Effects of Dietary Iodine Content on In Vitro Rumen Fermentation in Yaks (Postprint)

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Abstract

This study aimed to investigate the effects of dietary iodine content on in vitro rumen fermentation in yaks. Using potassium iodide as the additive form and yak diet as the substrate for in vitro fermentation, the substrate iodine contents were 0.1, 0.3, 0.5, 0.7, and 0.9 mg/kg, with a total fermentation period of 48 h. At the end of fermentation, total gas production, rumen fermentation characteristic indices, and digestive enzyme activities were measured. The results showed that when the substrate iodine content was 0.3 mg/kg, the concentrations of microbial crude protein (MCP), acetate (C2), propionate (C3), butyrate (C4), isovalerate (i-C5), valerate (C5), and total volatile fatty acids (TVFA) in the fermentation fluid, as well as the activities of amylase (AMS), lipase (LPS), and trypsin (TYS), all reached their maximum values, being 3.694 g/L, 56.286 mmol/L, 28.906 mmol/L, 9.507 mmol/L, 1.552 mmol/L, 0.919 mmol/L, 97.769 mmol/L, 1.567 U/mL, 0.453 U/mL, and 60.787 U/mL, respectively; when the iodine content was 0.5 mg/kg, dry matter digestibility (DMD) reached its maximum value of 69.39%, which was significantly higher than other treatments ($P<0.05$); at an iodine content of 0.7 mg/kg, cellulase (CLS) activity in the fermentation fluid reached its maximum value of 79.956 U/mL, and the acetate/propionate ratio in the fermentation fluid was the lowest at 1.636. Based on comprehensive analysis of all indices, it was concluded that under in vitro conditions, yak in vitro rumen fermentation was at a relatively high level when the substrate iodine content was 0.3–0.7 mg/kg.

Full Text

Effects of Dietary Iodide Content on Rumen Fermentation of Yaks *in Vitro*

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Abstract

This study investigated the effects of dietary iodide content on rumen fermentation of yaks *in vitro*. Potassium iodide was used as the additive form, with yak diet serving as the fermentation substrate at iodine concentrations of 0.1, 0.3, 0.5, 0.7, and 0.9 mg/kg. Fermentation was conducted for 48 hours, after which total gas production, rumen fermentation characteristics, and digestive enzyme activities were measured. The results demonstrated that when substrate iodine content was 0.3 mg/kg, concentrations of microbial protein (MCP), acetic acid (C2), propionic acid (C3), butyric acid (C4), isovaleric acid (i-C5), valeric acid (C5), and total volatile fatty acids (TVFA), as well as activities of amylase (AMS), lipase (LPS), and trypsin (TYS) in fermentation fluid reached their maximum values at 3.694 g/L, 56.286 mmol/L, 28.906 mmol/L, 9.507 mmol/L, 1.552 mmol/L, 0.919 mmol/L, 97.769 mmol/L, 1.567 U/mL, 0.453 U/mL, and 60.787 U/mL, respectively. When iodine content was 0.5 mg/kg, dry matter digestibility (DMD) peaked at 69.39%, significantly higher than other treatments ($P<0.05$). At 0.7 mg/kg iodine content, cellulase (CLS) activity in fermentation fluid reached its maximum of 79.956 U/mL, while the acetic/propionic acid ratio reached its minimum of 1.636. Based on comprehensive evaluation of all indicators, *in vitro* rumen fermentation of yaks maintained high levels when substrate iodine content ranged from 0.3 to 0.7 mg/kg.

Keywords: yak; potassium iodide; *in vitro* gas production technique; volatile fatty acids; digestive enzyme activity

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Introduction

Yaks are a bovine species that have evolved on the Qinghai-Tibet Plateau above 3,000 m elevation, primarily distributed in high-altitude mountainous regions of Qinghai and Tibet and adjacent alpine and subalpine areas far from coastal zones. They possess strong adaptability to harsh environmental conditions including cold climate, hypoxia, and forage scarcity, serving as irreplaceable productive assets and a pillar industry in alpine pastoral regions [1]. As an important ecological component of alpine grasslands, yaks primarily depend on natural pasture resources for grazing. However, recent years have witnessed intensified overgrazing, grassland degradation, and deepening forage-livestock conflicts [2], resulting in imbalanced nutrient supply for yaks, particularly severe

weight loss during winter, which significantly constrains yak industry development. Moreover, Qinghai and Tibet are regions with severe iodine deficiency, where iodized salt coverage has remained low for extended periods. Dietary surveys indicate that Tibetan populations predominantly consume air-dried yak meat, yak milk, and their products, suggesting alternative iodine intake pathways beyond iodized salt [3]. Consequently, iodine deficiency in yaks profoundly impacts both the industry's development and the health of Tibetan populations, necessitating urgent solutions through in-depth research on yak nutrition, formulation of appropriate feeding standards, and rational supplementation.

While studies on yak nutrition and feeding management have been reported [4-5], research on trace element nutrition in yaks remains scarce. Iodine participates in thyroid hormone synthesis [6], regulates animal metabolism [7], affects growth and development [8], and is an essential trace element for maintaining reproductive performance [9]. Iodine deficiency causes dry skin, loss of hair luster, thickened skin, and even generalized hair loss and fibrosis [10]. Excessive iodine supplementation adversely affects growth and health, yet dietary iodine often exists in a deficient state. Yang [11] noted that iodine is typically marginally deficient in animal diets. Although trace element complexes are commonly added as premixes in practical production to meet animal requirements, this practice, while beneficial, fails to maximize animal growth potential.

Guo et al. [12] reported that an adult cow weighing 600 kg requires 10 mg of iodine daily for thyroid hormone synthesis. Yang et al. [13] indicated that cattle typically require 0.5 mg/kg iodine with a tolerance of 20 mg/kg. According to NRC (2007) standards, iodine supplementation should be 0.25–0.50 mg/kg for cattle and 0.10–0.80 mg/kg for sheep per kg of dietary dry matter (DM), with a tolerance of 50.00 mg/kg for both [14]. Based on Chinese conditions, the appropriate iodine supplementation for cattle and sheep is 0.5 mg/kg DM [14]. Iodine' s physicochemical properties are extremely unstable, readily lost or transformed, making chelated iodine rarely synthesized as an iodine additive for animals. Potassium iodide provides stable iodine forms with accurate content determination and minimal variation. Therefore, this study utilized potassium iodide as the trace element iodine additive form to investigate the effects of substrate iodine concentrations ranging from 0.1 to 0.9 mg/kg on artificial yak rumen fermentation using *in vitro* gas production technology, aiming to determine optimal iodine levels in yak diets and provide references for improving yak feeding standards and scientific supplementation.

Materials and Methods

1.1 Experimental Animals and Management Three healthy adult castrated yaks with similar body condition and fitted with permanent rumen fistulas were selected as rumen fluid donors. The experimental diet consisted of concentrate (without any trace elements) and roughage (oat hay) at a 6:4 ratio,

fed individually twice daily (08:00 and 18:00) with free access to water. Rumen fluid was collected after 15 days of feeding during morning fasting.

1.2 Experimental Design Referencing China' s "Feeding Standard of Beef Cattle" (NY/T 815-2004) and energy and protein requirements for growing yaks [4,15], a basal diet formula for 150 kg yaks with 500 g daily gain was designed. Oat hay served as roughage to prepare fermentation substrate at a 6:4 concentrate-to-roughage ratio. A single-factor experimental design was employed with five treatments, each having three replicates. Potassium iodide (provided by Changsha Xingjia Bioengineering Co., Ltd., purity 77.28%) was added to the fermentation substrate of five treatments to achieve iodine concentrations of 0.1, 0.3, 0.5, 0.7, and 0.9 mg/kg. *In vitro* gas production technology was used to investigate the effects of different substrate iodine concentrations on yak rumen fermentation *in vitro*.

1.3 *In Vitro* Gas Production Technique After 15 days of feeding, rumen fluid was collected from three yaks before morning feeding, mixed, and filtered through four layers of gauze. Artificial rumen fermentation fluid was prepared following Menke et al. [16] with continuous CO infusion to maintain anaerobic conditions. Using a dispensing device, 30 mL of fermentation fluid was injected into each fermentation tube containing 200 mg substrate, then immediately transferred to an artificial rumen incubator [(39.0±0.5)°C, 40 r/min] for cultivation. At 2, 4, 6, 8, 12, 16, 20, 24, 30, 36, and 48 h of incubation, the scale (mL) of the piston in fermentation tubes (special glass syringes, D 89173 Lonsee-Ettlenschie type, Germany, 27 cm length, 3 cm inner diameter, with scale display within 100 mL range, minimum division 1 mL, silicone rubber tubing covering the 3.5 cm extension tube at the syringe front end, secured with special PVC water stop clamps) was quickly read and recorded. After 48 h of fermentation, gas production was measured, fermentation tubes were immediately transferred to an ice-water bath to terminate fermentation, and fermentation fluid was collected and frozen for storage.

1.4 Analytical Methods 1.4.1 Iodine Content

Iodine content was determined following GB/T 13882-2010 [17] using the potassium thiocyanate-nitrite catalytic kinetic method. The standard curve equation obtained in this study was:

Abs = -0.34Conc + 0.4756 (r = 0.9973, n = 5)

where Conc is iodine content (g/mL) and Abs is absorbance at 460 nm.

1.4.2 Total Gas Production, Gas Production Rate, and Dry Matter Digestibility (DMD)

Total gas production, methane production, and gas production rate were calculated based on recorded gas volumes at each time point.

Total gas production (mL) = Total gas production of test tube - Total gas production of blank tube
Methane production (mL) = Total gas production × Percentage of methane
Stage gas production rate (mL/h) = Stage gas production / Time interval

After *in vitro* fermentation, fermentation residues were collected, dried at 105°C for 12-24 h, and DMD was calculated as:

DMD (%) = [(Sample DM weight - Residue DM weight + Blank tube DM weight) / Sample DM weight] × 100

1.4.3 pH, Ammonia Nitrogen (NH -N), and Microbial Protein (MCP) Concentration

pH was measured using a HANNA HI221 benchtop pH meter. NH -N concentration was determined using the modified colorimetric method of Feng et al. [18]. MCP concentration was measured using a kit from Nanjing Jiancheng Bioengineering Institute (Cat. No.: A045-1) via the biuret method. Procedure: Accurately pipette 0.20 mL fermentation fluid, add 0.80 mL physiological saline, homogenize mechanically in an ice-water bath to prepare 20% homogenate, and centrifuge at 646×g for 10 min. Transfer 0.05 mL supernatant to a test tube, add 0.05 mL ultrapure water to blank tube, and 0.05 mL 56.3 g/L protein standard to standard tube. Add biuret reagent (prepared by diluting Reagent 1 powder to 100 mL and Reagent 2 powder to 200 mL with ultrapure water, then mixing diluted Reagents 1 and 2 at 1:2 ratio) 2.50 mL to each tube, mix well, incubate at 37°C for 10 min, and cool under running water. Absorbance was measured at 540 nm using a TU-1810 UV-Vis spectrophotometer (preheated for 30 min) with 1 cm cuvette and zeroed with ultrapure water. MCP concentration was calculated as:

MCP (g/L) = (Am - AK) / (As - AK) × Ck × w

where Am is absorbance of test tube, AK is absorbance of blank tube, As is absorbance of standard tube, Ck is protein standard concentration (56.3 g/L), and w is dilution factor.

1.4.4 VFA Concentration

VFA concentration was determined following references [19-20]. Sample pretreatment: Fermentation fluid was filtered through four layers of gauze, 5 mL was transferred to a clean centrifuge tube and centrifuged at 930×g for 10 min. Two mL of supernatant was transferred to a centrifuge tube, 0.2 mL of 25% metaphosphoric acid solution was accurately added, mixed well, reacted for 10 min, then centrifuged at 14,876×g, 4°C for 10 min. The supernatant was transferred to a new tube and stored at -80°C.

VFA analysis was performed using a Shimadzu 2014 gas chromatograph. Conditions: Flame ionization detector (FID), capillary column (30.00 m × 0.32 mm × 0.50 m); temperature program: initial 60°C, increased to 120°C at 10°C/min,

held for 2 min, then increased to 180°C at 15°C/min, held for 5 min; vaporization temperature 250°C; detection temperature 250°C; injection volume 1 L; carrier gas high-purity nitrogen (99.99%) at 0.7 MPa; detector hydrogen pressure 0.4 MPa; air pressure 0.4 MPa; capillary column pressure 0.6–0.8 MPa; split ratio 40:1.

1.4.5 Digestive Enzyme Activities

Activities of amylase (AMS) (Cat. No.: C016-1), lipase (LPS) (Cat. No.: A054), trypsin (TYS) (Cat. No.: A080-2), and cellulase (CLS) (Cat. No.: A138) were determined using kits from Nanjing Jiancheng Bioengineering Institute.

Unit definitions: One AMS activity unit is defined as the amount of enzyme that hydrolyzes 10 mg starch in 30 min at 37°C; one LPS activity unit is the amount that consumes 1 mol substrate per minute at 37°C; one TYS activity unit is the amount that changes reaction system absorbance (253 nm) by 0.003 per minute at 37°C, pH 8.0; one CLS activity unit is the amount that catalyzes production of 1 g glucose per minute.

1.5 Statistical Analysis Experimental data were analyzed using the ANOVA procedure of SAS 9.0 software for one-way analysis of variance, with Duncan' s multiple comparison test. $P<0.05$ was considered statistically significant. Data are presented as mean ± standard deviation (mean±SD).

Results

2.1 Total Gas Production, Methane Production, pH, and DMD As shown in Table 1 , total gas production, methane production, and DMD exhibited a trend of initial increase followed by decrease with increasing substrate iodine content, while pH showed the opposite trend. Total gas production peaked at 69.0 mL with 0.3 mg/kg iodine content, though differences among treatments were not significant ($P>0.05$). Methane production reached its maximum of 6.80 mL at 0.7 mg/kg iodine content, with no significant differences among treatments ($P>0.05$). pH reached its minimum of 7.08 at 0.3 mg/kg iodine content, with no significant differences among treatments ($P>0.05$), and all treatments maintained pH between 7.08 and 7.40. DMD peaked at 69.39% with 0.5 mg/kg iodine content, significantly higher than at 0.1 mg/kg ($P<0.05$), but not significantly different from 0.3, 0.7, and 0.9 mg/kg treatments ($P>0.05$).

Table 1 Effects of iodine content of substrate on total gas production, methane production, pH, and DMD of *in vitro* rumen fermentation

Items	Iodine content (mg/kg)	Total gas production (mL)	Methane production (mL)	pH	DMD (%)
	0.1	66.0±2.3	4.56±2.05	7.40±0.12	67.12±8.29
	0.3	69.0±0.7	5.68±2.41	7.08±0.39	68.36±2.59

Items	Iodine content (mg/kg)	Total gas production (mL)	Methane production (mL)	pH	DMD (%)
	0.5	65.7±0.8	6.54±0.35	7.17±0.09	69.89±11.23
	0.7	65.5±4.6	6.80±1.15	7.31±0.10	58.00±6.99
	0.9	67.8±2.8	5.78±0.25	7.30±0.06	59.62±10.82

In the same row, values with different letter superscripts mean significant difference (P<0.05), while with no letter or the same letter superscripts mean no significant difference (P>0.05). The same as below.

2.2 Gas Production Rate As shown in Figure 1 [Figure 1: see original paper], gas production rates for all five treatments exhibited an initial increase followed by a decrease with prolonged fermentation time. Gas production rate changed substantially during the initial cultivation stage (0–3 h), reaching maximum at 3 h, then continuously decreasing after 30 h.

Figure 1 Effects of iodine content of substrate on gas production rate of *in vitro* rumen fermentation

2.3 NH -N and MCP Concentration As shown in Table 2 , NH -N concentration in fermentation fluid showed a trend of initial decrease followed by increase with rising substrate iodine content, reaching its highest value of 10.168 mg/dL at 0.1 mg/kg iodine content, significantly higher than at 0.5 mg/kg (P<0.05), but not significantly different from other treatments (P>0.05). MCP concentration peaked at 3.694 g/L with 0.3 mg/kg iodine content, significantly higher than at 0.9 mg/kg (P<0.05), but not significantly different from other treatments (P>0.05).

Table 2 Effects of iodide content of substrate on NH -N and MCP concentrations of *in vitro* rumen fermentation

Items	Iodine content (mg/kg)	NH -N (mg/dL)	MCP (g/L)
	0.1	10.168±0.180	3.068±0.361
	0.3	9.835±1.011	3.694±0.206
	0.5	6.842±1.372	3.217±0.498
	0.7	8.706±1.341	3.098±0.137
	0.9	9.882±1.239	2.889±0.338

2.4 VFA Concentration As shown in Table 3 , concentrations of acetic acid (C2), propionic acid (C3), butyric acid (C4), isovaleric acid (i-C5), valeric acid (C5), and total volatile fatty acids (TVFA) in fermentation fluid all exhibited an initial increase followed by decrease with rising substrate iodine content, while isobutyric acid (i-C4) showed no clear pattern. C2, C3, C4, i-C5, C5, and TVFA concentrations peaked at 56.286, 28.906, 9.507, 1.552, 0.919, and

97.769 mmol/L, respectively, with 0.3 mg/kg iodine content, though differences among treatments were not significant (P>0.05). The acetic/propionic acid ratio showed an initial increase followed by decrease, reaching its minimum of 1.636 at 0.7 mg/kg iodine content, with no significant differences among treatments (P>0.05).

Table 3 Effects of iodide content of substrate on VFA concentrations of *in vitro* rumen fermentation

Items	Iodine content (mg/kg)	C2 (mmol/L)	C3 (mmol/L)	i-C4 (mmol/L)	C4 (mmol/L)	i-C5 (mmol/L)	C5 (mmol/L)	TVFA (mmol/L)	C2/C3
	0.1	41.048±2.364	8.408±0.478	0.719±0.028	3.192±0.517	0.765±0.034	2.904±0.182	56.820±0.318
	0.3	56.286±3.298	8.586±0.499	0.905±0.052	3.572±0.518	0.819±0.037	3.669±0.200	73.937±0.327
	0.5	43.061±2.653	8.871±0.387	0.748±0.032	3.385±0.887	0.747±0.035	2.799±0.183	59.712±0.375
	0.7	40.683±2.475	8.232±0.862	0.826±0.037	3.374±0.268	0.758±0.067	2.777±0.266	57.150±0.083
	0.9	46.189±2.437	8.871±0.723	0.748±0.030	3.293±0.087	0.716±0.080	2.771±0.308	64.788±0.223

2.5 Digestive Enzyme Activities As shown in Table 4 , activities of AMS, LPS, TYS, and CLS in fermentation fluid all exhibited an initial increase followed by decrease with rising substrate iodine content. AMS activity peaked at 1.567 U/mL with 0.3 mg/kg iodine content, significantly higher than at 0.1, 0.7, and 0.9 mg/kg (P<0.05), but not significantly different from 0.5 mg/kg (P>0.05). LPS activity reached its maximum of 0.453 U/mL at 0.3 mg/kg iodine content, though differences among treatments were not significant (P>0.05). TYS activity peaked at 60.787 U/mL with 0.3 mg/kg iodine content, with no significant differences among treatments (P>0.05). CLS activity reached its maximum of 79.956 U/mL at 0.7 mg/kg iodine content, significantly higher than at 0.1 mg/kg (P<0.05), but not significantly different from 0.3, 0.5, and 0.9 mg/kg (P>0.05).

Table 4 Effects of iodide content of substrate on digestive enzyme activities of *in vitro* rumen fermentation

Items	Iodine content (mg/kg)	AMS (U/mL)	LPS (U/mL)	TYS (U/mL)	CLS (U/mL)
	0.1	1.311±0.048	0.333±0.026	46.759±8.099	53.304±2.007
	0.3	1.567±0.051	0.453±0.053	60.787±12.586	68.368±1.175
	0.5	1.472±0.005	0.444±0.053	37.407±16.198	75.321±1.175
	0.7	1.294±0.196	0.419±0.030	18.704±8.099	79.956±3.953
	0.9	1.297±0.038	0.316±0.141	37.407±16.198	70.686±2.237

Discussion

In vitro fermentation gas production is an important indicator reflecting digestible nutrient content in ruminant fermentation substrates and rumen microbial metabolic status, often positively correlated with nutrient digestibility [15,21-22]. In this study, total gas production and methane production exhibited initial increase followed by decrease with rising iodine content when potassium iodide was used as additive, with total gas production peaking at 69.0 mL at 0.3 mg/kg substrate iodine content, and methane production remaining at relatively high levels of 6.80 mL at 0.5–0.7 mg/kg, reaching maximum at 0.7 mg/kg. These results indicate that potassium iodide supplementation can increase *in vitro* fermentation gas production and benefit rumen fermentation, consistent with findings by Wang [23].

Gas production rate showed a unimodal trend of initial increase followed by decrease, with rapid gas production in early fermentation due to readily utilizable soluble sugars in the substrate. As fermentation progressed, the rate gradually decreased, likely due to diminishing fermentable components, eventually approaching zero. pH comprehensively reflects rumen fermentation and is influenced by substrate type and organic acid precipitation [24]. Studies show that pH effects on rumen microbial fermentation depend on the pH range [25]; only when pH remains within normal ranges can rumen fermentation and feed degradation proceed normally. Under normal physiological conditions, rumen fluid pH in ruminants is approximately 5.6–7.5. Kopecny et al. [26] proposed that optimal pH for rumen microbial hydrolases is 5.5–7.0, while other research indicates ideal rumen pH is weakly acidic to neutral, at 6.4–6.8 [27]. In this study, *in vitro* fermentation pH across different potassium iodide concentrations ranged from 7.08 to 7.40, within the normal range.

DMD varies among different diets and represents the extent of dietary degradation by rumen microorganisms. In this study, DMD showed an initial increase followed by decrease with rising iodine content, remaining at relatively high levels at 0.5–0.7 mg/kg, indicating that diets are readily fermentable and degradable by rumen microorganisms at these iodine concentrations. Activities of AMS, LPS, and TYS peaked at 0.3 mg/kg iodine content, while CLS activity peaked at 0.7 mg/kg, with rumen digestive enzyme activities generally consistent with DMD changes. Therefore, from the perspectives of DMD and rumen digestive enzyme activities, iodine concentrations of 0.5–0.7 mg/kg appear optimal for microbial attachment and growth, most conducive to dietary degradation. The yak rumen digestive enzyme activities measured in this study were generally comparable to those reported by Zhang et al. [28] for calf AMS activity (0.29–1.74 U/mL) and Liu et al. [29] for dairy cow CLS activity (71.43–99.05 U/mL). Wang [30] reported TYS activity in sheep rumen as 18.09–24.56 U/mL, while this study measured TYS activity of 18.704–60.787 U/mL, possibly due to TYS activity being related to rumen pH, protein deposition rate, and protein content.

Rumen NH -N reflects the dynamic balance between dietary protein degradation, ammonia generation from microbial nitrogen source degradation, and ammonia utilization [31]. Murphy et al. [32] reported optimal NH -N concentration for microbial fermentation as 6.3–27.5 mg/dL, while Slyter [33] indicated rumen fluid NH -N ranges from 0.35–29 mg/dL. In this study, fermentation fluid NH -N concentrations across five treatments ranged from 6.842 to 10.168 mg/dL, within the normal range, showing an initial decrease followed by increase with rising substrate iodine content, peaking at 10.168 mg/dL with 0.1 mg/kg iodine addition. Thus, from the perspective of fermentation fluid NH -N concentration alone, microbial ability to degrade feed protein showed an initial decrease followed by increase with rising substrate iodine content. MCP reflects rumen microbial population and activity and is the primary nitrogen source provider for ruminants, supplying 40%–60% of protein requirements. In this study, fermentation fluid MCP concentration exhibited an initial increase followed by decrease with rising substrate iodine content, remaining at relatively high levels at 0.3–0.7 mg/kg. This suggests that substrate iodine content of 0.3–0.7 mg/kg favors rumen microbial population and activity for MCP synthesis; lower iodine content may reduce fermentable substrate utilization efficiency due to asynchronous ammonia and energy release, resulting in weaker MCP synthesis capacity and lower MCP concentration. Considering both fermentation fluid NH -N and MCP concentrations, NH -N showed an initial decrease followed by increase, while MCP showed the opposite trend with rising substrate iodine content, possibly because MCP synthesis consumes substantial NH -N, resulting in lower NH -N concentrations when MCP content is high.

VFA is the primary energy source for ruminant metabolism [19], providing 60–80% of digestible energy [34]. In this study, although differences in C2, C3, i-C4, C4, i-C5, C5, and TVFA concentrations among different iodine treatments were not significant, they generally showed initial increase followed by decrease with rising substrate iodine content, remaining at relatively high levels at 0.3–0.9 mg/kg. The acetic/propionic acid ratio was not significantly different among treatments but reached its minimum of 1.636 at 0.7 mg/kg iodine content. The carbohydrate fermentation level in ruminant rumen fermentation is reflected by the acetic/propionic acid ratio; lower values indicate a propionate-type fermentation pattern, beneficial for dietary energy utilization. In this study, the acetic/propionic acid ratio reached its minimum at 0.7 mg/kg iodine content. Therefore, from the perspective of VFA concentration, iodine content of 0.3–0.9 mg/kg favors rumen fermentation energy production and benefits yak growth.

Under *in vitro* conditions, for growing yaks supplemented with potassium iodide as the iodine source, rumen fermentation and dietary degradation remain at relatively high levels when dietary iodine content ranges from 0.3 to 0.7 mg/kg.

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Note: Figure translations are in progress. See original paper for figures.