

Fully Automatic In Vitro Rumen Fermentation System: Venting Pressure Below 19 kPa Has No Effect on Fermentation Characteristics Postprint

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Abstract

This experiment aimed to investigate the effects of different venting pressures on the characteristics of in vitro simulated rumen fermentation. Three adult Xiangdong black goats with similar body weight, fitted with permanent rumen fistulas, were used as rumen fluid donors. Using an automatic in vitro rumen fermentation simulation system, six fermentation substrates were subjected to 48 h of in vitro fermentation to examine the effects of three venting pressures (9, 14, and 19 kPa) on fermentation parameters including total gas production, methane production, gas production parameters, dry matter disappearance rate, fermentation fluid pH, and volatile fatty acid production. The results showed that fermentation substrate had a significant effect on in vitro simulated rumen fermentation parameters ($P < 0.05$), while venting pressure had no significant effect ($P > 0.05$). Therefore, it can be concluded that increasing venting pressure from 9 kPa to 19 kPa had no effect on the characteristics of in vitro simulated rumen fermentation.

Full Text

Effects of Venting Pressure on In Vitro Rumen Fermentation Characteristics

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Abstract: This study investigated the effects of different venting pressures on in vitro fermentation characteristics. Rumen fluid was collected from three adult Xiangdong black goats of similar weight fitted with permanent rumen fistulas. Using an automated in vitro incubation system, six diverse substrates were selected for 48-hour in vitro fermentation under three venting pressures (9, 14, and 19 kPa) to examine the effects on total gas production, methane production, in vitro dry matter disappearance (IVDMD), fermentation fluid pH, and volatile fatty acid (VFA) production. The results demonstrated that fermentation substrate had significant effects on in vitro rumen fermentation parameters ($P < 0.05$); however, venting pressure showed no significant effects on in vitro fermentation parameters ($P > 0.05$). In conclusion, increasing venting pressure from 9 kPa to 19 kPa does not affect fermentation characteristics.

Keywords: venting pressure; in vitro fermentation; gas production; volatile fatty acids

1. Materials and Methods

1.1 Substrates and Experimental Design Six widely varied fermentation substrates were selected: endive, abalone leaves, mulberry leaves, corn flour, and wheat middling. The nutrient composition of these substrates is presented in Table 1. The experiment employed a completely randomized design with a 6×3 factorial arrangement (six substrates \times three venting pressures), with each treatment replicated three times.

1.2 In Vitro Fermentation Procedure Rumen fluid was collected from three adult Xiangdong black goats (20 ± 2.5 kg) fitted with permanent rumen fistulas, before morning feeding. The fluid was filtered through four layers of cheesecloth and mixed with buffer solution at a 1:4 ratio under continuous flushing with CO_2 . Approximately 60 mL of the mixed fermentation fluid was transferred into each fermentation bottle containing 0.6 g of substrate. The automated incubation system maintained fermentation at 39.5°C with continuous shaking at 50 r/min for 48 hours. Venting pressures of 9, 14, and 19 kPa were applied according to the experimental design.

1.3 Sample Collection and Analysis Gas production was automatically recorded at 2-hour intervals. Methane content was determined using a gas chromatograph (Agilent 7890A) equipped with a thermal conductivity detector. After 48 hours, fermentation was terminated and pH was measured immediately. For VFA analysis, 2 mL of fermentation fluid was centrifuged at $10,000 \times g$ for

10 minutes at 4°C. The supernatant (1 mL) was mixed with 0.2 mL of 25% metaphosphoric acid and frozen at -20°C for subsequent VFA determination by gas chromatography. IVDMD was determined by filtering residual contents through pre-weighed filter bags, drying at 105°C for 8 hours, and calculating the disappearance rate.

1.4 Calculations and Statistical Analysis Gas production kinetics were modeled using the logistic-exponential equation: $GP(t) = Vf / (1 + \exp(-k \times (t - b)))$, where $GP(t)$ is cumulative gas production at time t , Vf is the final gas volume, k is the fractional rate of gas production, and b is the lag time. Statistical analysis was performed using SPSS 21.0 software. Data were analyzed by two-way ANOVA with substrate and venting pressure as main factors. Differences were considered significant at $P < 0.05$.

2. Results

2.1 Fermentation Kinetics and Gas Production The variation curve of bottle pressure during fermentation is shown in Figure 1 [Figure 1: see original paper]. Total gas production under different venting pressures is presented in Figure 2 [Figure 2: see original paper] and Table 2. While substrate type significantly affected total gas production ($P < 0.05$), venting pressure had no significant effect ($P > 0.05$). The kinetic parameters (Vf , k , and b) were similarly unaffected by venting pressure ($P > 0.05$).

2.2 Methane Production Methane production and content are illustrated in Figure 3 [Figure 3: see original paper] and Table 3. Substrate type significantly influenced methane production ($P < 0.05$), but venting pressure showed no significant effect on either methane production or methane content ($P > 0.05$).

2.3 IVDMD, pH, and VFA Production The effects of venting pressure on IVDMD, pH, and VFA production are summarized in Table 4. Substrate type significantly affected all these parameters ($P < 0.05$). However, venting pressure had no significant effect on IVDMD, pH, or total VFA production ($P > 0.05$). Similarly, individual VFA components (acetate, propionate, butyrate, and their ratios) were not significantly affected by venting pressure ($P > 0.05$).

3. Discussion

The present study demonstrates that venting pressure between 9-19 kPa does not significantly affect in vitro rumen fermentation characteristics. This finding is consistent with previous research showing that headspace pressure has minimal impact on fermentation parameters within this range. The dissolution of CO_2

in fermentation fluid and the buffering capacity of the system likely explain this insensitivity.

Carbon dioxide constitutes over 80% of fermentation gases and readily dissolves in the aqueous phase, establishing equilibrium with carbonic acid (H_2CO_3) and bicarbonate (HCO_3^-). This equilibrium is pH-dependent, and the buffer system in rumen fluid maintains relatively stable pH despite CO_2 accumulation. At higher venting pressures, more CO_2 remains dissolved rather than entering the headspace, but this does not substantially alter the fermentation environment.

Previous studies have reported conflicting results regarding pressure effects. Theodorou et al. [9] used 48 kPa without observing negative effects, while Cattani et al. [10] found reduced gas production at 45 kPa compared to 3.4 kPa. Our results support the conclusion that moderate pressure variations (9-19 kPa) do not affect fermentation, validating the use of automated gas production systems with periodic venting.

The significant effects of substrate type on all fermentation parameters confirm that the experimental system was sensitive enough to detect treatment differences. The lack of pressure effects across six diverse substrates further strengthens our conclusion that venting pressure is not a critical factor within the tested range.

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