leq 14.2\%$ may have met the NE requirement for both maintenance and 1-kg DG for growing Simmental male calves. Estimates for the ME ($\text{kJ kg}^{-0.75} \text{ BW day}^{-1}$) required for 1-kg DG in male calves (AFRC 1993) increased as AH intake increased, *i.e.*, from 845.0 (AH-intake ratio, 0%) to 946.3 (AH-intake ratio, 19.1%); whereas MEI decreased at the AH-intake ratios of $\leq 19.1\%$ (Table 18). An increased difference between MEI and the ME required might have caused the observed reduction in DG and increase in FCR when the AH-intake ratio reached approximately 19.1%. Methane production ($\text{L kg}^{-0.75} \text{ BW day}^{-1}$) at the AH-intake ratios of $\geq 19.1\%$ was higher than that with the AH-intake ratio of $\leq 14.2\%$ ($P < 0.01$), whereas CH_4 production did not differ among the latter three groups ($P = 0.23$). Furthermore, the ratios of energy of emitted CH_4 to total GE intake did not differ among these three groups (4.23%, 4.64%, and 4.51% at the AH-intake ratio of 0%, 8.1%, and 14.2%, respectively) ($P = 0.87$) and are consistent with the reported ratios of 3.0%–5.0% for cattle fed diets with $\geq 90\%$ concentrates (Dong *et al.* 2006). In addition, the CH_4 production of these three groups (13.1–15.9 $\text{g kg}^{-1} \text{ DMI}$) was lower than the reported value (19.6 $\text{g kg}^{-1} \text{ DMI}$) for cattle fed grass and grass mixed with legumes (Kennedy and Charmley 2012) and much lower than another (34 $\text{g kg}^{-1} \text{ DMI}$) for buffalo heifers fed soy-sauce by-product (Purnomoadi 2013). The AH-intake ratios of $\leq 14.2\%$ were not significantly detrimental in terms of CH_4 production. Economic benefit was higher when the AH-intake ratio was 8.1% or 14.2% than with a ratio of 19.1% ($P < 0.05$); this increase relative to the increased amount of AH (to a maximum of 14.2%) was similar to that of DG. I could not recommend a specific

range of AH-intake ratio effective for optimizing the digestion coefficients of DM, CP, and NDFom because of the lack of significant difference in these digestion coefficients among all T1 groups ($P = 0.68\text{--}0.88$).

In T2 (Table 19), DG was numerically the highest at the AH-intake ratio of 7.8%. The DG decreased and the FCR increased as the AH-intake ratio increased above 7.8%. The FCR values (3.55–3.71) associated with the AH-intake ratios of 7.8% and 21.1% indicate high feeding efficiency for Simmental crossbred beef cattle in T2, as seen in T1. The MEI at the AH-intake ratio of 7.8% and 21.1% was significantly higher than that at the ratio of 0% ($P < 0.05$) and slightly higher than that at the ratio of 22.6% ($P = 0.17$). In addition, all of the blood glucose, NEFA, and BHBA values in the T2 (2016) groups (Table 15), for which the AH-intake ratios exceeded 7.8%, were within or above standard ranges (National Agricultural Insurance Association 1997, Oikawa 2015). All three of the T2 (2016) groups achieved the target 1-kg DG. Energy supply in the groups whose AH-intake ratios exceeded 7.8% seems to have been sufficient to meet the calves' requirements for basic metabolism and essential growth (1-kg DG). The AH inclusion at 7.8%–21.1% of total DMI was therefore appropriate in terms of energy intake and efficiency. Economic benefit was higher at the AH-intake ratio of 7.8% than at ratios of 0% or 38.1% ($P < 0.05$) and did not differ among the groups with ratios of 7.8%–30.1%. This range included the ratio of 14.2%, which was associated with the highest economic benefit in T1 groups (in 2015 and 2016). In the T2 groups (in 2015 and 2016), the digestion coefficients of DM, CP, and GE decreased slightly at the AH-intake ratios of $\geq 7.8\%$, consistent with findings of previous studies (Sekine *et al.* 1986, Cowsert and Montgomery 1969), but the decrease was not significant ($P > 0.10$) and did not appear to affect growth performance (*i.e.*, DG and FCR).

Table 19 Ratio of alfalfa-hay intake to total dry-matter intake for Trial 2 in 2015 and 2016, and parameters for the preferred ratio for Simmental crossbred male calves

Item	Year						SEM	P
	2015	2016	2016	2015	2016	2015		
Trial	T2-CTRL	T2-LA	T2-LA	T2-MA	T2-LA	T2-HA	T2-HA	
AH-intake [§] total-DMI ⁻¹ , %	0	7.8	33.9	21.1	22.6	30.1	38.1	
Roughage-intake [§] total-DMI ⁻¹ , %	19.7			46.8	37.8	52.4	50.6	
Dairy gain, kg day ⁻¹	0.69 ^B	1.46 ^A		1.40 ^A	1.20 ^A	1.23 ^A	1.15 ^A	
Feed conversion ratio	5.83 ^A	3.55 ^B		3.71 ^{AB}	4.58 ^{AB}	4.31 ^{AB}	6.09 ^A	
ME intake, kJ kg ^{-0.75} BW day ⁻¹	744.3 ^C	1077.0 ^{A†}		997.2 ^{AB‡}	857.3 ^{BC}	1061.0 ^{A‡}	986.8 ^{AB}	
Methane production, L kg ^{-0.75} BW day ⁻¹	1.66 ^B	–		–	2.09 ^{AB}	–	2.63 ^A	
Methane production, g kg ⁻¹ DMI	19.1	–		–	17.3	–	18.1	
Economic benefit, US\$ day ⁻¹ head ⁻¹	0.68 ^D	3.28 ^{AB}		3.14 ^{BC}	1.96 ^{BCD}	2.58 ^{BC}	1.50 ^{CD}	

Trial 2, T2-CTRL, T2-LA, T2-MA, and T2-HA: for details, see Table 4, 5, 11 or the section on materials and methods in Chapters 2 and 4.

AH, alfalfa hay; DM, dry matter; DMI, dry matter intake; DG, daily gain; ME, metabolizable energy; BW, body weight; SEM, standard error of the mean.

^{A, B, C, D} Means with different superscripts within a row differ significantly ($P \leq 0.05$).

[†] $P < 0.0005$.

[‡] Calculated by using the ratio for converting DE into ME obtained in the trials in 2015.

[§] On a dry-matter basis.

Low-level AH feeding, which achieved effective N utilization for DG (see Chapter 3), was regarded also as the feeding with sufficient N supply; whereas high N retention appeared to be achieved with the AH feeding at higher level (see section 4.3.4).

Therefore, the values of parameters obtained in T1 and T2 lead to the following conclusions:

- 1) Appropriate AH-intake ratios to total DMI for male Simmental beef cattle according to the criterion that T2 groups received higher proportions of C than T1 groups were: DG, $\leq 14.2\%$ and $\geq 7.8\%$; FCR, 14.2% and 7.8%–30.1%; energy intake and utilization efficiency, $\leq 14.2\%$ and 7.8–21.1%; economic benefit, 8.1%–14.2% and 7.8%–30.1%.
- 2) The AH-intake ratios of $\leq 14.2\%$ are not significantly detrimental in terms of CH₄ production.
- 3) Sufficient N supply and effective N utilization for DG is achieved with the low-level AH feeding.

As reported in section 4.3.2, the MEm of crossbred Simmental calves appeared higher than that of other breeds. It was posited that the higher MEm estimate in this study could be compensated through the inclusion of AH or concentrate in feeding regimens. The MEm values estimated with the equation provided by AFRC (1993), using the mean BW of the calves in T1-2015 (209.5 kg) and T2-2015 (257.0 kg) with no DG (0 kg day⁻¹), were 514 and 507 kJ kg^{-0.75} BW day⁻¹, respectively. The differences of these estimated MEm values from those calculated in the current study (652 and 600 kJ kg^{-0.75} BW day⁻¹ for T1- and T2-2015) were 138 and 93 kJ kg^{-0.75} BW day⁻¹ for T1- and T2-2015, respectively. These values were equivalent to 7.60 and 5.97 MJ for 209.5 and 257.0 kg BW. Given that the ME concentrations of AH and C were 7.18 and 13.48 MJ kg⁻¹ DM (MOA 2004, NARO 2010),

the higher MEm values of Simmental crossbred male calves could have been compensated accordingly by including 1.06 (or 0.56) kg DM of AH (or C) in T1-2015, and 0.83 (or 0.44) kg DM of AH (or C) in T2-2015.

4.3.8. Relevance of the feeding standards applied in the trials

The results of a simple regression analysis of the NEmf intake (MJ day^{-1}) estimated using the tabular values in MOA (2004) with the required NEmf (MJ day^{-1}) demonstrated a slope close to one (0.99) (Figure 16). They revealed that the estimated NEmf intake corresponded closely to the required NEmf and entailed a certain correlation ($R^2 = 0.45$). Liu *et al.* (2013) reported that Xiangzhong Black cattle aged 13 months achieved 1-kg DG and that the NEmf listed by MOA (2004) closely reflected the animals' growth performance. The validity of tabular NEmf values and functions for estimating the NEmf intake (MOA 2004) was confirmed.

NEmf intake values calculated using the actual DE GE^{-1} ((2) in Table 20) were lower than those calculated using the tabular values in MOA (2004) ((1) in Table 20) in all of the trials conducted in 2015 and 2016. The lower values of the first set of NEmf intake values compared with the latter set of values were reflected by the slope of the regressed line of the required NEmf in relation to the first NEmf intake values (0.88) (Figure 16). Despite a certain linear correlation between the first and latter set of NEmf values ($R^2 = 0.39$), these findings suggest that the values of DE and/or DE GE^{-1} for feed ingredients used in this study (AH, CS, and C) were lower than those indicated in MOA (2004).

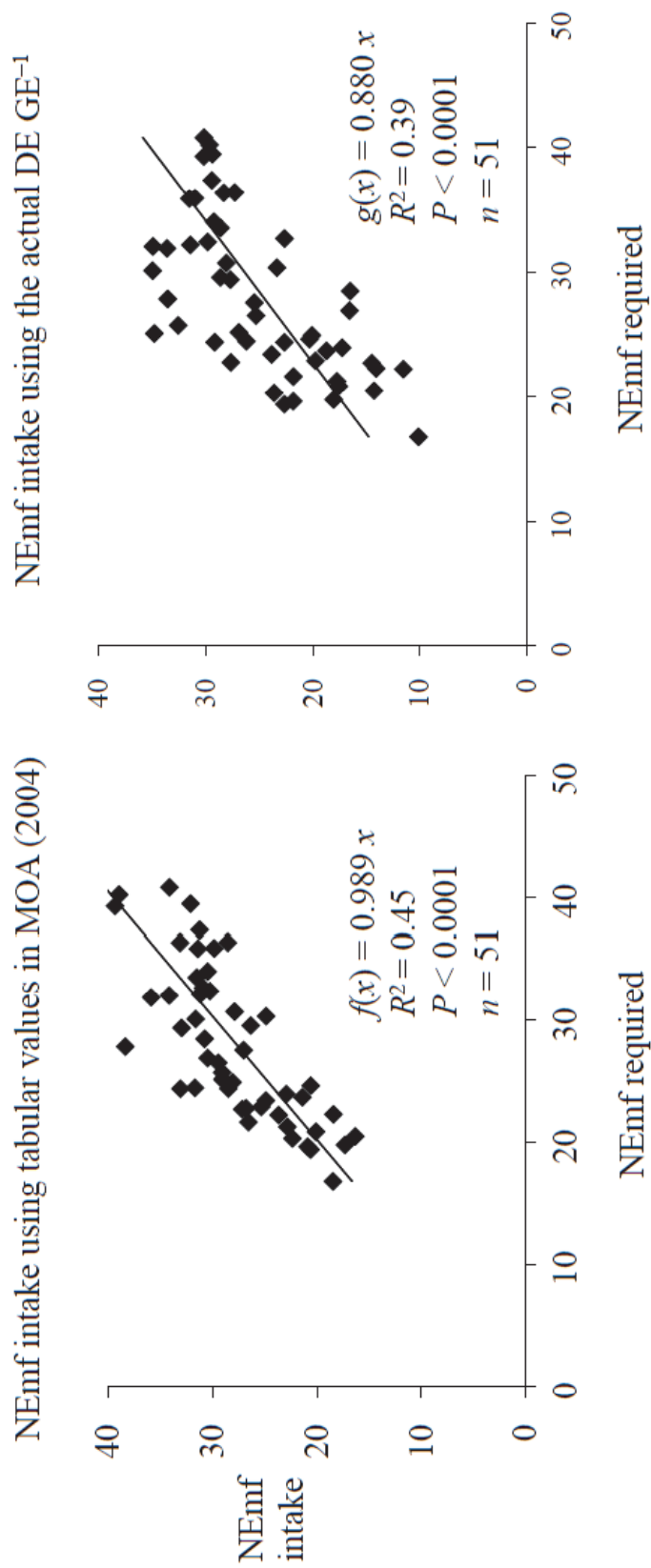


Figure 16 Linear regression of the intake of net energy for maintenance and fattening (NEmf) estimated using tabular values in MOA (2004) ($f(x)$, MJ day⁻¹) and that using the actual digestible-energy (DE) gross-energy (GE)⁻¹ ($g(x)$, MJ day⁻¹) with required NEmf (x , MJ day⁻¹). Mean BW = 190.0 kg.

Table 20 Estimated net energy intake for maintenance and fattening and the net energy required, and the sufficiency ratio of the net energy required to the net energy intake for Simmental male calves

Item	2015					
	Trial 1			Trial 2		
	T1-CTRL	T1-LA	T1-HA	T2-CTRL	T2-LA	T2-HA
Mean BW, kg	196.6	193.7	197.8	241.6	242.3	242.1
NEmf intake using tabular values [†] , MJ day ⁻¹ (1)	30.2	28.7	24.8	28.2	33.8	37.9
NEmf intake using actual DE GE ^{-1‡} , MJ day ⁻¹ (2)	24.8	23.5	21.9	27.2	28.9	32.4
NEmf required [§] for actual DG, MJ day ⁻¹ (3)	26.8	28.3	23.5	28.5	34.2	33.0

Item	2016					
	Trial 1			Trial 2		
	T1-LA	T1-MA	T1-HA	T2-LA	T2-MA	T2-HA
Mean BW, kg	130.6	139.3	132.3	185.8	188.9	183.4
NEmf intake using tabular values [†] , MJ day ⁻¹ (1)	20.5	21.9	19.2	33.1	30.5	29.5
NEmf intake using actual DE GE ^{-1‡} , MJ day ⁻¹ (2)	16.7	19.6	18.8	30.5	27.5	29.2
NEmf required [§] for actual DG, MJ day ⁻¹ (3)	21.7	20.7	21.5	34.9	33.7	30.1

Trials 1 and 2 comprised T1-CTRL, T1-LA, T1-MA, T1-HA, T2-CTRL, T2-LA, T2-MA, and T2-HA; for details, see Table 4, 5, 11 or the section on materials and methods in Chapter 2 and 4.

BW, body weight; NEmf, net energy intake for maintenance and fattening; DG, daily gain; DE, digestible energy; GE, gross energy.

[†] Calculated using the tabular values of feed ingredients (MOA 2004).

[‡] Calculated using the actual DE GE⁻¹ (Tables 7 and 14).

[§] Calculated using the estimation equation formulated by MOA (2004).

It was posited that the MEm requirement of crossbred Simmental bull calves for BW gain is relatively higher than that of other species (see section 4.3.2). The higher MEm requirement and the lower NEmf intake values calculated using the actual DE GE⁻¹ of Simmental calves may have been caused by their high heat increment for maintenance and fattening. This suggests a possible difference among breeds in the extent to which they obtain the NE for maintenance and BW gain. Such a difference could be attributed to the growing stage of the calves used in this study, during which their bodies were accumulating protein.

4.4. Conclusions

The results of the trials held in 2015 and 2016 suggested the following:

- 1) The optimal DG could be achieved with a ratio of roughage (DM) intake to total DMI of ~41% (*i.e.*, the ratio of concentrate to total DMI at ~59%).
- 2) The proper AH-intake ratios to total DMI, according to necessary criteria, are as follows:

Criteria	Season requiring minimal feeding of concentrate (warm season, T1 groups)	Season requiring increased concentrate (cool season, T2 groups)
DG	≤14.2%	≥7.8%
FCR	14.2%	7.8%–30.1%
Energy intake and utilization efficiency	≤14.2%	7.8–21.1%
Economic benefit	8.1%–14.2%	7.8%–30.1%

The ratios of 14.2% and 7.8–21.1% are appropriate for the seasons requiring minimal feeding of concentrate and increased concentrate, respectively.

- 2) The AH-intake ratios mentioned in 1) above (*i.e.*, 14.2% and 7.8–21.1%) are regarded as low-level AH feeding and appropriate in terms of sufficient N supply and effective N utilization for DG.

- 3) Low-level inclusion of AH (*i.e.*, 8–21% of total DM) in the diets of growing beef cattle is therefore recommended as a practical feeding method that can achieve more than 1-kg DG, and would promote subsequent robust growth performance during the fattening stage in dryland areas of Gansu Province, China.
- 4) The larger MEm requirement of crossbred Simmental male calves, which may differ according to their age or BW, may result in a higher ME requirement for BW gain than that for other breeds.
- 5) The MEm requirement may be compensated through supplementation with AH and/or concentrate feed. For a Simmental calf weighing 257.0 kg, the provision of 5.97 MJ day⁻¹ (0.83 kg or 0.44 kg DM of AH or C) would appear necessary in addition to the regimen calculated by AFRC (1993).

Chapter 5

General conclusions

5.1. Main findings

In this study, it was posited that low-level incorporation of an AH mixture could be introduced as a preferred and economically advantageous practice with an increase in DG but no decline in energy metabolizability and no significant increase in GHG emissions (Chapter 2). The practicability of low-level AH inclusion was further supported through the conduct of trials to assess the N balance, which demonstrated effective N utilization compared with high-level AH inclusion (Chapter 3). The optimal DG (about 1.5 kg) could be achieved by applying a ratio of roughage (DM) intake to total DMI at ~41%, which was found to be associated with the optimal DG. The incorporation of an AH mixture would bring economic benefits to farmers, increasing their incomes. The proper ratio of AH intake (on a DM basis) to total DMI can be selected according to the relevant criteria when the AH mix is substituted for concentrate feed. The energy allowance should be calculated accordingly to ensure the energy requirements of crossbred Simmental cattle. The large ME requirement for BW gain of crossbred Simmental male calves may be compensated through supplementation with AH and/or concentrate feed. Further studies on the applicability of actual feeding standards (AFRC 1993, MOA 2004) for Simmental male calves in Gansu Province are anticipated (Chapter 4).

One of the measures proposed in this study for improved feeding regimens for Simmental male calves is thus to design a diet that fulfills the following requirements.

- 1) A ratio of roughage (on a DM basis) to total DM allowance at ~41% (*i.e.*, the ratio of concentrate to total DM allowance at ~59%).

- 2) A ratio of AH (on a DM basis) to total DM allowance within a range considered appropriate in relation to the relevant criteria (see section 4.4).
- 3) Extra supplementation with AH or concentrate feed to meet the high MEM requirement, in addition to the feeding regimen estimated with the tabular values provided by MOA (2004) and the estimation equations of AFRC (1993).

5.2. Significance of the study

To my knowledge, this is the first study that has sought to determine energy metabolism using respiration trials in beef cattle fed on domestic basal forages in Gansu Province, China. This study's attempt to propose an improved feeding regimen combining both respiration and feeding trials entails an element of novelty, though further *in situ* and *in vivo* studies are expected to further elaborate an appropriate feeding design. The data obtained in this study regarding energy metabolism of Simmental crossbred male calves could be applied to update existing feeding standards such as MOA (2004). Possible differences among cattle breeds in the extent to which they meet the required NEmf (MOA 2004) could be incorporated into feeding standards so that NE requirements of beef cattle can be properly predicted at a given production level. MOA (2004) refers to NRC (2000) for some values because of the limited availability of *in situ* studies, and does not incorporate variations of breeds and feeding environment (air temperatures) (Yi and Xiong 2010). According to Patterson *et al.* (2006), incorporation of MP is critical in the application of guidelines in NRC (2000) when estimating nutrient requirements. This study makes a valuable contribution in this regard.

Pre-trial interviews conducted with farmers in the vicinity of the Linze Research Station in 2014 revealed that the farmers were burdened by the cost of concentrate for feeding beef

cattle. Jiang (2013) estimated a gradual increase in international corn prices by 2030. Gale *et al.* (2014) reported that corn consumption in China will outpace the growth in its domestic supply, requiring imports from other countries to address its deficit. Assuming that the demand in China for corn grain as an ingredient of concentrate feed does not change, the domestic price of concentrate varieties in China will continuously increase, reflecting international trends in corn prices. The conventional feeding regimen using concentrate feed purchased from the market needs to be scrutinized to promote more sustainable and stable livestock management among Chinese farmers.

A rapid increase in the demand for meat (see section 1.1.1) will require fattening performance that exceeds 1-kg DG, and early shipping of beef cattle. Low-level AH inclusion in the diet of beef calves, which comprised low-quality corn stover as basal forage in the current study (see section 4.4), would result in robust growth performance (*e.g.*, 2-kg DG) in Gansu Province by the high level of feeding for fattening during the period which succeeds the growing stage. In this context, the feeding regimen for Simmental male calves with AH inclusion, or optimal utilization of roughage locally produced by farmers, is a recommended farming practice for Chinese dryland regions.

5.3. Limitations of this work and suggestions for future research

A total of 126 species of leguminous forage resources have been identified in Gansu Province (Chen 2007). Most of these species are perennial and could constitute major sources of protein for animals. The introduction of these legumes as substitutes for concentrate will depend on their energy concentration. Avoiding any reduction in energy intake through substitution is a critical issue. Thus, legumes or other grasses with high ME content are required. Out of the 126 leguminous species, 58 are ranked above alfalfa based

on the scale used to evaluate legumes in Gansu Province (Chen 2007). Various legumes have different effects on *in vitro* ruminal fermentation, particularly CH₄ production, according to their tannin content (Williams *et al.* 2011). Some varieties of the 58 legumes may be comparable with alfalfa. An appropriate ratio of roughage in the diets appears essential to improve the DG, as a ratio outside of a suitable range will cause a decline in energy utilization efficiency and/or DG (see section 4.3.2). Given that roughage contains more NDF (fibers) than concentrate, the ratio of roughage allowance will vary in the NDF concentration of ingested feed and in the dietary NDF intake. The possible mixing ratios of other legumes with the recommended NDF content, or the feasibility of using these legumes as a substitute for concentrate deserves further study.

In addition, considering the high MEm of Simmental male calves (see section 4.3.2), feed improvement can be explored with breeding for the reduction of MEm and the increase in feed efficiency. Local breeds in China such as Yellow Cattle and Xiangzhong Black bulls have demonstrated relatively low MEm requirements (Liu *et al.* 2013). There are more than 50 local breeds nationwide, which are categorized in three groups (Chen *et al.* 1990, Chang 2001). These breeds, also, could be considered in further studies aimed at developing future crossbreds. Moreover, as the MEm requirement may vary with the growth stage (see section 4.3.2), *in situ* studies to determine an appropriate age for sale or slaughter will contribute to farming practices.

Other factors that could affect the growth performance of beef cattle such as environmental conditions could not be controlled in the trials conducted for this study, because of the limited capacity of the respiration chambers used in the trials. In this study, air temperatures in the chambers mostly corresponded to outside temperatures, and the objectives of the study were mostly achieved under this condition. Previous studies have

reported on the various effects of air temperature on the metabolism or growth performance of cattle (see section 4.3.2). Trials *in situ* according to local conditions in the study area are thus anticipated. However, some factors that may be affected by the substitution of AH for C were omitted from the parameters considered in this study. This omission was caused by the inevitable need to focus on available indicators of growth performance (such as DG) during the limited period for this study. The effects of the substitution of AH for C on these omitted factors, such as the distribution of energy and N intake in fat and protein content, have been reported through comparative slaughter technique by Hata *et al.* (2005). Trials based on feeding and slaughtering cattle, and using a number of animals, are anticipated to further investigate the effects of AH mixtures on beef quality. Previous studies that have attempted to establish models for estimating retained fat and protein (Williams and Jenkins 2003a, 2003b, Henrique *et al.* 2009), can be subsequently consulted to analyze the outputs of trials.

Simmental male calves appear to have a higher energy requirement for maintenance compared with the requirement in other breeds. The design of an improved feeding regimen that takes into consideration the energy utilization status (including the MEm requirement) should be further explored. The dietary ratios of AH and roughage mixtures recommended in the current study will require further verification. Feeding and respiration trials entailing a variety of feeding conditions would be required to establish more specific feeding regimens for farmers, thereby contributing to their need for early cattle sales.

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Summary

In China, beef consumption is rapidly increasing because of the population increase and shifting dietary preferences associated with economic growth. Effective foraging systems are required to handle the concurrent increase in feed requirements for cattle. In Gansu Province, which is a major beef cattle production area in China, a strategy of raising beef cattle in pens or feedlots to prevent the desertification of natural pastures has been promoted in accordance with the 2003 directive to “restore agricultural land to forest and pasture”. Alfalfa, a forage species that is commonly cultivated in the Province, appears to have considerable value as a source of nutrition for beef cattle because of its tolerance to drought and its high protein concentration. However, small-scale farmers in the Province use corn stover as the basal diet along with ample provision of commercial concentrate. This study was aimed at proposing measures for improved feeding regimens of beef cattle through the substitution of alfalfa hay (AH) for concentrate feed. Feeding trials were conducted in the Province to develop appropriate substitution levels of alfalfa for concentrate according to the energy and nitrogen utilization status of beef cattle. Additionally, respiration trials for estimating energy used for basic metabolism were conducted. A summary of the trial results is presented here.

1. Feeding and respiration trials were performed using crossbred male Simmental calves ($n = 18$) at Linze Research Station of the College of Pastoral Agriculture Science and Technology of Lanzhou University, Gansu Province. Trial 1 (T1) was performed in August and September 2015 during the warm season, entailing a minimal requirement of concentrate feed. Trial 2 (T2) was conducted from September to November in 2015 during the cool season, entailing an increased requirement of concentrate. The daily body-weight gain (DG) target was set at 1 kg for both trials. In each trial, animals (mean body weight (BW); 175.8 kg in T1, 218.8 kg in T2) were allocated to a conventional feeding group (CTRL), a low-level alfalfa-hay (AH) feeding group (LA), and a high-level AH feeding group (HA). The following experimental diets were applied within a one-way-layout design: harvested corn stover (CS) and concentrate (C) (T1-CTRL and T2-CTRL), 10–20% replacement of C content in the CTRL groups' diets with AH (T1-LA and T2-LA), and 20–40% replacement of C content in the CTRL groups' diets with AH (T1-HA and T2-HA). Feed intake, metabolizable energy (ME) intake, and BW changes were measured. The average DG was lower in T1-HA than in T1-CTRL and T1-LA and higher in T2-LA and T2-HA than in T2-CTRL. There were no significant differences in dry-matter intake (DMI) and ME intake (MEI) among the T1 groups. However, with increased

AH substitution, these intakes increased in T2 groups. Feed utilization efficiency was lower in the HA groups compared with the LA groups. This was evident in the DG decrease caused by a reduction in MEI in T1 and lack of the DG increase that reflected an increase in MEI in T2. By contrast, low-level AH inclusion in the calves' diet (LA groups) did not lead to reduced DG in comparison to that of calves in the CTRL groups. Feeding costs for achieving 1-kg DG in the LA groups were the lowest among all three groups in both T1 and T2. Therefore, low-level AH inclusion in the diets of beef cattle appears to benefit farmers.

At the conclusion of the trials, respiratory measurements (for CO₂ and CH₄ production, and O₂ consumption) were taken for 15 calves in T1 and 12 calves in T2 within ventilated open-circuit respiration chambers over 2 days, following a 5-day adaptation period. CH₄ production levels in the LA groups did not differ significantly from those in the CTRL groups. However, they increased with increases in dietary AH inclusion levels. The results of the respiration trials revealed higher MEm values for the crossbred Simmental male calves (600–652 kJ kg^{-0.75} BW day⁻¹) than for other species.

2. I evaluated the effects of dietary AH inclusion on DG in terms of N balance on the basis of the results of the trials conducted in 2015. Fecal and urine samples collected during the trials were used for the evaluation. Whereas N intake did not differ significantly among the T1 groups, this increased with increasing AH inclusion level in T2. Urinary excretion of N was slightly lower in the T1-HA group than in the T1-CTRL and T1-LA groups. However, there were no differences among the T2 groups. Thus, N retention increased in both trials with increasing AH inclusion levels. However, the reduction and lack of increase in DG in the respective HA groups in T1 and T2 (compared with the LA groups) were indicative of the advantage of low-level AH feeding for effective N utilization and growth performance (the ratio of AH intake to total DMI was 8.1% in the warm season and 22.6% in the cool season).
3. After analyzing the results of the trials in 2015, I performed two further feeding and respiration trials using crossbred male Simmental calves ($n = 12$) to develop an appropriate AH inclusion level to total DM allowance. The trials were performed in July and August 2016 (T1) and from August to October in 2016 (T2) with a DG target of 1 kg. The calves were allocated to low-level (LA), medium-level (MA), and high-level AH feeding groups. The T1-LA and T2-MA diets were based on the low-level AH mixtures used for the feeding trials (T1-LA and T2-LA) conducted in

2015. To assess the effect of augmenting the proportion of the AH mixture used in T1-LA, the calves in T1-MA received an increased amount of AH with CS and C, whereas those in T1-HA received a proportionately greater amount of AH with CS and C ($n = 4$ per group). To assess the effect of modifying the quantity of the AH mixture used in T2-MA, the calves received CS, C, and a decreased amount of AH in T2-LA but an increased amount of AH in T2-HA ($n = 4$ per group). AH proportions for calves in T1 (mean BW = 126.2 kg) and T2 (mean BW = 159.4 kg) were, respectively, 15% (T1-LA), 23% (T1-MA), and 31% (T1-HA) and 9% (T2-LA), 24% (T2-MA), and 34% (T2-HA) of their dietary DM allowances. Feed intake and BW changes were measured. At the end of T1, respiratory measurements were conducted over 2 days for all 12 calves within ventilated open-circuit respiration chambers. DMI did not differ significantly among the three groups in T1 and T2. The DG and estimated profit based on the DG were slightly higher for T1-LA than in the T1-MA and T1-HA groups, and higher in T2-LA and T2-MA than in the T2-HA group. Energy digestibility did not differ among the three groups in T1 and T2. The estimated value of required MEm ($737 \text{ kJ kg}^{-0.75} \text{ BW day}^{-1}$) exceeded previously reported values.

Data from the feeding trials conducted in 2015 and 2016 indicate the following ratios of AH intake to total DMI as being appropriate for male Simmental beef cattle, considering requirements of minimal and increased feeding of concentrate during the two respective seasons: DG, $\leq 14.2\%$ and $\geq 7.8\%$; feed conversion ratio, 14.2% and 7.8–30.1%; energy intake and utilization efficiency, $\leq 14.2\%$ and 7.8–21.1%; and economic benefit, 8.1–14.2% and 7.8–30.1%.

These findings suggested that low-level inclusion of AH (8–21% of the total DM) augments DG and farmers' incomes without causing significant increases in GHG emissions. Adjusting the feed amount to meet the high MEm requirements of growing Simmental calves on the basis of amounts estimated through actual feeding standards would enhance the effectiveness of feeding regimens with low AH inclusion.

To my knowledge, this is the first study in which analyses of outputs from both open-circuit respiration chambers and feeding trials were used to formulate improved feeding regimens in Gansu Province. ME values for maintaining and fattening calves estimated from the respiration trials were considered. The study's findings can be used to update the *Feeding Standard for Beef Cattle* in China (2004).

摘要

中国では、人口増加と経済成長に伴う食生活の変化によって牛肉消費量が急増しており、これに伴う飼料給与量の抑制のためには飼料の効率的な利用体系の構築が求められる。同国肉用牛生産の重点地域である甘粛省では、2000年代から推進する「退耕還林・退牧還草」政策に基づき、天然草地の砂漠化を防止するための舎飼い飼養が推奨されてきた。同省で一般的に播種・栽培されているアルファルファは、タンパク質含量や耐旱耐暑性が高いことから肉用牛飼料源としての利用価値はきわめて高いと考えられるが、同省の小規模農家は、トウモロコシ茎葉部(CS)を基礎飼料とし、市販濃厚飼料を多給して肉用牛の舎飼い飼養を行なっているのが現状である。本研究では、アルファルファ乾草(AH)を用い、濃厚飼料(C)代替による肉用牛の飼養法改善の方策を提示することを目的として、エネルギー・窒素出納成績に基づく適切な代替割合を設定するための飼養試験、および基礎代謝量を把握するための呼吸試験を、中国甘粛省で行なった。得られた試験結果は以下のように要約される。

1. 蘭州大学リンゼ試験場にてシンメンタール交雑育成雄牛を用い、日増体量(DG)を1kgに設定し、2015年8-9月(温暖期;濃厚飼料標準量給与期)および9-10月(冷涼期;同多給期)に飼養試験と呼吸試験を行なった。各季18頭の供試牛(開始時体重, 温暖期 175.8kg;冷涼期 218.8kg)を用い、CS-C 給与区(対照区)、対照区のC 給与量の10-20%相当量をAHで代替した区(LA区)、20-40%相当量を代替した区(HA区)の3処理区(各区6頭)を設け、試験期間中の乾物摂取量(DMI)、代謝エネルギー摂取量(MEI)、体重変化を測定した。DGは、温暖期では対照区、LA区よりHA区で低く、冷涼期では対照区よりLA区、HA区で高かった。DMIおよびMEIは、温暖期では各区間に有意差はなかったが、冷涼期ではAH給与割合の増加に伴って増加した。AH多給与区(HA区)では、MEI減少によるDGの減少やMEI増加を反映しないDGの停滞といった形で飼料効率の低下が見られたが、適切量のAH給与(LA区)ではDGの減少は見られなかった。1kg DGに必要な飼料コストは両期ともLA区で最も低く、一定程度のAH給与が営農上も望ましいと思われた。

さらに、飼養試験後に各区から5頭以上を選出し、3日間の馴致後、開放型呼吸試験装置を用いて呼吸量(CO₂・CH₄産生量、O₂消費量)を2日間測定した。AH給与に伴ってCH₄排出量は増加したが、LA区でのCH₄排出量は対照区との間に有意差がなかった。呼吸試験からは、シンメンタール交雑育成雄牛の維持ME量(600-652kJ/kg^{0.75}BW/day)が他種牛よりも高いことが明らかになった。

2. AH給与による増体効果について窒素出納成績から検討した。上記1の供試牛か

ら採取した糞・尿サンプルを分析に用いた。摂取窒素(N)量は、温暖期では各区間に有意差なく、冷涼期ではAH給与割合の増加に伴って増加した。尿中排泄Nは、温暖期には対照区、LA区よりHA区で低く、冷涼期は各区間での有意差はなかった。AH給与によって蓄積N量は増加したが、AH多給与(HA区)でのDGの減少(温暖期)や停滞(冷涼期)を考慮すると、適切量のAH給与(AH混合割合(乾物重ベース); $\geq 8.1\%$ (温暖期), $\geq 22.6\%$ (冷涼期))が、期待DGの達成と効率的な窒素出納に寄与することが示された。

3. 上記1、2の結果をふまえ、給与飼料中の適切なAH混合割合を明確にすべく、2016年7-8月(温暖期)および8-10月(冷涼期)に、上記1と同様の飼養試験(DG 1kg)と呼吸試験を実施した。温暖期では上記1のLA区(温暖期)の混合割合に加え、中・高水準AH混合区を設定、冷涼期では同割合(冷涼期)に加え、低・高水準AH混合区を設定した。各回12頭の供試牛(開始時体重、温暖期126.2kg;冷涼期159.4kg)を用い、AH混合割合(乾物重ベース)を15%, 9%(それぞれ温暖期, 冷涼期、以下同じ)とした低混合区、23%, 24%とした中混合区、31%, 34%とした高混合区を設け(各区4頭)、試験期間中のDMIと体重変化を測定した。また、温暖期試験後に開放型呼吸試験装置を用いて全12頭の呼吸量を2日間測定した。DMIは、両期とも各区で有意差がなかった。DGおよび増体による想定収益は、温暖期では低混合区が高く、冷涼期では低混合区および中混合区が高かった。エネルギー消化率は両期とも各区間での有意差はなかった。維持ME要求量($737\text{kJ/kg}^{0.75}\text{BW/day}$)は既報値より高かった。

上記1~3の結果を、各区での総飼料摂取量に占めるAH摂取量の割合と照らして比較したところ、生産成績および収益に応じた適切な割合として、DG; $\leq 14.2\%$, $\geq 7.8\%$ (それぞれ温暖期, 冷涼期、以下同じ)、飼料要求率; 14.2%, 7.8-30.1%、ME摂取量; $\leq 14.2\%$, 7.8-21.1%、収益; 8.1-14.2%, 7.8-30.1%を得た。

本研究結果から、適切量でのAHの給与(混合割合;温暖期14.2%、同多給期7.8-21.1%)により、温室効果ガス排出の増加を抑制し、日増体量および農家収入を増加させる可能性が示された。このための飼料設計は、飼養標準に基づく給与量にシメンタール育成牛の高い維持ME量に見合う量を上乘せしての給与が適切と考えられる。

飼養試験に加えて開放型呼吸試験装置での計測結果を用い、維持・増体のためのME量を考慮して肉用牛の飼養改善策を検討した本研究での取り組みは、甘粛省に先例がなく、中国飼養標準(2004)の検討作業に際しても有用な情報を提供しうると期待される。

List of publications

Nobuyuki Kobayashi, Fujiang Hou, Atsushi Tsunekawa, Xianjiang Chen, Tianhai Yan, Toshiyoshi Ichinohe (2017) Effects of substituting alfalfa hay for concentrate on energy utilization and feeding cost of crossbred Simmental male calves in Gansu Province, China. *Grassland Science* 63 (4): 245–254. (Published, this article covers Chapter 2).

Nobuyuki Kobayashi, Fujiang Hou, Atsushi Tsunekawa, Xianjiang Chen, Tianhai Yan, Toshiyoshi Ichinohe (2018) Appropriate level of alfalfa hay in diets for rearing Simmental crossbred calves in dryland China. *Asian-Australasian Journal of Animal Sciences* (Accepted, this article covers Chapter 4).