Front. Agric. China 2011, 5(4): 598–604
DOI 10.1007/s11703-011-1138-7

RESEARCH ARTICLE

Effects of rumen-degradable protein balance on rumen fermentation in continuous culture fermenters

Qiufeng LI, Yanxia GAO, Yufeng CAO, Zhihua FENG, Jianguo LI (✉)

College of Animal Science and Technology, Agricultural University of Hebei, Baoding 071001, China

Abstract **Twelve dual-flow continuous culture fermenters (culture 8 d with 5 d for adjustment and 3 d for sample collection) were used to evaluate the effects of rumen-degradable protein balance (RDPB) on rumen fermentation. The different RDPB levels in six diets were as follows: – 16.84, – 8.87, – 0.87, + 7.13, + 15.13, and + 23.12 g RDPB /kg DM. Results indicated that RDPB had a significant effect on fermenter NH_3-N ($P < 0.0001$), but had less effect on the fermenter pH ($P = 0.058$), total VFA concentration ($P = 0.57$), or acetate molar proportion ($P = 0.70$). The decrease in rumen-available N in the diets with the RDPB levels at + 15.13, + 7.13, – 0.87, – 8.87, – 16.84 g RDPB/kg DM resulted in NH_3-N concentration decreasing at 9%, 52%, 104% and 118% in rumen compared with the diet of + 23.12 g RDPB/kg DM. Total VFA concentration, acetic acid and valeric acid at different time points was altered ($P < 0.05$) by the treatment. With the increase of dietary RDPB, the total NH_3-N flowing out of fermenters linearly increased, and the N losses also increased.**

Keywords rumen-degradable protein balance, artificial rumen, rumen fermentation

Introduction

Nitrogen losses from dairy cows are inevitable, but N excretion can, to some extent, be prevented or at least controlled. Reducing N losses could be carried out by feeding and management to reduce N excretion (Satter et al., 2002). Dietary proteins are divided into rumen-degradable (RDP) and undegradable proteins (RUP). RDP is degraded as peptides, free amino acids, and ammonia for microbial growth and protein synthesis in rumen (NRC, 2001). The rate of degradation of dietary proteins is very important in supplying amino acids for ruminant animals. When dietary RDP is in excess of the required amount of ruminal microorganisms, the proteins are degraded to ammonia N. We should reduce N excretion by limiting the excess of proteins digested in the small intestine and balancing the level of rumen-degradable proteins with the available energy supplying for microbial protein synthesis (Colin-Schoellen et al., 2000; Børsting et al., 2003). Tamminga et al. (1994) reported that the rumen-degradable protein balance according to the Dutch system (OEB; Tamminga et al., 1994) could reduce N losses. Indeed, the OEB value estimates the difference in the potential microbial protein synthesis based on RDP and rumen fermented OM (Yu et al., 2003). Most researchers reported that the optimal OEB value of a diet was close to zero and corresponded to a well-balanced availability of energy and N to rumen (Bach et al., 2008).

The NRC-2001 dairy model (NRC, 2001) is used widely in the world, but there is no research report about the effect of RDPB (NRC-2001 Model). Therefore, the present study was conducted to determine the effects of different RDPB on ruminal pH, NH_3-N concentration and VFA concentration *in vitro*. Also, the correlations between RDPB and ruminal metabolism were investigated using a continuous culture fermenter system. We hypothesized that the RDPB value was positive for a diet, and N losses from the rumen occurred, whereas a negative value indicated a shortage of RDP, and, consequently, the microbial activity may be impaired.

Received June 12, 2011; accepted August 21, 2011
Correspondence: Jianguo LI
E-mail: jgli@hebau.edu.cn

Materials and methods

Animal and collection of rumen fluid

Three ruminally cannulated Holstein dairy cows, as donors of rumen fluid, were used as the inoculum for the dual-flow continuous culture fermenter system. The dairy cows were gradually adapted to an experimental diet (consisting of 42.7% corn silage, 10.3% hay, 24.7% corn, 7.7% cottonseed meal, 4.3% soybean meal, 6.8% wheat bran, 1.0% mineral-vitamin mix, 0.5% salt, 1.5% di-calcium phosphate, 0.5% $NaHCO_3$) and then had ad libitum access to this diet for 2 wk before and throughout the study. This diet was formulated to meet or exceed the nutrient requirement of Holstein dairy cows (NRC, 2001). The rumen fluid was collected (using a vacuum pump) from each dairy cow approximately 2 h after feeding and strained immediately after removal from the rumen through four layers of gauze into a prewarmed container, without oxygen. Upon arrival at the laboratory, the rumen fluids from three dairy cows were combined and used to inoculate the 12 fermenters.

Apparatus and experimental design

Twelve 760 mL dual-flow continuous culture fermenters were developed by the Agricultural University of China and were used in 3 replicated periods of 8 d each. On the first day of each period, all fermenters were inoculated with ruminal fluid obtained from 3 rumen-fistulated cows (600 kg BW). Each fermenter was supplied daily with 48 g DM of a ground diet by an automated feeding system controlled to deliver the diet in 2 equal portions over a 24-h period. The diets are shown in Table 1. The diets were designed to meet or exceed nutrient recommendations for a Holstein cow (650 kg BW) that produced 30 kg of milk daily (NRC, 2001). Temperature (39°C), liquid (10%/h) and solid (4%/h) dilution rates remained constant. Anaerobic conditions were maintained by infusion of CO_2 at a rate of 40 mL/min. Artificial saliva (Weller and Pilgrim, 1974) was continuously infused into flasks fermenters and contained 0.4 g/L urea to simulate recycled N.

The experimental design was a single-factorial experimental design and consisted of three repeated periods of 8 d

Table 1 Ingredient, chemical composition and nutritive value of the diets with different rumen-degradable protein balances (RDPB)

Items		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Diet composition	Steam-flaked corn (%)	5	5.6	5.6	5.6	5.6	5.6
	Soybean meal (%)	1.28	1.71	1.71	1.71	1.71	1.71
	DDGS(%)	1	1	1	1	1	1
	Brewers grain (%)	1	1	1	1	1	1
	Wheat bran (%)	1.5	1.5	1.5	1.5	1.5	1.5
	Soybean oil (%)	0.592	0	0	0	0	0
	Grass hay (%)	2.1	2	2	2	2	2
	Corn silage (%)	25	25	25	25	25	25
	Alfalfa hay (%)	3.7	3.45	3.45	3.45	3.45	3.45
	Urea (%)	0	0	0.0598	0.1195	0.1793	0.239
	Power (%)	0.1	0.1	0.1	0.1	0.1	0.1
	$CaHPO_4$ (%)	0.16	0.16	0.16	0.16	0.16	0.16
	NaCl (%)	0.05	0.05	0.05	0.05	0.05	0.05
	$NaHCO_3$	0.1	0.1	0.1	0.1	0.1	0.1
	Vitamin mixture[1] (%)	0.002	0.002	0.002	0.002	0.002	0.002
	Trace additives mixture[2] (%)	0.01	0.01	0.01	0.01	0.01	0.01
Feed nutrients levels	CP (%)	14.54	15.45	16.29	17.13	17.97	18.81
	NDF (%)	37.38	37.70	37.70	37.70	37.70	37.70
	ADF (%)	21.50	21.54	21.54	21.54	21.54	21.54
	RDP (%)	7.80	8.29	9.12	9.96	10.80	11.64
	RUP (%)	6.74	7.17	7.17	7.17	7.17	7.17
	RDPB (g/kg DM)	−16.84	−8.87	−0.87	+ 7.13	+ 15.13	+ 23.12
	TDN (%)	69.54	67.29	67.55	67.84	68.12	68.41
	Forage to concentrate ratio	49.97:50.03	50.07:49.97	50.08:49.92	50.08:49.92	50.08:49.92	50.08:49.92
	Ca (%)	0.71	0.71	0.71	0.71	0.71	0.71
	P (%)	0.40	0.41	0.41	0.41	0.41	0.41

[1] means per kilogram vitamin mixture supply contains vitamin A, 4750 IU; vitamin D, 1000 IU and vitamin E, 27.5 IU. [2] means per kilogram trace additives mixture supply contains 0.5 mg I, 20 mg Fe, 10 mg Cu, 12.5 mg Mn, 40 mg Zn, 0.03 mg Se, and 0.01 mg Co. [a,b,c] mean treatments with different letters were different at $P < 0.05$.

each. The first 5 days of each period were used for stabilization and the last 3 days were used for sample collection. The RDPB of the 6 diets was −16.84, −8.87, −0.87, + 7.13, + 15.13 and + 23.12 g /kg DM. The six diets were allocated randomly to fermenters, giving two replications for each diet per period.

Sample collection and preparation

On each sampling day, both overflow and the filtered fraction of the effluent for each fermenter were combined and homogenized for 10 min. A 200-mL sample was removed via vacuum aspiration during homogenization. At the end of each period, the samples of each fermenter from each of the 3 d were composited. During homogenization of the effluent, subsamples were taken for pH, NH_3-N, and VFA analyses. During each period, 10 mL filtered fermenter fluid was taken 2 h, 4 h, 6 h, 8 h, and 10 h after the morning feeding for determining VFA and ammonia N concentration. During the last 3 days of each period, 40 mL of filtered fermenter fluid was taken at 0 h, 2 h, 4 h, 6 h, 8 h, and 10 h after the morning feeding for ammonia N determination.

Chemical analyses

The DM content of diets was determined by oven drying at 105°C for 48 h (AOAC, 1990). Crude proteins were calculated as N × 6.25. Dietary ingredients were analyzed for fatty acid content and composition using a Soxlec system HT6 apparatus (Tecator, Fisher Scientific) according to the procedure of Sukhija and Palmquist (1988). NDF and ADF were determined using the modified filter bag method of Van Soest et al. (1991) and an ANKOM200 Fiber Analyzer (ANKOM Technology Corp., Fairport, NY).

Samples for VFA were prepared as described by Jouany (1982). A 1 mL solution comprising 0.2% (wt/wt) mercuric chloride, 0.2% (wt/wt) 4-methylvaleric acid (as an internal standard), and 2% (vol/vol) orthophosphoric acid was added to 4 mL filtered rumen fluid and frozen. Samples were centrifuged at 3000×*g* for 30 min and the supernatant fluid was analyzed by gas chromatography (model 6890; Hewlett Packard, Palo Alto, CA) using a polyethylene glycol nitroterephtalic acid-treated capillary column (BP21; SGE, Europe Ltd., Buckinghamshire, UK) at 275°C in the injector and at a gas flow rate of 29.9 mL/min. For ammonia N determination, a 4-mL sample of filtered fermenter fluid was acidified with 4 mL 0.2 mol/L HCl and frozen. Samples were centrifuged at 25000×*g* for 20 min, and the supernatant was analyzed by visible spectrophotometry (UV-120-01; Shimadzu, Kyoto, Japan) for ammonia N (Chaney and Marbach, 1962).

The NRC-2001 model

The ruminally undegraded feed CP was calculated as RDP = CP × %RDP, where, a Kp (ruminal passage rate) was 4%/h. Ruminally synthesized microbial CP was calculated as MCP = 0.13 × TDN, when RDP was less than 1.18 × TDN − predicted MCP. MCP was calculated as 0.85 × RDP, in g/kg DM. The factor 0.13 means that 130 g microbial protein CP/kg TDN is assumed to be synthesized.

The detailed concepts and formulas of RDPB were provided by NRC (2001). RDPB reflects the difference between the potential microbial protein syntheses based on ruminally degraded feed crude proteins and that based on TDN available for microbial fermentation in the rumen. The RDPB was calculated as RDP − 1.18MCP, in g/kg DM.

Statistical analysis

All statistical analyses were conducted using SAS (version 8.1; SAS Inst., Inc., Cary, NC). Results for the determination of VFA and ammonia N concentrations (during the adaptation days) and pH were analyzed using PROC MIXED for repeated measures (Littell et al., 1998). The model accounted for the effects of treatments and hours (for VFA and ammonia N concentration) of sampling, and the interaction of treatment and hours. Period was considered a random effect. Differences between treatments were declared at $P < 0.05$ using Tukey's multiple comparison test.

Results

Ruminal fermentation characteristics

Concentrations of VFA and NH_3-N in the effluent are presented in Table 2. Increasing RDPB improved the NH_3-N ($P < 0.0001$) of rumen (from 8.09 to 17.67 mg/dL), but it did not alter rumen pH ($P = 0.058$), total VFA concentration ($P = 0.57$), and acetate molar proportion ($P = 0.70$). The ruminal pH (Table 2) increased from the lowest RDPB level (−16.86 g of RDPB/kg DM) to the positive RDPB level (+ 23.12 g of RDPB/kg of DM). Total VFA concentration had an increasing trend ($P = 0.57$) as the rumen-degradable protein supply increased and resulted from a significant quadratic increase of the ruminal propionate, butyric acid and valeric concentrations. In contrast, increasing RDPB improved ($P = 0.12$) the butyrate molar proportion, but no change was observed for acetic acid ($P = 0.70$), propionic acid ($P = 0.51$), and valeric ($P = 0.34$) molar proportions.

Effects of different RDPB in the diet on pH of fermentation

No differences were detected among treatments for ruminal pH at different points (Table 3). In higher RDPB diets, the highest pH was greater, and the pH change tended to be greater for the lower RDPB diet in postprandial evolution for 2–4 h ($P = 0.27$).

Table 2 Effects of different RDPB in the diet on pH, ammonia concentration and VFA in the rumen

Item	Group 1 −16.87 g/kg DM	Group 2 −8.87 g/kg DM	Group 3 −0.87 g/kg DM	Group 4 + 7.13 g/kg DM	Group 5 + 15.13 g/kg DM	Group 6 + 23.12 g/kg DM	*P*
pH	6.05±0.06^{a}	6.09±0.06^{a}	6.10±0.07^{a}	6.13±0.06^{a}	6.13±0.05^{a}	6.15±0.04^{a}	0.0582
Ammonia N(mg/dL)	8.09±2.52^{B}	7.89±2.48^{B}	8.83±2.99^{B}	12.29±3.69^{AB}	16.57±4.03^{A}	17.67±5.28^{A}	< 0.0001
Acetic acid(μg/mL)	35.91±9.41^{a}	37.42±3.91^{a}	38.86±4.70^{a}	36.73±6.57^{a}	39.94±3.85^{a}	35.18±3.13^{a}	0.7003
Propionic acid(μg/mL)	12.90±3.26^{a}	14.47±1.61^{a}	14.47±2.08^{a}	14.29±2.28^{a}	15.48±1.52^{a}	13.96±1.94^{a}	0.5078
Butyric acid(μg/mL)	6.27±2.14^{a}	7.42±0.93^{a}	7.02±0.96^{a}	7.71±1.51^{a}	7.73±1.03^{a}	6.08±0.53^{a}	0.1288
Valeric(μg/mL)	0.80±0.31^{a}	0.92±0.17^{a}	0.97±0.20^{a}	0.91±0.17^{a}	0.99±0.16^{a}	0.76±0.12^{a}	0.3458
Total VFA(μg/mL)	55.92±15.07^{a}	60.22±6.46^{a}	61.30±7.85^{a}	59.64±10.23^{a}	64.14±6.45^{a}	55.47±5.16^{a}	0.5727

Means with different superscripts within the same row differ significantly ($P < 0.05$), and means with different capital superscripts within the same column differ significantly ($P < 0.01$).

Table 3 Effects of different RDPB level on pH in rumen

Item	Group 1 −16.87 g/kg DM	Group 2 −8.87 g/kg DM	Group 3 −0.87 g/kg DM	Group 4 + 7.13 g/kg DM	Group 5 + 15.13 g/kg DM	Group 6 + 23.12 g/kg DM	*P*
0	6.17±0.09^{a}	6.08±0.19^{a}	6.12±0.13^{a}	6.15±0.14^{a}	6.16±0.13^{a}	6.19±0.13^{a}	0.8460
2 h	5.97±0.19^{a}	6.05±0.14^{a}	6.00±0.14^{a}	6.05±0.03^{a}	6.08±0.12^{a}	6.13±0.11^{a}	0.2684
4 h	6.06±0.13^{a}	5.99±0.13^{a}	6.10±0.08^{a}	6.10±0.13^{a}	6.13±0.13^{a}	6.17±0.13^{a}	0.9429
6 h	6.07±0.15^{a}	6.01±0.13^{a}	6.09±0.08^{a}	6.09±0.14^{a}	6.14±0.14^{a}	6.21±0.12^{a}	0.6204
8 h	6.11±0.14^{a}	6.04±0.15^{a}	6.12±0.14^{a}	6.12±0.15^{a}	6.17±0.12a	6.23±0.10^{a}	0.8947
10 h	6.14±0.08^{a}	6.06±0.18^{a}	6.10±0.14^{a}	6.13±0.15^{a}	6.17±0.16^{a}	6.20±0.15^{a}	0.8938

Means with the different superscripts within the same row differ significantly ($P < 0.05$), means with the different capital superscripts within the same column differ significantly ($P < 0.01$).

Effects of different RDPB in the diet on rumen ammonia concentration of fermentation

Table 4 shows the effects of different RDPB diets on rumen ammonia concentration in fermentation. The NH_3-N concentration increased linearly ($P < 0.01$), from 8.09 to 17.67 mg N/100 mL, with increasing dietary RDPB. Thus, a positive RDPB diet will cause higher levels of rumen ammonia concentration. The dynamic ammonia concentration at different sampling times in rumen can be found in Table 4, ammonia concentration was up to the peak value in all treatments after feeding for 2 h ($P < 0.01$), and then gradually decreased to its lowest point at feed for 8 h, then gradually increased ($P < 0.01$) (Table 4). The temporal pattern of ruminal NH_3-N concentration was similar, but peak concentrations were influenced by RDPB. At 0, 2, 4, 6 and 8 h after the meal distribution, ruminal NH_3-N levels were significantly different among the treatments. The decrease in rumen-available N in the diet with the −16.84, −8.87, −0.87, +7.13, +15.13 g of RDPB/kg DM diets resulted in a reduction of 118%, 104%, 52% and 9% of ruminal NH_3-N concentration compared with the + 23.12 g of RDPB/kg DM diet.

Effects of RDPB on volatile fatty acid in the rumen

Total VFA, acetic acid, propionic acid, butyric acid and valeric concentrations at different time points in the rumen are presented in Table 5. The diets of different RDPB did differ in terms of total VFA concentration, acetic acid and valeric acid at different points. In contrast, the total VFA concentration was higher in the diet containing −0.87 and + 7.13 g/kg DM

Table 4 Different rumen degradable protein diet on rumen ammonia concentration (mg/dL)

Item	Group 1 RDPB (−16.87 g/kg DM)	Group 2 RDPB (−8.87 g/kg DM)	Group 3 RDPB (−0.87 g/kg DM)	Group 4 RDPB (+ 7.13 g/kg DM)	Group 5 RDPB (+ 15.13 g/kg DM)	Group 6 RDPB (+ 23.12 g/kg DM)	*P*
0 h	8.66±1.38^{C}	8.49±1.11^{C}	9.42±1.14^{Bd}	10.92±1.94^{Bc}	14.55±2.25^{Ab}	12.97±1.37^{Aa}	< 0.0001
2 h	12.39±2.03^{Cc}	12.38±3.30^{Cc}	14.24±2.03^{Cc}	19.19±2.95^{B}	23.65±3.15^{Ab}	26.07±2.19^{Aa}	< 0.0001
4 h	8.93±1.60^{D}	7.69±2.31Db	9.27±1.96^{Da}	13.13±3.00^{C}	18.78±1.76^{B}	22.05±0.98^{A}	< 0.0001
6 h	6.36±0.81^{C}	5.93±1.41^{C}	6.59±1.00^{C}	11.01±2.36^{B}	15.76±2.17^{A}	16.94±0.88^{A}	< 0.0001
8 h	5.31±1.58^{C}	5.38±1.34^{C}	5.94±2.08^{C}	8.47±2.55^{B}	13.77±2.10^{A}	14.13±1.73^{A}	< 0.0001
10 h	6.87±1.11^{C}	7.48±1.96^{C}	7.55±2.44^{C}	10.99±2.88^{Bb}	12.88±2.04^{ABa}	13.83±1.66^{A}	< 0.0001

Means with the different superscripts within the same row differ significantly ($P < 0.05$), means with the different capital superscripts within the same column differ significantly ($P < 0.01$).

Table 5 Rumen degradable protein balance of different diets on the on total VFA and proportions of individual VFA in continuous culture fermenters (μg/mL)

Item		Group 1 −16.87 g/kg DM	Group 2 −8.87 g/kg DM	Group 3 −0.87 g/kg DM	Group 4 + 7.13 g/kg DM	Group 5 + 15.13 g/kg DM	Group 6 + 23.12 g/kg DM	*P*
0 h	Acetic acid	30.59±2.33^{b}	35.15±4.03^{ab}	35.22±4.04^{ab}	39.61±3.54^{a}	35.20±5.05^{ab}	30.29±2.88^{b}	0.0178
	Propionic acid	10.87±1.14^{b}	13.17±1.35^{ab}	12.85±1.45^{ab}	15.42±1.42^{a}	13.65±2.03^{ab}	11.27±1.73^{b}	0.0041
	Butyric acid	4.84±0.89^{b}	6.52±0.83^{ab}	5.96±0.67^{ab}	7.63±2.08^{a}	6.28±1.33^{ab}	5.18±1.08^{ab}	0.0238
	Valeric	0.60±0.09^{b}	0.74±0.0^{5ab}	0.76±0.10^{ab}	0.94±0.14^{a}	0.78±0.18^{ab}	0.58±0.06^{b}	0.0016
	Total VFA	46.90±4.22^{b}	55.58±5.31^{ab}	54.79±6.16^{ab}	63.59±6.69^{a}	55.91±8.34^{ab}	47.32±5.67^{b}	0.0073
	Acetic/propionic	2.81	2.67	2.74	2.57	2.58	2.69	–
2 h	Acetic acid	32.04±3.84^{b}	32.71±0.61^{ab}	46.87±3.30^{a}	28.50±2.27^{b}	41.57±6.81^{a}	36.62±4.45^{ab}	0.0097
	Propionic acid	12.38±1.73^{b}	13.76±0.29^{ab}	18.11±1.57^{a}	11.82±1.54^{b}	16.49±2.24^{a}	16.60±1.66^{ab}	0.0139
	Butyric acid	5.35±1.40a	6.46±0.40^{a}	8.11±0.87^{a}	5.78±1.86^{a}	7.61±1.24^{a}	6.34±0.23^{a}	0.1828
	Valeric	0.74±0.19^{b}	0.86±0.05^{ab}	1.24±0^{a}	0.73±0.15^{b}	0.97±0.18^{ab}	0.80±0.16^{ab}	0.0398
	Total VFA	50.51±7.08^{b}	53.78±1.24^{ab}	74.33±5.74^{a}	46.82±9.89^{b}	66.64±9.89^{a}	57.36±6.03^{b}	0.0116
	Acetic/propionic	2.59	2.38	2.59	2.41	2.52	2.21	–
4 h	Acetic acid	51.19±0.67^{a}	44.14±6.52^{ab}	41.41±2.28^{ab}	39.45±4.49^{b}	44.09±4.46^{ab}	39.1±3.43^{b}	0.0158
	Propionic acid	18.04±2.25^{a}	17.5±1.28^{a}	15.38±1.36^{a}	15.97±1.80^{a}	16.83±1.14^{a}	15.74±0.24^{a}	0.2002
	Butyric acid	9.48±1.48^{a}	8.9±1.24^{a}	7.84±0.55^{a}	8.98±2.25^{a}	9.23±0.81^{a}	6.66±0.59^{a}	0.1525
	Valeric	1.31±0.07^{a}	1.25±0.0^{8ab}	1.15±0.03^{ab}	1.09±0.19^{ab}	1.23±0.15^{ab}	0.95±0.10^{b}	0.0275
	Total VFA	80.03±3.24^{a}	71.79±6.31^{ab}	65.78±3.09^{b}	65.49±7.65^{b}	71.38±5.66^{ab}	62.45±4.26^{b}	0.0132
	Acetic/propionic	2.84	2.52	2.69	2.47	2.62	2.48	–
6 h	Acetic acid	43.92±2.50^{a}	36.27±3.47^{a}	39.19±39.19^{a}	42.77±42.77^{a}	41.92±41.92^{a}	34.76±34.76^{a}	0.0954
	Propionic acid	15.68±1.69^{ab}	13.26±1.76^{b}	14.64±1.93^{ab}	17±0.92^{a}	16.34±0.77^{ab}	13.15±2.20^{b}	0.0105
	Butyric acid	8.48±1.80^{ab}	7.06±1.68^{ab}	7.67±1.17^{ab}	9.77±2.32^{a}	8.26±1.45^{ab}	6.25±0.84^{b}	0.0658
	Valeric	1.12±0.08^{ab}	0.92±0.13^{ab}	0.99±0.17^{ab}	1.09±0.12^{a}	1.06±0.14^{a}	0.77±0.16^{b}	0.0218
	Total VFA	69.19±6.07^{ab}	57.52±6.16^{ab}	62.5±8.44^{ab}	70.63±4.69^{a}	67.56±7.46^{ab}	54.93±8.98^{b}	0.0312
	Acetic/propionic	2.80	2.74	2.68	2.52	2.57	2.64	–
8 h	Acetic acid	30.05±3.66^{b}	39±3.87^{a}	35.17±3.37^{ab}	28.32±4.96^{b}	35.01±7.04^{ab}	37.08±2.54^{a}	0.0006
	Propionic acid	10.66±1.43^{b}	14.72±1.68^{a}	13.09±1.46^{ab}	11.37±2.58^{ab}	13.44±2.69^{ab}	13.91±1.12^{a}	0.0032
	Butyric acid	4.55±0.25^{b}	7.74±1.49^{a}	6.08±1.34^{ab}	6.39±2.36^{ab}	6.99±2.48^{ab}	6.27±1.14^{ab}	0.0361
	Valeric	0.57±0.07^{b}	0.89±0.11a	0.8±0.17^{ab}	0.71±0.18^{ab}	0.87±0.27^{a}	0.74±0.05^{ab}	0.009
	Total VFA	45.83±5.30^{b}	62.35±6.41^{a}	55.13±6.22^{ab}	46.79±9.73^{b}	56.31±12.28^{ab}	58.00±4.03^{ab}	0.0022
	Acetic/propionic	2.82	2.65	2.69	2.49	2.60	2.67	–
10 h	Acetic acid	27.68±2.44^{bc}	37.22±3.57^{abd}	35.28±3.28^{bd}	41.75±1.95^{a}	41.83±1.48^{a}	33.20±2.87^{bcd}	< 0.0001
	Propionic acid	9.78±1.00^{b}	14.39±1.27^{a}	12.72±1.79^{ab}	14.17±2.30^{a}	16.15±0.79^{a}	13.07±2.24^{ab}	0.0025
	Butyric acid	4.94±0.91^{b}	7.83±1.54^{a}	6.44±0.76^{ab}	7.68±2.42^{a}	8.06±0.46^{a}	5.76±0.39^{ab}	0.0070
	Valeric	0.64±0.07^{bd}	0.84±0.08^{abd}	0.85±0.07^{ad}	0.89±0.17^{ad}	1.01±0.05^{ac}	0.69±0.10^{bd}	0.0004
	Total VFA	43.03±4.15^{bd}	60.28±5.71^{a}	55.29±5.71^{bd}	64.50±6.24^{a}	67.06±1.29^{ac}	52.73±4.99^{b}	< 0.0001
	Acetic/propionic	2.83	2.59	2.77	2.95	2.59	2.54	–

Means with different superscripts within the same row differ significantly ($P < 0.05$), means with different capital superscripts within the same column differ significantly ($P < 0.01$).

(RDPB) ($P = 0.0073$ and 0.0116, respectively) at 0–2 h, and was the lowest in the diet containing RDPB over 23 g/kg DM. Total VFA concentration was decreased at 0–6 h. The dynamic value of volatile fatty acids in rumen fluid reached a peak after feeding for 2–4 h. Acetate-to-propionate ratio was reduced with decreasing RDPB. Acetate ($P < 0.05$), propionate ($P < 0.01$), butyrate ($P < 0.05$), valerate ($P < 0.01$) and total VFA ($P < 0.01$) improved quadratically in response to increasing RDPB at 0 h. At 2 h, butyrate was not affected ($P > 0.05$) by the treatments. Similarly, propionate and butyrate were not affected ($P > 0.05$) at 4 h. But after 6 h, each volatile fatty acid and total VFA was significantly affected by the treatments.

Discussion

The aim of the current research was to determine the effects of different RDPB diets on rumen metabolism and some variables such as total VFA concentrations, pH, and NH_3-N concentration in the effluent.

Effects of different rumen degradable protein balance diet on rumen pH

The pH of the rumen fluid is the most important factor affecting the production and speed of volatile fatty acids in the rumen. A decrease in pH in the rumen reduces appetite (Britton and Stock, 1987.), ruminal motility, fiber digestion, and microbial production. Reduced ruminal pH not only affects energy intake and microbial protein yield, but can also result in severe health problems, such as laminitis, ruminal ulceration, and liver abscesses. Ruminal pH for all treatments in the current experiment remained greater than the value for optimal rumen function (de Veth and Kolver, 2001), therefore, the slight difference in ruminal pH was detected in this study. In the trials dealing with different RDPBs which changed the proportion and quantity of VFA at different points, the pH values of the rumen fermentation in the six treatment groups, were significantly different.

Effects of different RDPB diet on rumen ammonia concentration

The concentration of NH_3-N in the rumen is an indicator of the rate of ruminal N degradation, the concentration of rumen-degraded N above microbial needs, and the amount of dietary energy available to the ruminal microorganisms. Increased consumption of NFC can decrease ruminal ammonia concentrations (Royes et al., 2001) for greater microbial protein synthesis (MPS) and concurrent higher demand for RDP. Rumen ammonia nitrogen comes mainly from feed material degradation and saliva (Klusmeyer et al., 1990). Ammonia is the main nitrogen source of microbial protein synthesis (Klusmeyer et al., 1990), rumen ammonia nitrogen concentration reflects the number of rumen microorganisms which can use nitrogen. Dietary crude protein content and rumen liquid ammonia nitrogen concentration were highly correlated (Klusmeyer et al., 1990). Reducing the RDP concentration or the concentration of dietary nitrogen can significantly reduce the ammonia concentration of rumen fluid. The ruminal NH_3-N concentration was highly influenced by the level of RDPB in the diet. The experimental results showed that with the increasing RDPB, the rumen ammonia concentration was significantly increased ($P < 0.01$), indicating that with the increase of the value in RDPB rumen fermentation in the rumen, the microbial N utilization can be increased gradually. When RDPB was from 0 down to -16, the concentration of ammonia nitrogen in the rumen fluid decreased from 12.29 to 8.09 mg/dL. When RDPB was positive, rumen ammonia concentration was greater.

In this study, NH_3-N concentrations were well above the minimum concentrations suggested for maximal microbial synthesis (5 mg/100 mL; Satter and Roffler, 1975). It is well known that high ruminal NH_3-N may slightly impair ruminal function because of the metabolic cost of synthesizing and additional urea excretion (Kolver and Muller, 1998). Most studies in dairy cattle reported lower NH_3-N concentrations in dairy diets, mainly because of the greater rate of TDN degradability of the diet and the greater incorporation of NH_3-N into microbial protein synthesis (McCarthy et al., 1989; Casper et al., 1990, 1999). Decreased dietary CP concentration or degradation of dietary N usually results in decreased NH_3-N concentrations in the rumen (Milton et al., 1997; Hristov et al., 2004; Ipharraguerre et al., 2005). Valkeners et al. (2008) found that increasing the OEB level from -23.7 to 5.3 g/kg DM in cattle diets caused an 80% increase in ruminal NH_3-N concentration. Their findings were in agreement with our results. With the positive RPDB diet, a large production of NH_3-N in the rumen occurred after the degradation of the urea included in the diet. It is very likely that large amounts of NH_3 was lost from the rumen fluid by absorption through the rumen epithelium and transferred by portal blood to the liver to produce urea.

Effects of different rumen degradable protein balance diet on rumen VFA

Volatile fatty acids (VFA) as the raw materials for the synthesis of milk fat and body fat are the main source of energy for ruminants and are affected by the impact of the diet. When the diet was of similar structure, the rumen contents of the various VFA and their proportions changed little. The changes in individual VFA are related to microbial protein supply, nitrogen losses, milk yield and composition. When RDP is adequate, TDN availability determines MPS in the rumen and the efficiency of ammonia use (Hristov et al., 2005). Synchronization of NSC and protein sources for rapid or slow fermentation tend to result in greater VFA production and flow of microbial N in a dual-flow continuous culture (Rotger et al., 2006). It is well documented that an excess of available TDN can enhance the concentration of lactic acid, which can result in a greater yield of microbial proteins (Ipharraguerre et al., 2002). In this experiment, the diet with the same structure as in each group had little effect on ruminal fermentation and proportion of total VFA concentration. However, this experiment revealed different ruminal degradation protein contents and composition of TDN, with significant effects at different time points on a variety of fatty acid compositions and proportions ($P < 0.05$). In this experiment, the main carbohydrate in Groups 2–6 was the same, so the increase in volatile fatty acids formed as RDPB was linear. Because of slightly different carbohydrates in the first group compared with the other groups, the trends of VFA were different. The difference in molar proportions of VFA might be expected because of the differences in CP degradation among treatments.

Conclusions

In the present study, increased RDPB had a slight effect on ruminal pH. Rumen ammonia concentration increased significantly with increasing RDPB. With the increasing RDPB, the total rumen VFA concentration had no significant changes. However, the proportion of volatile fatty acids at each time point was significantly different. The proportion of acetic acid and propionic acid decreased with the increase in RDPB. The total VFA concentration of six diets had a quadratic change with the increasing RDPB, with the highest value of total VFA obtained in diet 5.

Acknowledgements

This work was supported by the earmarked fund for Modern Agro-industry Technology Research System, China (No. CARS-37).

References

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

Bach A, Ruiz Moreno M, Thrune M, Stern M D (2008). Evaluation of the fermentation dynamics of soluble crude protein from three protein sources in continuous culture fermenters. J Anim Sci, 86(6): 1364–1371

Børsting C F, Kristensen T, Misciattelli L, Hvelplund T, Weisbjerg M R (2003). Reducing nitrogen surplus from dairy farms. Effects of feeding and management. Livest Prod Sci, 83(2–3): 165–178

Britton R A, Stock R A (1987). Acidosis, rate of starch digestion and intake. Feed Intake by Beef Cattle Symposium. Proc. MP 121: 125–137

Casper D P, Maiga H A, Brouk M J, Schingoethe D J (1999). Synchronization of carbohydrate and protein sources on fermentation and passage rates in dairy cows. J Dairy Sci, 82(8): 1779–1790

Casper D P, Schingoethe D J, Eisenbeisz W A (1990). Response of early lactation dairy cows fed diets varying in source of nonstructural carbohydrate and crude protein. J Dairy Sci, 73(4): 1039–1050

Chaney A L, Marbach E P (1962). Modified reagents for determination of urea and ammonia. Clin Chem, 8: 130–132

Colin-Schoellen O, Jurjanz S, Laurent F (2000). Metabolizable protein supply (PDIE) and restricted level of ruminally degradable nitrogen (PDIN) in total mixed rations: Effect on milk production and composition and on nitrogen utilization by dairy cows. Livest Prod Sci, 67(1–2): 41–53

de Veth M J, Kolver E S (2001). Digestion of ryegrass pasture in response to change in pH in continuous culture. J Dairy Sci, 84(6): 1449–1457

Hristov A N, Etter R P, Ropp J K, Grandeen K L (2004). Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. J Anim Sci, 82(11): 3219–3229

Hristov A N, Ropp J K, Grandeen K L, Abedi S, Etter R P, Melgar A, Foley A E (2005). Effect of carbohydrate source on ammonia utilization in lactating dairy cows. J Anim Sci, 83(2): 408–421

Ipharraguerre I R, Clark J H, Freeman D E (2005). Varying protein and starch in the diet of dairy cows. I. Effects on ruminal fermentation and intestinal supply of nutrients. J Dairy Sci, 88(7): 2537–2555

Ipharraguerre I R, Shabi Z, Clark J H, Freeman D E (2002). Ruminal fermentation and nutrient digestion by dairy cows fed varying amounts of soyhulls as a replacement for corn grain. J Dairy Sci, 85 (11): 2890–2904

Jouany J P (1982). Volatile fatty acids and alcohol determination in digestive contents, silage juice, bacterial cultures and anaerobic fermentor contents. Sci Aliments, 2: 131–144

Klusmeyer T H, McCarthy R D Jr, Clark J H, Nelson D R (1990). Effects of source and amount of protein on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J Dairy Sci, 73 (12): 3526–3537

Kolver E S, Muller L D (1998). Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. J Dairy Sci, 81(5): 1403–1411

Littell R C, Henry P R, Ammerman C B (1998). Statistical analysis of repeated measures data using the SAS procedures. J Anim Sci, 76:1216–1231

McCarthy R D Jr, Klusmeyer T H, Vicini J L, Clark J H, Nelson D R (1989). Effects of source of protein and carbohydrate on ruminal fermentation and passage of nutrients to the small intestine of lactating cows. J Dairy Sci, 72(8): 2002–2016

Milton C T, Brandt R T Jr, Titgemeyer E C (1997). Urea in dry-rolled corn diets: finishing steer performance, nutrient digestion, and microbial protein production. J Anim Sci, 75(5): 1415–1424

NRC (2001). Nutrient Requirements of Dairy Cattle: Seventh Revised Edition, Washington, DC: Natl Acad Press

Rotger A, Ferret A, Calsamiglia S, Manteca X (2006). Effects of nonstructural carbohydrates and protein sources on intake, apparent total tract digestibility, and ruminal metabolism in vivo and *in vitro* with high-concentrate beef cattle diets. J Anim Sci, 84(5): 1188–1196

Royes J B, Brown W F, Martin F G, Bates D B (2001). Source and level of energy supplementation for yearling cattle fed ammoniated hay. J Anim Sci, 79(5): 1313–1321

Satter LD, Klopfenstein T J, Erickson G E (2002). The role of nutrition in reducing nutrient output fromruminants. J Anim Sci, 80(E. Suppl 2): E143–E156.

Satter L D, Roffler R E (1975). Nitrogen requirement and utilization in dairy cattle. J Dairy Sci, 58(8): 1219–1237

Sukhija P S, Palmquist D L (1988). Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. Journal of Agricultural and Food Chemistry, 36(6): 1202–1206

Tamminga S, Van Straalen W N, Subnel A P J, Meijer R G M, Steg A, Wener C J G, Block M C (1994). The Dutch protein evaluation system: The DVE/OEB system. Livest Prod Sci, 40: 139–155

Valkeners D A, Théwis M, Van Laere, Beckers Y (2008). Effect of rumen-degradable protein balance deficit on voluntary intake, microbial protein synthesis, and nitrogen metabolism in growing double-muscled Belgian Blue bulls fed corn silage-based diet. J Anim Sci, 86: 680–690

Van Soest P J, Robertson J B, Lewis B A (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci, 74(10): 3583–3597

Weller R A, Pilgrim A F (1974). Passage of protozoa and volatile fatty acids from the rumen of the sheep and from a continuous in vitro fermentation system. Br J Nutr, 32 (02): 341–351

Yu P, Christensen D A, McKinnon J J (2003). Comparison of the NRC 2001 model with the Dutch System (DVE/OEB) in the prediction of nutrient supply to dairy cows from forages. J Dairy Sci, 86: 2178–2192