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Dose-Response of Fruit Oligosaccharides on Rumen Fermentation Parameters, CH_4 Emission and Skatole Content In Vitro

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Abstract: The purpose of this work was to study the dose effects of fruit oligosaccharide (FOS) supplementation on rumen fermentation parameters, methane (CH_4) production and skatole production. The rumen fluid of Hu sheep was collected through their fistula and immediately transferred to the laboratory for rumen fermentation in vitro. The experimental diet was supplemented with 0%, 0.2%, 0.8%, 1.2%, 1.8% and 2.4% FOS in the basal diet. Gas production (GP) and CH_4 production were measured and recorded at 2, 4, 6, 8, 10, 12, 24, 36 and 48 h. After 48 h of fermentation, degradation rates of nutritional components, fermentation parameters and skatole content were determined. The results showed that the GP, the nutrient degradation rates and the fermentation parameters of rumen linearly increased with increasing doses of FOS supplementation ($p < 0.05$). There was a quadratic trend between FOS addition and CH_4 production and skatole content in rumen fluid ($p < 0.05$). We also observed the CH_4 production in the 1.2% FOS-treated group was significantly lower than the other FOS-treated groups. Skatole content of the 0.2%, 0.8% and 1.2% FOS-treated groups were significantly lower than the other FOS-treated groups ($p < 0.05$). Our findings indicated that the effect of FOS on rumen fermentation parameters, CH_4 production and skatole production in vitro was dose-dependent. To improve the digestibility of nutrients and the fermentation parameters of rumen, a higher FOS dosage might be helpful. However, if CH_4 and skatole production is a concern, a dose of FOS at 1.2% is recommended.

Keywords: fructo-oligosaccharides; skatole; methane; rumen fermentation; in vitro

Citation: Wang, L.; Ji, S.; Yan, H.; Li, J.; Zhang, L.; Yan, D.; Duan, C.; Liu, Y.; Zhang, Y. Dose-Response of Fruit Oligosaccharides on Rumen Fermentation Parameters, CH_4 Emission and Skatole Content In Vitro. *Fermentation* **2023**, *9*, 428. https://doi.org/10.3390/fermentation9050428

Academic Editor: Guijie Chen

Received: 30 March 2023
Revised: 24 April 2023
Accepted: 26 April 2023
Published: 28 April 2023

1. Introduction

Rumen fermentation plays an important role in feed degradation and health condition of ruminants [1]. Rumen microorganisms convert low-quality protein and nonprotein nitrogen into high-quality microbial protein (MCP), and fibers into volatile fatty acids (VFAs) [2], thereby providing 50–80% of the total absorbable protein [3] and approximately 75% of the total metabolic energy [4] required for ruminants. Abnormal rumen function can lead to multiple disorders in ruminants such as acidosis and feed accumulation [1]. Targeting improvements in rumen fermentation is an efficient way to enhance feed digestion and health of ruminants.

During rumen fermentation, microbes in the rumen can also convert nutrients into CH_4 and skatole, which are a concern in ruminant agriculture [5]. CH_4 emissions account for about 44% of all greenhouse gases emissions from livestock agriculture [6], previous studies demonstrated that the average yield of CH_4 for dairy cattle under dietary patterns is 304.44 g/d [7] and that for sheep is 9.10 g/d [8]; therefore, reducing methane emissions

is an important goal in ruminant agriculture [9]. Skatole, also known as 3-methylindole, is produced by the degradation of tryptophan by rumen bacteria; tryptophan has moderate toxicity and can cause intestinal diseases, pulmonary edema and emphysema in ruminants [10]. However, skatole is one of the major contributors to the odor of feces and easily deposits in adipose tissue, thus, has a negative role in environmental and meat quality [11,12].

Fruit oligosaccharides (FOS) are a green and safe plant additive purified mainly from Jerusalem artichoke. They accounted for 23.93% of the global total production of prebiotics in 2019. Because of its benefits for animals, low cost and convenient use [13], there is increasing interest in the use of FOS as a feed additive [14,15]. Previous studies demonstrated that FOS could improve rumen fermentation performance, food intake [16], feed utilization efficiency [17], absorption and utilization of protein [18], production performance [19] and antioxidant capacity of ruminants [20]. It also has the ability to regulate the hormone neuroendocrine axis related to fat metabolism by affecting beneficial microorganisms and their metabolites in the intestine [21]. Some studies have shown that FOS supplementation in ruminant diets can also reduce CH_4 production [22] and fecal odor [23]. However, the dose effect of FOS on rumen fermentation on feed digestibility, CH_4 and skatole production is still unclear.

Therefore, this study adopts the method of in vitro rumen fermentation with Hu sheep to investigate the dose-response of FOS on nutrient degradation and CH_4 and skatole production by adding different FOS doses to the diet. The findings in the current experiment might provide a guide for FOS usage in ruminants by demonstrating the impact of FOS on rumen fermentation and environmental issues.

2. Materials and Methods

This study was conducted between December 2021 and August 2022 at the animal center of the College of Animal Science and Technology of Hebei Agricultural University, and the experimental protocol was approved by the Ethical Committee of Hebei Agricultural University (ID: 2021006).

2.1. Experimental Design and Experimental Diet

The Hu sheep (ewe, n = 6) in the current experiment had similar body conditions (2.80 ± 0.35 in body condition score, 49.74 ± 2.51 kg in body weight) and age (1.5 years old), and all were raised at the animal center of the College of Animal Science and Technology of Hebei Agricultural University. All sheep were prepared with a rumen fistula 3 months before the experiment, provided a formula diet following NRC 2007 (body weight 50 kg, daily gain 200 g) at 9:00 a.m. and 5:00 p.m. each day and given free access to fresh water. The composition and nutritional content of the diet was shown in Table 1.

2.2. In Vitro Rumen Fermentation

Rumen contents from six Hu sheep were collected through the fistula before morning feeding, and filtered using four layers of sterile cheesecloth. The fresh fluid was immediately transferred to the laboratory in heated vacuum flasks (39 °C) under anaerobic conditions. The composition of the basal diet for in vitro fermentation is presented in Table 1. The experimental diet was supplemented with 0%, 0.2%, 0.8%, 1.2%, 1.8% and 2.4% FOS in the basal diet.

The rumen fermentation experiment in vitro was performed following the method of Menke [24] and our previous study [25]. Briefly, rumen fluid from six sheep was mixed together, and the rumen fluid was then mixed with the buffer solution (pH = 6.86) at a ratio of 1:2 to achieve artificial rumen fluid. The artificial rumen fluid was then preheated to 39 °C and CO_2 was used to deoxygenate. Then, 1.5 g of feed was accurately weighed, and put into fiber packages with a pore size of 25 μm. Two fiber packages and 300 mL of artificial rumen fluid were added to each plastic incubation bag with a 500 mL capacity (Anscitech Company, Hangzhou, China). All incubation bags (6 treatment × 6 replicates)

were deoxygenated and sealed using a bag vacuum packer (Aodeju Company, Hu-zhou, China) to create anaerobic conditions. Then, the incubation bags were placed in a 39 °C thermostatic water bath (Jerriel Company, Changzhou, China) with a speed of 45 r/min for incubation. The gas production (GP) readings (mL) were measured and recorded by a graduated syringe, and CH_4 production was measured by a CH_4 detector (JQ-AZ-2(T), JingQi Company, Shanghai, China) at 2, 4, 6, 8, 10, 12, 24, 36, and 48 h after incubation. Additionally, three blank incubation bags with rumen fluid and buffer (without feed substrate) were used to correct the GP readings. After 48 h fermentation, all fermentation bags were immediately moved into ice water (0 °C) for 30 min to stop fermentation.

Table 1. Composition and nutrition content of the experimental diet.

Component	Contents
Ingredients, g/kg of DM	
Peanut seedling	344
Corn	508
Soybean meal (CP:44%)	138
Premix compound	10
Total	1000
Nutritive composition	
Metabolic energy, MJ/kg	11.00
CP, %	12.29
NDF, %	34.63
ADF, %	20.56
Ca, %	0.68
P, %	0.28

Note: Premixtures provided 26,000 IUs of Vitamin A, 7200 IUs of Vitamin D, 60 IUs of Vitamin E, 12.5 mg of copper, 160 mg of zinc, 106 mg of iron, 150 mg of manganese, 0.4 mg of selenium, 1.2 mg of iodine and 0.4 mg of cobalt per kilogram of body weight. The metabolic energy are calculated values, and the others are measured values. DM, dry matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; Ca, Calcium; P, Phosphorus.

2.3. Sampling

After fermentation, the fiber package was taken out, washed with distilled water, and dried at 65 °C for 48 h for further analysis. The artificial rumen fluid of each incubation bag was collected into eight 2 mL sterile tubes after fermentation. Two tubes were centrifuged by SIGMA 3K15 Centrifuge (4000× *g*, 15 min, 4 °C, SIGMA, Osterode am Harz, Germany) to obtain a supernatant, and mixed with a meta-phosphoric acid solution (0.2 mL, 250 g/L, 30 min, 4 °C). The mixtures were then centrifuged by SIGMA 3K15 Centrifuge (10,000× *g*, 10 min, 4 °C, SIGMA, Osterode am Harz, Germany) for VFA determination. The other six tubes were centrifuged by SIGMA 3K15 Centrifuge (3000 r·min^{-1}, 10 min, 4 °C, SIGMA, Osterode am Harz, Germany), and the supernatant was collected and stored at −20 °C for NH_3-N (two tubes), MCP (two tubes) and skatole (two tubes) analysis.

2.4. Chemical Analysis

For the diet substrate and undegraded residuals, DM content was determined by drying at 105 °C for 24 h; GE content was determined by an oxygen bomb calorimeter, model No.XRY-1A; CP content was determined by the Kjeldahl nitrogen determination method and NDF and ADF content were determined using a Ringbio fiber analyzer following the AOAC (2016) method.

For the fermented rumen fluid, the pH was measured with pH EL-20 acidometer (Lecurn Fllid Controls Company, Shanghai, China), NH_3-N was measured by phenol-hypochlorite colorimetry [26] and MCP was measured by Coomassie brilliant blue method [22].

VFAs in rumen fluid were measured by gas chromatography [27] (7890A, Agilent, Milton Keynes, UK). Briefly, H_2 was used as the carrier gas with a 30 m × 320 μm × 0.5 μm capillary column (AT-FFAP). The column temperature was set at 1-min hold (60 °C), increased 5 °C·per minute to 120 °C (not held) and then increased 10 °C·per minute to

180 °C. The detector temperature was set at 250 °C, and the injection port temperature was set at 220 °C.

Skatole content in rumen fluid was measured by the 4-Dimethylaminobenzaldehyde (DMAB) colorimetry method [28]. First, the standard curve of skatole solution was prepared: acetone and tris buffer were mixed in a 3:1 volume ratio for 3L as the mixed solution, 3-methylindole was added into the mixed solution with concentrations at 0.2 mg/L, 0.4 mg/L, 0.6 mg/L, 0.8 mg/L and 1 mg/L to achieve standard solutions. To achieve the color reagent, 480 mL 99.9% ethanol, 8 g DMAB, 240 mL H_2SO_4 (75%) and 80 mL distilled water were mixed. After adding 2.84 mL of the color reagent into 2 mL of the standard solution and incubation for 3–5 min with 3 repetitions, absorbance was measured by PowerWaveXS2 (580 nm, Biotek, Winooski, VT, USA). Finally, the skatole content in the rumen fluid was measured by mixing the rumen fluid with the color reagent solution with the proportion of 0.7:1, and incubated for 3–5 min until the absorbance measured by PowerWaveXS2 (580 nm, Biotek, Vermont, USA).

2.5. Data Processing and Analysis

Cumulative GP (or CH_4 production) was calculated using the GP (or CH_4 production) measurements at each time point. The gas production parameters were calculated by using the exponential model with discrete lag time [29] as below: GP (or CH_4 production) = v × (1 − exp(−k × (t − LAG)), where t is the time of fermentation (h), GP is the cumulative gas production (mL), v is the theoretical maximum GP (mL), k is the rate of GP (%/h) and LAG is the discrete lag time. The digestibility of nutrients is calculated as: degradation rate of certain nutrients (%) = [(certain nutrient content before fermentation − certain nutrient content after fermentation)/certain nutrient content before fermentation] × 100%.

All data were processed by Excel 2016, and ANOVA analysis was performed by IBM SPSS Statistics 21.0 (SPSS, Chicago, IL, USA). Multiple comparisons were performed using Duncan's multiple range test, and a significant difference was considered at $p < 0.05$.

3. Results

3.1. Effects of FOS Addition on GP In Vitro Rumen Fermentation

The cumulative GP at 2, 4, 6, 8, 10, 12, 24 and 48 h differed between groups (Figure 1A; $p < 0.05$). The 48 h cumulative GP of the 2.4% FOS-treated group was significantly higher than that of 0%, 0.2%, 0.8% and 1.2% FOS-treated groups (Figure 1B; $p < 0.05$). After calculating the GP parameters using the exponential model with discrete lag times, we observed that the parameters of lag time, potential total GP and GP rate were significantly affected by FOS supplementation. Furthermore, a quadratic trend was observed between lag time and FOS addition ($p < 0.05$; Table 2).

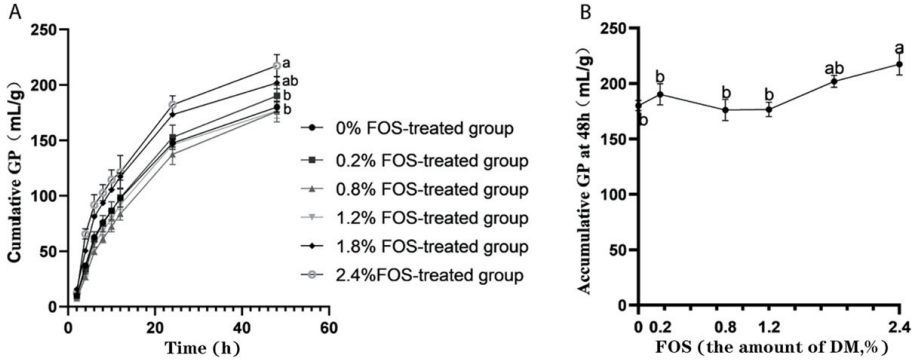

Figure 1. Effects of FOS addition on GP in vitro rumen fermentation. (**A**) dynamic trend in cumulative GP at different time points of rumen fermentation in vitro; (**B**) comparison of 48 h cumulative GP between groups of rumen fermentation in vitro. GP, gas production; FOS, fruit oligosaccharides; DM, dry matter. Different letters in the figure indicated significantly different ($p < 0.05$).

Table 2. Effects of FOS addition on GP parameters in vitro rumen fermentation.

Items	FOS (mg/g DM)						SEM	*p* Value		
	0	0.2	0.8	1.2	1.8	2.4		Trt	L	Q
Lag time, h	0.05 ^{d}	0.18 ^{c}	0.30 ^{a}	0.25 ^{b}	−0.06 ^{e}	−0.38 ^{f}	0.10	<0.01	0.08	0.02
Potential total GP, mL	189.70 ^{b}	204.50 ^{ab}	197.50 ^{b}	190.90 ^{b}	208.31 ^{ab}	221.64 ^{a}	6.48	<0.01	0.25	0.42
GP rate, (%/h)	0.06 ^{ab}	0.06 ^{ab}	0.05 ^{b}	0.06 ^{ab}	0.07 ^{a}	0.07 ^{a}	0.01	<0.01	0.22	0.23

Note: GP, gas production; FOS, fruit oligosaccharides; SEM, standard error of mean; Trt, treatment; L, linear; Q, quadratic. Different superscript letters in the same row indicated significantly different ($p < 0.05$).

3.2. Effects of FOS Addition on CH_4 Production In Vitro Rumen Fermentation

The cumulative CH_4 production at 2, 4, 6, 8, 10, 12, 24 and 48 h differed between groups (Figure 2A; $p < 0.05$). The 48 h cumulative CH_4 production of the 0% FOS-treated group was significantly higher than that of the 0.2%,0.8%,1.2% and 2.4% FOS-treated groups (Figure 2B; $p < 0.05$). In addition, the 1.2% FOS-treated group was significantly lower than that of the 0%, 0.2%, 0.8% and 2.4% FOS-treated groups (Figure 2B; $p < 0.05$). After calculating the CH_4 production parameters using the exponential model with discrete lag times, we observed that the parameters lag time, potential total CH_4 production and CH_4 production rate were significantly affected by FOS supplementation. Furthermore, there was a quadratic trend in potential total CH_4 production associated with FOS addition ($p < 0.05$; Table 3).

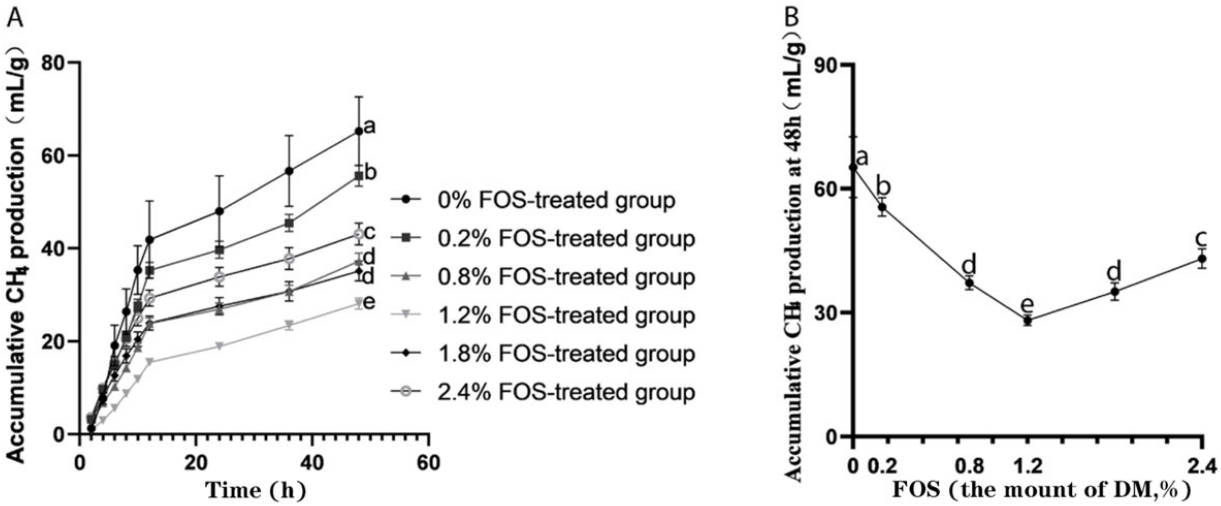

Figure 2. Effects of FOS addition on CH_4 production in vitro rumen fermentation. (**A**) dynamic trend in cumulative CH_4 production at different time points of rumen fermentation in vitro; (**B**) comparison of 48 h cumulative CH_4 production between groups of rumen fermentation in vitro. FOS, fruit oligosaccharides; DM, dry matter. Different letters in the figure indicated significantly different ($p < 0.05$).

Table 3. Effects of FOS addition on CH_4 production parameters in vitro rumen fermentation.

Items	FOS (mg/g DM)						SEM	*p* Value		
	0	0.2	0.8	1.2	1.8	2.4		Trt	L	Q
Lag time, h	0.57 ^{a}	0.29 ^{b}	0.27 ^{b}	0.60 ^{a}	0.17 ^{c}	0.18 ^{c}	0.08	<0.01	0.64	0.44
Potential CH_4 production, mL	66.32 ^{a}	57.17 ^{b}	38.20 ^{d}	31.34 ^{f}	34.82 ^{e}	42.80 ^{c}	5.61	<0.01	0.39	0.02
CH_4 production rate, (%/h)	0.07 ^{ab}	0.06 ^{bc}	0.06 ^{bc}	0.05 ^{c}	0.08 ^{a}	0.08 ^{a}	0.01	<0.01	0.18	0.34

Note: FOS, fruit oligosaccharides; SEM, standard error of mean; Trt, treatment; L, linear; Q, quadratic. Different superscript letters in the same row indicated significantly different ($p < 0.05$).

3.3. Effects of FOS Addition on Nutrient Degradation Rates of Rumen Fermentation

The degradation rate of DM, GE, CP, NDF and ADF increased linearly with increasing FOS additions ($p < 0.05$; Table 4). Degradation rates of CP, NDF and ADF with 1.2%, 1.8% and 2.4% FOS-treated group were significantly higher than those of 0%, 0.2% and 0.8% FOS-treated groups ($p < 0.05$; Table 4).

Table 4. Effects of FOS addition on nutrient degradation rates in vitro rumen fermentation.

Items	FOS (mg/g DM)						SEM	*p* Value		
	0	0.2	0.8	1.2	1.8	2.4		Trt	L	Q
DM	60.3	62.11	60.67	61.41	62.99	63.10	1.26	0.32	<0.01	0.70
GE	54.19	57.98	51.92	59.88	61.92	58.67	0.79	0.82	<0.01	0.95
CP	61.15 ^{b}	59.79 ^{b}	60.70 ^{b}	67.64 ^{a}	71.14 ^{a}	69.58 ^{a}	0.65	<0.01	<0.01	0.39
NDF	41.73 ^{b}	38.42 ^{b}	36.33 ^{b}	47.63 ^{a}	49.08 ^{a}	47.83 ^{a}	1.46	<0.01	<0.01	0.58
ADF	28.88 ^{b}	26.76 ^{c}	22.10 ^{d}	32.87 ^{a}	35.43 ^{a}	35.03 ^{a}	1.01	<0.01	<0.01	0.90

Note: FOS, fruit oligosaccharides; DM, dry matter; GE, gross energy; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; SEM, standard error of the mean; Trt, treatment; L, linear; Q, quadratic. Different superscript letters in the same row indicated significantly different ($p < 0.05$).

3.4. Effects of FOS Addition on the pH, and Contents of NH_3-N and MCP In Vitro Rumen Fermentation

With increasing FOS addition, pH and MCP content both increased linearly, but NH_3-N content decreased linearly ($p < 0.05$; Table 5). The content of MCP in the 2.4% FOS-treated group was significantly higher than that of 0%, 0.2%, 0.8%, 1.2% and 1.8% FOS-treated groups ($p < 0.05$; Table 5).

Table 5. Effects of FOS addition on the pH, contents of NH_3-N and MCP in vitro rumen fermentation.

Items	FOS (mg/g DM)						SEM	*p* Value		
	0	0.2	0.8	1.2	1.8	2.4		Trt	L	Q
pH	5.78	5.82	5.93	5.95	6.03	6.13	1.26	0.47	<0.01	0.64
NH_3-N, mg/dL	18.83	18.76	18.84	18.67	18.66	18.60	0.87	0.82	<0.01	0.80
MCP, μg/mL	56.35 ^{d}	62.93 ^{c}	64.64 ^{bc}	63.57 ^{c}	70.92 ^{ab}	74.73 ^{a}	1.01	<0.01	<0.01	0.33

Note: FOS, fruit oligosaccharides; DM, dry matter; NH_3-N, ammonia-N; MCP, microbial crude protein; SEM, standard error of the mean; Trt, treatment; L, linear; Q, quadratic. Different superscript letters in the same row indicated significantly different ($p < 0.05$).

3.5. Effects of FOS Addition on the Contents of Skatole In Vitro Rumen Fermentation

There was a quadratic trend between FOS addition and skatole content in rumen fluid ($p < 0.05$). Skatole content in 0.2%, 0.8% and 1.2% FOS-treated groups were significantly lower than that of 0%, 1.8% and 2.4% FOS-treated groups (Figure 3; $p < 0.05$).

3.6. Effects of FOS Addition on the Contents of VFA In Vitro Rumen Fermentation

The contents of acetic acid, propionic acid, butyric acid, valeric acid and total VFA linearly increased with increasing FOS addition; howver, acetic acid/propionic acid linearly decreased with increasing FOS addition ($p < 0.05$; Table 6). The contents in the 1.2%, 1.8% and 2.4% FOS-treated groups were significantly higher than those of the 0%, 0.2% and 0.8% FOS-treated groups ($p < 0.05$; Table 6).

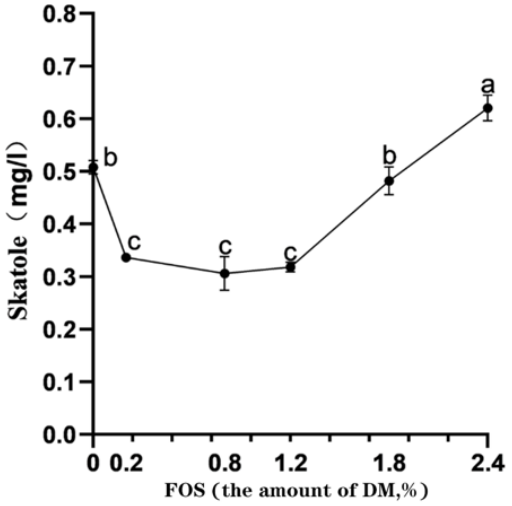

Figure 3. Effects of FOS addition on the contents of skatole in vitro rumen fermentation. FOS, fruit oligosaccharides; DM, dry matter. Different letters in the figure indicated significantly different ($p < 0.05$).

Table 6. Effects of FOS addition on the contents of VFA in vitro rumen fermentation.

Items	FOS (mg/g DM)						SEM	*p* Value		
	0	0.2	0.8	1.2	1.8	2.4		Trt	L	Q
Acetate, mmol/L	21.42 ^{b}	20.38 ^{b}	22.09 ^{ab}	23.60 ^{a}	23.61 ^{a}	23.95 ^{a}	0.34	0.01	0.02	0.10
Propionate, mmol/L	12.98 ^{bc}	12.13 ^{c}	14.02 ^{b}	15.67 ^{a}	15.68 ^{a}	16.05 ^{a}	0.45	0.03	<0.01	0.07
Butyrate, mmol/L	9.95 ^{c}	8.61 ^{d}	11.59 ^{b}	14.20 ^{a}	14.21 ^{a}	14.79 ^{a}	0.50	<0.01	0.01	0.07
Valeric acid, mmol/L	2.07 ^{cd}	1.97 ^{d}	2.19 ^{b}	2.38 ^{ab}	2.39 ^{ab}	2.43 ^{a}	0.04	0.01	0.01	0.07
Total VFA, mmol/L	46.43 ^{cd}	43.09 ^{d}	49.89 ^{bc}	55.86 ^{ab}	55.89 ^{ab}	57.22 ^{a}	1.25	<0.01	0.01	0.08
Acetate/Propionate	1.65 ^{ab}	1.68 ^{a}	1.58 ^{abc}	1.51 ^{bc}	1.51 ^{bc}	1.49 ^{c}	0.03	0.03	0.02	0.09

Note: FOS, fruit oligosaccharides; DM, dry matter; VFA, volatile fatty acid; SEM, standard error of the mean; Trt, treatment; L, linear; Q, quadratic. Different superscript letters in the same row indicated significantly different ($p < 0.05$).

4. Discussion

4.1. Effects of FOS Addition on GP and CH_4 Production In Vitro Rumen Fermentation

Fermentation gas is produced by microbial degradation of carbohydrates and proteins, and is positively correlated with rumen nutrient degradations [30], and CH_4 is one of the major fermentation gases [31]. Previous studies showed that the addition of functional oligosaccharides to the diet could increase GP and reduce CH_4 production in rumen fermentation by changing rumen microflora [32]. Our findings add to these studies by demonstrating that cumulative GP increases linearly with increasing doses of FOS supplementation. This means that a higher dose of FOS in diet might be helpful in promoting the degradation of nutrients in the rumen. Furthermore, cumulative CH_4 production and CH_4 production parameters has a quadratic relationship with the dose of FOS supplementation, and 1.2% FOS supplementation in diet achieved the lowest CH_4 production in the rumen. These findings indicate that different FOS levels should be applied when considering different target indicators in rumen fermentation.

4.2. Effects of FOS Addition on Nutrient Degradation Rates of Rumen Fermentation

Degradation rates of DM, GE, CP, NDF and ADF directly reflect the efficiency of nutrition usage in rumen fermentation [33,34]. Previous studies observed that the addition of oligosaccharides to the diet of sheep could increase the digestibility of DM and CP [35] and improve fiber degradation [36]. But with different FOS supplementation in the current study, we found that FOS significantly affected the degradation rates of CP, NDF and ADF; however, the degradation rates of DM and GE were not affected. Furthermore,

the degradation rates of DM, GE, CP, NDF and ADF linearly increased with the FOS supplementation level increased. These results were also consistent with our findings in gas production, indicating that a higher dose of FOS in diet might be helpful in promoting the degradation of nutrients in the rumen.

4.3. Effects of FOS Addition on the pH, Contents of NH_3-N and MCP In Vitro Rumen Fermentation

The rumen pH reflects in vitro fermentation characteristics and provides a suitable fermentation environment for rumen microorganisms; the optimum pH range is 5.5–7.5 [37]. Rumen pH might be associated with VFAs, lactic acid and NH_3-N content in rumen; the effects of FOS on rumen pH are still in dispute. Garcia et al. (2018) found that the addition of oligosaccharides could significantly increase pH in the rumen by altering the fermentation [38], while Sun et al. (2022) found that the addition of FOS had a tendency to reduce pH by enhancing VFAs production [39]. In current study, we did not observe a significant effect of FOS on rumen pH, but the rumen pH linearly increased as the dose of FOS addition increased.

The NH_3-N and MCP content in rumen fluid reflects the dynamic balance between microorganisms degrading CP and non-protein nitrogen of diet to produce NH_3-N and using NH_3-N to synthesize MCP in the rumen [40,41]. Previous studies demonstrated that the appropriate concentrations of NH_3-N in the rumen of sheep are in the range of 10–50 mg/dL [42,43], which might be conducive to the growth and reproduction of rumen microorganisms [44] which, in turn, produce more MCP [45]. The effects of FOS on rumen NH_3-N is still in dispute as it is the intermediate metabolite of conversion from protein or non-protein nitrogen to MCP [46,47], while a previous study observed that adding FOS to the diet of Holstein calves could increase the MCP content in rumen fluid [48]. Our results showed that FOS had no significant effect on NH_3-N content in the rumen but significantly and linearly increased MCP content with the increasing doses of FOS. These findings indicated that the effect of FOS on MCP production was also dose-dependent, and a higher dose of FOS could efficiently increase the transform rate from diet proteins or non-protein nitrogen to MCP.

4.4. Effects of FOS Addition on the Contents of Skatole In Vitro Rumen Fermentation

Skatole is produced by the degradation of tryptophan by rumen bacteria [10], and is one of the major contributors to the odor of feces and meat quality [11,12]. Previous studies observed that FOS could affect tryptophan degradation by altering anaerobic fermentation [49,50], and some studies also demonstrated that FOS added to the diet could reduce skatole content in the gut of broiler [51,52] or swine [53]. Our study showed that the supplementation of 0.2%, 0.8% and 1.2% FOS in diet could significantly reduce the skatole content in the rumen by 33.33%, 39.22% and 37.26%, respectively, but a higher level of FOS supplementation with 1.8% or 2.4% in diet could then increase the skatole content in the rumen. This finding suggests that FOS has a dose effect on skatole production, in which a lower level of FOS supplementation inhibited the conversion of tryptophan to skatole, but a higher level of FOS supplementation promotes the conversion of tryptophan to skatole; the mechanism needs study further.

4.5. Effects of FOS Addition on the Contents of VFA In Vitro Rumen Fermentation

VFAs which are mainly composed of acetic acid, propionic acid, butyric acid and valeric acid, are the main products of rumen fermentation by degrading diet carbohydrates [54]. VFAs provide about 70~80% of the required energy for ruminants [39], and VFAs content in rumen reflect the efficiency of rumen fermentation [55]. Our study showed that total VFAs, acetic acid, propionic acid, butyric acid and valeric acid content as well as the ratio of acetic acid/propionic acid in the rumen were significantly affected by the FOS addition, and the relationship is an upward linear trend, which was also consistent with our findings in gas production and nutrients degrading rate. These findings suggest

that a higher dose of FOS supplementation might be helpful in improving the efficiency of nutrient degrading and VFA production.

5. Conclusions

With a huge range of FOS dosages added to basal diet from 0% to 2.4%, we observed the effect of FOS on rumen fermentation parameters, CH_4 production and skatole production in vitro was dose-dependent. For improving the digestibility of nutrients, MCP and VFA production, a higher FOS dosage might be helpful, while considering CH_4 and skatole production, a dose of FOS at 1.2% was recommended.

Author Contributions: Conceptualization, S.J., H.Y. and Y.Z.; methodology, L.W., S.J. and H.Y.; validation, S.J. and C.D.; formal analysis, L.W., S.J. and J.L.; investigation, D.Y., L.Z. and Y.L.; resources, L.Z. and Y.L.; data curation, L.W., J.L. and S.J.; writing—original draft preparation, L.W. and S.J.; writing—review and editing, S.J. and H.Y.; supervision, H.Y. and S.J. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by Hebei Natural Science Foundation (C2021204147 and C2022204232), the Science and Technology Project of Hebei Education Department (QN2019134), and the Scientific Research Foundation of Hebei Agricultural University (YJ201846).

Institutional Review Board Statement: The study was approved by the Ethical Committee of Hebei Agricultural University (ID:2021006).

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflict of interest.

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