Effects of dietary carbohydrate composition on rumen fermentation characteristics and microbial population in vitro

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Abstract

The objective of the experiment was to evaluate the effects of dietary carbohydrate composition on rumen fermentation characteristics and microbial populations in vitro. The treatments were organized in three different carbohydrate composition diets: the wheat fibre rich diet (WF) (67.2% wheat bran and 25.7% corn grain in concentration), mixed diet (MD) (45.1% wheat bran and 34.3% corn grain) and corn starch rich diet (CS) (2.1% wheat bran and 63.8% corn grain), respectively (n=6). The results showed that consumption of CS diet led to a decrease in NH₃-N (ammonia nitrogen) concentration (P<0.05), and microbial protein concentration were increased (P<0.05). They indicated more efficient ruminal NH₃-N utilization and large amounts of N being excreted to the environment (Schoeder and Tilgemen, 2008).

Meanwhile, ruminal fermentation availability of dietary carbohydrate can differ greatly in response to various structure of carbohydrate (Villalba et al., 2006). Sniffen et al. (1992) has reported that composition of carbohydrate feed ingredients could be distinguished into non-structural carbohydrate (starch, sugar) and fibre. Existing literatures about the functions of nonstructural carbohydrate on rumen fermentation are decreasing ruminal pH (McAllan et al., 1994) and concentration of ammonia nitrogen (Hristov et al., 2005), increasing dry matter degradation of feed and volatile fatty acid production (Rotger et al., 2006) but also inhibiting fibre fermentation (Suarez et al., 2006). Regarding fibrous feedstuff, it can increase ruminal pH, provide microbial adherence (Krause et al., 2003), affect ruminal microbial community (Saro et al., 2012), ruminal mat formation and ruminination (Zebeli et al., 2012) and increase ammonia nitrogen (Belanche et al., 2012). While improvements of ruminal N utilization and transfer of microbial N into milk protein by fermentable fiber have been reported as well (Hristov and Ropp, 2003). Therefore, a better constitution of dietary carbohydrate supply could be suggested as an efficient way for enhancing microbial protein synthesis (Carruthers et al., 1997), thus maximizing the efficiency of N utilization and minimizing environmental issues. Our hypothesis was that the combined effect of carbohydrate ingredients with different ratio of starch to fibre on microbial protein synthesis and ruminal fermentation would differ. Hence, the objectives of this study were to evaluate the effects of dietary carbohydrate composition, with similar protein content, on in vitro fermentation characteristics, microbial protein synthesis and microbial community.

Introduction

Ruminants have the unique capacity to transform relatively low-quality dietary nitrogen [e.g., non-protein nitrogen (NPN)] into high-quality microbial protein. The microbial protein produced in rumen can supply more than 50% small intestinal digestible amino acids, depending on many factors such as the availability of carbohydrates and N in the rumen (Shabi et al., 1998), ruminal pH (Dehority, 2003), physiological effects (Hoover and Stokes, 1991), sources and levels of N components (Stern and Hoover, 1979) and stabilisation of ruminal fermentation (Khorasani et al., 1994). Carbohydrate and protein were reported as the major nutrients supporting microbial protein synthesis (Clark and Davis, 1980, 1983). For carbohydrate, it is an important constituent of animal diet being required for the vital body processes that are essential for life and the synthesis of body tissues and animal products (McDonald, 2002). In vitro and in vivo studies support that degradation rate of carbohydrate is the major factor of controlling the energy availability for microbial protein synthesis (Feng et al., 1993; Koenig et al., 2003; Hall and Herejak, 2001). Despite the significance of dietary carbohydrate composition, the importance of protein content is commonly overestimated in the Chinese livestock industry. The efficiency of N utilization is decreased with imbalance energy and protein type, leading to high feeding costs, low nitrogen utilization and large amounts of N being excreted to the environment (Schoeder and Tilgemen, 2008).

Materials and methods

Experimental design and diets

The experimental diets, designed in three levels of different carbohydrate composition diets with isonitrogenous protein, were the wheat fiber rich diet (WF) (67.2% wheat bran and 25.7% corn grain in concentration), mixed diet (MD) (45.1% wheat bran and 34.3% corn grain) and wheat bran rich diet (WF) (67.2% wheat bran and 25.7% corn grain in concentration), mixed diet (MD) (45.1% wheat bran and 34.3% corn grain).
grain) and corn starch rich diet (CS) (2.1% wheat bran and 63.8% corn grain), respectively. The nutrient level of experimental diets of concentrate was presented in Table 1.

Experimental methods

Fermentation substrates were weighed accurately into the culture glass syringes. There were 6 units per treatment at each sampling time point (0, 2, 4, 6, 8, 10, 12, 16 and 24 h). Incubation medium composition followed the method of Menke et al., (1988), and the component proportion of rumen fluid, coming from three healthy Xuanhan yellow cattle with permanent fistula and artificial saliva was 1:2. The incubation medium was placed in a water-bath to maintain at 39°C with continuous flux of carbon dioxide, and then 30 mL of incubation medium was added into the glass syringes, and the amounts of cumulative gas production (GP) were recorded at 0, 2, 4, 6, 8, 10, 12, 16 and 24 h. The data of cumulative GP were corrected through deducting the influence of control group.

Sampling and analysis

Experimental diets’ crude protein (CP) was determined by reference to AOAC (1990). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the method of Van Soest et al. (1991). Nutrient composition is shown in Table 1. The fermentation medium was analysed for ammonia nitrogen (NH3-N), volatile fatty acid [VFA, including total volatile fatty acid (TVFA), acetate, propionate and butyrate] and microbial protein (MCP) concentrations and pH. The concentration of NH3-N was determined by the method of Feng et al. (1993). Acetate, propionate, butyrate and TVFA concentrations were estimated by gas chromatograph (CP-3800GC, Varian). The pH was determined by pH meter (pHS-3D) and MCP concentration was determined by the method of Makkar et al. (1999).

DNA extraction, PCR primers and Real-time PCR

DNA extraction

Fermentation fluids collected at 0, 8, 16, 24 h were subjected to analysis of microbial population with Tissue Lyser II (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. Total DNA was extracted from the incubated rumen samples by using the modified bead-beating protocol with the QIAamp DNA mini kit (250) (QIAGEN).

PCR primers

The PCR primer sets used in this study for amplification of general bacteria, Protozoa, Bacteroides amylophilus, Streptococcus bovis and Butyricibrio fibrisolvens were shown in Table 2.

Real-time PCR

The methods of PCR assays for ruminal bacterial species (general bacteria, Protozoa, Bacteroides amylophilus, Streptococcus bovis and Butyricibrio fibrisolvens) were performed according to Denman and McSweeney (2006) and Denman et al. (2007). Ninety-six-well optical plates on CFX96TM Real Time System (Bio-Rad) was used for PCR assays with SYBR Premix Ex Taq™II (Takara, Dalian, China). The 25 µL real-time PCR reaction mixtures contained 1 µL 10 µmol/L of forward and reverse primers, respectively, 12.5 µL SYBR Premix Ex Taq™, 2 µL DNA template ranging from OD 1.6 to 1.8 and DNase/RNase free ddH2O up to 25 µL. The fold change (number of fold difference) of different microbial populations, compared to the control, was determined by cycle threshold (Ct) of real-time PCR. All samples were analysed in triplicate and mean Ct of triplicates of each sample was used for calculations. Each Ct of DNA template of our samples reacted with general bacterial primer was used as calibrator, and reaction of each primer without template DNA was used as negative control. The equation: relative quantification = 2(ΔCt(Target)-ΔCt(Control)) was used for calculation of relative abundance of the bacterial species.

Table 1. Nutrient levels of experimental concentrate (dry matter basis %).

| Ingredients                  | WF          | MD          | CS          |
|------------------------------|-------------|-------------|-------------|
| Corn grain                   | 25.7        | 45.1        | 63.8        |
| Wheat bran                   | 67.2        | 34.3        | 2.1         |
| Wheat middling               | 2.0         | 13.0        | 24.0        |
| Rapeseed meal                | 0.2         | 1.3         | 2.4         |
| Soybean meal                 | 0.2         | 1.3         | 2.4         |
| Limestone                    | 1.1         | 0.7         | 0.4         |
| Calcium phosphate dibasic    | 0.1         | 0.8         | 1.5         |
| Salt                         | 1.0         | 1.0         | 1.0         |
| Sodium bicarbonate           | 1.0         | 1.0         | 1.0         |
| Premix°                      | 1.5         | 1.5         | 1.5         |
| Total                        | 100.0       | 100.0       | 100.0       |

| Nutrient                     | WF          | MD          | CS          |
|------------------------------|-------------|-------------|-------------|
| CP                           | 13.0        | 12.9        | 13.0        |
| NDF                          | 31.5        | 22.7        | 14.0        |
| ADF                          | 11.1        | 7.9         | 4.8         |

| Table 2. Oligonucleotide primers used for real-time PCR assay. |
|------------------|-------------|-------------|
| Target group     | Primer      | Reference   |
| General bacteria | F           | 51.3        | McSweeney et al., 1988 |
| Protozoa         | R           | 59.5        | Denman et al., 2007 |
| Bacteroides amylophilus | R     | 64.1        | Wang et al., 2010 |
| Streptococcus bovis | R          | 62.5        | Khafipour et al., 2009 |
| Succinimonas amyloytica | F         | 50.0        | Tajama et al., 2001 |
| Butyricibrio fibrisolvens | F       | 59.5        | Denman et al., 2007 |
Statistical analyses

Statistical analysis was performed using SAS9.2. Sampling time was considered as a repeated measure. Data were analysed by repeated measures approach of the PROC MIXED (SAS) to account for the effects of treatment (WF, MD and CS), sampling time and their interaction. Kinetic parameters of gas production were calculated using NONLINEAR method (SAS). Duncan multiple comparisons were used to test the differences between treatments, which were denoted by different letter superscripts. Significant and extremely significant differences were set at P<0.05 and P<0.01, respectively.

Results and discussion

Fermentation characteristics

Significant treatment effect and time effect were observed for mean pH value and gas production, while no treatment × time interaction were observed for pH (Table 3). Hourly pH and gas production are presented in Figure 1 and Table 4. The gas production (from 10 to 24 h) increased continuously with CS diet containing high corn grain and there existed significant treatment differences (P<0.05), but no such differences (P>0.05) were observed from 0 to 8 h of incubation between treatments. The pH of WF (12 h and average) was higher than that of CS (P<0.01), and significant differences were also observed between the WF and MD (12 h and average) (P<0.05). The relatively high fermentation pH when supplied with WF diet was suggested to be attributed to high wheat fibre content in dairy cows’ diet (Bataajo and Shaver, 1994). However, there was no difference (P>0.05) in pH (from 0 to 10 h and 16 h) between treatments, as well. This finding was similar to those reported in in vitro study by other researchers (Sutton et al., 1993; McCarthy et al., 1989). Likely related to low dietary concentrate: roughage, pH values were relatively stable (6.0-6.7) and all treatment means were within the normal range pH (6.0-7.0) reported for optimal microbial digestion (Hoover, 1986).

As shown in Table 3, total and individual VFA concentrations were affected by treatments and sampling time (P<0.01), and interactions of treatment × time were observed for propionate, butyrate and total VFA. Acetate, propionate, butyrate and TVFA concentrations were increased with dietary corn starch increasing within most sampling time (Table 5). However, no difference was obtained in acetate: propionate ratio between treatments (P>0.05). The results showed that a higher ratio of dietary starch to fibre may lead to change of ruminal microbial fermentation producing more VFA. The availability of carbohydrate mainly attributes to proportion of non-structural and structural carbohydrate (Van Houtert, 1996). In current study, dietary energy level was increased by increasing the corn grain [starch (non-structural carbohydrate) NSC] rich,] (Philippeau et al., 1999) concentration and decrease wheat bran [fibre (structural carbohydrate, SC) rich] (Nandini and Salimath, 2000).
2001). Moreover, NSC digestion in rumen will be utilized initially by ruminal microbes under the action of enzyme degradation quickly for volatile fatty acid, acetic acid and butyric acid, propionic acid (Chesson and Forsberg, 1988). Sutton et al. (1993) confirmed that high NSC in concentrate resulted in higher propionate concentration and McCarthy et al., (1989) have also concluded that increasing the content of ruminal fermentable starch enhanced the TVFA concentration under in vivo condition.

Treatment, time and their interaction had effects on ammonia nitrogen (P<0.001) (Table 3). As shown in Figure 2, ammonia nitrogen of WF was higher than that of CS within sampling times (P<0.01), and higher values of ammonia nitrogen was observed in group supplied with MD diets at time points of 2, 10, 16h, compared with CS diet (P<0.01). The concentration of NH₃-N in rumen was reduced with greater starch inclusion, characterized by lowerized nitrogen in CS group. NH₃-N was regarded as one of the major nitrogen source for microbial protein synthesis in rumen. Suitable concentration of ruminal NH₃-N could stabilize rumen ecology and improve microbial protein synthesis (Preston et al., 1987). In present in vitro experiment, NH₃-N concentration was decreased with increasing dietary starch content, implying a more efficient capture of N for increased microbial protein synthesis (Joo et al., 2005). The result agrees with the statement of Lee et al. (2003) whose research indicated increasing availability of water-soluble carbohydrates may influence on utilization of ruminal ammonia nitrogen in the in vitro rumen fermentation. The in vitro technique we used has been widely accepted since it could be designed into sufficient number of treatments and repetitions with low-cost, and readily monitored and sampled in real time. Our data represented a continuous increasing and accumulation of ammonia nitrogen during in vitro fermentation. However, Chumpawadee et al. (2006) has reported contrary tendency that ammonia nitrogen would increase in first 2 h after feeding and then decrease until 10 h in beef cattle, which is mainly due to the difference between in vitro and in vivo system. The ammonia nitrogen produced by microbes within in vitro fermented fluid could not be absorbed by ruminal epithelium, and unlike in vivo study, fermentation substrate was not continuous and flowing in culture glass syringes (Seo et al., 2013).

Significant treatment, time and their interaction effects on microbial protein were noticed (Table 3). According to Figure 3, the microbial protein concentrations of WF, MD and CS diets increased as incubation progressed. Decreasing dietary fibre proportion promoted microbial protein synthesis, and WF diet showed the lowest microbial protein concentration within sampling time points (2, 4 and 8 h) than MD and CS diets (P<0.01). The highest average concentration of microbial protein was observed in the CS (P<0.05). Under the condition of ample nitrogen supply, ruminal carbohydrate fermentation would be the main influencing factor of microbial protein synthesis efficiency (Feng et al., 1993),

Table 5. Effect of different dietary carbohydrate composition on volatile fatty acids (mmol/L).

| Time, h | Acetate | Propionate | Butyrate | TVFA | Acetate/propionate |
|---------|---------|------------|----------|------|--------------------|
|         | WF      | MD         | CS       | SEM  | WF     | MD       | CS   | SEM  | WF   | MD   | CS   | SEM  | WF     | MD     | CS  | SEM  |
| 0       | 32.34   | 31.14      | 31.82    | 0.23  | 6.22a  | 6.23a    | 6.22a | 0.05  | 3.52a | 3.62a | 3.27b | 0.05  | 42.07  | 42.00  | 41.03| 0.26 |
| 2       | 16.49a  | 27.16b     | 31.12c   | 0.90  | 3.61a  | 3.63a    | 3.61a | 0.13  | 3.81  | 4.24  | 4.06  | 0.11  | 23.36a  | 35.30a  | 47.81b| 0.24 |
| 4       | 21.39a  | 28.45Ba    | 31.96Bb  | 0.56  | 4.65a  | 5.14a    | 4.65a | 0.14  | 5.21  | 5.47  | 5.33  | 0.08  | 46.07  | 51.73  | 53.08| 1.45 |
| 6       | 35.82   | 39.50      | 41.71    | 1.15  | 6.53a  | 7.65a    | 7.34b | 0.21  | 3.71  | 4.57  | 4.03b | 0.14  | 46.07  | 51.73  | 53.08| 1.45 |
| 8       | 39.02a  | 43.22a     | 45.43a   | 0.51  | 7.27a  | 7.93a    | 8.35a | 0.14  | 4.31  | 4.83a | 5.21a | 0.13  | 50.61a | 55.97b | 59.19b| 0.66 |
| 10      | 40.54a  | 41.50ab    | 43.12a   | 0.46  | 7.41a  | 7.50a    | 8.05a | 0.12  | 4.53  | 4.63  | 4.93  | 0.12  | 56.24  | 53.63  | 56.10| 0.53 |
| 12      | 43.36a  | 44.87a     | 47.38ab  | 0.38  | 7.92a  | 8.56a    | 9.17ab | 0.09  | 4.96  | 5.51a | 6.07b | 0.11  | 56.24a | 58.95ab | 62.63 | 0.40 |
| 14      | 44.97a  | 49.31ab    | 51.49a   | 0.54  | 8.09a  | 8.87a    | 9.41a | 0.18  | 4.99  | 5.56a | 6.00b | 0.15  | 58.05a | 63.74a | 66.89 | 0.79 |
| 24      | 53.82a  | 53.05a     | 57.09a   | 0.73  | 9.71   | 9.73a    | 10.21 | 0.15  | 6.24  | 6.37  | 6.78  | 0.14  | 69.77a | 69.14a | 74.08 | 0.93 |

TVFA, total volatile fat acid; WF, wheat fibre rich diet; MD, mixed diet; CS, corn starch rich diet. a,b,A,BIn the same row, values with different small letter superscripts mean significant difference (P<0.05), and with different capital letter superscripts mean extremely significant difference (P<0.01), while with the same or no letter superscripts mean no difference (P>0.05).
thereby, an adequate dietary carbohydrate of rapidly digestible NSC with sufficient nitrogen in CS diet may promote microorganism proliferation and usage of ammonia nitrogen, then increasing MCP synthesis (Blümmel et al., 2001; Stokes et al., 1991) As reported by Stokes et al. (1991), the study using an in vitro continuous culture technique showed that increasing the proportion of nonstructural carbohydrate (NSC) and reducing the NDF of diets could result in higher yield of microbial protein. The in vitro results of Carruthers et al. (1997) also suggested that ruminal nitrogen utilization and microbial protein synthesis could be improved effectively by increasing NSC: protein ratio of diet.

**Microbial populations in rumen**

The ruminal microbial population for each treatment are shown in Table 6. Treatment had significant effects on protozoa, *Streptococcus bovis* and *Butyrivibrio fibrisolvens* population. Similar effects of sampling time were observed for all four microbes. They were affected by interaction of treatment × time except *Bacteroides amylophilus*. At 8 h of incubation, WF diet showed less protozoa than other groups (P<0.01), and highest population was observed at 16, 24 h in CS, when compared with WF and MD diets (P<0.01). The current study is consistent with the findings in Holstein heifers that active protozoa population could be increased with increase of energy concentration proportion of diets (Dennis et al., 1983). However, there is negative effects of protozoa on ruminal nitrogen usage because of its phagocytosis and turnover of microbial protein, leading to ruminal protein outflow reduction (Faciola and Broderick, 2014). The unwanted high protozoa population with high starch diets could be due to high proportion of available carbohydrate. When protozoa was supplied sufficient nitrogen source, carbohydrate supply would be the main factor of its growth (Veira, 1986), indicating improved ruminal protozoa mainly attribute to the higher fermentation substrate of dietary rapidly fermentable starch supply. As shown in Table 6, CS diet containing high corn starch increased *Streptococcus bovis* at each sampling time point (P<0.01), in contrast, no significant observation of *Bacteroides amylophilus* among treatments was obtained during incubation. *S. bovis* is a rapidly growing bacterium that flourishes in the rumen when animals are fed with large amounts of starch (Owens et al., 1998). The proportion of NSC degradation bacterial including *S. bovis*, *B. amylophilus*, *Selenomonas ruminantium*, etc., which are able to utilize starch to maintain growth, could be enhanced to 85% of total bacterial when sheep consumed high concentration supplementation (Roxas, 1980). Our observations of increasing *S. bovis* are consistent with the findings in Holstein dairy cows, that diets would provide more readily fermentable energy to multiply *S. bovis* by the availability of increasing NSC during transition from forage to grain diets (Tajima et al., 2001). Dietary starch composition tended to increase the ruminal *B. amylophilus* population but not significantly, which is one of the few species in rumen reported to be able to degrade both starch and protein feeds (Hungate, 1966). This is consistent with Petri et al. (2012) who reported a trend of lower *B. amylophilus* population with replacement of high-starch barley with low-starch DDGS. Our result indicated that *B. amylophilus* seems to be less sensitive to change of dietary carbohydrate structure, while supplied with sufficient protein sources, although the reasons underlying this are still needed to be further researched.

There were significant treatment effects on *Butyrivibrio fibrisolvens* at 8 and 24 h of incubation (P<0.01) (Table 6). *B. fibrisolvens* of

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**Table 6. Effect of dietary carbohydrate composition on the ruminal microbial relative population.**

|                | WF       | MD       | CS       | SEM | P-value         | Time   | Treatment × Time |
|----------------|----------|----------|----------|-----|-----------------|--------|------------------|
| **Protozoa**   |          |          |          |     |                 |        |                  |
| 0              | 1.13     | 1.49     | 1.67     | 0.07| 0.001           | 0.001  | 0.027            |
| 8              | 1.00     | 1.31     | 1.03     | 0.07| 0.136           |        |                  |
| 16             | 1.34a    | 1.87a    | 1.84a    | 0.08| 0.001           |        |                  |
| 24             | 1.06ab   | 1.47ab   | 2.26b    | 0.14| 0.001           |        |                  |
| **Bacteroides amylophilus** | |          |          |     |                 |        |                  |
| 0              | 1.10     | 1.23     | 1.73     | 0.09| 0.006           |        |                  |
| 8              | 1.18     | 1.24     | 1.23     | 0.03| 0.768           | 0.009  | 0.34             |
| 16             | 1.00     | 1.15     | 1.16     | 0.05| 0.240           |        |                  |
| 24             | 1.56     | 1.39     | 1.45     | 0.11| 0.841           |        |                  |
| **Streptococcus bovis** | |          |          |     |                 |        |                  |
| 0              | 1.10     | 1.20     | 1.23     | 0.08| 0.473           |        |                  |
| 8              | 1.01     | 1.51     | 2.83     | 0.20| 0.001           | 0.001  | 0.001            |
| 16             | 1.04     | 1.04     | 1.04     | 0.04| 0.989           |        |                  |
| 24             | 1.49ab   | 2.33ab   | 5.77b    | 0.49| 0.001           |        |                  |
| **Butyrivibrio fibrisolvens** | |          |          |     |                 |        |                  |
| 0              | 0.56ab   | 1.63ab   | 2.83c    | 0.23| 0.001           |        |                  |
| 8              | 0.31     | 0.34     | 0.36     | 0.009| 0.041           | 0.001  | 0.001            |
| 16             | 0.06     | 0.05     | 0.07     | 0.01| 0.394           |        |                  |
| 24             | 0.05b    | 0.17b    | 0.19b    | 0.02| 0.004           |        |                  |
groups using a corn starch rich diet (CS) as energy supply was lower than other groups at 8 h of incubation (P<0.01), in contrast, the diets containing high fibre showed lowest population among treatments at 24 h (P<0.01). B. fibrisolvens plays multiple functions of degrading xylans (Hespell et al., 1987), monosaccharide (Miron and Ben-Ghedalia, 1993), fibre (McSweeney et al., 1998), etc. It has been reported to be restrained in the rumen of beef cattle fed with 75% barley diet (Klieve et al., 2003). Similarly, it was decreased at 8 h using high starch and low fibre diet in current study, indicating that it was possibly reduced by decrease of NDF and ADF fermentation substrates (Krause et al., 2003). Their degradation ability to maintain growth could be metabolically versatile due to different fermentation substrates including a wide range of sugars (Stewart et al., 1997). We observed the rise of B. fibrisolvens in CS treatment, compared with other diets, at 24 h incubation would therefore indicate it may be because of degradation of starch by nonstructural carbohydrate degrader in rumen and thereby more available intermediate products (glucose, fructose, xylose, etc.) after a series of former actions. The B. fibrisolvens growth condition was found to be more suitable while cattle was fed with relatively low level of grain (Klieve et al., 2003).

Conclusions

The present study demonstrated that the effect of dietary carbohydrate composition on rumen fermentation and a CS diet (high non-structural carbohydrate content) had relatively positive effects on in vitro fermentation characteristics, increasing carbohydrate fermentation and VFAs production. Particularly, decreasing ammonia nitrogen and increasing microbial protein when consumed high non-structural carbohydrate diet indicated better nitrogen utilization and microbial protein synthesis, because of the alteration of ruminal microbes in response to CS diet. Unlike fibrous diet, there was a transition of fibre degrading to starch degrading bacteria in rumen which could help ruminants to accommodate high grain diet. Supplementation with appropriate proportion of NSC and SC was beneficial to enhance the efficiency of ruminal carbohydrate and nitrogen utilization in vitro.

Our result implied the consideration that the dietary carbohydrate composition was necessary for ruminant diet formula optimization.

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