Effects of Starch Content on Hydrogen and Methane Productions, Rumen Fermentation and Microbial Protein Synthesis During in Vitro Ruminal Culture

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Research

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Abstract

Background: Starch has faster rate of rumen fermentation than fiber, and always causes a rapid increase in ruminal molecular hydrogen (H_2) partial pressure and microbial protein synthesis, which may promote other H_2 sinks to compete H_2 from methanogenesis. The study was designed to investigate the effects of increasing starch content on methane (CH_4), hydrogen gas (gH_2) production, rumen fermentation, metabolic hydrogen ([H]) production, microbial protein (MCP) synthesis through *in vitro* ruminal batch incubation.

Methods: Seven different treatments was prepared by replacing corn straw with corn grain, and starch content were 72, 185, 297, 410, 525, 634 and 747 g/kg DM.

Results: Elevating starch content increased DM degradation ($P_{linear} < 0.001$), and decreased the CH_4 (P_{linear} and $P_{quadratic} < 0.001$) and gH_2 ($P_{linear} < 0.001$) productions relative to DM degraded. Elevating starch content increased VFA concentration ($P_{linear} < 0.001$), propionate molar percentage ($P_{linear} < 0.001$; $P_{quadratic} = 0.001$) and MCP concentration (P_{linear} and $P_{quadratic} < 0.001$), and decreased acetate molar percentage ($P_{linear} < 0.001$), acetate to propionate ratio ($P_{linear} < 0.001$) and estimated net [H] production relative to DM degraded ($P_{linear} < 0.001$). Elevating starch content decreased molar percentage of [H] utilized for CH_4 ($P_{quadratic} = 0.003$) and gH_2 ($P_{linear} < 0.001$) production.

Conclusion: Increasing starch content alters rumen fermentation pathway from acetate to propionate production with reduction in efficiency of [H] production, promotes H_2 utilization with enhanced MCP synthesis and leads to the reduction in efficiency of CH_4 and gH_2 production.

Background

Methane (CH_4) is an important greenhouse gas, which receives great attention worldwide for its impact on global climatic change [1]. Ruminant CH_4 emissions accounts for approximately 17% of the global CH_4 emissions [2]. Furthermore, CH_4 emission is also an important energy loss which represents 2–12% of dietary gross energy and strongly associated with efficiency of ruminants production [3, 4]. Thus, CH_4 mitigation is beneficial to the environment and animal performance.

Molecular hydrogen (H_2) is the precursor of ruminal methanogenesis and mainly produced during the fermentation of carbohydrate to volatile fatty acid (VFA) [5]. Comparing with forage fiber, starch has faster rate of rumen fermentation and ATP production [6] and is always accompanied with a rapid increase in ruminal H_2 partial pressure [7]. Other H_2 sinks, such as reductive acetogenesis, biohydrogenation, propionate production and microbial protein (MCP) synthesis [8], can be promoted and serve as H_2 competitors in the rumen, when ruminal H_2 partial pressure is increased [9]. Studies have investigated relationship between fermentation pathways and methanogenesis [7, 10]. However, few studies have conducted to investigate contribution of H_2 utilization by methanogenesis on the metabolic hydrogen ([H]) generated by VFA production, when different types of carbohydrates was fermented.

We hypothesized that increasing starch content could decrease the contribution of H₂ disposal by methanogenesis, so that fermentation pathway might not be only cause of the decreased CH₄ production in starchy diet. *In vitro* ruminal batch culture was employed, as it is effective method to measure the actual net fermentation products. Increasing starch content was then achieved by replacing corn straw with corn grain. We measured the kinetics of total gas, CH₄ and H₂ gas (gH₂) production, fermentation end products, estimated net [H] production and MCP after 48-h *in vitro* ruminal fermentation.

Materials And Methods

The experiment was approved by the Animal Care Committee, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, China.

Experimental design

The corn grain and corn straw (Table 1) were employed to generate different starch contents for the incubated substrates. Seven treatments were ratios of corn grain to corn straw being 6:0, 5:1, 4:2, 3:3, 2:4, 1:5 and 0:6, and grounded to pass 1-mm aperture sieve. The starch content of seven treatments were 72, 185, 297, 410, 525, 634 and 747 g/kg DM.

Table 1
Chemical compositions of corn grain and corn straw.
Components are expressed in g kg⁻¹ of dry matter (DM).

Item	Corn grain	Corn straw
Organic matter	985	915
Crude protein	76.4	38.7
Neutral detergent fiber	132	792
Acid detergent fiber	34.5	482
Starch	747	72.4
Gross energy (MJ/kg DM)	16.5	14.8

In vitro ruminal batch incubation

Rumen fluid was collected from two of three Xiangdong black goats with permanent rumen cannula before morning feeding. Goats were fed a total mixed diet containing corn straw and concentrated mixture (1:1) containing CP 137 g kg⁻¹ of DM and NDF 380 g kg⁻¹ of DM, respectively. The rumen fluid was filtered through six layers of cheesecloth into a pre-warmed insulated bottle and taken to the laboratory.

About 0.6 g of substrate was accurately weighed into a 135-ml fermentation bottle. Then buffered rumen fluid containing 12-ml rumen fluid and 48-ml McDougall's buffer [11] were added into bottle under a stream of CO₂ at 39.5 °C. Bottles were immediately placed into the automatic incubation system described by Wang
Incubation bottle was in line with gas chromatograph

(GC, Agilent 7890 A, Agilent Inc., Palo Alto, California, USA) via a computer-controlled three way solenoid valve, the released gas was automatically vented into a GC for measuring CH₄ and hydrogen gas concentrations. Gas production (GP), CH₄ and gH₂ accumulations were calculated using the equation equation described by Wang et al. [13].

In vitro ruminal fermentation was stopped at 48 h. About 2 mL of liquid without visible particles were collected from each bottle and centrifuged at 15,000 g for 10 min at 4°C. The supernatant (1.5 mL) was acidified using 0.15 mL of 25% (w/v) metaphosphoric acid, and stored at -20°C for analysis of VFA and ammonia. The pH was measured immediately with a portable pH meter (Starter 300; Ohaus Instruments Co. Ltd., Shanghai, China). About 8 ml of samples were collected for measuring microbial protein after intense shaking of the bottle to ensure that representative portions of liquid and particle fractions. Solid residues were filtered into pre-weighed Gooch filter crucibles, dried at 105°C to constant weight and weighed to determine degradation of incubated substrates.

Each run had four replicates for each treatment. Two bottles were used for measuring pH and DM degradation, and the other two bottles were used for obtaining samples for measuring fermentation end-product and microbial protein. Each run was repeated three times, each on different days, so that each treatment was conducted in triplicate.

Sample analyses

The dry matter (DM) content was determined by drying at 105°C for 24 hours in an oven, and the organic matter (OM) content was determined by ashing at 550°C for 12 hours in a muffle furnace. Gross energy (GE) was measured using an isothermal automatic calorimeter (5EAC8018; Changsha Kaiyuan Instruments Co. Ltd, Changsha, China). The contents of crude protein (CP) ($N \times 6.25$) in feed samples were determined according to procedures of AOAC [14]. The contents of neutral detergent fiber (NDF) and acid detergent fiber (ADF) in feed samples were determined according to the methods described by Van Soest et al. [15] and expressed as inclusive of ash. Heat stable α -amylase was added to for NDF analysis.

Volatile fatty acid concentration was measured according to the procedure described by Wang et al. [16], using a GC (Agilent 7890 A, Agilent Inc., Palo Alto, California, USA). Ammonia concentration was measured colorimetrically according to Chaney and Marbach [17]. Rumen microorganisms was separated from feed particles according to Makkar et al. [18] with filtration (four layers of gauze), shaking (125 rpm/min for 1h) and centrifugation (150 × g for 10 min), and microbial nitrogen production was measured colorimetrically according to Bradford [19], Using a Coomassie brilliant blue kit (Build a biopharmaceutical research institute, Nanjing, China).

Calculations and statistical analysis

The logistic-exponential model [20] was employed to analyze the kinetics of total gas and CH₄ production by using the Nonlinear Regression Analysis Program (NLREG, version 5.4) [21], and was expressed as follows:

$$GP_t = VF \frac{1 - \exp(-kt)}{1 + \exp(b - kt)}$$

Where GP_t is the accumulated gas or CH_4 production at time t (ml/g); VF is the final asymptotic gas or CH_4 production (ml/g); k is the fractional rate of gas or CH_4 production (/h); b is the shape parameter.

The kinetics of gH_2 production (V_{H_2}) was analyzed using the equations provided by Wang et al. [22], which was expressed as follows:

$$V_{H_2t} = \frac{VF_{H_2} \left\{ 1 - \exp \left[-k_{H_2} (t - lag_{H_2}) \right] \right\} \{ 1 + c_{H_2} \exp \left[-\mu_{H_2} (t - lag_{H_2}) \right] \}}{1 + \exp[b_{H_2} - k_{H_2} (t - lag_{H_2})]}$$

Where V_{H_2t} is the accumulated H_2 gas production at time t (ml/g); VF_{H_2} is the final asymptotic H_2 gas volume (mL/g), b_{H_2} and c_{H_2} are shape parameters of H_2 gas curve without dimension, k_{H_2} is the fractional rate of H_2 gas formation (/h), μ_{H_2} is the fractional rate of H_2 gas re-resolution (/h), and lag_{H_2} is discrete lag time (h).

The stoichiometric equations developed by Wang et al. [16] was used to calculate the net [H] production (P_{NH_2} , mM) and estimated [H] production relative to the amount of total VFA produced (R_{NH_2} , mol/100 mol of VFA), which was expressed as follows:

$$P_{NH_2} = 2(\text{Ace} + \text{But} + \text{Isobut}) - (\text{Pro} + \text{Val} + \text{Isoval})$$

$$R_{NH_2} = 100 P_{NH_2} / (\text{Ace} + \text{But} + \text{Isobut} + \text{Pro} + \text{Val} + \text{Isoval})$$

where ace, but, pro, val, isobut and isoval were concentration (mM) of acetate, propionate, valerate, isobutyrate, and isovalerate respectively.

The data were analyzed using general linear model (GLM) with SPSS 26.0 (Chicago, IL, USA), and are presented as mean and SEM. The analytic model included treatment ($n = 7$) as fixed effect and run ($n = 3$) as random effect, and were analyzed for linear or quadratic responses to starch content using orthogonal contrasts. Statistical significance was considered at $P \leq 0.05$ with $0.05 < P \leq 0.10$ considered as a tendency.

Results

Elevating starch content increased DMD ($P_{linear} < 0.001$) and altered kinetic of gas production, with increase in 48-h gas production (P_{linear} and $P_{quadratic} < 0.001$), final asymptotic gas production ($P_{linear} < 0.001$; $P_{quadratic} = 0.007$) and fractional rate of gas production ($P_{linear} < 0.001$; $P_{quadratic} = 0.005$) (Fig. 1A and Table 2). Elevating starch content altered kinetics of CH_4 accumulation, with increase in 48-h CH_4 production (P_{linear} and $P_{quadratic} < 0.001$), final asymptotic CH_4 production ($P_{quadratic} < 0.001$), fractional rate of CH_4 production ($P_{linear} < 0.001$; $P_{quadratic} = 0.007$), and reduction in 48-h CH_4 production relative to DM degraded (P_{linear} and $P_{quadratic} < 0.001$) (Fig. 1B and Table 2). Elevating starch content altered kinetics of gH_2

accumulation, with reduction in 48-h gH₂ production ($P_{linear} = 0.011$; $P_{quadratic} = 0.003$), final asymptotic gH₂ production ($P_{linear} = 0.03$; $P_{quadratic} = 0.002$), and 48-h gH₂ production relative to DM degraded ($P_{linear} < 0.001$), and increase in fractional rate of gH₂ resolution ($P_{linear} = 0.035$) (Fig. 1C and Table 4).

Table 1 Chemical compositions of corn grain and corn straw. Components are expressed in g kg⁻¹ of dry matter (DM).

Item	Corn grain	Corn straw
Organic matter	985	915
Crude protein	76.4	38.7
Neutral detergent fiber	132	792
Acid detergent fiber	34.5	482
Starch	747	72.4
Gross energy (MJ/kg DM)	16.5	14.8

Table 2

Effects of starch content on DMD, FRD₀ and the kinetic parameters of total gas production (GP) after 48-h of *in vitro* ruminal incubation.

Items ¹	Starch content (g/kg)							SEM	P-value	
	72	185	297	410	522	634	747		Linear	Quadratic
DMD (g/kg)	511	579	645	706	777	854	916	8.4	< 0.001	0.592
GP (ml/g DM)	270	305	360	384	412	428	444	5.8	< 0.001	< 0.001
VF _{GP} (ml/g DM)	307	338	384	397	411	418	432	9.4	< 0.001	0.007
k _{GP} (10 ⁻² /h)	5.43	5.22	5.84	6.97	9.65	12.81	16.08	1.059	< 0.001	0.005
¹ DMD = dry matter degradation; VF _{GP} = the final asymptotic volume of total gas production; k _{GP} = the fractional rate of gas production.										

Table 3

Effects of starch content on the kinetic parameters of methane (CH₄) production after 48-h of *in vitro* ruminal incubation.

Items ¹	Starch content (g/kg)							SEM	P-value	
	72	185	297	410	522	634	747		Linear	Quadratic
CH ₄										
ml/g DM	44.8	49.4	53.4	57.7	58.9	57.7	52.5	0.88	< 0.001	< 0.001
ml/g of DDM	88.0	85.6	82.9	81.7	75.8	67.6	57.3	1.71	< 0.001	< 0.001
VF _{CH₄} (ml/g DM)	46.1	53.4	55.2	56.2	56.0	51.9	46.8	1.18	0.970	< 0.001
k _{CH₄} (10 ⁻² /h)	8.40	6.70	7.21	8.48	9.81	12.76	16.54	1.320	< 0.001	0.007
¹ DDM = degraded dry matter; VF _{CH₄} = the final asymptotic volume of CH ₄ production; k _{CH₄} = the fractional rate of CH ₄ production.										

Table 4

Effects of starch content on the kinetic parameters of hydrogen gas (gH₂) production after 48-h of *in vitro* ruminal incubation.

Items ¹	Starch content (g/kg)							SEM	P-value	
	72	185	297	410	522	634	747		Linear	Quadratic
gH ₂										
ml/g DM	0.097	0.110	0.102	0.108	0.102	0.098	0.076	0.005	0.011	0.003
ml/g of DDM	0.19	0.19	0.16	0.15	0.13	0.12	0.08	0.012	< 0.001	0.344
VF _{H₂} (ml/g DM)	0.095	0.110	0.103	0.110	0.104	0.100	0.076	0.006	0.030	0.002
k _{H₂} (10 ⁻² /h)	34.0	43.3	30.3	26.7	27.1	28.5	33.9	5.52	0.281	0.252
μ _{H₂} (h)	4.16	3.89	5.56	5.96	6.68	6.54	8.00	1.429	0.035	0.986
¹ DDM = degraded dry matter; VF _{H₂} = the final asymptotic volume of hydrogen gas production; k _{H₂} = the fractional rate of hydrogen gas production; μ _{H₂} = the fractional rate of hydrogen gas utilization.										

Elevating starch content decreased pH ($P_{linear} < 0.001$) and increased total VFA concentration ($P_{linear} < 0.001$). Elevating starch content decreased acetate ($P_{linear} < 0.001$), butyrate ($P_{quadratic} = 0.002$) and isobutyrate ($P_{quadratic} = 0.02$) molar percentage and acetate to propionate ratio ($P_{linear} < 0.001$), and increased propionate ($P_{linear} < 0.001$; $P_{quadratic} = 0.001$), valerate ($P_{linear} < 0.001$; $P_{quadratic} = 0.009$) and isovalerate ($P_{linear} < 0.001$ and $P_{quadratic} < 0.001$) molar percentage (Table 5). Elevating starch content increased estimated net [H] production ($P_{linear} < 0.001$; $P_{quadratic} = 0.005$), and decreased the estimated net [H] production relative to DM degraded ($P_{linear} < 0.001$) and estimated net [H] production relative to total VFA produced ($P_{linear} < 0.001$; $P_{quadratic} = 0.004$). Elevating starch content decreased molar percentage of [H] utilized for CH₄ ($P_{quadratic} = 0.003$) and gH₂ ($P_{linear} < 0.001$) production. Elevating starch content increased MCP (P_{linear} and $P_{quadratic} < 0.001$) and ammonia concentration ($P_{quadratic} = 0.004$) (Table 6).

Table 5

Effects of starch content on ruminal pH and the profile of volatile fatty acids (VFA) after 48-h of *in vitro* ruminal incubation.

Items	Starch content (g/kg)							SEM	P-value	
	72	185	297	410	522	634	747		Linear	Quadratic
pH	6.58	6.53	6.49	6.44	6.40	6.34	6.27	0.010	< 0.001	0.067
Total VFA (mM)	56.8	64.4	66.3	72.0	79.0	84.9	86.0	1.61	< 0.001	0.490
Molar percentage of individual VFA (mol/100 mol)										
Acetate	71.0	69.0	68.1	66.3	64.4	62.4	60.4	0.35	< 0.001	0.122
Propionate	19.8	20.9	21.5	22.9	24.6	27.0	29.6	0.43	< 0.001	0.001
Butyrate	5.89	6.49	6.52	6.67	6.61	6.30	5.82	0.200	0.647	0.002
Isobutyrate	1.04	1.09	1.10	1.12	1.13	1.07	0.99	0.042	0.445	0.020
Valerate	0.86	0.97	1.04	1.12	1.20	1.22	1.26	0.022	< 0.001	0.009
Isovalerate	1.37	1.60	1.75	1.92	2.04	2.02	1.93	0.042	< 0.001	< 0.001
Acetate to propionate ratio	3.61	3.30	3.17	2.89	2.62	2.32	2.04	0.081	< 0.001	0.361

Table 6

Effects of starch content on P_{NH_2} , R_{NH_2} , molar percentage of metabolic hydrogen ([H]) utilized, ammonia concentration, and MCP after 48-h of *in vitro* ruminal incubation.

Items ¹	Starch content (g/kg)							SEM	P-value	
	72	185	297	410	522	634	747		Linear	Quadratic
P_{NH_2}										
mM	76.1	83.5	84.2	87.9	92.0	92.9	87.4	1.98	< 0.001	0.005
mM/g of DDM	149.7	144.9	130.8	124.3	118.3	108.8	95.4	4.26	< 0.001	0.642
R_{NH_2}	134	130	127	122	116	109	102	1.3	< 0.001	0.004
Molar percentage of [H] utilized (mol/100 mol P_{NH_2})										
CH ₄	49.9	49.5	52.5	53.8	51.7	49.5	47.2	1.28	0.227	0.003
gH ₂	0.053	0.055	0.050	0.050	0.045	0.042	0.034	0.003	< 0.001	0.076
Others	50.1	50.4	47.4	46.1	48.2	50.4	52.8	1.28	0.222	0.003
Ammonia (mM)	17.2	19.0	19.0	21.1	21.0	20.0	17.8	0.90	0.262	0.004
MCP (mg/ml)	0.75	0.73	0.78	0.81	0.84	0.91	1.08	0.022	< 0.001	< 0.001
¹ P_{NH_2} = estimated net [H] production; DDM = degraded dry matter; R_{NH_2} = estimated net [H] production relative to the amount of total VFA produced; gH ₂ = hydrogen gas; MCP = microbial protein.										

Discussion

It's well-known that corn grain and corn straw have different types of carbohydrate components and are rich in starch and fiber respectively. Ruminal degradation rate of carbohydrates depends on their monosaccharide and bond composition, molecular size, sugar arrangement at molecular level and physical morphology [23, 24]. Starch is mainly formed by α -1,4 glycosidic bond and easily hydrolyzed by enzyme [6, 25], while fiber is the major component of cell wall, formed by β -1,4 glycosidic bond, and contains "crystals" which formed by the connection of cellulose macromolecules through hydrogen bonds and resistant to acid and enzyme hydrolysis [26]. Comparing with starch, cellulose and hemicellulose are less susceptible to microbial degradation in rumen [5]. In our study, increasing starch content linearly increased substrate degradation, gas production and fractional rate of gas production during *in vitro* 48-h batch incubation. These results were consistent with previous studies [27, 28], which report that starch has greater and faster rumen degradability in comparison with fiber.

Methane is the end-product during ruminal carbohydrate fermentation [3]. It is not surprising that elevated starch content linearly increased fractional rate of CH_4 production, as starch has faster rate of fermentation than straw fiber. The amount of CH_4 produced is related to the degree of substrate degradation and efficiency of CH_4 produced (i.e. CH_4 produced per unit of substrate degraded) [5]. Starch and fiber fermentation have different efficiency of CH_4 produced. Readily fermentable feed high in starch leads to lower CH_4 production, while slowly fermentable feed in cellulose and hemicellulose causes higher CH_4 production [29–31]. In the present study, the elevated starch content quadratically increased the 48-h and final asymptotic volume of CH_4 production, but linearly decreased amount of CH_4 produced per unit of DM degraded. Although starch had greater fermentation rate than straw fiber, reduced CH_4 production in starchy treatment could be caused by the reduction in efficiency of CH_4 produced.

Hydrogen is produced during carbohydrate fermentation to VFA and mainly consumed by methanogens to produce CH_4 as the end product [32]. The unused H_2 will be evolved from liquid to gas phase in the headspace and vent to air. Normally, the ruminal H_2 partial pressure is very low to facilitate the rumen fermentation [5]. Rooke et al. [33] report that H_2 emitted just accounts for less than 2% of the estimated total H_2 production from fermentation in beef cattle. In our study, both 48-h and final asymptotic gH_2 production was less than 1 ml/g DM, indicating that most of H_2 produced were utilized by methanogens to produce CH_4 . Furthermore, starch and fiber had different profile of gH_2 production. For example, elevated starch content increased fractional rate of gH_2 consumption, although fractional rate of gH_2 production was not altered. Elevated starch content also quadratically decreased the 48-h and final asymptotic volume of gH_2 production and linearly decreased amount of gH_2 produced per unit of DM degraded. We proposed that the starch exhibited lower efficiency of H_2 production than straw fiber, which can be likely to be related to their different pathways of rumen fermentation.

Fermentation of feed rich in starch produces more propionate and butyrate, and less acetate than feed rich in cellulose and hemicellulose [5, 34]. In our study, elevated starch content linearly increased propionate molar percentage and reduced acetate molar percentage and acetate to propionate ratio. Formation of acetate and butyrate from carbohydrates is the net $[\text{H}]$ production, while formation of propionate from pyruvate causes the net $[\text{H}]$ utilization [35]. Elevated starch content linearly decreased estimated net $[\text{H}]$ production per units of DM degraded. Increased estimated net $[\text{H}]$ production could be caused by the greater extent of degradation, when starch content was increased. Furthermore, less than 55% of estimated net $[\text{H}]$ produced was used for CH_4 and gH_2 synthesis. This suggests that a huge amount of $[\text{H}]$ were redirected into other fermentation products other than CH_4 and gH_2 . Elevated starch content quadratically decreased proportion of $[\text{H}]$ produced for CH_4 production, indicating that elevated starch content may enhance $[\text{H}]$ incorporation into other H_2 sinks. Rapid starch fermentation causes a fast increase in H_2 partial pressure [7, 36], which can energetically promote other H_2 utilization pathways, such as microbial protein synthesis, biohydrogenation of unsaturated fatty acid, acetogenesis and etc [9].

Microbial protein can be a alternative ruminal H_2 sink, and synthesized by utilizing ammonia or amino acids in the rumen [37]. Microbial growth and protein synthesis requires utilization of ATP generated during rumen

fermentation [6, 38]. Non-fiber carbohydrates is the major energy substrate for ruminal microorganism, and thus could promote the incorporation of N into MCP synthesis [37, 39]. In our study, elevated starch content linearly increased MCP concentration, leading to a quadratical change in ammonia concentration. Ammonia concentration is determined by the balance between its production and utilization, and thus related to the substrate degradation rate and MCP synthesis. Thus, increasing starch content can result in a more rapidly available energy source for microorganisms, leading to enhanced utilization of ammonia for MCP synthesis [27, 40]. Other studies also proposes that more [H] can be incorporated into microbial biomass when available H_2 is increased [8]. Therefore, in comparison with straw fiber, starch fermentation is beneficial to MCP synthesis, which also contributed to the reduction in methanogenesis in starchy treatment.

Conclusion

In summary, comparing with fiber, starch has faster and greater rumen degradability. Increasing starch content alters rumen fermentation pathway from acetate to propionate production with reduction in efficiency of [H] production, increases MCP synthesis and decreases efficiency of CH_4 and gH_2 production. Such enhanced MCP synthesis is accompanied with increased molar percentage of [H] not for CH_4 and gH_2 production, and thus contributes to reduction in methanogenesis in starchy treatment.

Abbreviations

CH_4 , methane; H_2 , molecular hydrogen; [H], metabolic hydrogen; gH_2 , hydrogen gas; MCP, microbial protein; DMD, dry matter degradation; VF_{GP} , the final asymptotic volume of total gas production; b, shape parameter of gas production; k_{GP} , the fractional rate of gas production; VF_{CH_4} , the final asymptotic volume of CH_4 production; k_{CH_4} , the fractional rate of CH_4 production; DDM, degraded dry matter; VF_{H_2} , the final asymptotic volume of hydrogen gas production; b_{H_2} , shape parameter of hydrogen gas; k_{H_2} , the fractional rate of hydrogen gas production; μ_{H_2} , the fractional rate of hydrogen gas utilization. P_{NH_2} , estimated net [H] production; R_{NH_2} , estimated net [H] production relative to the amount of total VFA produced.

Declarations

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Authors' contributions

SY, XMZ designed the study; SY, XZC, JWZ and CG performed the research; SY analyzed data and wrote the paper; XMZ, MW, CXZ, BL and ZLT contributed to revision of the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on request.

Ethics approval and consent to participate

The experiment was approved by the Animal Care Committee, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, China.

Consent for publication

Not applicable.

Competing interests

The authors declare that no competing interests exist. The manuscript has not been published previously.

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Figures

Figure 1

Effect of starch content on total gas (A), methane (CH₄, B) and hydrogen gas (gH₂, C) production through 48-h in vitro ruminal fermentation. Values are normalized by incubated dry matter (DM) weight.