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The relationship between odd- and branched-chain fatty acids and microbial nucleic acid bases in rumen

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Objective: This study aims to identify the relationship between odd- and branched-chain fatty acids (OBCFAs) and microbial nucleic acid bases in the rumen, and to establish a model to accurately predict microbial protein flow by using OBCFA.
Methods: To develop the regression equations, data on the rumen contents of individual cows were obtained from 2 feeding experiments. In the first experiment, 3 rumen-fistulated dry dairy cows arranged in a 3×3 Latin square were fed diets of differing forage to concentration ratios (F:C). The second experiment consisted of 9 lactating Holstein dairy cows of similar body weights at the same stage of pregnancy. For each lactation stage, 3 cows with similar milk production were selected. The rumen contents were sampled at 4 time points of every two hours after morning feeding 6 h, and then to analyse the concentrations of OBCFA and microbial nucleic acid bases in the rumen samples.
Results: The ruminal bacteria nucleic acid bases were significantly influenced by feeding diets of differing forge to concentration ratios and lactation stages of dairy cows ($p<0.05$). The concentrations of OBCFAs, especially odd-chain fatty acids and C15:0 isomers, strongly correlated with the microbial nucleic acid bases in the rumen ($p<0.05$). The equations of ruminal microbial nucleic acid bases established by ruminal OBCFAs contents showed a good predictive capacity, as indicated by reasonably low standard errors and high R-squared values.
Conclusion: This finding suggests that the rumen OBCFA composition could be used as an internal marker of rumen microbial matter.

Keywords: Rumen Odd- and Branched-chain Fatty Acids; Microbial Nucleic Acid Bases; Regression Equations

INTRODUCTION

Many studies have examined the effects of microbial protein synthesis and microbial nucleic acid composition in the rumen on protein nutrition [1]. Specifically, nucleic acids or their constituent purine or pyrimidine bases act as internal microbial markers of microbial protein synthesis [2]. Broderick and Merchen [3] regarded purine bases as one of the best internal markers. However, the protein synthesis of rumen bacteria and protozoa differed between the solid and liquid phases in the rumen due to the nucleic acid content of the diet. Therefore, this method needed to clarify the composition of undetached microbes associated with rumen particles [4] and confirm variations in non-renal and endogenous purine losses. Therefore, the search for an alternative internal marker continues.

The microbial fatty acid composition, especially odd- and branched-chain fatty acids (OBCFAs), has been examined in studies of the rumen. These fatty acids are mainly present in bacterial membrane lipids as stable compounds [5] that are easy to measure. Conversely, only trace levels of OBCFAs are found in most plants [6]. Recently, OBCFAs have been used to study rumen fermentation [7,8], rumen bacteria [9,10] and the duodenal flow of microbial protein [10,11]. Specifically,

several studies have examined the relationship between the rumen and milk OBCFA concentrations and the liquid- (LAB) and solid-associated bacteria (SAB) in both the rumen and duodenum [11]. Vlaeminck et al [10] accurately correlated the C17:0 and total OBCFA contents with the rumen uracil and purine levels. However, these findings were limited to variations in dietary treatment. Vlaeminck et al [12] determined adenine, cytosine and odd and branched-chain fatty acids both in SAB and LAB and used them to estimate bacterial N flow in duodenal. The results suggested that, depending on the marker used, changes in the proportions of SAB and LAB can have a substantial impact on estimated duodenal flow of bacterial N. Vlaeminck et al [8] found that a large and independent dataset of OBCFAs could accuracy predict the molar proportions of volatile fatty acids in the rumen. Thus, larger datasets will likely result in more accurate prediction equations.

In conclusion, there is insufficient information about the changes of bacteria nucleic acid bases in rumen fluid of different diets and lactation stages from previous studies. The objectives of this research were to analysis the changes of bacteria nucleic acid bases in rumen and the relationship of OBCFA to bacteria nucleic acid bases. For these purposes, data from 2 feeding experiments were used with various dietary treatments and different lactation stages.

MATERIALS AND METHODS

Experimental design, diets and sampling

Animal and basal diets: Experiment 1. Three rumen-fistulated dry Holstein cows (600±24 kg body weight) were arranged in a 3×3 Latin square and offered three total mixed rations (TMRs) of differing forage to concentration ratios (F:C): 30:70, 50:50, and 70:30 on a dry matter (DM) basis. TMRs were formulated according to the dairy nutrient requirements of National Research Council [13]. The compositions and nutrition levels in the diets are shown in Table 1. The basal diets were offered in equal amounts twice daily at 06:00 and 18:00 h. Each experimental period lasted for 3 weeks, and the first 2 weeks consisted of adaptation.

Experiment 2. Nine lactating Holstein dairy cows of similar body weights (650±33 kg body weight) were examined at the same stage of pregnancy. For each lactation stage, 3 cows with similar milk production were selected. The milk yield in the early, middle and late stages were 35.44±2.63 kg/d, 37.62±2.85, and 26.98±2.79, respectively. The cows were fed according to routine dairy cattle practice and offered TMR diets at 06:00 and 18:00 h. The total mixed F:C ratio was 55:45 on a DM basis. The compositions and nutrition levels in the diets are shown in Table 2.

Chemical composition analysis: Firstly, all the TMR samples were air-dried at 60°C±5°C, and then analyzed for DM. The dietary crude protein (CP), calcium (Ca), and total phosphorus (TP) were estimated by the AOAC 1990 method. The acid detergent fibre (ADF) and neutral detergent fuber (NDF) contents were analyzed according to Van Soest et al [14] using the Ankom system

Table 1. Feed ingredients and chemical composition (g/kg DM) of the rations of experiment 1

Items	F:C		
	30:70	50:50	70:30
Ingredients			
Alfalfa hay	12	24	161
Chinese wildrye	156	272	255
Corn silage	132	204	284
Distillers dried grains with soluble (DDGS)	164	112	68
Wheat bran	60	50	28
Corn grain	405	229	105
Soybean meal	51	92	89
$CaHPO_4$	1.2	2.2	5.2
Limestone	14	10	0
NaCl	1.6	1.6	1.6
Premix[1)]	3.2	3.2	3.2
Chemical composition			
NEL[2)] (MJ/kg)	7.27	7.19	7.10
CP	152	153	147
NDF	436	530	564
ADF	181	266	327
Ca	6.3	6.2	6.2
TP	3.7	3.7	3.6

DM, dry matter; F:C, forage to concentration ratios; NEL, net energy for lactation; CP, crude protein; NDF, neutral detergent fuber; ADF, acid detergent fibre; TP, total phosphorus.
[1)] Provided per kilogram of premix: Cu 4 560 mg, Mn 4,590 mg, Zn 12,100 mg, I 270 mg, Co 60 mg, Vit A 2,000,000 IU, Vit D_3 450,000 IU, Vit E 10,000 IU, Vit E 3,000 mg.
[2)] NEL is calculated value [13] and the other nutrient levels are measured values.

Table 2. Feed ingredients and chemical composition (g/kg DM) of the rations of experiment 2

Items	Content
Ingredients	
Alfalfa hay	73
Chinese wildrye	43
Corn silage	334
Corn	220
Soybean meal	41
Distillers dried grains with soluble (DDGS)	213
Cottonseed meal	58
Molasses	5
NaCl	3
$CaHPO_4$	2
Limestone	5
Premix[1)]	3
Nutrient levels	
NEL/(MJ/kg)[2)]	8.71
CP	162
NDF	312
ADF	183
Ca	8.7
TP	4.6

DM, dry matter; NEL, net energy for lactation; CP, crude protein; NDF, neutral detergent fuber; ADF, acid detergent fibre; TP, total phosphorus.
[1)] The premix provided the following per kg of diets: Vit A 330,000 IU, Vit D 60,000 IU, Vit E 1,000 IU, Zn 2,100 mg, Mn 1,500 mg, Cu 535 mg, Se 12 mg, I 45 mg.
[2)] NEL was estimated value NRC [13].

(Ankom 220 Fiber Analyzer; Ankom, New York, USA) with a heat-stable α-amylase and expressed exclusive of residual ash. Net energy for lactation (NEL) at a production level was calculated using a NRC summative approach from the dairy nutrient requirement [13].

Sampling and analyses

The rumen contents were evacuated via the rumen fistula (in experiment 1) or gastric canal (in experiment 2) at 6, 8, 10, and 12 h after morning feeding (represented by 6 h, 8 h, 10 h, and 12 h). The rumen contents were passed through two layers of cheesecloth to remove particulate matter and then freeze-dried before analysis. Although the feeding particles were not isolated from the rumen contents before sample analysis, contribution of nonmicrobial nucleic acids was negligible 4 h after feeding [15].

The rumen OBCFAs in freeze-dried samples were described by Zhang et al [16]. The rumen nucleic acid bases were extracted from freeze-dried samples using perchloric acid, as described by Zinn and Owens [17]. In detail, the lyophilized rumen fluid samples (50 mg) were placed in screw-cap tubes and added 2.5 mL 0.6 M $HClO_4$, and then incubated for 1 h. After cooling, the pH was adjusted to between 6.6 and 6.9 with KOH (8 M), and combined with 10 mM $NH_4H_2PO_4$ to 10 mL. After centrifugation of samples at 500 g for 10 min, the supernatant was filtered through a 0.45 μm filter, and then analyzed by high performance liquid chromatography (HPLC) using a C18 column (5 μ, 250× 4.6 mm, Diamonsil, Beijing, China). The buffer solution (20 mM $NH_4H_2PO_4$) was run isocratically at 1 mL/min, and the effluent was monitored at 254 nm. The concentrations of individual nucleic acid bases were determined from standard curve equations. The standards of individual nucleic acid bases (≥99.5%, Aladdin, Shanghai, China) were formulated to a concentration of 50 mg/L. The mixed solution and the standards nucleic acid bases solutions with same volume were serially diluted into 5 gradients and subsequently analyzed by HPLC which was same as the analysis method of rumen samples. The standard curve equations were calculated from data of analyzed by HPLC and their correspondent concentrations.

Statistical analyses

All statistical analyses were performed using SAS 9.2.

Univariate analysis. The rumen base data were analyzed using the MIXED procedure of SAS in a model that included the cow, diet treatment or lactation stage, experimental period, and random error in the experiment according to the following:

$$Y_{ijkl} = \mu + T_i + C_k + S_l + TS_{ij} + CS_{kl} + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the individual observation, μ is the overall mean, T_i is the effect of dietary treatment or lactation stage, C_k is the effect of the cow, S_l is the effect of the sample time, TS_{ij} is the interaction between treatment and sampling time, CS_{kl} is the interaction between cow and sampling time, and ε_{ijkl} is the residual error. Duncan's multiple range tests were performed to evaluate differences between dietary treatments or lactation stages.

Linear regression analyses. A multiple regression was applied using the STEPWISE method of REG procedure of SAS to develop equations to predict microbial nucleic acid bases in the rumen.

The regression equations were evaluated based on the root mean square error (RMSE) and coefficients of variation (CV). The accuracy of the prediction was also evaluated using the PRESS statistic, which is calculated as follows:

$$\text{PRESS} = \sum_{i=1}^{n} (y_i - \hat{y}_{i,-i})$$

Where y_i is the ith observation for the dependent variable, $\hat{y}_{i,-i}$ is the prediction of observation i using a model estimated without the ith observation, and n is the number of observation in the dataset used for parameter estimation.

Independent datasets to evaluate accuracy of prediction. The predicted equations were further evaluated used the concordance correlation coefficient (CCC), which is computed as follows:

$$\rho_c = \rho \times C_b$$

Where ρ_c is the concordance correlation coefficient, ρ is the Pearson correlation coefficient, and C_b the bias correction factor, which is calculated as follows:

$$C_b = \frac{2\sigma_0\sigma_p}{\sigma_0^2 + \sigma_p^2 + (\mu_0 - \mu_p)^2}$$

Where σ_0 and σ_p are the standard deviations of the observed and predicted values, respectively, and μ_0 and μ_p are the observed and predicted means.

RESULTS

Data file description

The experimental data used for model development occurred over a wide range and the data of OBCFA contents were more variable than the nucleic acid bases value in rumen (Table 3). In regard to ruminal nucleic acid bases, the variation coefficient of uracil bases was highest but that of purine was lowest. The concentrations of C15:0 and its isomers were highest, followed by C17:0 and its isomers.

The changes in the base concentrations in the rumen in response to differences in the dietary F:C ratios and lactation stages.

The interaction of dietary F:C ratios and time of sampling significantly influenced the nucleic acid bases concentrations with

Table 3. Summary statistics of experimental data (n = 72)

	Mean	SD	Minimum	Maximum	CV
Ruminal bases value (g/kg DM)					
Cytosine	1.11	0.16	0.84	1.48	0.14
Uracil	0.86	0.23	0.34	1.31	0.27
Guanine	2.26	0.29	1.53	2.82	0.13
Adenine	1.84	0.19	1.43	2.17	0.10
Total bases	6.07	0.68	4.80	7.40	0.11
Pyrimidine	1.97	0.36	1.25	2.69	0.18
Purine	4.10	0.38	3.27	4.95	0.09
Rumen odd and branched-chain fatty acids (g/kg DM)					
C11:0	0.02	0.01	0.004	0.06	0.45
C13:0	0.04	0.02	0.01	0.11	0.54
Iso-C15:0	0.18	0.08	0.05	0.41	0.46
Anteiso-C15:0	0.33	0.15	0.07	0.93	0.47
C15:0	0.85	0.44	0.10	2.45	0.52
Iso-C16:0	0.18	0.08	0.02	0.40	0.45
Iso-C17:0	0.18	0.06	0.03	0.31	0.35
Anteiso-C17:0	0.05	0.02	0.01	0.15	0.48
C17:0	0.16	0.07	0.03	0.35	0.42
TOBCFA	1.97	0.66	0.33	3.61	0.34

SD, standard deviation; CV, coefficients of variation; DM, dry matter; TOBCFA, total odd- and branched-chain fatty acids.

the exception of guanine bases (Table 4). But the concentrations of guanine were influenced by different diets and time of sampling. Furthermore, the ruminal nucleic acid bases concentrations with the exception of adenine positively related to the dietary F:C ratios. The cytosine and total nucleic acid bases concentrations were not influenced by the time of sampling in rumen. The nucleic acid bases concentrations were minimized at after feeding 8 h for a 50:50 F:C ratio and maximized at after feeding 6 h or 12 h for a 50:50 F:C ratio. However, there was not a clear rhythm for the other two dietary F:C ratios.

The variations of nucleic acid bases in rumen with lactation stages and time of sampling are shown in Table 5. The concentrations of cytosine, total nucleic acid bases and pyrimidine bases were significantly affected by the interaction of lactation stages and time of sampling. The uracil and pyrimidine contents were significantly increased with lactation stages. The bases concentrations with exclusion guanine and purine were changed by time of sampling.

Relationship of nucleic acid bases and OBCFA concentrations in rumen content

The regression models of ruminal nucleic acid bases based on rumen odd and branched-chain fatty acids are shown in Table 6. Ruminal C13:0 was positively related to ruminal cytosine, whereas a negative relation was observed with C15:0 concentrations in rumen. The concentrations of uracil were positively correlated with *iso*-C15:0 and C17:0, but also negatively associated with C15:0 concentrations. There was a positive relation between *anteiso*-C15:0 and guanine in rumen. Meanwhile, a positive relationship was found between adenine and *iso*-C17:0 in rumen. As for total nucleic acid bases, it was positively related to C13:0 and *anteiso*-C15:0 but negatively correlated with C15:0.

Table 4. The changes in the base concentrations in rumen content of dairy cows fed different F:C dietary (g/kg DM)

Items	F:C ratios												SEM	p value		
	30:70				50:50				70:30							
	6 h	8 h	10 h	12 h	6 h	8 h	10 h	12 h	6 h	8 h	10 h	12 h		Diet	Time	D×T[1)]
Cytosine	1.06	1.06	0.99	1.00	1.08	0.89	0.98	1.02	0.93	1.00	0.96	1.05	0.04	0.27	0.31	0.04
Uracil	1.05	0.85	0.77	0.67	0.85	0.51	0.64	0.72	0.70	0.74	0.47	0.75	0.06	0.001	0.001	0.005
Guanine	2.28	2.33	2.22	2.44	2.10	2.09	2.28	2.45	1.81	1.99	1.69	2.12	0.09	<0.0001	0.004	0.21
Adenine	1.83	2.05	2.08	1.72	2.00	1.45	1.69	1.76	1.96	1.76	1.86	1.67	0.05	0.0001	0.0001	<0.0001
Total bases	6.21	6.28	6.06	5.82	6.02	4.95	5.59	5.95	5.41	5.49	4.99	5.60	0.17	<0.0001	0.06	0.005
Pyrimidine	2.10	1.91	1.77	1.67	1.92	1.40	1.62	1.74	1.64	1.73	1.43	1.81	0.08	0.004	0.004	0.004
Purine	4.11	4.38	4.29	4.16	4.10	3.54	3.97	4.21	3.77	3.75	3.55	3.80	0.11	<0.0001	0.31	0.01

F:C, forage to concentration ratios; DM, dry matter; SEM, standard error of the mean.
[1)] D × T, the interaction of dietary F:C ratios and time of sampling.

Table 5. The changes in the base concentrations in rumen content of dairy cows at different lactation stages (g/kg DM)

Items	Lactation stages												SEM	p value		
	Early				Middle				Late							
	6 h	8 h	10 h	12 h	6 h	8 h	10 h	12 h	6 h	8 h	10 h	12 h		Stage	Time	S×T[1)]
Cytosine	1.22	1.28	1.22	0.97	1.11	1.32	1.26	1.09	1.27	1.23	1.25	1.29	0.07	0.11	0.02	0.048
Uracil	1.08	1.03	0.91	0.66	0.80	1.14	1.12	0.93	1.06	1.12	1.14	1.01	0.08	0.04	0.01	0.055
Guanine	2.33	2.47	2.36	2.01	2.04	2.48	2.67	2.34	2.50	2.31	2.50	2.40	0.16	0.49	0.22	0.25
Adenine	1.90	1.92	1.99	1.67	1.56	1.92	1.96	1.83	1.80	1.96	1.95	1.91	0.09	0.43	0.04	0.24
Total bases	6.52	6.70	6.49	5.30	5.51	6.87	7.01	6.19	6.62	6.62	6.83	6.62	0.29	0.14	0.01	0.04
Pyrimidine	2.30	2.31	2.13	1.62	1.91	2.46	2.38	2.02	2.32	2.35	2.39	2.30	0.12	0.03	0.01	0.03
Purine	4.22	4.39	4.35	3.68	3.60	4.40	4.63	4.17	4.30	4.27	4.44	4.32	0.23	0.54	0.07	0.19

DM, dry matter; SEM, Standard error of the mean.
[1)] S × T, the interaction of lactation stage and time of sampling.

The concentrations of C13:0, *iso*-C15:0, and C17:0 were significantly and positively linked with pyrimidine contents in rumen. However, there was a negative correlation that was existed between pyrimidine and C15:0 in rumen. As well as, the concentrations of purine in rumen were positively related C13:0 and ruminal *anteiso*-C15:0 contents.

The concentrations of OBCFAs, especially C15:0 and its isomers, significantly correlated with the bacterial nucleic acid bases in the rumen (Table 6). The predicted models reflected the relationship between rumen nucleic acid bases and the OBCFA concentrations in rumen digest. The nucleic acid bases concentrations were negatively correlated with C15:0, while positively related to C15:0 isomers and other odd-chain fatty acids. All models showed a good predictive capacity, as indicated by reasonably low standard errors and variation coefficients and high R-squared values. Residual plots for the model are shown in Figure 1. The plots trended random distribution at two sides of x-axis and concentrate in the scope 2 to –2.

DISCUSSION

C15:0 and its isomers accounted for most of the OBCFAs in this research, which is similar to the results obtained with Vlaeminck et al [9]. Among dietary components, the starch and fiber proportions play a critical role in the rumen OBCFA content [7,18] by influencing the composition and development of the microbial

Table 6. Equations to predict rumen proportions of bases (g/kg DM) from rumen odd and branched-chain fatty acids (g/kg DM) (n = 72)

	Variables	Parameter estimate	SE	p value	RMSE	R2	CV	PRESS	CCC
Cytosine	Intercept	1.00	0.03	<0.0001	0.10	0.57	9.46	0.82	0.73
	C13:0	5.71	0.63	<0.0001					
	C15:0	–0.12	0.03	<0.0001					
Uracil	Intercept	0.66	0.06	<0.0001	0.16	0.51	18.89	2.02	0.68
	Iso-C15:0	0.98	0.31	0.002					
	C15:0	–0.26	0.05	<0.0001					
	C17:0	1.63	0.37	<0.0001					
Guanine	Intercept	1.91	0.07	<0.0001	0.24	0.32	10.64	4.25	0.57
	Anteiso-C15:0	1.07	0.19	<0.0001					
Adenine	Intercept	1.60	0.06	<0.0001	0.17	0.20	9.25	2.14	0.45
	Iso-C17:0	1.39	0.33	<0.0001					
Total bases	Intercept	5.29	0.17	<0.0001	0.47	0.54	7.77	16.80	0.74
	C13:0	18.59	3.91	<0.0001					
	Anteiso-C15:0	1.08	0.51	0.04					
	C15:0	–0.31	0.13	0.02					
Pyrimidine	Intercept	1.63	0.08	<0.0001	0.23	0.61	11.87	4.22	0.78
	C13:0	6.98	2.05	0.001					
	Iso-C15:0	1.07	0.47	0.03					
	C15:0	–0.39	0.07	<0.0001					
	C17:0	1.42	0.62	0.03					
Purine	Intercept	3.56	0.08	<0.0001	0.29	0.43	7.19	6.44	0.60
	C13:0	7.47	2.38	0.003					
	Anteiso-C15:0	0.80	0.31	0.01					

DM, dry matter; SE, standard error; RMSE, root mean square erro; CV, variable coefficient; CCC, concordance correlation coefficient.

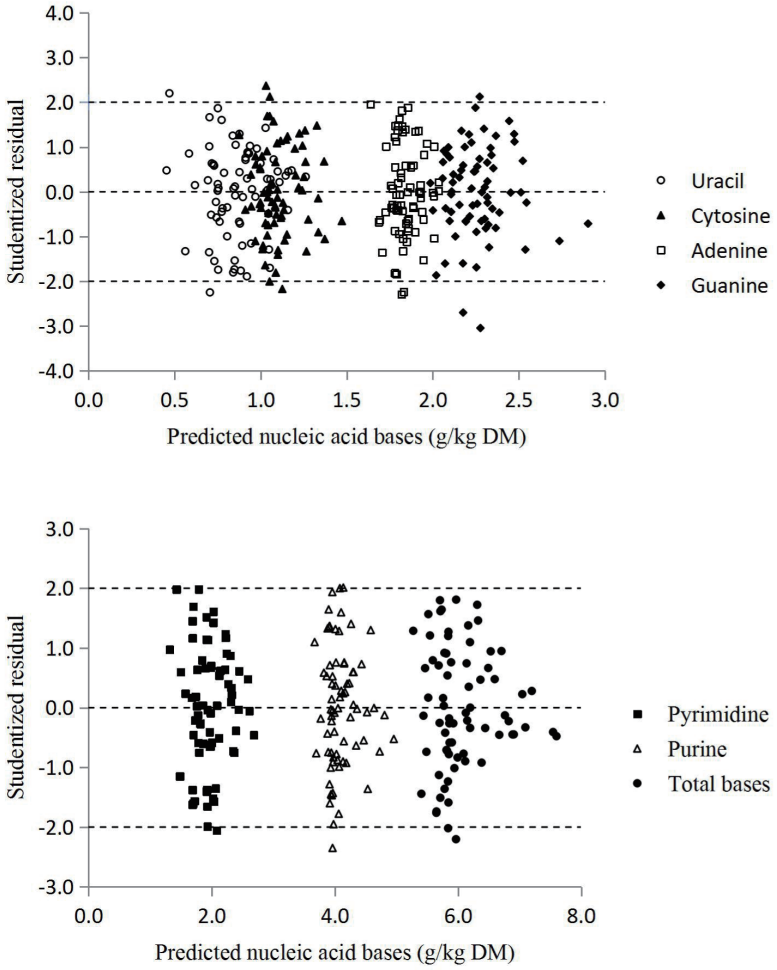

Figure 1. Plot of studentized residual value vs predicted nucleic acid bases value. DM, dry matter.

populations and, in particular, the number of cellulose-fermenting bacterial strains [9]. Because purine bases and cytosine are found in both RNA and DNA, the concentrations of purine and cytosine are higher than that of uracil [17]. A wide range purine bases and uracil data have been previously reported [19,20]. The total amounts of RNA synthesized in the rumen depend on the amounts of bacterial growth, which is determined by a number of factors [21]. Condon and Hatfield [22] found that the ruminant uses absorbed nucleic acids precursors for nucleic acid synthesis rather than synthesizing them *de novo*. Analysis of the OBCFA profile of cultivable rumen bacteria found that cellulolytic bacteria contain high levels of odd-chain *iso*-fatty acids and amylolytic bacteria are particularly enriched in linear odd-chain fatty acids [23]. The total nitrogen and RNA-N contents were higher in gram-negative bacteria than in gram-negative. Protozoa are unable to synthesize purines or pyrimidines, but can incorporate free adenine, guanine and uracil into their nucleic acids. In a word, the concentrations of OBCFA and nucleic acid bases both influenced by rumen bacterial growth.

About 30% to 50% of microbial N is bound in cell wall structure and nucleic acids [24]. Therefore, the factors which effect the rumen microbial growth should change the nucleic acid base concentrations in rumen fluid. Physiological and nutritional components all influence growth of rumen microbes. The rumen microbes clearly responded to the fermentation characteristics of the rumen, and increasing the F:C ratios increases the number of fibrolytic bacteria that can attach to forage particles [25]. Forage concentrations in the diet influence the concentration of nucleic acid bases in the rumen because the diet affects the rumen microbiome. Merry and Mcallan [26] reported that the purine contents were higher in amylolytic bacteria than in cellulolytic bacteria, which was associated with differences in bacteria species and changes in the metabolic activity of bacteria. In this research, the concentrations of adenine were increased with increased dietary F:C ratios. The metabolic processes of individual nucleic acid bases in the rumen are unique and allow them to be converted to other nucleic acid bases, which results in different nucleic acid bases concentrations. For adenine, this phenomenon is observed at the nucleoside stage and results in the final liberation of hypoxanthine. Guanine is first liberated and then delaminated to

xanthine. Cytosine is converted to uracil, most probably at the nucleotide or nucleoside stage [15]. During the first couple of weeks after parturition, a negative energy balance is common in dairy cows [27]. This situation could cause a deficiency of major nutrients supporting microbial growth. This may be one of primary reasons causing the contents of ruminal nucleic acid bases to be at their lowest at early lactation in this study.

The diurnal rhythms in the number of feed bunk visits, duration of each visit, nutrient intake per visit, and eating rate will lead to circadian rhythms of nutrient intake [28]. The concentrations of rumen nucleic acid bases were a function of the diurnal rhythms in this work because the digestion of nutrients in the rumen changed the microbial rhythms. This study showed that the timing of eating alters N metabolism, whereas the microbial protein synthesis in lactating dairy cows was maintained under thermoneutral conditions. Nikkhah et al [29] suggested that lactating cows can consume up to 30% to 70% of their daily intake within only 3 h of feed delivery. The contents of individual nucleic acid bases increased at 2 h after feeding time and then decreased at 4 to 6 h after feeding time. Some ruminal bacteria uncoupled their metabolism to adapt to changing ruminal conditions, allowing for accelerated bacterial reproduction and substrate production. After food entered the rumen, the bacterial populations quickly proliferated and then traveled to the duodenum following digestion, which reduced bacteria in the rumen contents.

The regression equations showed that C15:0 and its isomers clearly correlated with the pyrimidine and total nucleic acid bases contents in the rumen. Viviani et al [30] reported that restraining the growth of rumen microbes can affect the biosynthesis of branched C15:0 and C15:0 in the rumen ecosystem. The *iso*-acids (*iso*-C14:0, *iso*-C16:0) and odd iso-acids (iso-C15:0, iso-C17:0) should be distinguished using different processes based on the use of unique branched-amino acids as primers [23]. Increasing the dietary F:C ratios increased the *iso*-C14:0 [31] and *iso*-C15:0 levels to similar degrees [11]. The ratio of *iso*- to *anteiso*-odd branched-chain fatty acids is related to the growth of cellulolytic bacteria [8]. This finding suggested that branched C15:0 and C15:0 may relate to the well-known change in the rumen microbiome. Vlaeminck et al [10] found that purine and uracil bases positively correlated with the level of C17:0 in the rumen. The metabolism of microbial nitrogen in ruminants involves the degradation of dietary nitrogen and synthesis of microbial protein. All previous studies showed that the concentrations of branched-fatty acids and C17:0 related to the rumen microbial protein contents. In this study, the predicted model showed a strong relationship between individual nucleic acid bases and the OBCFA concentrations in the rumen, which corroborates previous findings.

The higher R^2 value indicates a more accurate equation. Based on this theory, the prediction equations of cytosine, uracil and pyrimidine bases were more valid than others in this work. The results suggested that the ruminal OBCFAs had the potential to predict the bacterial pyrimidine bases. The CCC reflects the degree to which individual predictions adhere to the concordance line. The lower CCC and higher RMSE values are mainly due to a lower accuracy of equations, which can be used to compare the validation of different prediction models. However, there was not sufficent data obtained from previous research. In order to improve the availability of prediction equation, further research is needed to collect more data.

CONCLUSION

The bacteria nucleic acid bases were significantly influenced by feeding diets of differing forge to concentration ratios and by the lactation stage of cows. The changes of bacteria nucleic acid bases and OBCFAs were linked with the bacteria growth during ruminal fermentation. The OBCFAs strongly correlated with the base contents in the rumen. Odd-chain fatty acids and C15:0 isomers showed potential to predict microbial protein in rumen. Furthermore, measurements of the ruminal OBCFAs could be developed as a new internal marker of microbial protein in the rumen.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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