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SPECIAL ISSUE ARTICLE

Substitution of leguminous forage for oat hay improves nitrogen utilization efficiency of crossbred Simmental calves

Wuchen Du[1] | **Fujiang Hou**[2] | **Atsushi Tsunekawa**[3] | **Nobuyuki Kobayashi**[3] | **Fei Peng**[4] | **Toshiyoshi Ichinohe**[5]

[1]The United Graduate School of Agricultural Sciences, Tottori University, Tottori, Japan

[2]State Key Laboratory of Grassland Agro-Ecosystems, Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture, College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, Gansu, China

[3]Arid Land Research Center, Tottori University, Tottori, Japan

[4]International Platform for Dryland Research and Education, Tottori University, Tottori, Japan

[5]Faculty of Life and Environmental Science, Shimane University, Matsue, Japan

Correspondence

Fujiang Hou, State Key Laboratory of Grassland Agro-Ecosystems; Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture; College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, Gansu, 730020, China.
Email: cyhoufj@lzu.edu.cn

Atsushi Tsunekawa, Arid Land Research Center, Tottori University, Tottori, 680-0001, Japan.
Email: tsunekawa@tottori-u.ac.jp

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Abstract

Low nitrogen (N) utilization efficiency (NUE, the ratio of retained N to N intake [NI]) of ruminants is always a potential dietary protein wastage as well as a global environmental problem, and dietary N manipulation is the most effective way to improve NUE. We conducted 2 experiments to investigate the effects of replacing oat hay by leguminous forages (alfalfa hay [AH] in experiment [Exp] 1 and common vetch hay [CVH] in Exp 2) with 4 levels (0%, 8%, 16% or 24% AH and 0%, 10%, 20% or 30% CVH on dry matter [DM] basis) at the same crude protein (135 g/kg DM) and metabolizable energy (10.1 MJ/kg DM) on feed intake, N metabolism, NUE and blood composition of crossbred Simmental calves. Sixteen calves of each Exp were assigned to the four diets in a randomized block design. Faecal N (FN) output and the ratio of FN to NI increased with increasing AH/CVH proportions, whereas urinary N (UN) output, the ratio of UN to NI, and the ruminal ammonia N concentration gradually decreased in both experiments. Nutrient digestibility (DM, organic matter [OM] and neutral detergent fibre [NDF]) of calves showed a parabolic trend with gradually increasing AH/CVH proportions. The highest values of nutrient digestibility (DM, OM and NDF) of calves were observed in 16% AH in Exp 1 and 20% CVH in Exp 2. Our findings suggest that 16% and 20% substitution (as a percentage of the total DM allowance) of AH and CVH, respectively, for oat hay are optimal diets to improve NUE and reduce the potential impact of N excretion from livestock farming on the environment through shifting routes of N from urine to faeces without negative effects on live weight gain and nutrient digestibility of crossbred Simmental calves in dryland environments.

KEYWORDS

alfalfa, common vetch, dryland, nitrogen utilization efficiency, Simmental calf

1 | INTRODUCTION

More than 70% of feed nitrogen (N) is excreted (such as in faeces and urine) from livestock farming into the environment (Chowdhury, Khan, Mahfuz, & Baset, 2018; Ghelichkhan, Eun, Christensen, Stott, & MacAdam, 2018), which is always perceived to be a major global environment problem (NRC, 2003). For example, nitrous oxide (N_2O) from solid manure heaps contributes to global warming (Chadwick, 2005). Ammonia (NH_3) emissions from the manure and urine not only indirectly contribute to N_2O emission (Kebreab, Dijkstra, Bannink, & France, 2009) but also lead to water eutrophication (Chadwick, 2005) and soil nitrification and acidification (Oonincx et al., 2010). In beef cattle feeding systems, approximately 60%–80% of total N intake (NI) was excreted in urine, which has great potential to aggravate NH_3 emissions, and only 20%–40% was excreted in faeces (Dong, Zhao, Chai, & Beauchemin, 2014; Kebreab et al., 2009). Therefore, there is increasing interest in improving N utilization efficiency (NUE, the ratio of retained N [RN] to N intake I [NI]) and reducing N excretion from livestock farming systems, especially the urinary N (UN, Chowdhury et al., 2018; Kebreab et al., 2009). Dietary crude protein (CP) manipulation was believed to have the potential to improve NUE and influence N partitioning to faeces and urine in ruminants (Yan, Frost, Keady, Agnew, & Mayne, 2007).

Generally, higher NUE would stimulate livestock growth and reduce the influence of large N excretion on the environment. For the past 2 decades, NUE has been extensively studied in beef cattle (Dong et al., 2014; Yan et al., 2007). For example, increasing feeding level (FL, metabolizable energy [ME] intake divided by ME requirement for maintenance from the AFRC (1993); Yan et al., 2007) and energy supplementation (Titgemeyer, Spivey, Parr, Brake, & Jones, 2012) could improve NUE. Higher diet quality (such as high ME; Zhao, Gordon, O'Connell, & Yan, 2016) also leads to high NUE. However, most studies focused on improving NUE through manipulating diets to reach the goal of promoting the rumen fractional passage rate or balancing energy and protein supply to maximize the microbial CP synthesis in the rumen. Few studies emphasized the N partitioning of livestock fed on forage legumes in intensive livestock farming, which is estimated to be responsible for 64% of all anthropogenic NH_3 emissions (Oonincx et al., 2010).

In general, leguminous forage can offer higher nutritive value and raise the efficiency of conversion to livestock production compared with grass (Patra, 2010). Previous studies have shown that CP, digestible organic matter (OM) intake, and in vitro OM digestibility are significantly higher with oat–common vetch mixture diets than with oat-only diets for cattle (Assefa & Ledin, 2001). The addition of alfalfa to a grass hay basal diet also increased the digestibility of CP and the disappearance rate of dry matter (DM) and neutral detergent fibre (NDF) in the rumen in beef cattle diets (Bhatti, Bowman, Firkins, Grove, & Hunt, 2008). However, little information is available on N metabolism and partitioning of N excretion under oat–alfalfa or –common vetch mixture diets. What is more, too high a proportion of leguminous forage in the diet may cause adverse effects. For example, although a high alfalfa hay (AH) diet (34%) for growing Simmental calves could improve NUE, it led to a decreased DM and CP digestibility compared with a low proportion of AH (9%) in the diet (Kobayashi et al., 2018). Therefore, the objective of the present study was to investigate the effects of different levels of oat–alfalfa, and oat–common vetch mixture feeding diets on nutrient digestibility, N metabolism, and form of N excretion as well as NUE in crossbred Simmental male calves, and to determine the optimal proportion of AH and common vetch hay (CVH) in feeding diets in dryland environments.

2 | MATERIALS AND METHODS

This study was conducted at the Linze Grassland Agriculture Trial Station, Lanzhou University, Zhangye City, Gansu Province, China (39.24°N, 100.06°E, and 1,390 m a.s.l.), the climate of which is characterized as a typical temperature continental climate because average annual precipitation is 121.5 mm and the annual average temperature is 7.7°C. In this study, AH was the second cut and

TABLE 1 Nutrient value of feeding ingredients

Item[a]	Alfalfa hay	Oat hay	Common vetch hay	Soybean meal	Wheat bran	Maize
OM, g/kg DM	905	942	918	935	931	982
CP, g/kg DM	168	60	177	465	182	83
NDF, g/kg DM	458	559	413	166	454	100
ADF, g/kg DM	347	407	302	102	186	20
Ether extract, g/kg DM	22	18	23	26	55	44
GE, MJ/kg DM	17.9	16.8	17.7	19.6	19.4	18.5
MEC, MJ/kg DM	8.7	9.0	9.5	13.0	10.9	13.4
MPC, g/kg DM	62	68	71	87	73	90

Abbreviations: ADF, acid detergent fibre; CP, crude protein; GE, gross energy; MEC, metabolizable energy concentration; MPC, metabolizable protein concentration; NDF, neutral detergent fibre; OM, organic matter.

[a] MEC and MPC were calculated by the equations from Agricultural and Food Research Council (1993) and Chinese Feeding Standard for Beef Cattle (2004), see details in Methods and Materials. Other chemical compositions were measured based on the methods in Chemical Analysis.

common vetch was harvested at the podding stage and stored as CVH. Oat hay (OH) was bought from a forage company near the station (Sanbao Agricultural Company). The ingredients of the concentrate (maize, soybean meal and wheat bran) were from a local source. The chemical composition of forage and concentrate is shown in Table 1.

2.1 | Animals, treatments and diets

Two experiments were conducted, and the forage-to-concentrate ratio was fixed (60:40, DM basis) for all diets in both experiments. In experiment (Exp) 1, 16 crossbred male Simmental calves with an initial BW of 134 kg (*SD* 6.9; 5 month of age) were assigned to 4 diets with different OH:AH ratios (60:0 in Diet-1, 52:8 in Diet-2, 44:16 in Diet-3, and 36:24 in Diet-4) in a randomized block design. In Exp 2, the same 16 crossbred male Simmental calves with BW of 206 kg (*SD* 14.3) were also assigned to 4 diets with different OH:CVH ratios (60:0 in Diet-1, 50:10 in Diet-2, 40:20 in Diet-3, and 30:30 in Diet-4) in a randomized block design. Both feeding experiments lasted for 50 days, with an initial 14 days for diet acclimation.

The target daily weight gain (DWG) for each calf was set at 1.0 and 1.3 kg/day for Exp 1 and 2, respectively. All experimental diets were designed to provide sufficient ME and metabolizable protein (MP) to meet the target DWG for a calf according to the published estimation equations and values of the Agricultural and Food Research Council (AFRC, 1993) and BW of calves (measured every 9 days). The ME and MP concentrations of OH, AH and concentrate were calculated based on the tabulated values, which were established by the Chinese Feeding Standard for Beef Cattle (Ministry of Agriculture of the People's Republic of China, 2004). The digestibility of ruminal CP and energy for CVH were from Larbi, El-Moneim, Nakkoul, Jammal, and Hassan (2011). The CP, ME and MP levels of all diets in each experiment are shown in Table 2. All calves were housed in individual pens and given free access to water and a mineral mixture. The daily mixed forage was divided into 2 equal parts and offered as separate meals twice daily (8:00 a.m. and 8:00 p.m. in Exp 1, 8:00 a.m. and 7:00 p.m. in Exp 2). The mixed concentrate was fed once daily (2:00 p.m.).

TABLE 2 Composition of feeding ingredients and the target metabolizable energy concentration and metabolizable protein concentration of all diets in Exp 1 and 2

	Diet[a] (Exp 1)				Diet[a] (Exp 2)			
Feed formula	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Forage								
Leguminous forage, g/kg DM	0	80	160	240	0	100	200	300
Oat hay, g/kg DM	600	520	440	360	600	500	400	300
Concentrate								
Maize, g/kg DM	48	78	110	140	55	69	81	95
Soybean meal, g/kg DM	107	86	68	48	112	75	38	1
Wheat bran, g/kg DM	245	236	222	212	233	256	281	304
Nutrient value[b]								
CP, g/kg DM	135	134	135	135	135	135	135	135
OM, g/kg DM	940	939	938	936	941	939	937	935
NDF, g/kg DM	469	457	442	429	465	456	448	439
ADF, g/kg DM	302	294	285	277	300	290	281	271
MEC, MJ/kg DM	10.1	10.1	10.1	10.1	10.2	10.1	10.1	10.1
MPC, g/kg DM	99	97	95	94	100	96	91	86

Abbreviations: ADF, acid detergent fibre; CP, crude protein; MEC, metabolizable energy concentration; MPC, metabolizable protein concentration; NDF, neutral detergent fibre; OM, organic matter.

[a] In Exp 1, Diet-1 contained 60% oat hay, Diet-2 contained 52% oat hay and 8% alfalfa hay, Diet-3 contained 44% oat hay and 16% alfalfa hay, and Diet-4 contained 36% oat hay and 24% alfalfa hay. In Exp 2, Diet-1 contained 60% oat hay, Diet-2 contained 50% oat hay and 10% common vetch hay, Diet-3 contained 40% oat hay and 20% common vetch hay and Diet-4 contained 30% oat hay and 30% common vetch hay. In addition, 10 g/day of mineral mixture fed to each calf throughout the feeding period contained (minimum values) 720 mg manganese, 30 mg copper, 0.05 mg biotin, 0.4 mg folic acid, 50 mg vitamin B_1, 2.5 mg vitamin B_2, 0.5 mg vitamin B_6 and 0.1 mg vitamin B_{12}.

[b] These values were calculated by the equations from Agricultural and Food Research Council (1993) and the Chinese Feeding Standard for Beef Cattle (2004); see details in Methods and Materials.

2.2 | Measurement and sampling procedure

The amount of forage and concentrate offered and all leftovers were weighed daily throughout the experimental period to calculate daily DM intake (DMI). After the 14-day acclimation period, on day 15, a randomly selected cattle from each diet group were moved to the four individual open-circuit respiration chambers for 9 days. On day 24, they were removed to the individual pens in the cowshed, and another 4 cattle, randomly selected from the remaining cattle of these four diet groups, entered the chambers and left on day 33, it continued until all 16 cattle finished measurements for each Exp. The BW of all calves was measured in the morning with an empty stomach to calculate ADG (kg/day) when exchanged cattle between chambers and cowshed. During the first 2 days of the 9 days' measurements in chamber, the calves were kept for acclimation. We collected the digestibility data over the following 4 days and gas exchange data (O_2 consumption, and CH_4 and CO_2 emissions) over the remaining 3 days. During the digestibility data collection period, the total weight of faeces and urine excreted daily was recorded. Faeces, which were excreted on a plastic mat placed under the cattle, were collected immediately with a shovel into a plastic container and weighted, mixed and sampled once daily. About 10% of each faeces sample was stored at −20°C for later chemical analysis. Total urine was collected through handmade urine bag into a bucket containing 200 ml 10% v/v H_2SO_4 to reduce ammonia loss once daily. Acidified urine was checked for pH with a portable pH instrument (PHBJ–260, Shanghai INESA Scientific Instrument). About 20% of the daily urine was stored at −20°C for chemical analysis.

The four indirect open-respiration calorimeter chambers used in the present study were equipped with a computer-controlled air-handling system with air conditioning units set to a temperature of 18 ± 1°C and relative humidity of 60% ± 10%. The calorimeter chambers were built with double Perspex walls fitted in aluminium frames, with a total volume of approximately 18 m^3 (4.2 m long, 1.95 m wide and 2.2 m high); Each chamber was equipped with a gas flow metre (GFM57, Aalborg) at the outflow site for recording total airflow and an engine ensures a slight negative pressure within the chamber. All chambers were ventilated by suction pumps with a flow rate of 45–50 m^3/hr. The exhaust air was removed for volume, temperature and humidity measurement and analysis in the bottom, middle and upper respectively inside each chamber. During the 3 days for gas exchange measurement, the concentrations of CO_2, CH_4 and O_2 in the air into and out of each chamber were measured every 16 min (the interval for each chamber) using a multigas analyzer (VA-3000, Horiba) in a general control room. The analyzer was calibrated using standard gases (O_2-free N_2 and a known quantity of CH_4, CO_2 and O_2 [span gas], Dalian Special Gases) at the beginning of the gas exchange collection period in each Exp. This determined an absolute range of 0–500 μl/L for CH_4, 0–2,000 μl/L for CO_2 and 0%–25% v/v for O_2, and the linearity within this range. The recovery rate of CH_4 was determined by comparing the CH_4 releasing into the chamber with a given concentration and the CH_4 concentration at the outlet. The gas recovery rate was approximate 100% ± 2% for all chambers. The data collection period for each chamber lasted for 4 min, which includes the time for gas mixture flowing from inside the chamber to the analyzer and the time for gas concentration stabilization. The concentrations of CO_2, CH_4 and O_2 were recorded in the last 3 s of this 4-min period. The O_2 consumption, CH_4 and CO_2 emissions were calculated based on the concentration differences of CO_2, CH_4 and O_2 between the air into and out of each chamber, and the total volume of gas exchange (flow rate × interval time). Each chamber was designed with a dedicated door, which was next to the animal trough. The staff only opened the door to feed animal immediately after the completion of data collection in the chamber. This minimized the effects of feeding activity (<1 min) on gas concentrations inside.

Rumen fluid samples were taken from each calf 4 hr after the morning feeding using stomach tubing on the first- and ninth-day during the 9 days' measurement in the chamber of each Exp. The digesta was immediately strained through 2 layers of muslin, and 5 ml of strained ruminal fluid was mixed with 1 ml of 6 M HCL and frozen at −20°C for analysis of ruminal ammonia N (Nguyen & Hegarty, 2017). Blood was collected into a 10-ml glass vacuum tube with anticoagulant from individual animals at the same time with collecting rumen fluid samples, and then all blood was immediately centrifuged for 15 min at 3,000 × g to obtain serum for blood urea N (BUN), glucose and triglyceride measurements. Plasma urea N concentration was assumed to be equivalent to the BUN concentration in the serum considering that urea diffuses freely into and out of blood cells (Kohn, Dinneen, & Russek-Cohen, 2005).

2.3 | Chemical analysis

The stored faeces samples were thawed at room temperature for 12 hr, and then the faeces samples from each calf from the 4 days were mixed. A portion of the thawed faeces sample was used for the N measurement according to the Association of Official Analytical Chemists method 976.05 (AOAC, 1990). The remaining samples were oven-dried at 65°C for 48 hr and then ground to pass through a 1-mm screen for N, NDF, ADF and OM measurements. Urine samples of each calf from the 4 days were also thawed at room temperature for 12 hr and then mixed before determining N using the Kjeldahl procedure previously described by the Association of Official Analytical Chemists (AOAC, 1990).

The NDF and ADF concentrations were sequentially analysed in an ANKOM 2000 fibre analyzer (ANKOM Technology) following the protocol described by Van Soest, Robertson, and Lewis (1991). The OM content was determined through ashing for 6 hr using a muffle furnace at 550°C (method 942.05; AOAC, 1990). The CP concentration was calculated by multiplying the N concentration by 6.25.

2.4 | Statistical analysis

One-way ANOVA was used to analyse the effects of diets on DMI, ADG, nutrient digestibility, N balance and NUE. Linear regression analysis was used to investigate the relationship between ruminal ammonia N and UN, between ruminal ammonia N and the ratio of UN to NI and between ruminal ammonia N and BUN. Differences among the means were considered significant at the $p \leq .05$ level on the basis of the Tukey's test. All data obtained from each experiment were subjected to the General Linear Model procedure for orthogonal polynomial analysis. The statistical program used in the current study was IBM SPSS Statistics for Windows, version 19.0 (IBM Corp).

3 | RESULTS

3.1 | Feed intake and nutrient digestibility

In Exp 1, forage DMI and total DMI of calves increased from the Diet-1 group to the Diet-4 group linearly ($p < .05$, Table 3), and total DMI was significantly higher (13.7%) in the Diet-4 group than in the Diet-1 group ($p < .05$; Table 3), corresponding to a greater ADG ($p < .05$; Table 3). No differences were found in digestibility of DM and NDF ($p > .05$, Table3) whereas OM digestibility was reduced 2.6% in the Diet-4 group than in the Diet-1 group ($p < .05$, Table 3). N digestibility tended to decrease linearly ($p < .05$, Table 3), and it was significantly reduced by 4.1% in the Diet-4 group compared with Diet-1 group ($p < .05$; Table 3).

In Exp 2, there were no differences in forage DMI, concentrate DMI, total DMI and ADG among the four diet groups ($p > .05$; Table 3). But, digestibility of DM, OM, NDF and N showed a quadratic trend from the Diet-1 group to the Diet-4 group ($p < .05$, Table 3) and was significantly higher in the Diet-2 group than in the Diet-4 group ($p < .05$; Table 3).

3.2 | Nitrogen balance and N utilization efficiency

In Exp 1, although there were no differences in NI, manure N (MN) and retained N for the 4 diet groups ($p > .05$; Table 4), FN and UN of calves significantly differed between the Diet-1 group and the Diet-4 group ($p < .05$; Table 4). In detail, FN tended to increase linearly ($p < .05$) with increasing AH proportions and it was significantly higher in the Diet-4 group than in the Diet-1 group by 38% ($p < .05$). However, UN tended to decrease with an increase in AH proportions and it was significantly lower in the Diet-4 group than in the Diet-1 group by only 8.3% ($p < .05$).

In Exp 2, no differences were found in NI and retained N among the four diet groups; but FN tended to increase quadratic ($p < .05$) and it was 18.8% higher in the Diet-4 group than in the Diet-2 group ($p < .05$; Table 4) despite there was no significant difference from Diet-1 group to Diet-3 group ($p > .05$, Table 4). UN and MN decreased from the Diet-1 group to the Diet-4 group linearly ($p < .05$), and they were significantly lower in the Diet-4 group than in the Diet-1 group by 19.3% and 10.8% respectively ($p < .05$; Table 4).

The FN:NI and UN:NI ratios showed a tendency similar to those of FN and UN, respectively, in both Exp 1 and Exp 2 ($p < .05$; Table 4). What is more, in Exp 1, the FN:NI ratio increased by 4.1% from the Diet-1 group to the Diet-4 group whereas the UN:NI ratio decreased by 7.2%. In Exp 2, although the FN:NI ratio increased 3.6% from the Diet-1 group to the Diet-4 group, the UN:NI ratio decreased by 8.5%.

3.3 | Blood urea N and ruminal ammonia N

In Exp 1, BUN and ruminal ammonia N concentrations tended to decrease from the Diet-1 group to the Diet-4 group linearly ($p < .05$, Table 5) and they were significantly lower in the Diet-4 group than in the Diet-1 group by 36.7% and 17.3% respectively ($p < .05$; Table 5). In Exp 2, BUN and ruminal ammonia N concentrations showed a quadratic tendency from the Diet-1 group to the Diet-4 group ($p < .05$, Table 5) and they were significantly greater in the Diet-2 group than in the Diet-4 group by 37.6% and 23.3% respectively ($p < .05$; Table 5). There were no differences in glucose concentration among the four diet groups in both experiments (Table 5).

3.4 | Relationship between ruminal ammonia N and urinary N output, the ratio of urinary N to N intake and blood urea N

Ruminal ammonia N was positively significantly correlated with UN output, the ratio of UN to NI, and BUN pooled from each Exp ($p < .05$; Figure 1). In detail, there was a higher slope of the linear regression between UN output and ruminal ammonia N in Exp 2 than that in Exp 1 (Figure 1a). For linear regression between the ratio of UN to NI and ruminal ammonia N, there was a similar value for the slope of the linear regression between Exp 1 and 2, but the ratio of UN to NI was relatively higher in Exp 1 than it in Exp 2 (Figure 1b). The slope of the linear regression between BUN and ruminal ammonia N was higher in Exp 1 than in Exp 2 (Figure 1c).

4 | DISCUSSION

4.1 | Feed intake and nutrient digestibility

Inclusion of legumes in diets affects the DMI through its influence on DM digestibility (McDonald, Edwards, Geenhalgh, & Morgan, 2002). In Exp 1, the linearly increasing forage DMI with the same concentrate DMI from the Diet-1 group to the Diet-4 group led to the difference in the total DMI (Table 3). This was in agreement with the results of Osuji and Odenyo (1997) that supplement of leguminous forage in low-quality forages could increase total DMI. A more important reason is that legumes are more easily digested than grass, which may reduce the ruminal fill of livestock (Allen,

TABLE 3 Effects of diet on DMI, ADG and nutrient digestibility in Exp 1 and 2 for Simmental crossbred calves

	Diet[a] (Exp 1)						Polynomial contrast[b]			Diet[a] (Exp 2)						Polynomial contrast[b]		
Item	**1**	**2**	**3**	**4**	***SEM*[b]**	***P***	**L**	**Q**	**C**	**1**	**2**	**3**	**4**	***SEM*[b]**	***P***	**L**	**Q**	**C**
BW and feed intake																		
Initial BW, kg	134	135	134	135	5.6	.991	NS	NS	NS	213	200	201	209	14.8	.749	NS	NS	NS
Forage DMI, kg/day	1.74^{ab}	1.63^{b}	2.01^{ab}	2.13^{a}	0.124	.013	0.035	NS	NS	4.22	3.83	4.05	4.03	0.716	.958	NS	NS	NS
Concentrate DMI, kg/day	1.69	1.80	1.74	1.76	0.082	.598	NS	NS	NS	2.73	2.73	2.73	2.73	–	1.000	NS	NS	NS
Total DMI, kg/day	3.42^{b}	3.43^{b}	3.75^{ab}	3.89^{a}	0.145	.028	0.044	NS	NS	6.53	6.18	6.38	6.37	0.647	.959	NS	NS	NS
ADG, kg/day	1.04^{b}	1.17^{ab}	1.18^{ab}	1.26^{a}	0.057	.019	0.002	0.08	0.015	1.32	1.29	1.32	1.33	0.039	.797	NS	NS	NS
Nutrient digestibility, %																		
DM	78.9	78.7	79.4	77.0	1.17	.493	NS	NS	NS	69.3^{ab}	70.9^{a}	70.7^{a}	65.6^{b}	1.43	.020	NS	0.036	NS
OM	80.0^{a}	79.5^{ab}	80.1^{a}	77.4^{b}	0.70	.017	NS	NS	NS	70.6^{ab}	72.4^{a}	71.7^{ab}	67.5^{b}	1.51	0.046	NS	0.047	NS
NDF	70.6	68.2	70.0	67.6	2.1	.468	NS	NS	NS	56.0^{ab}	60.5^{a}	59.6^{a}	51.7^{b}	1.84	.005	NS	0.010	NS
Nitrogen	86.2^{a}	85.5^{a}	84.4^{ab}	82.1^{b}	0.01	.022	0.034	NS	NS	78.9^{ab}	79.4^{a}	78.4^{ab}	75.3^{b}	1.15	.030	0.002	<0.001	<0.001

Note: Superscripts in lower case letters mean significant statistical difference at $p \le .05$.

Abbreviations: BW, body weight; DMI, dry matter intake.

[a] In Exp 1, Diet-1 contained 60% oat hay, Diet-2 contained 52% oat hay and 8% alfalfa hay, Diet-3 contained 44% oat hay and 16% alfalfa hay and Diet-4 contained 36% oat hay and 24% alfalfa hay. In Exp 2, Diet-1 contained 60% oat hay, Diet-2 contained 50% oat hay and 10% common vetch hay, Diet-3 contained 40% oat hay and 20% common vetch hay and Diet-4 contained 30% oat hay and 30% common vetch hay.

[b] *SEM*, total standard error of means, NS, not significantly different ($p > .05$); L, Linear; Q, Quadratic; C, Cubic.

TABLE 4 Effects of diet on N intake, N excretion and N utilization efficiency (NUE) in Exp 1 and 2 for Simmental crossbred calves

	Diet^{a} (Exp 1)						Polynomial contrast^{b}			Diet^{a} (Exp 2)						Polynomial contrast^{b}		
Item	1	2	3	4	*SEM*^{b}	*P*	L	Q	C	1	2	3	4	*SEM*^{b}	*P*	L	Q	C
N balance, g/day																		
NI	83.2	83.9	85.3	86.4	2.61	.633	NS	NS	NS	145	139	141	139	3.1	.273	NS	NS	NS
FN	11.3^{b}	12.1^{b}	13.6^{ab}	15.6^{a}	0.88	.006	<0.001	<0.001	<0.001	30.5^{ab}	28.8^{b}	30.4^{ab}	34.2^{a}	1.55	.041	NS	0.017	0.050
UN	50.3^{a}	48.7^{ab}	47.8^{ab}	46.1^{b}	0.98	.017	<0.001	0.002	0.008	79.8^{a}	78.4^{a}	69.3^{ab}	64.4^{b}	3.29	.004	<0.001	<0.001	<0.001
MN	61.6	60.9	61.4	61.7	1.73	.963	NS	NS	NS	111^{a}	106^{ab}	100^{b}	99^{b}	3.2	.013	<0.001	0.004	0.008
RN	21.6	23.0	24.0	24.7	1.87	.443	0.021	NS	NS	34.7	32.1	40.9	40.3	5.89	.420	NS	NS	NS
NUE, g/g																		
FN:NI ratio	0.138^{b}	0.145^{ab}	0.156^{ab}	0.179^{a}	0.0120	.022	0.034	NS	NS	0.211^{ab}	0.206^{b}	0.216^{ab}	0.247^{a}	0.0115	.030	0.002	<0.001	<0.001
UN:NI ratio	0.611^{a}	0.583^{b}	0.565^{b}	0.539^{b}	0.0193	.032	0.041	NS	NS	0.552^{a}	0.566^{a}	0.495^{ab}	0.467^{b}	0.0303	.009	0.011	0.030	0.045
MN:NI ratio	0.750	0.728	0.721	0.719	0.0201	.492	NS	NS	NS	0.762	0.773	0.711	0.713	0.0274	.113	NS	NS	NS
RN:NI ratio	0.251	0.272	0.279	0.282	0.0201	.492	NS	NS	NS	0.238	0.228	0.289	0.287	0.0274	.113	NS	NS	NS

Note: Superscripts in lower case letters mean significant statistical difference at $p \le .05$.

Abbreviations: FN, faecal N; MN, manure N (FN + UN); NI, N intake; RN, retained N; UN, urinary N.

[a] In Exp 1, Diet-1 contained 60% oat hay, Diet-2 contained 52% oat hay and 8% alfalfa hay, Diet-3 contained 44% oat hay and 16% alfalfa hay and Diet-4 contained 36% oat hay and 24% alfalfa hay. In Exp 2, Diet-1 contained 60% oat hay, Diet-2 contained 50% oat hay and 10% common vetch hay, Diet-3 contained 40% oat hay and 20% common vetch hay and Diet-4 contained 30% oat hay and 30% common vetch hay.

[b] *SEM*, total standard error of means; NS, not significantly different ($p > .05$); L, Linear; Q, Quadratic; C, Cubic.

TABLE 5 Effects of diets on composition of blood serum and ruminal ammonia concentration in Exp 1 and 2 for Simmental crossbred calves

Item (mmol/L)	Diet[a] (Exp 1)						Polynomial contrast[b]			Diet[a] (Exp 2)						Polynomial contrast[b]		
	1	2	3	4	*SEM*[b]	*P*	L	Q	C	1	2	3	4	*SEM*[b]	*P*	L	Q	C
BUN	7.09^{a}	6.23^{ab}	5.02^{b}	4.49^{b}	0.569	.007	<0.001	<0.001	<0.001	6.90^{ab}	7.06^{a}	6.77^{ab}	5.13^{b}	0.557	.034	0.019	<0.001	0.002
Glucose	6.53	6.71	6.77	6.98	0.374	.690	NS	NS	NS	6.27	6.34	6.40	6.29	0.187	.900	NS	NS	NS
Ruminal ammonia N	4.79^{a}	4.46^{ab}	4.23^{a,b}	3.96^{b}	0.313	.047	<0.001	0.003	0.010	4.63^{ab}	4.93^{a}	4.27^{ab}	4.00^{b}	0.228	.016	0.002	0.002	<0.001

Note: Superscripts in lower case letters mean significant statistical difference at $p \le .05$.

Abbreviation: BUN, blood urea N.

[a] In Exp 1, Diet-1 contained 60% oat hay, Diet-2 contained 52% oat hay and 8% alfalfa hay, Diet-3 contained 44% oat hay and 16% alfalfa hay and Diet-4 contained 36% oat hay and 24% alfalfa hay. In Exp 2, Diet-1 contained 60% oat hay, Diet-2 contained 50% oat hay and 10% common vetch hay, Diet-3 contained 40% oat hay and 20% common vetch hay and Diet-4 contained 30% oat hay and 30% common vetch hay.

[b] *SEM*, total standard error of means, NS, not significantly different ($p > .05$); L, Linear; Q, Quadratic; C, Cubic.

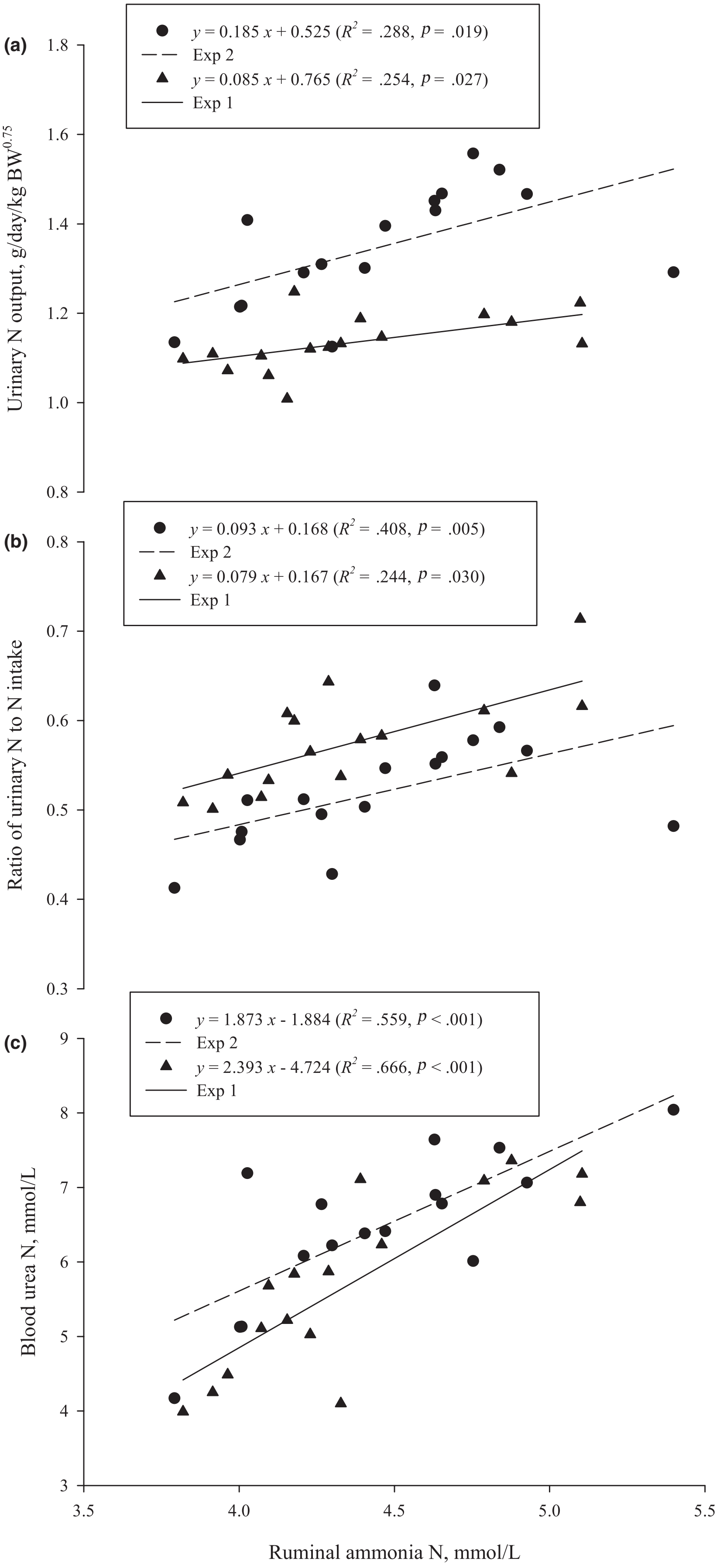
1996; Niederecker, Larson, Kallenbach, & Meyer, 2018), thereby increased AH proportions in the diet, which promoted total intake (Bhatti et al., 2008; Zhao, Aubry, O'Connell, Annett, & Yan, 2015). The ADG linearly increased with increasing forage DMI in Exp 1 (Table 3). However, the DM digestibility slightly decreased from the Diet-3 group to the Diet-4 group, although a higher forage DMI with the same concentrate DMI was observed in the Diet-4 group than in the Diet-3 group (Table 3). The much higher total DMI, which is negatively correlated with digestibility (Zhao et al., 2017), could be partially responsible for the decreased digestibility in Exp 1. In Exp 2, there were no differences in feed intake (forage DMI and concentrate DMI), which is due to the limited supplementation of feed. Despite this, the DM digestibility also showed a quadratic trend from Diet-1 group to Diet-4 group (Table 3). This indicates that there is a tipping point in substituting AH/CVH for OH for digestibility, and an appropriate proportion of leguminous forage in the diet would be beneficial for feed utilization, which was in agreement with a previous study (Kobayashi et al., 2018).

The average lower nutrient digestibility, including DM, OM and NDF, in Exp 2 than in Exp 1 was attributed to a higher FL in Exp 2 than in Exp 1 (average 1.91 vs. 2.44 in Exp 1 vs. Exp 2, respectively), which increased the fractional passage rate (AFRC, 1993); therefore, high FL depressed digestibility. The digestibility of a feed is influenced by the composition of other feeds consumed with it (Zhao et al., 2015). In Exp 1, digestibility of DM, OM and NDF was relatively stable from the Diet-1 group to the Diet-3 group and then decreased in the Diet-4 group, whereas in Exp 2, they showed parabolic tendencies from the Diet-1 group to the Diet-4 group (Table 3). The tendencies are probably due to an increasing proportion of maize in diets in both experiments (Table 2), which could provide more rapid fermentation of starch to VFA to depress rumen pH (McDonald et al., 2002). When the pH reached a threshold, it would inhibit microorganism activity and depress fibre digestibility (Zhao et al., 2015). Titgemeyer et al. (2012) also demonstrated that ruminal infusions of VFA could lead to slight decreases in fibre digestion and digestibility. Therefore, the present study found that 16% AH and 20% CVH were the highest levels of substitution for oat hay in the diet that did not suppress nutrient digestibility (DM, OM, NDF and N).

4.2 | Mitigation strategies to reduce N excretion

Nitrogen excretion in faeces and urine represents a considerable N loss in livestock farming (Castillo, Kebreab, Beever, & France, 2000; Zhao et al., 2016). In the present study, MN output is in a range of 71 to 76%, pooled from both experiments, which was consistent with the results (74%) of Dong et al. (2014) in beef cattle but less than that (78%) reported by Yan et al. (2007) in growing to finishing beef cattle. This difference is likely due to the animal breeds, the forage-to-concentrate ratio, the different ingredients in the concentrate offered and the various ages and BW. All of these factors likely affect N excretion per gram NI. Although the MN:NI ratio did not differ among the 4 diet groups in each

FIGURE 1 The relationship between ruminal ammonia nitrogen (N) and urinary N output (a), the ratio of urinary N to N intake (b) and blood urea N (c) in crossbred Simmental calves. The data were pooled from each experiment (Exp). $p \le .05$ Means a significant difference



experiment, the route of N excretion was altered. For example, more N was lost in faeces than in urine (Table 4), which was in agreement with the study of Zhao et al. (2016) and Ghelichkhan et al. (2018). UN is usually more volatile than FN because most of the urinary urea N is inorganic N and can be rapidly hydrolysed to ammonium and then converted to NH_3, which is more likely to lead to N lose from the farm system to the environment (Koenig & Beauchemin, 2018). In contrast, faecal NH_3 production is generally low due to slow mineralization rates of organic nitrogenous compounds (Kebreab et al., 2009). In the present study, the linearly increasing FN:NI ratio corresponded to a linearly decreasing UN:NI ratio (Table 4) in both experiments, which indicated that substitution of AH/CVH for OH could reduce UN loss and indirectly mitigate NH_3 emissions (Du et al., 2019).

Increasing FL (which is indicative of growth rate) could proportionally reduce N loss in urine more than in faeces (Yan et al., 2007), because high feed intake can contribute to a high ruminal fractional outflow rate, which leaves less time for rumen microorganisms to ferment the feedstuffs, consequently leading to a reduction in ammonia N absorbed in the rumen and subsequently reducing N excreted in urine (Zhao et al., 2015). In Exp 1, the increased total DMI from the Diet-1 group to the Diet-4 group (Table 3) and the linearly decreased ruminal ammonia N concentration (Table 5) and UN:NI ratio (Table 4) support the previous findings of Zhao et al. (2015) and Yan et al. (2007). In Exp 2, although there was no difference in total DMI (Table 3), the ruminal ammonia N concentration and UN:NI ratio were still significantly lower in the Diet-4 group than in the Diet-2 group (Table 4). The low DM digestibility in the Diet-4 group compared with that in the Diet-2 group could be partially responsible for this.

4.3 | Nitrogen metabolism, ME supply and N utilization efficiency

This study examined the effect of substitution of AH/CVH by OH on N metabolism and its partitioning, with the same NI (Table 4). In general, there is a positive relationship between N digestibility and the proportion of urine N loss per gram NI (Dong et al., 2014). A linearly decreased N digestibility from the Diet-1 group to the Diet-4 group in both Exp 1 and Exp 2 (Table 3), corresponding with a linearly decreasing UN output and UN:NI ratio (Table 4), was in line with the finding of Dong et al. (2014). This is likely due to high N digestibility usually being associated with a greater proportion of N absorbed as ammonia N from the rumen above the requirements of microbial activity. Therefore, higher N digestibility in the rumen would result in N being excreted more in urine than in faeces (Zhao et al., 2016).

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

Generally, high ruminal ammonia N concentration for optimal OM degradation will result in more N loss through urine (Ipharraguerre & Clark, 2005). In Exp 1, a decreasing ruminal ammonia N concentration (Table 5) under a relatively stable OM digestibility from the Diet-1 group to the Diet-3 group (Table 3) associated with a decreasing UN:NI ratio from the Diet-1 group to the Diet-3 group (Table 4), supporting the previous finding. In Exp 2, although OM digestibility and ruminal ammonia N concentration and the UN:NI ratio showed parabolic trends, they were still consistent with the previous finding. There is usually a strong positive correlation between ruminal ammonia N and BUN (Aboagye et al., 2018; Kohn et al., 2005), and the same strong positive correlation was found in the present study (Figure 1). In addition, BUN could be used as an indicator of the protein status of the animal (Aboagye et al., 2018). In the present study, BUN decreased with increasing AH/CVH proportions, with the range of BUN being between 7.09 and 4.49 mmol/L. These values were higher than the average value of 4.04 mmol/L reported by Aboagye et al. (2018) for weaned crossbred steers fed alfalfa and barley silages. The optimal BUN concentration for protein deposition by beef steers is around 2.49 mmol/L (Johnson & Preston, 1995). Usually, the greater BUN values could be attributed to the age and breed of animals (Kohn et al., 2005). However, the higher BUN concentration coupled with small age of animals in the present study (Table 5) compared with a lower BUN coupled with older age of animals in the study of Aboagye et al. (2018) was unexpected (5 and 9 months of age in Exp 1 and 2 respectively vs. 12 months of age), given that older age of animals had a higher BUN than small age of animals. This might be due to that the lower ruminal ammonia N concentration for animals in the present study corresponded to greater post-ruminal MP, and greater intestinal absorption of amino acids, leading to an increase in BUN and then excreted in urine when dietary protein supply exceeded the requirement of animals (Aboagye et al., 2018; Reynolds & Kristensen, 2008). The higher BUN has been reduced by using less degradable protein sources in feed (Ipharraguerre & Clark, 2005). However, less degradable protein in feed could also reduce the ruminal ammonia N concentration and thus decrease the available N supply for microbial growth

(Ipharraguerre & Clark, 2005). In the present study, substituting less degradable protein sources in legumes for high degradable protein sources in the concentrate (Table 2) decreased ruminal ammonia N concentration. However, no reduction in ADG was observed (Table 3), which suggests that ruminal available N was adequate for microbial growth (Prakash et al., 2013), and the ammonia N concentration of the rumen fermentation was around 4.0 mmol/L in the current diets.

5 | CONCLUSION

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

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ANIMAL WELFARE STATEMENT

The Animal Ethics Committee of Lanzhou University approved all animal management and experimental procedures according to the rules and regulations of experimental field management protocols (file No. 2010–1 and 2010–2) in accordance with the Guides for Management of Laboratory Animals in Gansu Province, China (Gansu Provincial Department of Science & Technology, 2005).

ORCID

Wuchen Du https://orcid.org/0000-0002-2379-1146

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