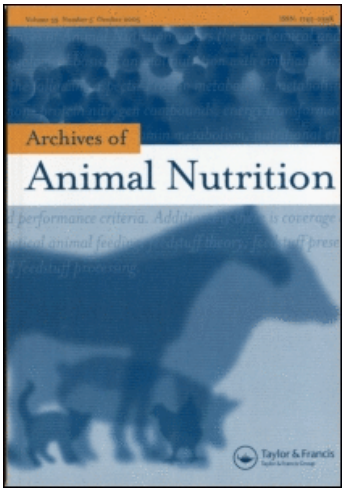

Archives of Animal Nutrition
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713453455

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First published on: 08 April 2011

To cite this Article Zeng, Bo , Tan, Zhiliang , Tang, Shaoxun , Han, Xuefeng , Tan, Chuanyan , Zhong, Rongzhen , He, Zhixiong and Arigbede, Oluwasanmi Moses(2011) 'Effects of alkyl polyglycoside, a nonionic surfactant, and forage-to-concentrate ratio on rumen fermentation, amino acid composition of rumen content, bacteria and plasma in goats', Archives of Animal Nutrition, 65: 3, 229 — 241, First published on: 08 April 2011 (iFirst)
To link to this Article: DOI: 10.1080/1745039X.2011.559729
URL: http://dx.doi.org/10.1080/1745039X.2011.559729

Archives of Animal Nutrition
Vol. 65, No. 3, June 2011, 229–241

Taylor & Francis
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Effects of alkyl polyglycoside, a nonionic surfactant, and forage-to-concentrate ratio on rumen fermentation, amino acid composition of rumen content, bacteria and plasma in goats

Bo Zeng[a,b], Zhiliang Tan[a]*, Shaoxun Tang[a], Xuefeng Han[a], Chuanyan Tan[a,b], Rongzhen Zhong[a,b], Zhixiong He[a,b] and Oluwasanmi Moses Arigbede[c]

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(*Received 27 July 2010; accepted 18 January 2011*)

In the present study, the effects of different forage-to-concentrate ratios (F:C) and an alkyl polyglycoside (APG) supplementation on parameters of rumen and blood metabolism were investigated in goats. A 2 × 2 factorial experiment was arranged within a 4 × 4 Latin square design (four 22-day periods), using four wether goats equipped with permanent ruminal cannulas. The experimental diets included two F:C levels (40:60 vs. 60:40), and two APG supplementation levels (None or 13 ml APG daily per animal). Rumen contents and blood samples were collected at the end of each period. Dietary F:C alteration affected plasma urea and influenced the proportions of leucine, histidine, arginine, glycine, proline, alanine, valine, phenylalanine, cysteine and tyrosine in rumen content, and the proportions of methionine, threonine and proline in solid-associated bacteria (SAB) significantly. Dietary APG decreased the proportions of valine and phenylalanine in rumen content, and the histidine content of liquid-associated bacteria. The interaction between dietary F:C and APG was significant for the proportions of glycine and alanine in rumen content, and the proportions of lysine and threonine in SAB. The proportion of lysine was greater, but the proportion of threonine was less in SAB for goats fed high F:C diet without APG supplementation. The proportions of plasma free amino acids and glucose concentration were not affected by experimental treatments. These results indicated that dietary APG addition affected the amino acid composition of the rumen content and ruminal bacteria, but this depended on the dietary F:C ratio. It is necessary to validate the effectiveness of dietary APG supplementation in further studies with more animals.

Keywords: concentrates; bacteria; forage; goats; nonionic surfactants; rumen fermentation

1. Introduction

The ruminal microbial cell matter, especially amino acids and fatty acids, is an important nutrient source for ruminants. Ørskov (1982) reported that bacterial protein synthesised in the rumen could meet 50% or more of the amino acid requirement of ruminants under various production states. Ruminal bacteria can

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ISSN 1745-039X print/ISSN 1477-2817 online

DOI: 10.1080/1745039X.2011.559729
http://www.informaworld.com

catabolise dietary protein and form all of their amino acids *de novo* or by incorporation, with different turnover rates for individual amino acids (Atasoglu et al. 2001, 2004). Numerous factors alter the amino acid composition of bacteria by affecting the microbial population (Wallace 1994), such as forage particle size and forage to concentrate ratio (F:C) (Hussein et al. 1995; Yang et al. 2001; Zebeli et al. 2008). Hussein et al. (1995) reported that the concentration of total, essential, non-essential and individual amino acid were higher in mixed ruminal bacteria from steers fed low-forage diets than from steers fed high-forage diets. Yang et al. (2001) indicated that high-F:C diets increased the compositions of cysteine and tyrosine, but reduced the compositions of methionine and valine in the ruminal bacteria of dairy cows when compared with a low-F:C diet. Zebeli et al. (2008) reported that in cows fed a low-forage diet, the proportions of phenylalanine and threonine in the ruminal bacteria tended to be greater, whereas the proportions of glutamine and lysine tended to be less. However, in the reported studies the effects of dietary factors on the amino acid composition of ruminal bacteria were not always consistent.

Alkyl polyglycosides (APG), based on glycoside and fatty alcohol, are a new category of mild nonionic surfactants. APG are characterised in terms of their emulsifying and dispersing physicochemical properties (Nickel et al. 1992, 1996). Therefore, APG are used for cosmetic lotions and creams, detergent and cleaning agents, and other medical applications (Weuthen et al. 1995; Nickel et al. 1996). However, only few studies reported the effectiveness of APG application as a ruminant feed additive. Cong et al. (2009) reported that dietary APG supplementation might increase *in vitro* dry matter (DM) and organic matter (OM) disappearances of low quality roughages. In a study by Yuan et al. (2009) the concentrations of ruminal ammonia N (NH_3-N), total volatile fatty acids and the ratio of acetate to propionate, were increased in goats fed diets containing APG. In addition, a previous study by our research group (Yuan et al. 2010) demonstrated that direct dietary supplementation of APG could increase the total tract digestibility of OM, neutral detergent fibre (NDF), the duodenal microbial N flow and the efficiency of microbial protein synthesis. However, there was no available data to illustrate the effect of APG, or even other nonionic surfactants, on the ruminal bacterial amino acid composition.

Therefore, in the current paper, a preferable concentration of dietary APG was designed, which was based on results of Cong et al. (2009) and Yuan et al. (2009), to study the effects of APG supplementation and F:C alteration on rumen fermentation and the amino acid composition of the rumen content, rumen bacteria and plasma in goats.

2. Materials and methods

This experiment was approved by the Animal Care Committee, Institute of Subtropical Agriculture (ISA), the Chinese Academy of Sciences, Changsha, China.

2.1. Experimental design, diets and sampling

Four Liuyang black wether goats (a local breed in southern China) with permanent rumen cannulas, initial body weights of 19.2 $\pm$ 1.1 (SD) kg and ages of 18 months, were assigned in a 4 $\times$ 4 Latin square design with four 22-day experimental periods.

The first 18 days were used for adaptation to their diets and the last 4 days for sample collection in each period. Treatments were arranged as a 2 × 2 factorial experiment. The main effects were two diets with different F:C (40:60 vs. 60:40, on air dry basis; Diet LF (low forage) and Diet HF (high forage) respectively) and two APG supplementation levels (None or daily 13 ml APG per animal). This factorial arrangement of the treatments was used to study any possible interactions between APG supplementation and dietary F:C. Corn stover (91% DM and 85.0% OM, 72.3% NDF, 47.5% acid detergent fibre (ADF), 6.8% crude protein (CP) and 1.3% ether extract on DM basis) was supplied as forage. In order to ensure similar levels of protein, finely ground corn, soya bean meal and wheat bran were used in different proportions to formulate the Diets LF and HF. The ingredients and chemical composition of the experimental diets are presented in Tables 1 and 2.

Table 1. Ingredients and chemical composition of the experimental diets.

Ingredient [g/kg air dry basis]	Diet		Composition[◇] [g/kg DM]	Diet	
	LF*	HF[†]		LF	HF
Corn stover	400	600	Dry matter	886 ± 0.9	895 ± 2.3
Ground corn	450	300	Organic matter	894 ± 2.3	869 ± 2.2
Soya bean meal	25	56	Crude protein	99 ± 7.5	100 ± 4.2
Wheat bran	81	0.6	Neutral detergent fibre	437 ± 16	525 ± 10
Urea	5.0	5.0	Acid detergent fibre	231 ± 3.6	315 ± 8.3
$CaCO_3$	4.7	0.5	Ether extract	18 ± 0.4	17 ± 0.4
CaH_2PO_4	8.6	12.5	TAA[§]	89 ± 4.7	85 ± 6.4
NaCl	5.1	5.1	ME [MJ/kg DM][‡]	7.22	6.21
Premix[#]	20	20			

Notes: *LF, Low forage diet; [†]HF, High forage diet; [◇]Values are means of four samples; [§]TAA, Total analysed amino acids; [#]Contained per kg premix: 571.4 g $NaHCO_3$, 2 g $FeSO_4 \cdot H_2O$, 1 g $CuSO_4 \cdot 5H_2O$, 0.01 g $CoCl_2 \cdot 6H_2O$, 0.1 g KIO_3, 7.5 g $MnSO_4 \cdot H_2O$, 4 g $ZnSO_4 \cdot H_2O$, 0.0025 g $NaSeO_3$, 371.7 g carrier, 250 mg vitamin E, 25000 IU vitamin A, and 50000 IU vitamin D; [‡]Calculated according to Zhang and Zhang (1998), other nutrient compositions were measured.

Table 2. Amino acid composition of the experimental diets [% of total analysed amino acids].

Essential amino acids (EAA)	Diet		Non-essential amino acids (NEAA)	Diet	
	LF*	HF[†]		LF	HF
Sum of EAA	38.3 ± 0.04	39.9 ± 0.07	Sum of NEAA	61.7 ± 0.04	60.1 ± 0.07
Met	1.09 ± 0.09	0.88 ± 0.17	Asp	8.18 ± 0.12	9.42 ± 0.14
Lys	4.11 ± 0.08	4.83 ± 0.14	Ser	4.74 ± 0.09	4.88 ± 0.01
Val	5.37 ± 0.16	5.74 ± 0.10	Glu	17.2 ± 0.22	16.3 ± 0.18
Ile	3.24 ± 0.04	3.51 ± 0.03	Gly	4.44 ± 0.09	4.64 ± 0.01
Leu	8.91 ± 0.03	8.66 ± 0.24	Ala	6.14 ± 0.15	6.14 ± 0.19
Phe	4.91 ± 0.04	5.19 ± 0.28	Cys	3.79 ± 0.84	3.07 ± 0.02
Thr	4.14 ± 0.08	4.46 ± 0.01	Tyr	3.50 ± 0.05	3.33 ± 0.04
His	2.44 ± 0.04	2.37 ± 0.01	Pro	13.7 ± 0.09	12.4 ± 0.13
Arg	4.09 ± 0.11	4.25 ± 0.22			

Notes: Individual amino acids in the mixed diet were calculated based on the measured values of concentrate and stover, values are means of four samples; *LF, Low forage diet; [†]HF, High forage diet.

The commercially available APG liquid (carbon number in alkyl was 12–14; appearing yellowish liquid; containing 50% solid content; hydrophilic-hydrophobic balance, 12–14, provided by Hunan Diyuan Co., Ltd. China) was carefully mixed by hand together with the concentrate according to the designed amounts.

All of the goats were kept in individual pens and had free access to fresh water. Feed intake was restricted to 540 g DM/d. Dietary forage and concentrate as mash were offered simultaneously in two equal portions at 08:00 and 19:00 h. To minimise the potential digestive disorders of goats resulting from the rapid switching of low or high F:C diets, concentrate or corn stover was gradually increased or decreased over the first 3 d of each adaptation period respectively. No refusal of the supplied diet occurred after the adaptation period. At the beginning and end of each experimental period, all of the goats were weighed before the morning feeding.

Feed samples were collected on days 4, 13 and 22 of each experimental period, and dried at 55°C in a forced-air oven to a constant weight and then ground to pass a 1-mm screen (DF-2, Changsha Instrument Factory, China). Finally, all the collected feed samples were pooled for every period resulting in four samples for each diet.

On day 19 of each period, before and 2 h after morning feeding, blood samples (8 ml) were taken from the jugular vein, collected into heparinised tubes and centrifuged for 15 min at 1500 *g* at 4°C. The collected plasma was stored at –20°C. About 80 ml of rumen contents were taken from each goat through the rumen cannula at 0 (before morning feeding, 08:00 h), 1, 2, 3, 6 and 9 h after morning feeding. pH-values were measured immediately using a pH-meter (model 2000, Beckman Instruments Inc., Fullerton, CA). Immediately after collection, the rumen contents were strained through four layers of cheesecloth for the analysis of NH_3-N.

On days 20 to 22, about 100 ml of rumen contents were sampled 2 h before and after feeding (morning and evening) every day during each period. The microbial fractions were separated by differential centrifugation according to the method of Legay-Carmier and Bauchart (1989). Briefly, rumen contents were squeezed through eight layers of cheesecloth. Total particles were washed in saline solution (0.9% cold NaCl, 100 ml/100 g particles) and squeezed again to remove free-floating bacteria of the liquid phase associated with the particles. The filtrates were pooled and centrifuged at 500 *g* for 10 min at 4°C to remove feed particles and protozoa. The supernatant obtained was centrifuged at 27,000 *g* for 30 min at 4°C to obtain rumen liquid-associated bacteria (LQB). The resultant pellet was washed twice (once with 0.9% NaCl and once with distilled water, respectively), and centrifuged at 27,000 *g* for 30 min at 4°C. The particles retained on the cheesecloth were suspended in 0.5 litre bottles by 0.9% cold NaCl (300 ml/100 g particles), and shook violently for 6 min. The homogenate was squeezed through eight layers of cheesecloth. Solid residue was rinsed in saline, shook and squeezed again as described previously. Filtrates were pooled and centrifuged to obtain solid-associated bacteria (SAB) by the same procedure of differential centrifugation as LQB. Finally, all the obtained bacterial sediments were gathered by animal per period, freeze-dried (GLZY-0.5B, Shanghai Pudong Freeze Dryer Equipment Co. Ltd., China) and stored at –20°C until analysis.

2.2. Analytical procedures

The analytical procedures determining the DM, OM and CP of diet and bacterial fractions were described by the Association of Official Analytical Chemists (AOAC

1990). Dietary NDF and ADF were determined according to the method of van Soest et al. (1991). The NH_3-N concentration was determined by the method of Chaney and Marbach (1962). For amino acid analysis, about 150 mg of finely ground bacterial samples and 300 mg of milled feed samples and rumen content were hydrolysed with 5 ml of 6 *N* HCl at 110°C for 24 h. Plasma was deproteinised by 7% trichloroacetic acid. The amino acid concentrations were measured by an Amino Acid Analyser (Hitachi L-8800, Japan). The glucose and urea in plasma were determined by an automated biochemistry analyser (Beckman Synchron CX4/Pro, America) using kits supplied by Leadman Co., Ltd. (Beijing, China).

2.3. Statistical analysis

The data on organic matter and nitrogen (N) of LQB and SAB, amino acid composition of rumen content, LQB and SAB were analysed using the MIXED procedures (SAS 9.0) for a 4 × 4 Latin square design according to the following:

$$Y_{ijkl} = \mu + A_i + P_j + F_k + S_l + (F \cdot S)_{kl} + e_{ijkl},$$

where: μ is the overall mean; Y_{ijkl} is the dependent variable; A_i is the random effect of goat; P_j, F_k, and S_l are the fixed effect of period F:C and APG respectively; $(F \cdot S)_{kl}$ is the interaction between F:C and APG; and e_{ijkl} is the random residual error. Least squares means were reported.

NH_3-N and pH in the rumen fluid, and metabolites and free amino acids (FAA) in the plasma at different sampling time-points were analysed by using the statement "repeated" of PROC MIXED (SAS 9.0) according to the following:

$$\begin{aligned} Y_{ijkl} = \mu &+ A_i + P_j + F_k + S_l + T_m + (F \cdot S)_{kl} + (F \cdot T)_{km} \\ &+ (T \cdot S)_{lm} + (F \cdot T \cdot S)_{klm} + e_{ijkl}, \end{aligned}$$

where: μ, A_i, P_j, F_k, S_l and $(F \times S)_{kl}$ are as described previously; T_m is the effect of sampling time; $(F \cdot T)_{km}$, $(T \cdot S)_{lm}$ and $(F \cdot T \cdot S)_{klm}$ are the interactions of F:C and sampling time, sampling time and APG, and F:C, sampling time and APG respectively. The unstructured covariance structure was determined based on the low values received for goodness of the Akaike Information Criterion (AIC) and Schwarz's Bayesian Information Criterion (BIC). Differences among treatments were determined using LSmeans with PDIFF and adjusted by the Tukey's procedure. Values were considered statistically significance at $p < 0.05$, and tendency was declared at $0.05 \leq p \leq 0.10$.

3. Results

3.1. Rumen fermentation and blood metabolites

The rumen fluid pH and NH_3-N concentrations are shown in Figures 1(a) and 1(b) respectively. The ruminal pH was relatively stable and tended to be higher ($p = 0.080$) when goats were fed Diet HF compared with Diet LF. No interaction between dietary F:C and APG supplementation was noted. However, with APG supplementation the ruminal mean pH values of goats fed Diet HF were less than 6.3

from 2 h to 9 h after morning feeding. The ruminal NH_3-N concentrations were greater than 5.0 mg/dl, and were not significantly affected by dietary treatment.

The plasma urea and glucose concentrations are shown in Table 3. Plasma urea concentration was greater when goats were fed Diet HF compared with Diet LF ($p = 0.030$), but was not affected by the APG supplementation. Neither dietary F:C nor APG supplementation affected the plasma glucose concentration.

3.2. Amino acid compositions of rumen content

The amino acid compositions of rumen content are given in Table 4. The concentration of total analysed amino acid (TAA) of rumen content was greater when goats received Diet LF. However, no significant effect of APG on TAA concentration of rumen content was observed. The proportions of leucine, histidine, arginine, glutaminc acid, proline, alanine and total non-essential amino acids (NEAA) were significantly greater when goats were fed Diet LF, whereas the proportions of essential amino acids (EAA), valine, phenylalanine, cystine and tyrosine were lower when goats received Diet LF. APG supplementation reduced the

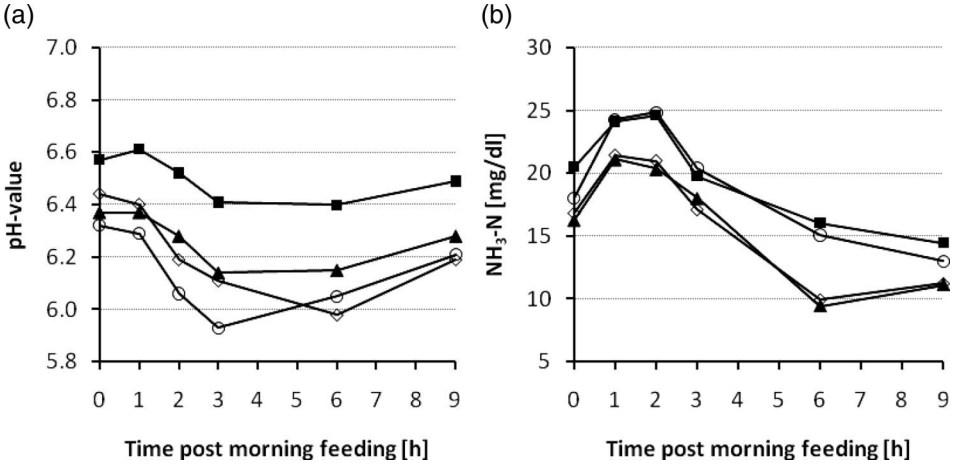

Figure 1. Changes of ruminal pH-value (a) and NH_3-N concentration (b) in goats receiving a high forage diet with and without supplementation of APG (▲ and ■ respectively), or a low forage diet with and without APG supplementation (□ and ○ respectively) ($n = 4$).

Table 3. Plasma urea and glucose concentrations in goats in response to the forage-to-concentrate ratio (F:C) and alkyl polyglycoside (APG) supplementation ($n = 8$).

	Diet LF*		Diet HF†			*p*-value		
	None	APG	None	APG	SEM#	FC	APG	FC × APG
Urea [mM]	4.43	3.94	5.96	4.81	0.475	0.030	0.116	0.502
Glucose [mM]	4.19	3.89	4.10	4.30	0.207	0.455	0.821	0.245

Notes: *Diet LF, Low forage diet; †Diet HF, High forage diet; #SEM, Pooled standard error of means.

Table 4. Amino acid composition of the rumen content in response to the forage-to-concentrate ratio (F:C) and alkyl polyglycoside (APG) supplementation ($n = 4$).

	Diet LF*		Diet HF†			*p*-value		
	None	APG	None	APG	SEM #	FC	APG	FC × APG
TAA§ [mg/g DM]	175	182	160	161	4.6	0.003	0.395	0.499
Amino acids [% of TAA]								
Sum of EAA‡	44.6	44.7	45.8	45.0	0.24	0.012	0.195	0.068
Met	2.10	2.19	2.34	2.68	0.179	0.071	0.261	0.503
Lys	8.16	8.55	8.91	8.35	0.262	0.330	0.760	0.105
Val	5.58	5.24	5.95	5.47	0.113	0.026	0.006	0.551
Ile	4.72	4.88	5.00	4.84	0.090	0.205	0.978	0.104
Leu	7.52	7.53	7.21	7.33	0.074	0.007	0.377	0.447
Phe	5.63	5.60	6.01	5.83	0.047	<0.001	0.049	0.125
Thr	5.45	5.42	5.45	5.46	0.050	0.753	0.903	0.753
His	1.99	1.94	1.86	1.88	0.041	0.038	0.768	0.385
Arg	3.44	3.39	3.12	3.17	0.065	0.003	0.970	0.442
Sum of NEAA+	55.4	55.3	54.2	55.0	0.24	0.012	0.195	0.068
Asp	10.6	10.8	11.1	10.8	0.19	0.177	0.889	0.236
Ser	4.58	4.52	4.53	4.61	0.034	0.500	0.747	0.064
Glu	13.6	13.8	13.5	13.4	0.04	0.000	0.181	0.064
Gly	4.65	4.56	4.46	4.60	0.042	0.116	0.669	0.025
Ala	6.07	5.82	5.45	5.85	0.122	0.037	0.568	0.027
Cys	4.52	4.34	4.69	4.70	0.093	0.021	0.390	0.338
Tyr	4.32	4.35	4.58	4.46	0.038	0.001	0.259	0.096
Pro	7.14	7.17	5.95	6.57	0.290	0.013	0.292	0.332

Notes: *Diet LF, Low forage diet; †Diet HF, High forage diet; #SEM, Pooled standard error of means; §TAA, Total analysed amino acids; ‡EAA, Essential amino acids; +NEAA, Non-essential amino acids.

proportions of valine and phenyalanine. There were interactive effects on the proportions of glycine and alanine ($p < 0.05$), and EAA, serine, glutamic acid, and tyrosine ($p < 0.10$) between dietary F:C and APG supplementation.

3.3. Chemical composition of rumen bacterial fractions

The OM, N and TAA concentrations of LQB and SAB are shown in Table 5. Neither APG supplementation nor dietary F:C affected these parameters.

The amino acid compositions of ruminal bacterial fractions are presented in Tables 6 and 7. No effect of dietary F:C on amino acid proportion of LQB was observed (Table 6). The proportions of histidine ($p < 0.05$), Arg and Pro ($p < 0.10$) of LQB were decreased by APG supplementation. In comparison, the proportions of threonine and tyrosine of LQB ($p < 0.10$) were increased by APG supplementation. The proportions of methionine and threonine ($p < 0.05$), and Arg ($p < 0.10$) in SAB were greater when the goats were fed Diet LF (Table 7). Conversely, the proportions of proline ($p < 0.05$) and lysine ($p < 0.10$) in SAB were less when goats received Diet LF. The interaction between dietary F:C and APG supplementation was significant ($p < 0.05$) for the lysine and threonine proportions of SAB. The lysine proportion of SAB was greater for goats fed Diet HF without APG compared

Table 5. Contents of organic matter (OM), nitrogen and composition of total analysed amino acids (TAA) of rumen bacterial fractions in response to the forage-to-concentrate ratio (F:C) and alkyl polyglycoside (APG) supplementation ($n = 4$).

	Bacterial fractions	Diet LF*		Diet HF†		SEM	*p*-value		
		None	APG	None	APG		FC	APG	FC × APG
OM [% of DM]	LQB§	0.89	0.90	0.88	0.90	0.013	0.571	0.203	0.453
	SAB‡	0.89	0.89	0.88	0.88	0.008	0.326	0.667	0.885
N [mg/g DM]	LQB	83.0	89.8	88.9	90.7	3.31	0.469	0.384	0.803
	SAB	86.2	79.7	71.2	80.5	7.20	0.495	0.711	0.697
TAA [mg/g DM]	LQB	453.3	473.7	471.6	490.1	13.7	0.236	0.190	0.947
	SAB	432.8	427.7	380.0	426.3	15.4	0.113	0.216	0.131

Notes: *Diet LF, Low forage diet; †Diet HF, High forage diet; #SEM, Pooled standard error of means; §LQB, Liquid-associated bacteria; ‡SAB, Solid-associated bacteria.

Table 6. Amino acid composition of liquid-associated bacteria in response to the forage-to-concentrate ratio (F:C) and alkyl polyglycoside (APG) supplementation [% of total analysed amino acids] ($n = 4$).

	Diet LF*		Diet HF†		SEM#	*p*-value		
	None	APG	None	APG		FC	APG	FC × APG
EAA‡	45.5	45.2	45.3	45.2	0.28	0.885	0.569	0.830
Met	2.69	2.92	2.87	3.04	0.146	0.335	0.206	0.822
Lys	8.14	8.16	8.07	7.85	0.123	0.150	0.421	0.341
Val	5.86	5.80	5.78	5.79	0.061	0.456	0.691	0.554
Ile	5.11	5.00	5.08	5.01	0.076	0.924	0.266	0.798
Leu	7.13	7.03	7.10	7.04	0.075	0.948	0.299	0.770
Phe	5.04	5.02	5.05	5.14	0.084	0.474	0.665	0.509
Thr	5.58	5.66	5.58	5.66	0.043	0.933	0.093	0.933
His	1.73	1.63	1.72	1.65	0.033	0.796	0.031	0.632
Arg	4.16	4.01	4.10	4.06	0.048	0.919	0.077	0.258
NEAA+	54.6	54.8	54.7	54.8	0.28	0.885	0.569	0.830
Asp	11.3	11.2	11.3	11.2	0.10	0.829	0.537	0.829
Ser	4.41	4.48	4.35	4.44	0.044	0.295	0.106	0.846
Glu	12.4	12.7	12.4	12.5	0.19	0.797	0.314	0.778
Gly	5.13	5.09	5.12	5.10	0.031	0.969	0.302	0.835
Ala	7.18	7.01	7.22	7.18	0.118	0.390	0.401	0.615
Cys	3.59	3.84	3.59	3.78	0.225	0.897	0.354	0.914
Tyr	4.69	4.87	4.62	4.71	0.069	0.132	0.078	0.474
Pro	5.91	5.67	6.10	5.80	0.127	0.251	0.060	0.841

Notes: *Diet LF, Low forage diet; †Diet HF, High forage diet; #SEM, Pooled standard error of means; ‡EAA, Essential amino acids; +NEAA, Non-essential amino acids.

with the other treatments. In comparison, the Thr proportion of SAB was less for goats fed Diet LF without APG compared with the other treatments.

Additionally, the TAA concentration in LQB was greater than in SAB (472 vs. 417 mg/g DM, $p < 0.001$). The amino acid compositions of LQB differed from those of SAB for most of the detected amino acids ($p < 0.10$, results not shown) except for valine, isoleucine, serine and glutamic acid. Compared with SAB, the LQB fraction had greater proportions of methionine, lysine, threonine, asparagine, glycine

Table 7. Amino acid composition of solid-associated bacteria in response to the forage-to-concentrate ratio (F:C) and alkyl polyglycoside (APG) supplementation [% of total analysed amino acids] ($n = 4$).

	Diet LF*		Diet HF†			*p*-value		
	None	APG	None	APG	SEM#	FC	APG	FC × APG
EAA‡	44.7	45.3	45.3	45.0	0.23	0.517	0.466	0.099
Met	2.71	2.69	2.42	2.51	0.060	0.005	0.556	0.344
Lys	7.76	7.88	8.18	7.79	0.073	0.051	0.099	0.007
Val	5.72	5.70	5.61	5.72	0.063	0.483	0.530	0.305
Ile	5.00	5.13	5.10	5.09	0.050	0.613	0.291	0.189
Leu	7.24	7.22	7.28	7.21	0.038	0.771	0.227	0.464
Phe	5.34	5.42	5.70	5.51	0.148	0.161	0.713	0.382
Thr	5.55	5.51	5.36	5.57	0.026	0.031	0.012	0.001
His	1.73	1.76	1.74	1.70	0.026	0.302	0.890	0.231
Arg	4.08	3.97	3.87	3.91	0.070	0.080	0.652	0.280
NEAA+	55.3	54.7	54.8	55.0	0.23	0.517	0.466	0.099
Asp	10.9	10.9	11.0	11.0	0.06	0.133	0.415	0.658
Ser	4.44	4.37	4.41	4.40	0.033	0.913	0.274	0.450
Glu	12.8	12.5	12.5	12.5	0.15	0.376	0.207	0.292
Gly	5.01	4.99	4.88	4.98	0.046	0.149	0.407	0.262
Ala	6.69	6.67	6.38	6.72	0.148	0.395	0.302	0.250
Cys	4.41	4.20	4.22	4.16	0.173	0.524	0.456	0.645
Tyr	4.88	4.92	4.88	4.87	0.056	0.651	0.778	0.682
Pro	6.19	6.21	6.50	6.34	0.087	0.031	0.402	0.320

Notes: *Diet LF, Low forage diet; †Diet HF, High forage diet; #SEM, Pooled standard error of means; ‡EAA, Essential amino acids; +NEAA, Non-essential amino acids.

and alanine ($p < 0.05$). In contrast, the SAB fraction had greater proportions of leucine, phenylalanine, cystine, tyrosine and proline ($p < 0.05$).

3.4. Compositions of free amino acids in plasma

The compositions of free amino acids in plasma are presented in Table 8 as LSmean values. Neither dietary F:C nor APG supplementation affected the proportions of plasma free amino acids significantly. In addition, there was also no treatment effect on the concentration of total free amino acid in plasma. However, APG supplementation tended to decreased TFAA concentration when goats were fed Diet LF, whereas it tended to increased TFAA when goats were fed Diet HF.

4. Discussion

In the present study, the ruminal pH of goats fed Diet HF with APG dropped to less than 6.3 from 2 h to 9 h after feeding. According to previous reports, the optimal pH value for fibre digestion in the rumen is greater than 6.3 (Stewart 1977). Thus the decline of pH value in this study might have a negative effect on ruminal fibre digestion when goats were fed Diet HF with APG supplementation. Ahn et al. (2009) reported that the ruminal pH was also decreased after feeding with another type of nonionic surfactants (Tween 80, polyoxyethylene sorbitan monostearate), but the lowest value was still greater than 6.3 when were steers fed a high F:C diet (60:40).

Table 8. Composition of free amino acid in plasma in response to the forage-to-concentrate ratio (F:C) and alkyl polyglycoside (APG) supplementation [% of total free amino acids] ($n = 8$).

	Diet LF*		Diet HF†			*p*-value		
	None	APG	None	APG	SEM#	FC	APG	FC × APG
TFAA§ [mg/g DM]	380	355	355	378	11.9	0.924	0.924	0.074
EAA‡	38.9	36.2	39.2	39.2	1.29	0.234	0.304	0.322
Met	1.30	1.21	1.35	1.22	0.091	0.764	0.247	0.878
Lys	7.51	6.48	7.79	7.10	0.612	0.484	0.190	0.789
Val	6.64	5.90	6.37	6.79	0.502	0.541	0.758	0.276
Ile	3.21	2.92	3.44	3.28	0.186	0.149	0.247	0.729
Leu	3.58	3.29	3.80	3.88	0.347	0.272	0.772	0.611
Phe	2.59	2.77	2.81	2.68	0.149	0.658	0.861	0.321
Thr	2.87	3.22	3.23	3.03	0.355	0.822	0.827	0.449
His	3.30	3.28	3.12	3.36	0.509	0.927	0.829	0.798
Arg	7.95	7.13	7.32	7.84	0.904	0.962	0.869	0.478
NEAA+	61.1	63.8	60.8	60.8	1.29	0.234	0.304	0.322
Asp	1.18	1.27	1.22	1.11	0.134	0.653	0.924	0.469
Ser	11.8	11.2	11.7	11.4	0.87	0.935	0.620	0.829
Glu	10.2	10.3	9.28	9.98	1.15	0.598	0.745	0.789
Gly	16.7	20.4	17.4	17.6	1.59	0.539	0.247	0.288
Ala	5.83	5.76	6.76	6.07	0.333	0.094	0.280	0.376
Cys	3.26	3.20	3.20	3.13	0.145	0.651	0.663	0.946
Tyr	4.54	4.25	4.55	4.50	0.350	0.715	0.642	0.733
Pro	7.55	7.44	6.63	6.99	0.409	0.127	0.761	0.584

Notes: *Diet LF, Low forage diet; †Diet HF, High forage diet; #SEM, Pooled standard error of means; ‡EAA, Essential amino acids; +NEAA, Non-essential amino acids; §TFAA, Total free amino acids.

On the other hand, the ruminal pH in the current study was slightly higher in goats receiving Diet LF with APG than in those fed same diet without APG. This was different from the previous findings of Lee et al. (2004), in which cows fed a low F:C ratio diet (40:60) with SOLFA-850 (Span85, sorbitan trioleate, another type of nonionic surfactant) had a lower ruminal pH. The differences between this study and previous ones were probably due to the different experimental animals and properties of the nonionic surfactants used.

Regardless of the treatment and sampling time in the current experiment, the ruminal NH_3-N concentrations were greater than 5 mg/dl, which was indicated as the minimum concentration for microbial growth and microbial protein synthesis (Satter and Slyter 1974). According to the present results, APG supplementation slightly decreased the concentrations of ruminal NH_3-N and plasma urea. However, previous studies demonstrated that NH_3-N concentration in the rumen was higher when animals were fed diets supplemented with APG (Yuan et al. 2009) or other nonionic surfactants (i.e. Tween 80 or SOLFA-850; Lee et al. 2004; Ahn et al. 2009). The differences might be ascribed to the variation in diets or changes of the experimental conditions.

Amino acids in the rumen content usually originate from dietary crude protein, endogenous protein and microbial protein. Generally, the concentration of total detected amino acids in the rumen content was about two times higher than that of the goats consuming the diets in the current study. The proportion of lysine

increased greatest when compared with other EAA in the rumen content, which was probably caused by high levels of synthesis by the rumen bacteria, because the ruminal bacteria often contain high concentrations of lysine. This finding was in agreement with the result of Hussein et al. (1995), who reported that lysine was the most abundant amino acid in mixed ruminal bacteria. The higher amino acid concentration in the rumen content observed for goats fed Diet LF in this study light probably be not only related to the greater amino acid concentration in diet, but also attributed to the higher synthesis efficiency of micro-organisms or the greater amount of bacteria. Diet LF would release energy more quickly to couple with the utilisation of NH_3-N by the rumen bacteria to synthesise amino acids and protein (Clark et al. 1992). Lascano et al. (2009) reported that the total counts of rumen bacteria in dairy heifers fed with a high concentrate diet (F:C, 40:60) was higher than in those who received a medium concentrate diet (F:C, 60:40).

Clark et al. (1992) summarised more than 70 observations from about 20 studies and found that the OM and N composition of rumen bacteria widely varied from 60.8 to 92.2% of DM and from 7.35 to 13.23% of OM respectively. The contents of OM and N of rumen bacteria in the current study were within this reported range. Dietary F:C alteration did not affect the composition of OM and N in rumen bacteria, which was in agreement with the previous findings of Czerkawski (1976) and Yang et al. (2001), but differed from the finding of Hussein et al.(1995) who reported that the OM and N concentrations in rumen bacteria were higher in steers fed a low-forage diet when compared with those fed a high forage diet.

Most of the bacterial (LQB and SAB) amino acids measured in this study were not affected by either dietary F:C or APG supplementation, although the proportions of methionine, lysine, threonine and proline in SAB were affected by alteration of the F:C ratio, and histidine in LQB and threonine in SAB were influenced by APG supplementation. Hussein et al. (1995), reported a lower dietary forage level was followed by a greater TAA concentration in the mixed ruminal bacteria. Our results showed similar effects on the amino acid concentration of SAB. However, contrary to LQB, the differences were quantitatively minimal. Our results were not in complete agreement with the results of Yang et al. (2001), who reported that the proportions of tyrosine and cystine were higher, but methionine and valine were lower under the condition of feeding dairy cows a high F:C diet. They suggested that these differences in the amino acid composition of rumen bacteria might be the result of a shift of bacterial species. There was no specific information available about the effects of APG on rumen bacteria. However, previous studies indicated that dietary Tween 80 supplementation appeared to increase the growth of non-cellulolytic bacteria, but not of cellulolytic bacteria (Ha et al. 2002; Goto et al. 2003; Lee et al. 2003). In the present study, APG supplementation might have reduced the population of cellulolytic bacteria due to the observed low ruminal pH, which could limit growth of these bacteria when APG were added to Diet HF. On the other hand, nonionic surfactants would alter cell membrane permeability and affect bacteria strains (Reese and Maguire 1969; King et al. 1991). The APG were characterised with the same nonionic hydrophilic head and hydrophobic portion as other nonionic surfactants (Weuthen et al. 1995). The amphiphilic activity and interfacial tense existing between APG molecules and bacterial surfaces might affect the bacterial growth by influencing the cell membrane lipid balance. In addition, the properties of APG might also affect the adhesion of bacteria to dietary particles. Further confirmatory studies are hereby recommended to ascertain this.

Dietary APG and F:C ratio had no effect on the proportions of plasma free amino acids, but interaction between the dietary F:C and APG supplementation was observed for the concentration of total free amino acids. The results implied that APG probably affected digestion and absorption of amino acid in the intestine depending on dietary F:C level. However, it is necessary to confirm this in future studies.

5. Conclusion

The results of this study indicated that both dietary APG supplementation and the alteration of forage-to-concentrate ratio partly influenced amino acid proportions of the rumen content and ruminal bacteria, but had minimal effects on ruminal pH value, ruminal NH_3-N concentration and plasma urea. The effects of APG on amino acid concentrations and proportions were dependent on dietary F:C level. APG probably affected rumen fermentation through reducing the ruminal pH when goats were fed with the high F:C diet. More studies might be needed to understand the effects of APG on rumen bacterial strains and performance of ruminants.

Acknowledgements

We wish to express our appreciation to the Chinese Academy of Sciences (grant number kscx2-YW-N-022) and the Ministry of Science and Technology of China (grant numbers KZCX2-YW-T07 and KZCX2-YW-JS407) for providing the financial support for this study.

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