The effect of starch, inulin, and degradable protein on ruminal fermentation and microbial growth in rumen simulation technique

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Abstract

A rumen simulation technique apparatus with eight 800 mL fermentation vessels was used to investigate the effects of rumen degradable protein (RDP) level and non-fibre carbohydrate (NFC) type on ruminal fermentation, microbial growth, and populations of ruminal cellulolytic bacteria. Treatments consisted of two NFC types (starch and inulin) supplemented with 0 g/d (low RDP) or 1.56 g/d (high RDP) sodium caseinate. No significant differences existed among dietary treatments in the apparent disappearance of dietary nutrients except for dietary N, which increased with increased dietary RDP (P<0.001). Compared with starch, inulin treatments reduced the molar proportion of acetate (P<0.001) and methane production (P=0.006), but increased the butyrate proportion (P<0.001). Increased dietary RDP led to increases in production of total volatile fatty acid (P=0.014) and methane (P=0.050), various measures of N (P=0.046), and 16s rDNA copy numbers of Ruminococcus flavefaciens (P=0.010). Non-fibre carbohydrate source did not affect daily microbial N flow regardless of dietary RDP, but ammonia N production was lower for inulin than for starch treatments under high RDP conditions (P<0.001). Compared with starch treatments, inulin depressed the copy numbers of Fibrobacter succinogenes in solid fraction (P=0.023) and R. flavefaciens in liquid (P=0.017) and solid fractions (P=0.007), but it increased the carboxymethylcellulase activity in solid fraction (P=0.045). Current results suggest that starch and inulin differ in ruminal volatile fatty acid fermentation but have similar effects on ruminal digestion and microbial synthesis in vitro, although inulin suppressed the growth of partial ruminal cellulolytic bacteria.

Introduction

Dietary non-fibre carbohydrates (NFC) are the major source of energy for high-producing dairy cattle, providing 30 to 45% of the diet on a dry matter (DM) basis (Hall et al., 2010). They are a very diverse group of carbohydrates, which include starch, sugars, fructans, and pectic substances (Hall, 2003). Different feed sources vary greatly in the major components of NFC, although NFC has been represented as a single value for feeds or diets. The NFC in corn grain is mostly starch, citrus pulp provides sugars and pectic substances, and sugars are predominant in molasses. Fructans are polymers of fructose with glucose (sucrose) usually as an end group and are found widely distributed in many feeds as important storage carbohydrates (Nelson and Spollen, 1987; Kasperowicz and Michalowski, 2002). Wheat, wheat bran, wheat germ, wheat middling, alfalfa meal, barley and peanut hulls contain significant amount of fructans (1.36 to 5.07 mg/g DM; Flickinger et al., 2003). Fructofuranosyl units in fructans are linked by either -2-1 or -2-6 glycosidic bonds and termed accordingly inulins or levan (Kasperowicz and Michalowski, 2002). Inulin is present in greater than 36,000 plant species, especially composite, as plant storage carbohydrates (Flickinger et al., 2003). However, fermentation of different NFC sources varies in digestion characteristics and the profiles of organic acids produced, which influences the effect of NFC on animal performance. Sucrose is rapidly fermented in the rumen compared with corn starch (Weisbjerg et al., 1998). Inulin also can be rapidly metabolised in the ruminal liquid from cow (<3 h) and sheep (<8 h) (Biggs and Hancock, 1998). Nocek and Russell (1988) reported that the rate of substrate fermentation was roughly proportional to the rate of microbial growth, with more rapidly fermented substrates yielding more microbial mass. Many studies have examined the effect of adding sucrose to dietary rations on microbial synthesis, but results remain inconclusive, with microbial protein production increased in some studies (Huhtanen, 1988; Chamberlain et al., 1993) but reduced in other studies (Hall and Herejk, 2001; Sannes et al., 2002). However, little data are available on the effect of inulin on microbial synthesis, though Rosendo et al. (2003) compared the effects of starch and inulin on microbial synthesis in vitro using isolated forage neutral detergent fibre (NDF) and the effects of inclusion of inulin in diets on ruminal fermentation and production of ammonia nitrogen (N) were investigated in some studies (Öztürk, 2008; Poulsen et al., 2012). Heldt et al. (1999) found that the impact of starch and sugars on low-quality forage utilisation may interact with the amount of supplemental rumen degradable protein (RDP). Considering the fact that inulin is rapidly fermented in the rumen as with sucrose, we speculate that the difference in ruminal fermentation and microbial synthesis between inulin and starch may be affected by dietary RDP level. However, little relevant information is available regarding the speculation. The objective of the present study was to evaluate the effects of starch, inulin, and dietary RDP on ruminal fermentation, microbial synthesis, and populations of ruminal cellulolytic bacteria using the rumen simulation technique.

Materials and methods

This study was approved by the Animal Care and Use Committee of the College of Animal Science and Technology of the Northwest A&F University, Xianyang, China.
Apparatus, animals, and diet

This study was carried out using the rumen simulation technique (Rusitec) (SanShin Co. Ltd., Tokyo, Japan) as described by Kajikawa et al. (2003). The fermentation equipment included eight fermenters with an effective volume of 800 mL each. The general incubation period was established following Kajikawa et al. (2003).

The inoculum used in the fermenters was obtained from four ruminally fistulated goats (40 kg mean body weight) fed two equal meals at 8:00 a.m. and 8:00 p.m. daily containing alfalfa hay and concentrate (40:60, DM basis). Rumen content was collected through the ruminal fistula before the morning feeding and strained through two layers of surgical gauze to separate the liquid and solid fractions. Squeezed solid inoculum (70 g wet weight) was enclosed in a nylon bag (14×7 cm with 100 µm pore size). On the first day, 400 mL of liquid inoculum was dispensed to each fermenter under CO2 flux, two bags were placed in the fermenter, one with feed and the other with solid inoculum, then McDougal’s buffer (McDougal, 1948) was used to fill the vessel. After 24 h, the bag with the solid inoculum was withdrawn and a new bag with feed was supplied. On subsequent days, the bag containing the feed which had been incubated for two days was replaced by a new feed bag. Therefore, each fermenter always had two bags, one of which was removed each day allowing feed to be incubated for 48 h. A continuous infusion of artificial saliva at a rate of 600 mL/d (3.1%/h) was maintained in each fermenter.

Experimental procedure and sampling

The experiment had a 2×2 factorial design with RDP levels, low and high, and two types of NFC including corn starch (Shanghai Jingchun reagent Co., Ltd., Shanghai, China) and inulin (Beneo, Tienen, Belgium). The level of RDP was regulated by adding 0 or 1.56 g/d of sodium caseinate (Sigma C8654; Sigma-Aldrich, St. Louis, MO, USA) into the two diets containing corn starch and inulin, respectively. Fermenters in low RDP and high RDP treatments received approximately 16.0 and 17.6 g DM diet daily from nylon feed bags, respectively (Table 1). Alfalfa hay and concentrate were ground through 4 and 2 mm sieves, respectively.

Experimental treatments were randomly assigned to one of eight fermenters. This experiment was conducted in two independent 15-day incubation periods, with 7 days for stabilisation and 8 days for sample collection.

On days 8, 9, and 10, the pH of the fluid from each fermenter was determined immediately before exchanging the feed bags, and the following samples were collected. The gas produced was collected in Tedlar bags (Tokyo Deodorant Inc., Tokyo, Japan) to determine gas production and concentrations of CH4. Liquid effluent was collected in effluent-collection bottles containing a solution of H2SO4 (20%; vol/vol) to maintain pH values below 2. One millilitre of effluent was preserved by adding 1 mL of deproteinising solution (100 g/L metaphosphoric acid and 0.6 g/L crotonic acid) to determine volatile fatty acid (VFA). Five millilitres of effluent were preserved to determine ammonia N concentration. The samples were frozen at -40°C until analysis. One feed bag from each vessel was collected, washed once with 100 mL of artificial saliva, washed in the cold rinse cycle (10 min) of a washing machine, dried at 60°C, and stored to determine DM disappearance. The residues were also analysed for organic matter (OM), NDF, neutral detergent fibre (ADF), and N.

On day 11, 4 mL of each fermenter fluid was collected at 0, 3, 6, 9, and 12 h after replacing the feed bag, and the pH was measured immediately. On days 12 and 13, 5 mL of saturated HgCl2 was added to the effluent-collection bottles, which were held in an ice-bath to impede microbial growth. On day 13 and 14, the effluent was collected, mixed and homogenised in a blender. One sample (300 mL) was frozen and lyophilised for determination of non-ammonia N (NAN) and total purines (adenine and guanine) and their metabolites (xanthine and hypoxanthine). Approximately 400 mL of effluent was collected for isolation of liquid-associated microbial pellets (LAM) according to Ranilla and Carro (2003). The contents of the nylon bags removed on day 13 and 14 were collected and mixed to determine the solid-associated microbial pellets (SAM). Approximately 20% of solids content was frozen and lyophilised for determination of DM, NAN, and purines concentration. The bacterial samples from LAM and SAM were lyophilised, ground using a mortar and pestle, and analysed for N and total purines concentration. On day 15, 4 mL of fermenter fluid as liquid fraction and one feed bag containing undigested feed as solid fraction from each vessel were collected and were frozen at -80°C for DNA extraction and determination of carboxymethylcellulase and xylanase following Pan et al. (2003).

Analytical procedures

Samples were analysed for DM by drying at 135°C in an airflow-type oven for 2 h, for OM by ashing at 550°C for at least 4 h, and for N using the Kjeldahl procedure (AOAC, 1990). The NDF and ADF content in all samples were analysed according to Van Soest et al. (1991). Heat-stable α-amylase (Sigma A3306; Sigma-Aldrich) and sodium sulfithe were used for NDF determination, with NDF not corrected for ash content. Ammonia N in samples was analysed according to Weatherburn (1967). To determine total and individual VFA, acidified samples were centrifuged at 11,000×g for 10 min, and the supernatant fraction was filtered.

Table 1. Daily quantity of the diets supplied to individual fermenters.

| Item           | Low RDP | High RDP |
|----------------|---------|----------|
|                | Starch  | Inulin   | Starch  | Inulin   |
| Diet ingredient supply, g/d |         |          |         |          |
| Alfalfa hay    | 6.40    | 6.40     | 6.40    | 6.40     |
| Ground corn    | 5.36    | 5.36     | 5.36    | 5.36     |
| Wheat bran     | 1.60    | 1.60     | 1.60    | 1.60     |
| Dicalcium phosphate | 0.06  | 0.06     | 0.06    | 0.06     |
| Sodium chloride| 0.08    | 0.08     | 0.08    | 0.08     |
| Mineral-vitamin premix* | 0.10  | 0.10     | 0.10    | 0.10     |
| Corn starch    | 2.40    | -        | 2.40    | -        |
| Inulin         | -       | 2.40     | -       | 2.40     |
| Sodium caseinate | -     | 1.56     | -       | 1.56     |
| Nutrients supply, g/d |         |          |         |          |
| DM             | 16.00   | 16.00    | 17.56   | 17.56    |
| OM             | 15.28   | 15.28    | 16.78   | 16.78    |
| CP             | 1.40    | 1.40     | 2.81    | 2.81     |
| NDF            | 6.26    | 6.26     | 6.26    | 6.26     |
| ADF            | 4.19    | 4.19     | 4.19    | 4.19     |

RDP, low rumen degradable protein; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre. *Vitamin-Mineral- mix (per kg): nicotinic acid, 450 mg; Mn, 600 mg; Zn, 950 mg; Fe, 440 mg; Cu, 650 mg; Se, 30 mg; I, 45 mg; Co, 20 mg; vitamin E, 800 mg; vitamin D3, 45,000 I.U.; vitamin A, 120,000 I.U.
through a 0.45 μm filter. The VFA concentrations in the filtered samples were determined by high-performance liquid chromatography (HPLC) (model L-2000; Hitachi, Tokyo, Japan) with a reversed-phase Agilent TC-C18 column (4.6x250 mm; 5 μm; Agilent Technologies, Santa Clara, CA, USA) according to Akalin et al. (2002). Crotonic acid was used as an internal standard. The concentration of CH4 was determined using carboxymethylcellulose sodium salt (Sigma Cat. 4888; Sigma-Aldrich) and beechwood xylan (Sigma X4252; Sigma-Aldrich) as substrates, respectively, with a modification that the incubation reaction was terminated by adding 3.0 mL of alkaline 3, 5-dinitrosalicylic acid reagent and heating at 100°C for 5 min (Zhao et al., 2013). The absorbance was read at 540 nm using a spectrophotometer (U-3900; Hitachi). Enzymatic activities were expressed as micromoles of glucose or xylose released that the incubation reaction was terminated by adding 3.0 mL of alkaline 3, 5-dinitrosalicylic acid reagent and heating at 100°C for 5 min (Zhao et al., 2013). The absorbance was read at 540 nm using a spectrophotometer (U-3900; Hitachi). Enzymatic activities were expressed as micromoles of glucose or xylose released from the corresponding substrates by 1 mL of ruminal fluid, for the liquid fraction, or 1 g of undigested feed (DM basis), for the solid fraction, in 1 min at 39°C and pH 6.5. For microbial determination, total genomic DNA was extracted and purified from fermenter liquid (220 µL) and digested feed (220 µg) samples using the QIAamp DNA stool Mini Kit (Qiagen China, Shanghai, China). The bacterial species determined were Fibrobacter succinogenes, Ruminococcus albus, and Ruminococcus flavefaciens as representatives of fibrolytic (cellulolytic and hemicellulolytic) species. The 16S rDNA copy numbers of three fibrolytic bacterial species were determined according to Zhao et al. (2013).

Calculations and statistical analyses

The proportion of digesta NAN (liquid or solid) of microbial origin were estimated in each fermenter by dividing the ratio total purines/N of the NAN portion of digesta by the ratio total purines/N in the corresponding microbial pellets [liquid-(LAB) or solid-(SAB) associated bacterial]. Daily microbial N production (mg/d; LAM or SAM) in each fermenter was calculated by multiplying the proportion of NAN of microbial origin by the amount of NAN in the corresponding digesta (liquid or solid). Total daily microbial production was calculated as the sum of the flows of LAM and SAM. Data were analysed by the PROC MIXED (SAS Inst. Inc., Cary, NC, USA) according to a randomised complete block design (blocking by incubation) (Hindrichsen and Kreuzer, 2009). The model included treatments (NFC and RDP) and the interaction between NFC and RDP as fixed effects and incubation period (block) as a random effect. Significance was declared at P≤0.05, and trends were discussed at P≤0.10. When a significant effect of treatment was detected, differences among means were tested using Tukey’s multiple comparison test.

Results and discussion

No effect of dietary treatments was observed for those data expect for apparent disappearance of N (P≤0.001), which increased with increased dietary RDP, but was unaffected by dietary NFC type (Table 2). There was a NFC×RDP interaction for 16s rDNA copy numbers of F. succinogenes in liquid fraction (P=0.012), which was lower for inulin than for starch treatments under high RDP conditions (Table 3). The effects of increased dietary RDP included increased 16s rDNA copy numbers of R. albus in solid fraction (P=0.056) and those of R. flavefaciens both in liquid and solid fractions (P=0.005 and P=0.010, respectively). Compared with starch treatments, 16s rDNA copy numbers of R. succinogenes in solid fraction (P=0.023) and R. flavefaciens both in liquid and solid fractions (P=0.017 and P=0.007, respectively) were lower for inulin treatments. The xylanase activity both in liquid and solid fractions and carboxymethylcellulase activity in liquid fraction were not affected by dietary treatments (Table 4). The carboxymethylcellulase activity in solid fraction was greater for inulin than for starch treatments (P=0.045).

The current study compared the effects of starch and inulin in the diets using mixed cultures of ruminal microorganisms from goat under two levels of RDP. The ruminal pH did not differ between starch and inulin, which is similar to results reported by Rosendo et al. (2003). There were no differences in the apparent disappearance of OM, NFC, and N due to dietary NFC type in the present study, which mean that starch and inulin also had similar extent of ruminal digestion. However, this result contradicts Biggs and Hancock (1998), in which inulin could be readily and completely digested and metabolised in cow and sheep rumen fluids. The disparities between the two studies may be due to differences in inulin source used. In the latter study, inulin from chicory root could be dissolved in artificial saliva and rumen fluid, but the water solubility of inulin used in the current study was very low (only 1%) according to the technical properties of Beneo-Orafti ingredients. Öztürk (2009) reported that the effects of inulin from chicory roots on in vitro ruminal fermentation were affected by its origins. In

| Item | Low RDP | High RDP | SEM | P |
|------|---------|----------|-----|---|
| pH, before feeding | 6.77 | 6.76 | 6.71 | 6.76 |
| pH, 0 to 12 h | 6.61 | 6.58 | 6.57 | 6.67 |
| Apparent disappearance, % | | | | |
| DM | 35.2 | 36.5 | 36.9 | 38.0 | 1.74 | 0.299 | 0.434 | 0.950 |
| OM | 36.2 | 37.2 | 38.2 | 39.3 | 2.31 | 0.182 | 0.483 | 0.947 |
| NDF | 19.9 | 19.6 | 18.1 | 18.7 | 1.97 | 0.245 | 0.906 | 0.703 |
| ADF | 12.1 | 14.0 | 10.8 | 11.2 | 1.70 | 0.203 | 0.455 | 0.622 |
| N | 38.1 | 38.8 | 54.5 | 49.7 | 1.88 | <0.001 | 0.425 | 0.090 |

RDP, rumen degradable protein; NFC, non-fibre carbohydrates; DM, dry matter; OM, organic matter; NDF, neutral detergent fibre; ADF, acid detergent fibre; N, nitrogen. *Within the same row, means without a common superscript letter are significantly different (P<0.05).
addition, concurred with our results, Dewhurst et al. (2000) reported that the rate and extent of gas production from fructan under simulated ruminal \textit{in vitro} conditions were similar to that of starch but considerably slower than for sucrose and glucose. In the present study, supplemental RDP did not affect nutrients disappearance except N, which is consistent with the report by Brun-Bellut et al. (1990) in dairy goats. Few data are available on the effects of inulin on ruminal NDF digestibility \textit{in vivo} and \textit{in vitro}. Rosendo et al. (2003) compared the effects of starch, inulin, and pectins on forage NDF digestion kinetics using \textit{in vitro} digestion technique and observed no difference among treatments. Similarly, the apparent disappearance of NDF did not differ due to NFC type in the current study, which may be because that the difference in fibrolytic enzyme activities among treatments was minor and slightly changes of carboxymethylcellulase activity in solid fraction may be not obvious enough to affect the NDF disappearance. No significant effects of inulin on the fibrolytic enzyme activities of caecal microflora were observed in rabbits. Bonai et al. (2008) found that replacing starch with inulin in the rabbit diets did not affect the activity of caecal cellulose and xylanase. Unlike fibrolytic enzyme activities, however, we found some differences in the ruminal cellulolytic bacteria in the present study. A significantly lower 16s rDNA copy numbers of \textit{F. succinogenes} and \textit{R. flavefaciens} was observed in fermenters fed inulin diets, which indicates that inulin may be not a stimulant for these fibrolytic bacterial species in the rumen. Starch and inulin were degraded in the rumin to glucose and fructose, respectively (Biggs and Hancock, 1998). Previous research has shown that \textit{F. succinogenes} are capable of using glucose and celllobiose but not fructose as growth substrates (Weimer, 1996), which may have resulted in a suppression of \textit{F. succinogenes} fed inulin diets. Similarly, \textit{R. flavefaciens} also cannot utilise fructose (Weimer, 1996), but there are conflicting reports regarding its ability to utilise glucose, which may be related to the strains used. \textit{R. flavefaciens} strain FD-1 and \textit{R. flavefaciens} C94 cannot utilise glucose as an energy source in some studies (Hiltner and Dehority, 1983; Gokarn et al., 1997), but Sijpesteijn (1951) reported that certain strains of \textit{R. flavefaciens} from the rumen of a slaughtered cow used glucose as carbon sources and grew readily. Current findings suggest that glucose hydrolysed from starch possibly stimulated the growth of partial \textit{R. flavefaciens} compared with inulin. No suppression in NDF disappearance with reduced 16s rDNA copy numbers of \textit{F. succinogenes} and \textit{R. flavefaciens} and slightly increased fibrolytic enzyme activity in inulin treatments may relate to variation in protozoa populations. Some researches found that protozoa showed maximum activity to produce -fructofuranosidase and utilise inulin compared with bacteria (Punj et al., 1970; Ziolecki et al., 1992). Jouany and Senaud (1983) reported that the ciliate population including \textit{Polyplostra multivesibulatum} and \textit{Entodinium sp.} was improved with the inulin diet and \textit{P. multivesiculatum} can ferment cellulose. Sixty-two percent of total rumen cellulase has been found in the protozoal fraction in normal sheep (Coleman, 1985). Jouany and Senaud (1982) noted that with a diet rich in cellulose and hemicellulose, bacterial cellulolytic activity was improved by the presence of ciliates in the rumen. Therefore, the combined effect of cellu-

### Table 3. Effect of experimental treatments on 16S rDNA gene copy numbers of three predominant ruminal cellulolytic bacteria from liquid and solid fractions in Rusitec fermenters.

| Item                          | Low RDP | High RDP | SEM  | P    |
|-------------------------------|---------|----------|------|------|
|                               | Starch  | Inulin   | Starch| Inulin|
| \textit{F. succinogenes}      | 7.00b   | 7.11a    | 7.32c| 6.25c|
| \textit{R. albus}             | 3.39    | 3.55     | 4.20 | 3.70 |
| \textit{R. flavefaciens}      | 4.71b   | 4.52a    | 5.47c| 4.84c|
| Solid fraction\textsuperscript{a}, log\textsubscript{10} of 16S rDNA gene copy numbers per g digested feed | 9.38    | 9.01     | 9.33 | 8.51 |
| \textit{F. succinogenes}      | 4.42    | 4.30     | 5.64 | 5.50 |
| \textit{R. flavefaciens}      | 7.24b   | 6.30a    | 7.75c| 7.19c|

RDP, rumen degradable protein; NFC, non-fibre carbohydrates. \textsuperscript{a}Dry matter basis. \textsuperscript{b}Within the same row, means without a common superscript letter are significantly different (P<0.05).

### Table 4. Effect of experimental treatments on carboxymethylcellulase and xylanase activity from liquid and solid fraction in Rusitec fermenters.

| Item                          | Low RDP | High RDP | SEM  | P    |
|-------------------------------|---------|----------|------|------|
|                               | Starch  | Inulin   | Starch| Inulin|
| Carboxymethylcellulase        | 0.099   | 0.095    | 0.088| 0.093|
| Xylanase                      | 1.49    | 1.42     | 1.28 | 1.27 |
| Solid fraction                | 1.54    | 1.70     | 1.40 | 2.85 |
| Carboxymethylcellulase        | 12.03   | 11.10    | 10.35| 11.81|
| Xylanase                      | 0.349   | 0.183    | 0.945| 0.946|

RDP, rumen degradable protein; NFC, non-fibre carbohydrates. Carboxymethylcellulase and xylanase activities are as micromoles of glucose or xylose released from the corresponding substrates by 1 mL of ruminal fluid, for liquid fraction, or 1 g of undigested feed (dry matter basis), for solid fraction, in 1 min at 39°C and pH 6.5.
loolytic bacteria and protozoa may be responsible for the NDF digestion in the present study. Increased dietary RDP increased production of total VFA (P=0.014) and methane (P=0.050) (Table 5). Compared with starch treatment, inulin treatment reduced the molar proportion of acetate (P<0.001), the acetate:propionate ratio (P<0.001), and methane production (P=0.006), but increased the butyrate proportion (P<0.001). Significant NFC×RDP interactions were observed for the propionate proportion (P=0.005) and methane:VFA ratio (P=0.005), which did not differ under low RDP conditions, but inulin produced greater propionate proportion and less methane:VFA ratio than starch with increased RDP.

The total amount of VFA in this trial was apparently not modified by dietary NFC type, which may be due to the similar OM disappearance between starch and inulin treatments. However, we observed substantial differences in the relative composition of the VFA and the molar ratio of acetate to butyrate was modified significantly. Compared with starch, inulin treatment reduced the molar proportion of acetate and increased the butyrate proportion, which was in agreement with previous results obtained by Poulsen et al. (2012) in in vitro ruminal fermentations and by Maertens et al. (2004) in rabbits. These results may be due to the fermentation of fructose resulting from inulin degradation. When compared with starch, fructose fermentation in the rumen produce less acetate and more butyrate (Heldt et al., 1999). In the study by Golder et al. (2012), butyrate concentrations were higher for the cathed fed fructose than those fed corn. Increased butyrate may be due to increased lactate production during fructose fermentation or differences in the microbial species responsible for the fermentation of starches and fructose (Heldt et al., 1999; Golder et al., 2012). However, Chamberlain et al. (1993) observed that supplemental fructose in sheep diets did not affect the molar proportions of butyrate compared with starch. The inverse relationship observed between acetate and butyrate is reasonable given that they both are derived from acetyl-CoA. The effect of dietary RDP supplementation on the rumen fermentation parameters has been extensively investigated but remains variable. Increasing dietary RDP did not affect the VFA profile in some studies (Gressley and Armentano, 2007; Hall et al., 2010), but reduced the molar proportion of acetate and butyrate and increased propionate molar proportion in the study by Mathis et al. (2000). Supplemental RDP increased the molar proportion of acetate and reduced that of butyrate in the present study, which is partially consistent with the results observed by McCormick et al. (2001). Although some studies have compared the effect of starch and inulin on the production of methane, results remain inconclusive. Czerkawski and Breckenridge (1969) quantified in vitro that 1.5 g of inulin substrate produced 4.2 mL of methane in the first hour, while starch produced only 0.6 mL of methane. However, Poulsen et al. (2012) found no differences between inulin, wheat and corn starch in methane production rates and total methane production after 48 h. Similar results were also observed in the study by Hindrichsen et al. (2004). In the present study, methane production was lower for inulin compared with starch, which is different from above studies, suggesting that production of methane from inulin fermentation need further research. As expected, increased dietary RDP led to increases (P≤0.046) in various measures of N and efficiency of microbial synthesis (EMS) (P=0.002), expressed as g microbial N/kg OM fermented (Table 6). Significant NFC×RDP interaction (P=0.013) was observed for ammonia N production.

| Item | Low RDP | High RDP | SEM | RDP | NFC | NFC×RDP |
|------|---------|----------|-----|-----|-----|----------|
| Total VFA, mmol/d | 38.4<sup>a</sup> | 34.6<sup>b</sup> | 40.6<sup>a</sup> | 40.0<sup>a</sup> | 1.46 | 0.014 | 0.145 | 0.284 |
| Individual VFA, mol/mol total VFA | | | | | | | | |
| Acetate | 50.3<sup>a</sup> | 41.5<sup>b</sup> | 53.2<sup>a</sup> | 43.8<sup>b</sup> | 0.94 | 0.005 | <0.001 | 0.660 |
| Propionate | 35.2<sup>a,b</sup> | 35.0<sup>ab</sup> | 33.5<sup>b</sup> | 37.0<sup>a</sup> | 0.65 | 0.820 | 0.013 | 0.005 |
| Butyrate | 14.4<sup>c</sup> | 23.6<sup>a</sup> | 13.5<sup>c</sup> | 19.4<sup>a</sup> | 0.78 | 0.002 | <0.001 | 0.066 |
| Acetate:propionate, mol/mol | 1.43<sup>a</sup> | 1.19<sup>c</sup> | 1.59<sup>a</sup> | 1.18<sup>a</sup> | 0.047 | 0.054 | <0.001 | 0.045 |
| Methane, mmol/d | 6.89<sup>a,b</sup> | 6.38<sup>b</sup> | 8.06<sup>a</sup> | 6.69<sup>b</sup> | 0.335 | 0.050 | 0.006 | 0.265 |
| Methane/VFA, mol/mol | 0.18<sup>a,b</sup> | 0.19<sup>ab</sup> | 0.20<sup>a</sup> | 0.17<sup>b</sup> | 0.006 | 0.820 | 0.013 | 0.005 |

RDP, rumen degradable protein; NFC, non-fibre carbohydrates; VFA, volatile fatty acids. *Within the same row, means without a common superscript letter are significantly different (P<0.05).

| Item | Low RDP | High RDP | SEM | RDP | NFC | NFC×RDP |
|------|---------|----------|-----|-----|-----|----------|
| Ammonia N, mg/d | 11.0<sup>a</sup> | 2.1<sup>c</sup> | 11.0<sup>a</sup> | 82.5<sup>a</sup> | 7.66 | <0.001 | <0.001 | 0.013 |
| Total NAN flow, mg/d | 209.9<sup>a</sup> | 220.3<sup>c</sup> | 340.3<sup>a</sup> | 362.6<sup>a</sup> | 7.13 | <0.001 | 0.011 | 0.223 |
| Microbial N flow, mg/d | 81.6<sup>b</sup> | 84.2<sup>a</sup> | 105.2<sup>ab</sup> | 116.9<sup>a</sup> | 6.15 | 0.004 | 0.287 | 0.489 |
| Total microorganisms | | | | | | | | |
| LAM | 30.0 | 35.3 | 37.3 | 38.9 | 2.17 | 0.046 | 0.162 | 0.429 |
| SAM | 51.6<sup>a</sup> | 48.9<sup>b</sup> | 67.9<sup>a</sup> | 78.0<sup>a</sup> | 5.16 | 0.005 | 0.496 | 0.263 |
| EMS, mg | | | | | | | | |
| microbial N/kg OM fermented | 14.7<sup>a</sup> | 14.5<sup>c</sup> | 21.6<sup>a</sup> | 23.2<sup>c</sup> | 1.77 | 0.002 | 0.651 | 0.533 |

RDP, rumen degradable protein; NFC, non-fibre carbohydrates; N, nitrogen; NAN, non-ammonia nitrogen; LAM, liquid-associated microbial pellets; SAM, solid-associated microbial pellets; EMS, efficiency of microbial synthesis; OM, organic matter. *Within the same row, means without a common superscript letter are significantly different (P<0.05).
duction, which did not differ under low RDP conditions, but was lower for inulin treatment than for starch treatment under high RDP conditions. Compared with starch treatment, inulin had greater daily production of NAN (P=0.011). Non-fibre carbohydrate source did not affect daily microbial N flow and EMS.

In the Rusitec system, ammonia N concentration is determined by the protein breakdown and by the ammonia N utilisation of rumen microorganisms. A large increase was observed in the production of ammonia N with supplemental RDP, which is in agreement with previously reported observations (Heldt et al., 1999). This is attributable to the direct provision of ruminally available N from supplemental casein. Supplementation of RDP tended to increase EMS in the present study, which is in agreement with results obtained by Grisswold et al. (2003). The production of ammonia N was lower for inulin than for starch under high RDP conditions in the present study, which is in agreement with previous reports of reduced ammonia accumulation by dietary inulin supplementation (Biggs and Hancock, 1998; Öztürk, 2008). Compared with starch, inulin treatment had numerically but not statistically lower N disappearance and greater microbial N flow in the present study, which maybe contributes to the lower ammonia N production. Inulin reduced the true digestibility of dietary protein or increased non microbial as well as microbial endogenous losses (Gressley and Armentano, 2007). Although some researchers deduced that the decrease in ammonia N concentration in response to inulin inclusion might have been the result of increased incorporation of ammonia into microbial protein (Biggs and Hancock, 1998; Öztürk, 2008, 2009), no direct data on the microbial protein synthesis could be found in these studies. Rosendo et al. (2003) found that compared with starch, inulin stimulated microbial protein synthesis when added to alfalfa NDF but the failure when added to either h Bermudagrass or timothy NDF, suggesting the effect of inulin was affected by dietary ingredients. No significant difference existed between inulin and starch in microbial N synthesis when the experiment diets contained various feed ingredients in the present study. Supplementation of RDP significantly increased microbial N flow including LAM and SAM in the present study, which is in agreement with the continuous culture results obtained by Grisswold et al. (2003). We have hypothesised that inulin is more effective in promoting bacterial growth than starch when rumen available N was sufficient, however, no NFC×RDP interaction was observed for microbial N flow in the present study. These results may be ascribed to the similar digestion characteristics between starch and inulin used in the current study.

Conclusions

Compared with starch, inulin fermentation produced more butyrate and less acetate. Inclusion of inulin in diets appeared to have some negative effects on the growth of F. succinogenes and R. flavefaciens but did not depress the fibrolytic enzyme activities. No significant differences existed between starch and inulin treatments in dietary nutrients digestibilities and microbial N synthesis regardless of dietary RDP, although lower ammonia N production was observed in inulin treatments. Overall, current results suggest that starch and inulin differ in ruminal VFA fermentation but have similar effects on ruminal digestion and microbial synthesis in vitro, although inulin suppressed the growth of partial ruminal cellulolytic bacteria.

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