Partially replacing cornstarch in a high-concentrate diet with sucrose inhibited the ruminal $\text{trans}-10$ biohydrogenation pathway \textit{in vitro} by changing populations of specific bacteria

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Abstract

Background: The positive influence of replacing dietary starch with sugar on milk fat production has been proposed to be partially attributed to the inhibition of the rumen $\text{trans}-10$ biohydrogenation pathway. However, whether and how sucrose inhibits the rumen $\text{trans}-10$ biohydrogenation pathway remains elusive.

Results: A batch \textit{in vitro} incubation system was used to evaluate effects of replacing cornstarch in a high-concentrate diet (forage to concentrate ratio = 40:60) with 0 (control), 3, 6 and 9 \% of sucrose on rumen fermentation pattern, fatty acid (FA) biohydrogenation pathways and bacterial populations relating to $\text{trans}-11$ to $\text{trans}-10$ biohydrogenation pathways. Replacing dietary cornstarch with sucrose did not alter rumen pH or concentrations of total volatile fatty acids (VFA) in comparison with the control but significantly influenced the profiles of individual VFA. The molar proportions of butyrate and valerate were linearly increased, while that of acetate was quadratically decreased and those of propionate, isobutyrate and isovalerate were linearly decreased with increasing concentrations of sucrose in the diet. Furthermore, replacing cornstarch with sucrose led to a linear decrease in C18:1 \textit{trans}-10, linear increases in the proportions of C18:1 \textit{trans}-11, C18:2n-6 and the ratio of $\text{trans}-11$ to $\text{trans}-10$, and linear decreases in biohydrogenation of C18:2n-6 and C18:3n-3. The abundance of \textit{Butyrivibrio fibrisolvens}, a butyrate and CLA \textit{cis}-9, \textit{trans}-11 producer, was increased with the increasing inclusion of sucrose in the diet, while the population of \textit{Megasphaera elsdenii}, a CLA \textit{trans}-10, \textit{cis}-12 producer, was significantly decreased by all levels of sucrose replacements.

Conclusions: These results indicate that replacing starch in a high-concentrate diet with sucrose increased butyrate production and inhibited the rumen $\text{trans}-10$ biohydrogenation pathway, which was at least partially due to increased abundance of \textit{Butyrivibrio fibrisolvens} and decreased abundance of \textit{Megasphaera elsdenii}.

Keywords: Bacterial populations, Cornstarch, Rumen fermentation, Sucrose, $\text{trans}-10$ shift

Background

Feeding high-concentrate diets is a commonly used strategy to increase energy intake and support high milk yield in the intensive dairy production system. However, it is also a well-known factor inducing rumen acidosis and milk fat depression (MFD). In order to maintain animal energy intake and simultaneously reduce the risks of rumen acidosis and MFD, sugar has been used as an alternative energy source. Partially replacing dietary starch or corn grain with sugar did not decrease rumen pH in several studies [1, 2] or even increased rumen pH in others [3]. More importantly, feeding sugar improved milk fat content and milk fat yield [3, 4]. However, the action mechanism for the observed results is not well understood. For example, it is not known why feeding sugar potentially increases rumen pH despite its
rapid rumen fermentation relative to starch. One plausible reason could be increased production of butyrate by sugar fermentation. However, effects of sugar addition on profiles of rumen volatile fatty acids (VFA) have been considerably variable. Most in vitro studies reported increased production of butyrate by including sugar in the diet [2, 5, 6]. As for in vivo studies, some reported increased butyrate concentrations by sugar-feeding [1, 3], while others showed no influence on concentrations of acetate, propionate, butyrate [7] or all individual VFA [4, 8]. Concentrations of VFA in the rumen reflect a balance between acid production and disappearance. Actual production of butyrate in the rumen is likely underestimated under in vivo conditions [9], due to the faster absorption rate of butyrate than those of acetate and propionate. Thus, in vitro studies might better reflect the actual fermentation characteristics of sugar-containing diets.

The positive influence of sugar on milk fat has been proposed to be partially attributed to inhibition of the rumen trans-10 biohydrogenation pathway [3, 9]. Occurrence of MFD induced by high-concentrate diets is known to be associated with the shift of rumen biohydrogenation pathways from trans-11 to trans-10 [10], which produce trans-10 fatty acids (FA), specifically C18:1 trans-10 and CLA trans-10,cis-12 at the expense of C18:1 trans-11 and CLA cis-9,trans-11. However, direct investigation of sugar on rumen biohydrogenation of FA is limited.

Variations in rumen VFA production and biohydrogenation of FA are associated directly with rumen microflora. However, influence of sugar on rumen microbial populations has not been extensively studied. The lactate-producing (Streptococcus bovis, S. bovis) or utilizing bacteria (Megasphaera elsdenii, M. elsdenii; Propionibacterium acnes, P. acnes) that proliferate in the rumen of animals receiving high-concentrate diets are major CLA trans-10, cis-12 producers [11–13]. In contrast, the well known CLA cis-9,trans-11 producer [14, 15], Butyrivibrio fibrisolvens (B. fibrisolvens), is very sensitive to high-starch diets and its population in the rumen declined considerably with concentrate feeding [16]. It is likely that replacing dietary starch with sugar could inhibit the proliferating of the CLA trans-10, cis-12 producers, reducing the production of the antilipogenic trans-FA and consequently improving milk fat synthesis. Unfortunately, no study, to the best of our knowledge, has investigated the effect of sugar feeding on rumen microbial community, especially the species related to rumen trans-11 to trans-10 biohydrogenation pathways.

The objective of the current in vitro study, therefore, was to evaluate the responses of partially replacing cornstarch in a high-concentrate diet with sucrose on rumen fermentation, biohydrogenation of FA and bacterial populations related to the rumen trans-10 shift.

**Methods**

**Experimental design**

A completely random design was used in an in vitro batch incubation system. Cornstarch in a basal diet (Table 1) with a concentrate to forage rate of 60:40 was replaced by four levels of sucrose: 0 (control), 3, 6 and 9 % of dry matter (DM).

**In vitro incubation and sampling**

The batch incubation was carried out with 1.0 g of the four experimental diets with 30 mL of buffer [17] and 30 mL of rumen fluid at 39 °C, shaking at 165 rpm. The rumen fluid was collected from four ruminally-cannulated dairy goats before morning feeding. The whole procedure was kept under strict anaerobic conditions by gassing the buffer with CO₂ and using resazurin as a redox indicator, flushing the rumen fluid during handling with CO₂ and gassing the headspace of incubation bottles before sealing with CO₂ as well. Samples from each treatment at 0 h incubation were frozen and freeze-dried for FA analyses and biohydrogenation calculation. The incubation periods

**Table 1** Ingredients and chemical composition of the basal diet (DM basis)

| Ingredient               | Content, % |
|--------------------------|------------|
| Alfalfa hay              | 14.50      |
| Corn silage              | 25.50      |
| Corn                     | 28.20      |
| Soybean meal             | 18.60      |
| Cornstarch               | 9.00       |
| Corn gluten meal         | 2.50       |
| Sodium bicarbonate       | 0.40       |
| Mineral and vitamin premix | 0.20     |
| Dicalcium phosphate      | 0.20       |
| Limestone                | 0.46       |
| Salt                     | 0.44       |
| Chemical composition (% DM) |           |
| Organic matter           | 94.3       |
| Crude protein            | 15.5       |
| Non fiber carbohydrates  | 49.9       |
| Neutral detergent fibre  | 25.1       |
| Acid detergent fibre     | 16.6       |
| Starch                   | 38.7       |
| Sugar                    | 3.96       |

1Providing Mn 63 mg, Zn 4,640 mg, Fe 972 mg, Cu 265 mg, Se 155 mg, 1,251 mg, Co 57 mg, nicotinic acid 450 mg, VE 800 mg,VD 450 KIU and VA 1,860 KIU per kilogram

2Calculated by 100 – (% crude protein + % ether extract + % neutral detergent fibre)
Chemical analysis
Nitrogen, DM, acid detergent fibre, ether extract and ash in the basal diet were analyzed according to the methods described by AOAC [18]. Crude protein was determined by multiplying nitrogen by 6.25. Neutral detergent fibre was assayed with a heat stable α-amylase and sodium sulfite according to Van Soest et al. [19], expressed inclusive of residual ash. Non structural carbohydrate was calculated by 100 – (% crude protein + % ether extract + % neutral detergent fibre). Starch was analyzed according to the enzymatic-colorimetric method [20]. Sugar was extracted with ethanol/water solution (v/v = 80:20) and followed by the phenol-sulfuric acid assay [21].

Samples for VFA and ammonia-N analyses were thawed, centrifuged at 20,000 × g for 20 min at 4 °C, and supernatant filtered through a 0.45 μm syringe filter for analysis. The pellets were freeze-dried and weighed for biohydrogenation estimation. VFA was determined by gas chromatography (GC-7890, Agilent Technologies, Hewlett Packard Co., Avondale, PA) fitted with a flame-ionization detector using a 30 m × 0.25 mm × 0.33 μm fused silica column (AE-FFAP; Atech Technologies Co. Ltd., Taipei, Taiwan, China) according to Khorasani et al. [22]. Ammonia-N was determined by colorimetry as described by Fawcett and Scott [23].

The composition of FA was analyzed as described by Sun and Gibbs [24]. Briefly, the freeze-dried samples were directly methylated with 4 mL of 0.5 mol/L NaOH/methanol (15 min at 50 °C) followed by 4 mL of 5 % HCl/methanol (1 h at 50 °C), extracted with 2 mL of heptane and then introduced to the GC equipped with a fused silica capillary column (SP-2560, 100 m × 0.25 mm × 0.2 μm; Supelco Inc., Bellefonte, PA). Nitrogen was used as the carrier gas. The split ratio was 50:1, and the injector and detector were held at 250 °C. The temperature gradient was 166 °C for 39 min, increased by 10.0 °C/min to 240 °C, held for 10 min, increased by 3.0 °C/min to 245 °C, and held for 10 min. C19:0 was used as an internal standard, and ME 61, ME93, BR2, BR3, CLA cis-9, trans-11 and CLA trans-10, cis-12 methyl esters were used as external standards (Larodan Fine Chemicals AB, Malmo, Sweden). Peaks of four C18:1 (trans-4, trans-5, trans-6/7/8, trans-10) and six C18:2 (trans-9, trans-12, cis-9, trans-13, trans-8, cis-13, cis-9, trans-12, trans-9, cis-12, trans-11, cis-15) isomers without commercial external standards were identified by the order of elution with respect to those FA with external standards and interpolation from the peaks reported [25].

Total microbial genomic DNA was extracted according to Kittelmann et al. [26]. Briefly, microbial cells were disrupted by bead-beating and microbial genomic DNA was extracted by phenol-chloroform extraction. DNA was subsequently precipitated by adding 1/10 volume of 3 mol/L sodium acetate (pH 5.2) and 2 volumes of 70 % ice-cold ethanol. The precipitate was then resuspended in 100 μL of an elution buffer (10 mmol/L Tris–HCl, pH 8.5). RNA was eliminated by adding 10 mg/mL of RNase A, and DNA was subsequently cleaned up using the EasyPure PCR purification kit (Transgen, China) and eluted in 50 μL EB. The concentration and purity of the extracted DNA were determined by a NanoDrop spectrophotometer (Thermo Scientific, USA).

Quantitative real-time PCR was performed on a CFX96 Real-Time PCR Detection System (Bio-Rad, USA) to investigate the abundance of B. fibrisolvens, S. bovis, M. elsdenii and P. acnes as described by Li et al. [27]. Briefly, a 25-μL PCR mixture was prepared using 12.5 μL of SYBR Premix Ex TaqII (TAKARA, China), 1 μL of forward primer (10 μmol/L), 1 μL of reverse primer (10 μmol/L), 1 μL of DNA, and 9.5 μL of double-distilled water. The thermal cycling parameters were programmed at 95 °C for 30 s for initial denaturation, 40 cycles of 95 °C for 5 s, and 60 °C for 30 s for primer annealing and product elongation. Fluorescence detection was performed at the end of each extension step. The 16S rRNA gene-targeted primer sets are listed in Table 2. Samples were run as triplicate. Amplicon specificity was determined via melt curve analysis of PCR end products by raising the temperature at the rate of 1 °C per 30 s from 60 to 95 °C. The population sizes of specific bacterial groups were expressed as a percentage relative to the abundance of the general bacterial 16S rRNA gene.

Calculation and statistical analysis
Biohydrogenation of C18:2n-6 and C18:3n-3 was estimated based on disappearance of these fatty acids from 0 h incubation to the end of incubation. Total weights of the residues after incubation were estimated by summing up the freeze-dried weights of contents in the incubation bottles and those pellets from VFA and ammonia-N samples.
Data were analyzed using One-way ANOVA of SPSS 17.0 (SPSS software for Windows, release 17.0, SPSS Inc., Chicago, IL). Tukey’s test was used for multiple comparisons, and orthogonal polynomials were used to test linear and quadratic responses for increasing levels of sucrose in the diet. Significance was declared at $P < 0.05$.

**Results**

**Rumen fermentation**

As shown in Table 3, replacing dietary cornstarch with 3 to 9% of sucrose had no significant influence on rumen pH ($P = 0.971$) and total VFA concentrations ($P = 0.056$) after 6 h in vitro incubation. However, the profiles of individual VFA were affected significantly ($P < 0.05$) by sucrose treatment. The molar proportion of acetate decreased quadratically ($P < 0.001$), while those of propionate, isobutyrate and isovalerate decreased linearly ($P < 0.001$) as the inclusion of sucrose increased. The molar proportion of butyrate increased quadratically ($P < 0.001$) with increasing sucrose addition, while the molar proportion of valerate increased linearly ($P = 0.002$). Concentrations of ammonia-N decreased linearly ($P < 0.001$) with the inclusion of sucrose.

**Fatty acids composition and biohydrogenation**

Effects of replacing dietary cornstarch with sucrose on FA composition and biohydrogenation after 6 h in vitro incubation were presented in Tables 4 and 5. Sucrose inclusion had no effect ($P > 0.05$) on the proportions of the medium-chain FA and long-chain saturated FA (Table 4). However, the proportions of some C18 FA isomers were influenced ($P < 0.05$) (Table 5). For C18:1 isomers, the proportions of C18:1$\text{-trans}\,-5$, C18:1$\text{-trans}\,-10$ and C18:1$\text{-trans}\,-11$ were affected significantly ($P < 0.05$) by dietary treatments. C18:1$\text{-trans}\,-10$ decreased linearly ($P < 0.05$) while C18:1$\text{-trans}\,-11$ increased linearly ($P < 0.05$) with the increasing inclusion of sucrose. For the two major dietary poly-unsaturated FA, the proportion of C18:2 n-
6 was influenced ($P < 0.05$) by inclusion of sucrose, while proportion of C18:3 n-3 was not affected ($P > 0.05$) by the dietary treatment. The ratio of trans-11 to trans-10 was linearly increased ($P > 0.05$) by increasing inclusion of sucrose in the diet. Biohydrogenation of C18:2 n-6 and C18:3 n-3 both decreased linearly ($P < 0.05$) by increasing inclusion of sucrose.

**Bacterial populations**

Replacing dietary cornstarch with sucrose did not influence ($P > 0.05$) the numbers of *B. fibrisolvens*, *M. elsdenii*, *S. bovis* or *P. acnes* after 6 and 12 h incubations (Table 6). Results from 24 h incubation indicated that sucrose inclusion linearly increased ($P = 0.005$) the abundance of *B. fibrisolvens*, whose numbers were greater ($P < 0.05$) in the 6 and 9 % sucrose treatments in comparison with the control and the 3 % treatment. The numbers of *M. elsdenii* after 24 h incubation were lower ($P < 0.05$) in three sucrose treatments compared to the control diet, however, no difference ($P > 0.05$) was observed among these treatments. The 3 % sucrose treatment had greater ($P < 0.05$) *S. bovis* populations than other treatments after 24 h incubation. However, the numbers of *P. acnes* were not affected ($P = 0.227$) by sucrose treatments even after 24 h incubation. The changing patterns of the measured bacterial groups were similar among 4 treatment groups, with the numbers of *B. fibrisolvens*, *S. bovis* and *P. acnes* increased and the number of *M. elsdenii* decreased over the time. However, sucrose inclusion caused larger changes than the control treatment.

**Discussions**

Results of the current study showed significant influences on all individual VFA by partially replacing dietary cornstarch with sucrose, with the molar proportions of butyrate and valerate were significantly increased ($P < 0.05$), while those of acetate, propionate, isobutyrate and isovalerate were significantly decreased ($P < 0.01$). This variation pattern was quite similar to the previous in vitro [2, 6] and in vivo [28] studies, except that the molar proportion of propionate was not affected in the study of Ribeiro et al. [6], while valerate was linearly reduced with the increasing level of dietary sucrose in the study of Vallimont [2]. The increased molar proportion of butyrate observed in the present study was consistent to the earlier in vitro [2, 6] and in vivo [3, 29] studies, confirming that fermentation of sucrose in place of starch increased butyrate production. Ribeiro et al. [6] suggested that the greater butyrate concentration could be attributed to a change in fermentation pathways to accommodate the higher flux of hydrogen from the rapidly fermented sugar source or the synthesis of this acid from lactate by *M. elsdenii*. In our study, the numbers of *M. elsdenii* were lower ($P < 0.05$) in three sucrose treatments after 24 h incubation compared to the control diet and it seems that *M. elsdenii* was not responsible for increased butyrate production. Instead, abundance of *B. fibrisolvens* increased after 24 h incubation, which might be at least partially responsible for increased butyrate production. Doubling ruminal butyrate concentrations by ruminal infusion of butyrate increased the relative abundance of *B. fibrisolvens* by 65 % [30]. In addition, oral administration of *B. fibrosolvens* in mice resulted in a significant increase in the rate of fecal butyrate production [31]. Butyrate is one of the major precursors of milk fat and increase of this acid may help explain the increased milk fat production observed in sugar feeding studies in vivo [3, 8].

The reduced production of branched-chain VFA (isobutyrate and isovalerate) found in the present study was consistent with the earlier studies [1, 6, 7], suggesting the deamination and decarboxylation of branched-chain amino acid formation system might be reduced by sucrose fermentation, because this is the producing

**Table 4** Composition of the medium- and long-chain fatty acid (FA) after 6 h in vitro incubation

| Fatty acid, % of total FA | Sucrose level | SEM 1 | P-value 2 |
|--------------------------|--------------|-------|-----------|
|                          | 0      | 3 %   | 6 %   | 9 %   | T   | L   | Q   |
| C12:0                    | 0.48   | 0.58  | 0.42  | 0.67  | 0.058| 0.507| 0.456| 0.563|
| C13:0 anteiso            | 0.36   | 0.25  | 0.34  | 0.29  | 0.024| 0.409| 0.569| 0.622|
| C13:0                    | 0.21   | 0.18  | 0.26  | 0.23  | 0.015| 0.324| 0.321| 0.970|
| C14:0 iso                | 0.71   | 0.57  | 0.56  | 0.69  | 0.033| 0.214| 0.825| 0.044|
| C14:0                    | 2.62   | 2.28  | 2.19  | 2.62  | 0.090| 0.218| 0.879| 0.044|
| C14:1 trans-9            | 2.21   | 1.83  | 2.12  | 2.19  | 0.087| 0.367| 0.783| 0.209|
| C15:0 anteiso            | 3.12   | 2.53  | 2.33  | 3.15  | 0.167| 0.228| 0.946| 0.047|
| C14:1 cis-9              | 1.85   | 1.61  | 1.42  | 1.65  | 0.073| 0.271| 0.219| 0.122|
| C15:0                    | 1.23   | 1.02  | 1.11  | 1.40  | 0.078| 0.333| 0.375| 0.124|
| C16:0 iso                | 1.22   | 1.04  | 1.01  | 1.12  | 0.040| 0.126| 0.785| 0.022|
| C16:0                    | 27.6   | 25.1  | 25.8  | 26.1  | 0.492| 0.294| 0.366| 0.158|
| C16:1 trans-9            | 0.53   | 0.49  | 0.46  | 0.48  | 0.021| 0.771| 0.395| 0.551|
| C17:0 anteiso            | 1.47   | 1.24  | 1.15  | 1.50  | 0.073| 0.271| 0.982| 0.060|
| C16:1 cis-9              | 0.69   | 0.68  | 0.63  | 0.80  | 0.034| 0.391| 0.358| 0.218|
| C17:0                    | 0.58   | 0.63  | 0.53  | 0.61  | 0.018| 0.251| 0.958| 0.630|
| C18:0 iso                | 0.05   | 0.06  | 0.12  | 0.11  | 0.015| 0.261| 0.077| 0.896|
| C20:0                    | 0.45   | 0.53  | 0.47  | 0.55  | 0.021| 0.229| 0.151| 1.000|
| C20:1 trans-11           | 0.19   | 0.20  | 0.21  | 0.20  | 0.007| 0.771| 0.576| 0.396|
| C22:1 trans-13           | 0.04   | 0.05  | 0.05  | 0.05  | 0.004| 0.470| 0.225| 0.526|
| C24:0                    | 0.36   | 0.60  | 0.50  | 0.65  | 0.051| 0.176| 0.089| 0.648|
| C26:0                    | 0.13   | 0.18  | 0.14  | 0.20  | 0.020| 0.632| 0.388| 0.909|

1 SEM standard error of mean

2 Probability of a significant effect of treatment (T), or linear (L) and quadratic (Q) orthogonal contrasts for dietary sucrose levels
pathway of rumen branched-chain VFA [32]. Besides, *M. elsdenii* were capable of deaminating branched-chain amino acid and producing branched-chain VFA [33], thus, the decreased branched-chain VFA proportion observed by sugar fermentation might be partially due to reduction in the population of *M. elsdenii* in the present study.

The unaffected \( P > 0.05 \) pH and total VFA concentrations (Table 3) observed in the present study were consistent with the earlier findings based on sucrose replacing for starch [1, 2] or supplementing to alfalfa hay [6]. Our results support the suggestion that the rapid disappearance of sugar *per se* does not necessarily mean extensive fermentation, leading to increased acid production and low rumen pH [9]. To our knowledge, studies [29, 34] that reported decreased rumen pH by sucrose inclusion in the diets were not starch replacing experiments, and reasons for reduced rumen pH observed in those studies might be attributed to either extremely low-forage rations (only 10 % of DM) [34], or extremely high sucrose amount (approximately 20 % of DM) dosed to the rumen [29]. Thus, the present results indicated that replacing dietary cornstarch in a high-concentrate diet with up to 9 % of sucrose might not increase the risk of rumen acidosis. Decreased proton production by increased butyrate and valerate production and less carbon provided by sucrose fermentation compared to starch [35] could be the most possible explanations for the unaffected pH observed in the present study. As 1 mole of hexose ferments to 1 mole of butyrate or valerate, or to 2 moles of propionate or acetate [36], the greater butyrate and valerate production in the rumen from feeding sugar would decrease proton production per unit of organic matter fermented in the rumen compared with acetate or propionate production.

To our knowledge, this is the first study investigating the effects of partially replacing dietary starch with sucrose on the rumen *trans*-10 shift with a high-

### Table 5 C18 fatty acids (FA) composition and biohydrogenation of C18:2 n-6 and C18:3 n-3 after 6 h in vitro incubation

| Fatty acid, % of total FA | Sucrose level | SEM† | P-value‡ |
|--------------------------|--------------|------|----------|
| C18:0                    |              |      |          |
| 0                        | 22.6         | 1.146| 0.252    |
| 3                         | 23.2         | 0.011| 0.150    |
| 6                         | 19.3         | 0.020| 0.567    |
| 9                         | 17.7         | 0.135| 0.475    |
| C18:1 *trans*-4           |              |      |          |
| 0.13                      | 0.20         | 0.18 | 0.015    |
| 0.17                      | 0.11         | 0.14 | 0.008    |
| 0.15                      | 0.14         | 0.14 | 0.021    |
| 0.14                      | 0.11         | 0.14 | 0.020    |
| 0.12                      | 0.20         | 0.14 | 0.072    |
| 0.20                      | 0.14         | 0.20 | 0.020    |
| 0.17                      | 0.11         | 0.15 | 0.020    |
| 0.20                      | 0.14         | 0.14 | 0.072    |
| 0.22                      | 0.20         | 0.20 | 0.020    |
| 0.16                      | 0.15         | 0.16 | 0.123    |
| 0.15                      | 0.18         | 0.18 | 0.313    |
| 0.18                      | 0.18         | 0.18 | 0.386    |
| 0.20                      | 0.20         | 0.20 | 0.259    |

Note: Means within the same row with different superscripts differ significantly \((P < 0.05)\)

†SEM = standard error of mean

‡Probability of a significant effect of treatment (T), or linear (L) and quadratic (Q) orthogonal contrasts for dietary sucrose levels

††Trans-11/trans-10 = ratio of total trans-11 C18 isomers (C18:1 *trans*-11 + CLA *cis*-9, *trans*-11) to trans-10 isomers (C18:1 *trans*-10 + CLA *trans*-10, *cis*-12)
concentrate diet. Results of the current study demonstrated that replacing cornstarch in a high-concentrate diet with sucrose inhibited the rumen trans-10 shift by reducing C18:1 trans-10 production and increasing C18:1 trans-11 production. The variations in these two C18:1 trans isomers occurred in the absence of changes in their major C18:2 precursors, CLA trans-10, cis-12 and CLA cis-9, trans-11. The trans-11 to trans-10 ratio in rumen fluid has been considered as an indicator of changed rumen microbial populations [37]. However, although the ratio of trans-11 to trans-10 in the present study increased linearly from 19.1 to 40.0 with the increasing inclusion of sucrose in the diet after 6 h in vitro incubation, we failed to detect any statistical difference in the relative abundance of the four groups of bacteria after 6 or 12 h of incubation. Results from 24 h incubation showed that replacing cornstarch for sucrose substantially increased the populations of rumen CLA cis-9, trans-11 producing bacteria (B. fibrisolvens) and reduced numbers of one species (M. elsdenii) of the reported CLA trans-10, cis-12 producers. These results were in accordance with observed variations in the proportions of C18:1 trans-11 and C18:1 trans-10, indicating that the inhibition of rumen trans-10 shift by sucrose might be mainly related to a shift of these bacteria. It has been suggested that trans-10 shift could result from a dysbiosis in the rumen in favor of trans-10-producing bacteria at the expense of those producing trans-11 or a modification of bacterial activities [38]. Thus, the change of rumen bacteria by sucrose addition could be due to higher activities at the beginning and higher bacterial numbers later.

As a major cellulolytic microorganism and butyrate producer in the rumen, B. fibrisolvens has a high affinity toward maltose and sucrose utilization [39], which could be a possible explanation for the increased number of this bacterium by sucrose inclusion in the diet. However, B. fibrisolvens is also a major microbe related to rumen biohydrogenation [40], and the increase of its population theoretically means greater biohydrogenation of dietary unsaturated FA. However, biohydrogenation of C18:2 n-6 and C18:3 n-3 in the present study decreased linearly with increasing sucrose inclusion in the diet. Similar observation was also reported when sucrose was added to alfalfa hay [6]. Why biohydrogenation of C18:2 n-6 and C18:3 n-3 did not increase in this study warrants further investigation.

### Conclusions
Replacing cornstarch in a high-concentrate diet with sucrose in vitro did not alter rumen pH and total VFA concentration, but increased molar proportions of butyrate and valerate. Sucrose inclusion in the diet inhibited the rumen trans-10 biohydrogenation pathway as evidenced by decreased proportion of C18:1 trans-10 and increased proportion of C18:1 trans-11. Biohydrogenation of C18:2 n-6 and C18:3 n-3 was decreased by sucrose inclusion. These changes may be at least partially due to increased abundance of B. fibrisolvens, and decreased abundance of M. elsdenii.

### Abbreviations
CLA: conjugated linoleic acid; DM: dry matter; FA: fatty acids; MFD: milk fat depression; VFA: volatile fatty acid.
Competition interested

The authors declare that they have no competing interests.

Authors' contributions

XQZ participated in the experimental design, carried out the experimental trial and the paper writing. YPW carried out the whole experimental trial, and helped to draft the manuscript. BC participated in the sampling and measured rumen fermentation parameters. XZ made crucial contributions to the experimental design and finalized the manuscript. All authors read and approved the final manuscript.

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