Effects of degradable protein and non-fibre carbohydrates on microbial growth and fermentation in the rumen simulating fermenter (Rusitec)

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Abstract

A rumen simulation technique (Rusitec) 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 pectin) supplemented with 0 g/d (low RDP) or 1.56 g/d (high RDP) sodium caseinate. Apparent disappearance of dry matter and organic matter was greater for pectin than for starch treatment (P<0.01) with low or high RDP. A NFC × RDP interaction was observed for neutral detergent fibre disappearance (P=0.01), which was lower for pectin than for starch only under low RDP conditions. Compared with starch, pectin treatment increased the copy numbers of Ruminococcus albus (P=0.01) and Ruminococcus flavefaciens (P=0.09), the molar proportion of acetate (P<0.01), the acetate:propionate ratio (P<0.01), and methane production (P<0.01), but reduced the propionate proportion (P<0.01). Increasing dietary RDP increased the production of total VFA (P=0.01), methane (P<0.01), ammonia N (P<0.01), and microbial N (P<0.01). Significant NFC × RDP interaction and interaction tendency were observed for ammonia N production (P=0.01) and daily N flow of total microorganisms (P=0.07), which did not differ under low RDP conditions, but pectin produced greater microbial N and less ammonia N than starch with increased RDP. Results showed NFC type, RDP level, and their interaction affected ruminal fermentation and microbial growth, and under sufficient ruminal degradable N pectin had greater advantage in microbial N synthesis than starch in vitro.

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. However, fermentation of different NFC sources varies in digestion characteristics and the profiles of organic acids produced, which influences the effects of NFC on animal performance. Sucrose and pectin are rapidly fermented in the rumen compared with corn starch (Van Soest et al., 1991; Weishjerg et al., 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 pectin on microbial synthesis, though inconsistent results have also been reported in other studies (Huhtanen, 1988; Chamberlain et al., 1993) but reduced in other studies (Hall and Herejk, 2001; Sannes et al., 2002). This study was approved by the Animal Care and Use Committee of the College of Animal Science and Technology of the Jiangxi Agricultural University.

Materials and methods

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 procedure was per 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 0800 and 2000 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 (14x7 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, and 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 has been incubated for two days was replaced by a new feedbag. 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 approximately 600 mL/d (3.1%/h) was maintained in each fermenter.

**Experimental procedure and sampling**

The experiment had a 2×2 factorial design with two RDP levels, low and high, and two types of NFC including corn starch (Shanghai Jingchun Reagent Co., Ltd., Shanghai, China) and citrus pectin (P9135, Sigma-Aldrich, Shanghai, China). The level of RDP was regulated by adding 0 or 1.56 g/d of sodium caseinate (C8654, Sigma-Aldrich) into the two diets containing corn starch and pectin, respectively. Fermenters in the low RDP and high RDP treatments received approximately 16.0 and 17.6 g of 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 stabilization 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 feedbags, and the following samples were collected. The gas produced was collected in Tedlar bags (Tokyo Deodorant Co., 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 milliliter 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 milliliters of effluent was preserved to determine ammonia nitrogen (N) concentration. The samples were frozen at -40°C until analysis. One feedbag 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), neutral detergent fibre (NDF), acid 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 feedbag, 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. The effluent on day 13 and 14 was collected, mixed and homogenized in a blender. One sample (300 mL) was frozen and lyophilized 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) (Ranilla and Carro, 2003). Approximately 20% of solids content was frozen and lyophilized for determination of DM, NAN, and purines concentration. The bacterial samples from LAM and SAM were lyophilized, ground using a mortar and pestle, and analyzed for N and total purines concentration. On day 15, 4 mL of fermenter fluid as liquid fraction and one feedbag containing undigest feed as solid fraction from each vessel were collected and were frozen at -80°C for deoxyribonucleic acid (DNA) extraction.

**Analytical procedures**

Samples were analyzed for DM by drying at 135°C in an air-flow-type oven for 2 h (AOAC, 1990; method 930.15), for OM by ashing at 550°C for at least 4 h, and for N using the Kjeldahl procedure (AOAC, 1990; method 984.13). The NDF and ADF content in all samples were analyzed according to Van Soest et al. (1991). Heat-stable α-amylase (Sigma A3306, Sigma-Aldrich, Shanghai, China) and sodium sulfite were used for NDF determination. 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,

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

| Diet ingredient supply, g/d | Starch | Pectin | Starch | Pectin |
|----------------------------|--------|--------|--------|--------|
| 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   | -      |
| Pectin§                    | -      | 2.55   | -      | 2.55   |
| Sodium caseinate§          | 1.56   | 1.56   |        |        |

| Nutrients supply, g/d | Starch | Pectin | Starch | Pectin |
|-----------------------|--------|--------|--------|--------|
| DM                    | 16.00  | 16.15  | 17.56  | 17.71  |
| 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   |

Low RDP, low rumen degradable protein; High RDP, high 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, 430 mg; Cu, 650 mg; Se, 30 mg; I, 45 mg; Co, 20 mg; vitamin E, 800 mg; vitamin D, 45,000 U; vitamin A, 120,000 U. §Pectin: the ash content is 5.9%. #Sodium caseinate: the CP content is 90.4%.
and the supernatant fraction was filtered through a 0.45 µm filter. The VFA concentrations in the filtered samples were determined by HPLC (model L-2000; Hitachi High-Technologies Corporation, Tokyo, Japan) with a reversed-phase Agilent TC-C18 column (4.6 mm x 250 mm; 5 µm, Agilent Technologies, Santa Clara, CA) according to Akalin et al. (2002). Crotonic acid was used as an internal standard. The concentration of CH₄ was analysed by GC (model 663-30; Hitachi High-Technologies Corporation) equipped with a flame ionization detector. Total purines in the NAN fraction of the digesta and bacterial pellets were quantified by HPLC (Reynal and Broderick, 2009). The volume of total gas produced was measured by the displacement of water as previously described (Soliva and Hess, 2007).

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 Co., Ltd., Shanghai, China). The bacterial species determined were *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* as representatives of fibrolytic (cellulolytic and hemicellulolytic) species. The 16s ribosomal deoxyribonucleic acid (rDNA) copy numbers of three fibrolytic bacterial species were determined according to Zhao et al. (2014).

### 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 (LAB or SAB). 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 procedure (SAS Inst. Inc., Cary, NC, USA) according to a randomized 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 tests.

### Results and discussion

Data on pH and apparent disappearance of diet are presented in Table 2. The pH before feeding was not affected by dietary treatments and averaged 6.65 across treatments. A NFC x RDP interaction was observed for mean pH over 12 h after the feeding (P=0.01), with greater values found for pectin treatment.

### Table 2. Effects of experimental treatments on pH and apparent disappearance of diet in the Rusitec fermenters.

| Item                      | Low RDP | High RDP | SEM | P             |
|---------------------------|---------|----------|-----|---------------|
|                           | Starch  | Pectin   | Starch | Pectin |         | RDP | NFC | NFC×RDP |
| pH before feeding         | 6.67    | 6.64     | 6.62 | 6.66       | 0.038 | 0.77 | 0.89 | 0.27    |
| pH, 0 to 12 h             | 6.60ab  | 6.58ab   | 6.52b| 6.62a      | 0.027 | 0.33 | 0.05 | 0.09    |
| DM                        | 38.7    | 42.9b    | 40.6c| 48.4a      | 0.88  | <0.01| <0.01| 0.09    |
| OM                        | 39.9    | 44.8b    | 42.0c| 50.3a      | 1.58  | <0.01| <0.01| 0.14    |
| NDF                       | 20.4    | 18.2b    | 17.8c| 19.3ab     | 0.82  | 0.17 | 0.49 | 0.01    |
| ADF                       | 13.1    | 8.8      | 12.7| 11.4       | 1.26  | 0.46 | 0.12 | 0.32    |
| N                         | 38.1    | 39.4b    | 54.5a| 56.5a      | 1.42  | <0.01| 0.27 | 0.82    |

Low RDP: low rumen degradable protein; High RDP: high rumen degradable protein; NFC, non-fibre carbohydrates; DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; N, nitrogen. a-cWithin a row, means without a common superscript letter differ (P≤0.05).

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

| Item                      | Low RDP | High RDP | SEM | P             |
|---------------------------|---------|----------|-----|---------------|
|                           | Starch  | Pectin   | Starch | Pectin |         | RDP | NFC | NFC×RDP |
| Liquid fraction, log₁₀ of 16S rDNA gene copy numbers per mL fermenter liquid | | | | | | | | |
| *F. succinogenes*         | 7.50    | 7.52     | 7.82 | 7.35       | 0.277 | 0.78 | 0.43 | 0.39    |
| *R. albus*                | 4.18ab  | 5.79a    | 4.68c| 5.39b      | 0.263 | 0.49 | <0.01| 0.14    |
| *R. flavefaciens*         | 4.83    | 5.33bc   | 5.64b| 6.27a      | 0.157 | <0.01| 0.01 | 0.70    |
| Solid fraction, log₁₀ of 16S rDNA gene copy numbers per g digested feed | | | | | | | | |
| *F. succinogenes*         | 9.57    | 9.31     | 9.52 | 9.17       | 0.193 | 0.64 | 0.16 | 0.81    |
| *R. albus*                | 4.50ab  | 5.64b    | 5.81ab| 7.25a      | 0.319 | <0.01| 0.01 | 0.65    |
| *R. flavefaciens*         | 7.36ab  | 7.89ab   | 7.88ab| 8.91a      | 0.258 | 0.01 | 0.09 | 0.09    |

rDNA, ribosomal deoxyribonucleic acid; Low RDP, low rumen degradable protein; High RDP, high rumen degradable protein; NFC, non-fibre carbohydrates; * dry matter basis. a-cWithin a row, means without a common superscript letter differ (P≤0.05).
under high RDP conditions. Apparent disappearance of DM and OM was greater for fermenters fed pectin diets than those fed starch diets (P<0.01). Supplementation with casein increased apparent DM, OM, and N disappearance (P<0.01). There were NFC × RDP interactions for apparent disappearance of NDF (P=0.01), which was lower for pectin than for starch treatment under low RDP conditions. Data on cellulolytic bacterial populations in liquid and solid fractions are shown in Table 3. The 16s rDNA copy numbers of *F. succinogenes* both in liquid and solid fractions were not affected by dietary treatments. Increased dietary RDP increased 16s rDNA copy numbers of *R. albus* in solid fraction (P<0.01) and those of *R. Flavefaciens* both in liquid and solid fractions (P<0.01 and P=0.01, respectively). Compared with starch treatments, 16s rDNA copy numbers of *R. albus* and *R. Flavefaciens* both in liquid and solid fractions were greater or tended to be greater (P<0.09) for pectin treatments.

A previous study reported that although pectin substances are readily degraded in the rumen, they do not mimic the pH-lowering effect of starch because they generally are not fermented to lactate (Hall et al., 1998). Ben-Ghedalia et al. (1989) observed that the sheep fed pectin-rich diets maintained a higher rumen pH than the ones fed starch-rich diets. In the present study, higher pH found for pectin treatment under high RDP conditions is in agreement with above results. The apparent disappearance of DM and OM was greater for pectin treatment than for starch treatment, which may relate to the differences in digestibility between pectin and starch. Pectin is a rapidly degraded complex carbohydrate (Van Soest et al., 1991) and is almost completely degraded in the rumen (Hall et al., 1998). However, the *in situ* digestibility of corn starch is only about 60% (Sauvant et al., 2004). Few data are available on the effect of purified pectin on ruminal NDF digestibility. Rosendo et al. (2003) compared the effects of starch, inulin, and pectins on forage NDF digestion kinetics using *in vitro* digestion technique and observed no difference among treatments. Ben-Ghedalia et al. (1989) studied the effects of pectin-rich vs starch-rich diets on quantitative aspects of digestion in sheep and found that the cell walls in the pectin-rich diet were 16% more digestible than in the starch-rich diet. In the present study, the difference in the apparent disappearance of NDF between starch and pectin treatments was affected by dietary RDP. The apparent disappearance of NDF was lower for pectin than for starch treatment when dietary RDP was low, and increasing dietary RDP increased NDF disappearance for pectin and reduced that for starch treatment. The results, however, while not unexpected, remains unexplained, because they were not supported by the observation of fibrolytic bacterial numbers, which were only data related to the NDF disappearance in the present study. The 16s rDNA copy numbers of *R. albus* and *R. Flavefaciens* were increased by increased dietary RDP and were greater for pectin than for starch treatment. Greater numbers of *R. albus* and *R. Flavefaciens* for pectin treatment may be due to their degradation and utilization of pectin (Gradel and Dehority, 1972). Current results suggest that the variation in numbers of three predominant fibrolytic bacteria may be not better indicator for fibre digestibility, because other ruminal microorganism, such as protozoa and fungi populations (Windham and Akin, 1984; Coleman, 1985), may also degrade and utilize feed fibre.

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### Table 4. Effects of experimental treatments on daily production of methane and volatile fatty acids in the Rusitec fermenters.

|                        | Low RDP |                | High RDP |                | SEM | P      |
|------------------------|---------|----------------|----------|----------------|-----|--------|
|                        | Starch  | Starch         | Pectin   | Pectin         |     |        |
|                        |         |                |          |                |     |        |
| Total VFA, mmol/d      | 39.8a   | 40.1a          | 41.4ab   | 48.2a          | 1.83| 0.01   |
| Individual VFA, mol/100 mol total VFA |         |                |          |                |     |        |
| Acetate                | 51.5b   | 56.1b          | 52.9b    | 55.0b          | 0.97| 0.07   |
| Propionate             | 35.4ab  | 32.1ab         | 33.9b    | 30.9b          | 1.24| <0.01  |
| Butyrate               | 13.9    | 11.9           | 13.7     | 13.7           | 0.93| <0.01  |
| Acetate:propionate     | 1.44a   | 1.75a          | 1.56b    | 1.80a          | 0.089| 0.16   |
| Methane, mmol/d        | 7.5b    | 8.0b           | 8.8b     | 10.4a          | 0.52| <0.01  |

VFA, volatile fatty acids; Low RDP, low rumen degradable protein; High RDP, high rumen degradable protein; NFC, non-fibre carbohydrates. *Within a row, means without a common superscript letter differ (P<0.05).*

### Table 5. Effects of experimental treatments on daily production of ammonia and non-ammonia nitrogen, daily nitrogen flow of liquid-associated and solid-associated microorganisms, and efficiency of microbial synthesis in the Rusitec fermenters.

|                        | Low RDP |                | High RDP |                | SEM | P      |
|------------------------|---------|----------------|----------|----------------|-----|--------|
|                        | Starch  |                | Starch   |                |     |        |
|                        |         |                |          |                |     |        |
| Ammonia N, mg/d        | 10.9c   | 10.6c          | 131.2a   | 106.8a         | 9.79| <0.01  |
| Total NAN flow, mg/d   | 205.6b  | 213.0b         | 331.5a   | 331.3a         | 8.89| <0.01  |
| Microbial N flow, mg/d |         |                |          |                |     |        |
| Total microorganisms   | 86.6b   | 92.7b          | 108.5b   | 138.0b         | 5.30| <0.01  |
| LAM                    | 30.0b   | 35.9b          | 40.6b    | 45.8b          | 2.89| <0.01  |
| SAM                    | 56.8b   | 56.9b          | 67.9b    | 92.2b          | 6.18| <0.01  |
| EMS, mg microbial N/g OM fermented | 14.3   | 13.6           | 15.4     | 16.6           | 1.03| 0.05   |

Ammonia N, ammonia nitrogen; NAN, non-ammonia nitrogen; EMS, efficiency of microbial synthesis; Low RDP, low rumen degradable protein; High RDP, high rumen degradable protein; NFC, non-fibre carbohydrates; LAM, liquid-associated microbial pellets; SAM, solid-associated microbial pellets. *Within a row, means without a common superscript letter differ (P<0.05).*
fraction. The discrepancy between the population of fibrolytic bacteria and fibre digestibility was also found in other studies (Barrios-Urdaneta et al., 2003).

Data on the daily production of methane and VFA are presented in Table 4. No NFC × RDP interaction was observed for those data expect for total VFA production (P<0.09), which tended to be greater for pectin treatment under high RDP conditions. Compared with starch treatment, pectin treatment increased the molar proportion of acetate (P<0.01), the acetate:propionate ratio (P<0.01), and methane production (P<0.01), but reduced the propionate proportion (P<0.01). The molar proportion of butyrate was unaffected by dietary factors. Increased dietary RDP increased production of total VFA (P<0.01) and methane (P<0.01). Total VFA and methane production were greater for pectin and high RDP treatments, which is in agreement with the observed increases of feed disappearance. Compared with starch treatment, pectin treatment produced greater molar proportion of acetate and lower proportion of molar proportion, which is consistent with results obtained in previous studies (Marounek et al., 1985; Strobel and Russell, 1986). The methane production was greater for pectin treatment may also relate to the hydrolysis of methyl esters from pectin to produce methanol, which can be exclusively converted into methane and increase the rate of methanogenesis (Pol and Demeyer, 1988).

Data on microbial N, ammonia N and NAN fractions of the digesta are presented in Table 5. As expected, increased dietary RDP led to increases (P<0.05) in the ammonia N production (P<0.01) and the flow of total NAN (P<0.01) and microbial N (P<0.01). Significant NFC RDP interaction (P<0.01) was observed for ammonia N production, which did not differ under low RDP conditions, but was lower for pectin treatment than for starch treatment under high RDP conditions. Carbohydrate source did not affect daily production of NAN and efficiency of microbial synthesis (EMS), expressed as mg microbial N/g OM fermented. There were NFC × RDP interaction tendencies for daily N flow of total microorganisms (P=0.07) and SAM (P=0.10), and the values were greater and tended to be greater for pectin than for starch treatment under high RDP conditions. Daily N flow of LAM was greater (P=0.02) for pectin than for starch treatment.

The daily production of ammonia-N in the fermenters depends on the extent of CP degradation and N uptake by ruminal bacteria. 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 mainly attributable to the direct provision of rumenally available N from supplemental casein. The greater production of ammonia-N in the starch treatment under high RDP conditions may have related to lower observed microbial N synthesis compared with pectin treatment. Ben-Gheldalia et al. (1989) also found that ammonia-N concentration of pectin-rich diets was lower than that of starch-rich diets. The optimal concentrations of ruminal ammonia N for microbial growth are controversial, but 5 mg/dL of ammonia N maximized microbial protein synthesis in vitro in previous research (Satter and Slyter, 1974). The concentrations of ammonia N for starch and pectin treatments were 1.8 and 1.8, respectively, under low RDP conditions and were 22.4 and 16.3 mg/dL, respectively, under high RDP conditions, which suggest that microbial growth may be limited by low supplemental RDP. Greater 16s rDNA copies of R. albus and R. flavefaciens were observed under high RDP conditions partly supported this speculation.

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 Griswold et al. (2003). Compared with starch, pectin produced similar microbial N to several previous studies (Strobel and Russell, 1986; Chester-Jones et al., 1991). However, tendencies of NFC × RDP interaction were observed for microbial N flow in the present study. Microbial N production did not differ under low RDP conditions, but was greater for pectin treatment than for starch treatment when more supplemental RDP was provided. This result may relate to the synchrony between ruminal protein and carbohydrate digestion. Microbial protein synthesis depends largely on the available amount and fermentation rate of carbohydrates and N in the rumen (NRC, 2001). The substrate fermentation rate was roughly proportional to the rate of microbial growth, with more rapidly fermented substrates yielding more microbial mass (Nocek and Russell, 1988). Pectin has a higher rumen degradability rate and extent compared with starch. In addition, diets containing high levels of pectin may have higher efficiency of available N use or create more favourable conditions for microbial utilisation of other carbohydrates (cellulose) in the rumen than that observed with starch diets (Ariza et al., 2001). However, current results contradict Hall and Herejk (2001), in which maximal microbial N yield was greatest for starch compared to pectin in an in vitro fermentation with mixed ruminal microorganisms. These disparities may have resulted from the fact that ammonia-N could be removed from the fermenters by the effluent in the present research, but not in the system used by Hall and Herejk (2001). Therefore, the synchrony between ruminal protein and carbohydrate digestion may not be exhibited in the latter's research, in which the net microbial synthesis between starch and pectin was compared under quiescent conditions. In addition, compared with starch, greater apparent disappearance of OM for pectin treatment in current study also resulted in greater available carbohydrates/energy for microbial synthesis. The efficiency of microbial synthesis expressed as g microbial N/kg OM fermented was not affected by the type of carbohydrate, which is consistent with some studies on sugar beet pulp substitution for starch (Chester-Jones et al., 1991). However, Ariza et al. (2001) reported that EMS was greater for citrus pulp treatment than for hominy feed treatment. Supplementation of RDP tended to increase EMS in the present study, which is in agreement with results obtained by Griswold et al. (2003).

Conclusions

Compared with starch, pectin treatment has greater apparent disappearance of DM and OM and produced more acetate and less propionate. When rumen degradable N was deficient, disappearance of fibre was lower for pectin than for starch treatment and no differences in microbial N production was observed between the two treatments. When rumen degradable N was sufficient, apparent disappearance of fibre did not differ, but microbial N production was greater for pectin than for starch treatment. These results indicate that RDP level, NFC type, and their interaction affected ruminal fermentation and microbial growth, and under sufficient ruminal degradable N and pectin had greater advantage in microbial N synthesis than starch in vitro.

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