

Effective Rumen Degradation Rate of Total Mixed Rations with Different Levels of Grape Seed Winery By-product in Fattening Sheep (Postprint)

Authors: GAO Xinmei, Zhang Fan, Tang Fu, Jia Chunyun, Yang Zhenhua, Gao Wei

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Abstract

This experiment was designed to evaluate the effective degradability in the rumen of finishing sheep fed total mixed ration (TMR) containing varying levels of grape seed wine residue. Three Kazakh castrated rams fitted with permanent rumen fistulas, with an average body weight of (35.0 ± 3.7) kg, were used as experimental animals. The trial consisted of 5 periods, each employing TMR with one of five grape seed wine residue levels [0 (TMR1), 4.17% (TMR2), 8.33% (TMR3), 12.50% (TMR4), and 16.67% (TMR5)] for rumen infusion tests, conducted sequentially from low to high levels. Each period lasted 15 days, comprising days 1-7 as the adaptation period, days 8-12 as the continuous infusion period, and days 13-15 as the sampling period. Nylon bag trials were simultaneously conducted on days 13-15 of each period. Ytterbium acetate (Yb-ac) was utilized as a digesta marker to generate concentration decay curves, and nonlinear regression was employed to fit outflow rates; degradation kinetic parameters for dry matter (DM), organic matter (OM), and crude protein (CP) were assessed to calculate rumen effective degradability. The results demonstrated a significant positive linear relationship between grape seed wine residue level and rumen digesta outflow rate ($r=0.6070$, $P<0.05$). The effective degradability of DM, OM, and CP initially decreased then stabilized or slightly increased with rising TMR grape seed wine residue levels, with both TMR4 and TMR5 groups being significantly lower than TMR1 ($P<0.05$). All five TMRs were of the rumen energy-nitrogen negative balance type, with rumen energy-nitrogen balance (RENB) values decreasing linearly as grape seed wine residue levels increased ($P=0.0055$). In summary, the appropriate grape seed wine residue level in TMR for finishing sheep ranges from 8.33% to 12.5%.

Full Text

Effective Degradability of Total Mixed Ration with Different Levels of Brewing Residue of Grape Seed in Rumen of Fattening Sheep

GAO Xinmei, ZHANG Fan, TANG Fu, JIA Chunyun, YANG Zhenhua, GAO Wei* (College of Animal Science and Technology, Shihezi University, Shihezi 832000, China)

Abstract: This experiment aimed to evaluate the effective degradability of total mixed ration (TMR) with different levels of brewing residue of grape seed (GSBR) in the rumen of fattening sheep. Three Kazakh wethers weighing (35.0 ± 3.7) kg and fitted with permanent rumen cannulas were used as experimental animals. The trial consisted of five periods, with each period conducting rumen perfusion tests using TMRs containing five different GSBR levels [0 (TMR1), 4.17% (TMR2), 8.33% (TMR3), 12.50% (TMR4), and 16.67% (TMR5)], sequentially administered from low to high GSBR levels. Each period lasted 15 days, comprising a 7-day pre-test phase (days 1-7), a 5-day continuous perfusion phase (days 8-12), and a 3-day sampling phase (days 13-15). Concurrently, nylon bag tests were performed on days 13-15 of each period. Ytterbium acetate (Yb-ac) served as the digesta marker, and its concentration decay curve was fitted using nonlinear regression to estimate outflow rate. Degradation kinetic parameters of dry matter (DM), organic matter (OM), and crude protein (CP) were evaluated to calculate ruminal effective degradability. The results demonstrated a significantly positive linear relationship between GSBR level and ruminal digesta outflow rate ($r=0.6070$, $P<0.05$). The effective degradability of DM, OM, and CP initially decreased and then stabilized or slightly increased with rising GSBR levels in TMR, with TMR4 and TMR5 groups being significantly lower than TMR1 ($P<0.05$). All five TMRs exhibited ruminal energy-nitrogen negative balance, with RENB values showing a linear decreasing trend as GSBR level increased ($P=0.0055$). In conclusion, the optimal GSBR level in TMR for fattening sheep ranges from 8.33% to 12.5%.

Keywords: outflow rate; ruminal degradability; effective degradability; ruminal energy and nitrogen balance; fattening sheep

Introduction

With the development of animal husbandry, feed resources have become widely scarce despite being largely utilized. Grape is one of the most widely cultivated fruits globally, with China's annual grape production reaching approximately 5 million tons, primarily concentrated in the Xinjiang Uygur Autonomous Region. Approximately 80% of harvested grapes are used for wine production, 13% for fresh consumption, and 7% for juice processing, leaving grape seeds as an industrial byproduct. Grape seed dry matter (DM) accounts for 4-7% of fresh

fruit weight, translating to approximately 3.8 million tons of grape processing byproducts worldwide (calculated at 5%) and about 485,000 tons in China [1], representing substantial utilization potential.

Numerous studies have reported on the development and utilization of grape pomace and grape seeds and their processed products. Several reports have also addressed the application of grape seed residue in animal husbandry. Wu et al. [2] conducted feeding trials substituting grape seed residue for portions of corn, wheat bran, and soybean meal in dairy cows, finding that the appropriate inclusion level of grape seed residue in dairy concentrate was approximately 10%. Zhang et al. [3] added different levels of grape seed powder to replace corn in diets and observed that replacing 7% of corn with grape seed powder in basal diets yielded optimal weight gain and economic benefits in adult ewes. The grape seed brewing residue used in this experiment was a byproduct provided by Xinjiang Western Animal Husbandry Company in Shihezi City, Xinjiang Uygur Autonomous Region, following white liquor production from grape seeds using the “solid-state fermentation of grape seeds—comprehensive extraction tank—extraction, fractionation, and chromatographic purification” process. Our research group previously evaluated its nutritional value, finding that non-fibrous carbohydrate content was only 2.64%, unavailable fiber content reached 34.18%, and the available energy value was correspondingly low. Crude protein (CP) content was 14.2%; while a small portion of soluble protein could be degraded by rumen microbes, the majority remained as bound protein (58.17% of CP), with very low amino acid (AA) digestibility after entering the small intestine [4]. These findings indicate that grape seed brewing residue can be developed as a roughage source for ruminants, though its optimal inclusion level remains unclear. Therefore, this experiment designed five TMRs with varying GSBR levels to measure ruminal digesta outflow rate and nutrient degradation kinetic parameters in fattening sheep, investigating the effects of GSBR level on effective degradability of TMR nutrients to determine the appropriate inclusion level for fattening sheep and provide experimental basis for its utilization.

Materials and Methods

1.1 Grape Seed Brewing Residue The grape seed brewing residue used in this experiment was provided by Xinjiang Western Animal Husbandry Company in Shihezi City, Xinjiang Uygur Autonomous Region. It was the remaining residue after producing white liquor from grape seeds using the “solid-state fermentation of grape seeds—comprehensive extraction tank—extraction, fractionation, and chromatographic purification” process. Its nutrient levels are presented in Table 1 .

1.2 Experimental TMRs Based on the nutritional requirements for fattening sheep recommended by NRC (1985), five isoenergetic and isonitrogenous TMRs with different GSBR levels [0 (TMR1), 4.17% (TMR2), 8.33% (TMR3), 12.50% (TMR4), and 16.67% (TMR5)] were formulated. The composition and

nutrient levels of experimental TMRs are shown in Table 2 .

1.3 Experimental Animals and Management Three Kazakh wethers born in the same year, weighing (35 ± 3.7) kg, were individually housed in metabolic cages. After isolation, quarantine, and deworming with ivermectin injection, they were surgically fitted with permanent rumen cannulas. The experiment was conducted at the Nutrition and Metabolism Laboratory of the College of Animal Science and Technology, Shihezi University. Prior to the experiment, all lambs were allowed ad libitum access to TMR1, with daily voluntary intake exceeding 1,400 g (DM). To avoid feed refusal, fattening sheep were fed 1,200 g/d during the experimental period, with equal portions provided at 08:00 and 20:00, and free access to water.

1.4 Experimental Design Using the three wethers, five experimental periods were conducted, with each period performing rumen perfusion tests using one of the five TMRs with different GSB levels, sequentially administered from low to high GSB levels. Each period lasted 15 days, including a 7-day pre-test phase (days 1-7), a 5-day continuous perfusion phase (days 8-12), and a 3-day sampling phase (days 13-15). Nylon bag tests were simultaneously conducted on days 13-15 of each period.

1.5.1 Rumen Perfusion Test During the continuous perfusion period, ytterbium acetate tetrahydrate [purity 99.99%, purchased from Saen Chemical Technology (Shanghai) Co., Ltd.] was used as the rumen digesta marker. A Yb-ac solution of 500 mL was prepared at a perfusion rate of 100 mg/d ytterbium (Yb) (with 1 mL taken for Yb concentration measurement) and continuously infused into the rumen via the cannula using a peristaltic pump for 24 h. During the sampling period, 50 mL of rumen digesta was collected at 0, 2, 4, 8, 12, 24, 36, 48, 60, and 72 h after stopping perfusion. Samples were dried at 60°C, ground, and analyzed for Yb concentration in the prepared Yb-ac solution and digesta samples using inductively coupled plasma atomic emission spectrometry (ICP-AES). The concentration decay curve was plotted, and nonlinear regression (NLIN) was used to fit the ruminal digesta outflow rate.

1.5.2 Nylon Bag Test The nylon bag method was used to determine the kinetic parameters of DM, organic matter (OM), and CP degradation of the five TMRs in fattening sheep. Nylon bags measuring 5 cm × 8 cm with pore size of 40-50 μm were used, with the test samples being the experimental TMRs. Exactly 2.5 g of sample was weighed into each nylon bag, sealed with nylon thread, and attached to an iron chain. Each chain held nine nylon bags, which were placed in the rumen ventral sac before morning feeding on day 13 of each period. The terminal nylon thread of the chain was fixed to the wool with a clip to prevent loss into the rumen. After placement, one nylon bag was removed from each of the three experimental sheep at 2, 4, 8, 12, 24, 36, 48, 60, and 72 h, rinsed with tap water, and stored at -20°C. After all bags were retrieved,

they were repeatedly rinsed under running tap water until the water became clear. The 0-h nylon bags were not placed in the rumen but were rinsed using the same method, frozen, and stored for analysis. DM, OM, and CP contents in nylon bag residues at each time point were determined according to Yang [5].

1.6.1 Rumen Digesta Outflow Rate Rumen digesta outflow rate was determined from the Yb concentration decline curve in rumen contents using the following mathematical model:

$$b = C_0 \times e^{-kt}$$

where:

- b = Yb concentration in rumen contents at time t (mg/kg)
- C_0 = Yb concentration in rumen contents at time zero (mg/kg)
- t = time after stopping perfusion (h)
- k = rumen digesta outflow rate (%/h)
- e = base of natural logarithm

Based on Yb concentrations at different time points, the DUD (Doesn't Use Derivatives) method in the NLIN procedure of SAS 8.01 software [6] was used to fit the Yb concentration decline curve equation and determine parameters.

1.6.2 Rumen Degradation Kinetic Parameters Degradation kinetic parameters of DM, OM, and CP in TMR were fitted using the Marquardt method in the NLIN procedure of SAS 8.01 software [6]. Effective degradability of DM, OM, and CP in the rumen was calculated according to Ørskov et al. [7] using the formula:

$$ED = a + \frac{b \times K_d}{K_d + K_p}$$

where:

- ED = effective degradability (%)
- a = rapidly degradable fraction content (%)
- b = slowly degradable fraction content (%)
- K_d = degradation rate of slowly degradable fraction (%/h)
- K_p = rumen digesta outflow rate (%/h)

The same formula applies below. Rumen degradation kinetic parameters were fitted using the Marquardt method in the NLIN procedure of SAS 8.01 software [6].

The following formula was used to calculate effective residence time of DM in the rumen:

$$\text{Effective residence time of DM in rumen (h)} = \frac{100 - a - b}{K_p} + \frac{b}{K_d + K_p}$$

1.6.3 Rumen Energy-Nitrogen Balance (RENB) The French National Institute for Agricultural Research (INRA) indicates that each kilogram of fermentable organic matter (FOM) can produce 23.2 g of microbial nitrogen or 145 g of microbial crude protein (MCP). Based on RENB theory and assuming an efficiency of 0.9 for converting rumen degradable protein (RDP) to MCP, the RENB value was calculated as:

$$\text{RENB value (g/kg)} = \text{MCP}_{\text{FOM}} - \text{MCP}_{\text{RDP}} \quad [8]$$

The MCP conversion rate was calculated as:

$$\text{MCP conversion rate (\%)} = 3.5685 - 0.8414 \ln \left[\frac{\text{RDP (g)}}{\text{FOM (kg)}} \right]$$

1.7 Data Processing and Statistical Analysis The CORR procedure in SAS 8.01 software [6] was used for linear correlation analysis between GSB, NDF, and ADF levels and outflow rate, followed by linear regression analysis using the REG procedure. Dynamic degradation rates of DM, OM, and CP of TMR in the rumen were analyzed using the PROC MIXED procedure for repeated measures data. Rumen degradation kinetic parameters and effective residence time of DM were analyzed using the PROC ANOVA procedure. Differences were considered significant at $P < 0.05$ and highly significant at $P < 0.01$. FOM, RDP, RENB values, and effective degradability were analyzed for linear and quadratic trends using GLM CONTRAST statements.

Results and Analysis

All experimental sheep remained healthy throughout the trial, consumed the provided TMR normally with minimal refusal, and had an average final body weight of (39.0 ± 2.3) kg.

2.1 Rumen Digesta Outflow Rate

Yb concentration in rumen digesta showed a declining trend over time, approaching zero after 48 h (Figure 1 [Figure 1: see original paper]). A significantly positive linear relationship existed between GSB level and outflow rate ($r = 0.6070$, $P < 0.05$), described by the model $y = 0.42861x$ ($R^2 = 0.7360$). Outflow rate increased with higher GSB levels, with a significant difference between TMR1 and TMR5 groups ($P < 0.05$), while other TMR groups did not differ significantly ($P > 0.05$) (Table 3). NDF level also showed a significantly positive linear relationship with outflow rate ($r = 0.6070$, $P < 0.05$), modeled as $y = 0.09862x$

($R^2=0.9872$), indicating that outflow rate increased with NDF level. ADF level exhibited a significantly positive linear relationship with outflow rate as well ($r=0.6050$, $P<0.05$), with the model $y=0.13058x$, $R^2=0.9871$.

Dynamic Degradation Rates of DM, OM, and CP of Five TMRs in the Rumen

As shown in Table 4, DM degradation rates of all five TMRs increased significantly with prolonged rumen retention time ($P<0.01$). GSBR level exerted a highly significant effect on DM degradation rate ($P<0.01$), which gradually decreased and then increased with higher GSBR levels. The mean value across all time points was lowest in TMR4 at 39.45%, which was significantly lower than the other four groups ($P<0.01$), while no significant differences existed among the remaining groups ($P>0.05$).

Table 5 reveals that OM degradation rates of all five TMRs increased significantly with extended rumen retention time ($P<0.01$). Mean OM degradation rates across time points in TMR4 and TMR5 were significantly lower than in the other three groups ($P<0.01$), though these two groups did not differ from each other ($P>0.05$).

Table 6 shows that CP degradation rates of all five TMRs increased significantly with longer rumen retention time ($P<0.01$), stabilizing after 60 h as degradation reached a plateau. No significant differences were observed in mean CP degradation rates across time points among TMR groups ($P>0.05$).

Degradation Kinetic Parameters of DM, OM, and CP of Five TMRs in the Rumen

Table 7 indicates that rapidly degradable fraction contents and degradation rates of DM, OM, and CP did not differ significantly among TMR groups ($P>0.05$). Effective residence time of DM in the rumen was significantly higher in TMR1 than in the other four groups ($P<0.05$). Effective degradability of DM was significantly higher in TMR1 than in TMR4 and TMR5 ($P<0.05$). Effective degradability of OM was significantly higher in TMR1 than in TMR3, TMR4, and TMR5 ($P<0.05$). Effective degradability of CP was significantly higher in TMR1 than in all other groups ($P<0.05$).

As shown in Table 8, effective degradability of DM decreased linearly with increasing GSBR level ($P=0.011$), OM effective degradability decreased highly significantly in a linear trend ($P=0.002$), while CP effective degradability decreased significantly in a quadratic trend ($P=0.020$). No significant differences were observed in rumen kinetic parameters among the five groups, indicating that variations in outflow rate were the primary factor causing significant changes in effective degradability.

Table 9 demonstrates that FOM, RDP, and RENB values all decreased linearly with increasing GSBR level ($P<0.001$, $P=0.006$, $P=0.005$, respectively). All

TMR groups showed negative RENB values, indicating RDP excess and FOM deficiency that increased with GSBR level, requiring FOM supplementation to further improve RDP utilization efficiency. MCP conversion rates calculated from regression formulas were higher in TMR1 and TMR2 groups.

Discussion

Du et al. [9] replaced alfalfa hay with grape seed meal in dairy cow diets and found that 2-4% grape seed meal inclusion had no adverse effects on production performance or individual condition. Sun et al. [10] reported that adding 8% grape pomace to basal diets yielded the best weight gain in adult ewes. Zhang et al. [3] found that replacing 7% of corn with grape seed powder in diets improved weight gain and reduced feed costs in adult ewes. In this experiment, GSBR inclusion in TMR had no significant effect on feed intake in fattening sheep, possibly due to the restricted feeding regimen employed.

Effects of Different GSBR Levels on Rumen Digesta Outflow Rate

Rumen digesta outflow rate in fattening sheep refers to the percentage of solid or liquid digesta volume flowing out of the rumen per unit time relative to total rumen content volume, expressed as %/h. Outflow rate is a crucial parameter for evaluating effective degradability of feed DM and CP in the rumen. Accurate assessment of outflow rate under specific physiological, feeding, and environmental conditions is scientifically significant and practically valuable for precisely evaluating feed nutritional value and scientifically formulating diets. Increased digesta outflow rate reduces the extent of roughage digestion in the rumen and overall digestive tract digestibility in ruminants, while decreased outflow rate enhances them. Moreover, passage rate is a dynamic value. Previous studies have shown that numerous factors influence outflow rate, including animal breed [11-12], feed intake [13-14], dietary concentrate-to-forage ratio and NDF level [15], and feeding level [17-18].

Under isoenergetic and isonitrogenous conditions among the five TMRs in this experiment, NDF level significantly affected rumen digesta outflow rate, contrary to Wang et al. [19] who found that TMR fiber level did not significantly affect digesta outflow rate at the same digestive tract location in sheep consuming similar energy and nitrogen amounts. This discrepancy may be attributed to different fiber sources used in TMRs, as fiber level [20-21], fiber quality [22-23], and fiber length [24-25] all influence fiber utilization, leading to different experimental results. Chase et al. [26] observed that passage rate decreased linearly with reduced dietary fiber level, from 3.90%/h to 3.68%/h, consistent with our findings.

Effects of Different GSBR Levels on Rumen Kinetic Parameters of DM, OM, and CP

Li et al. [27] found that dietary degradation rate increased with prolonged rumen microbial action time. This experiment yielded similar results, with DM, OM, and CP degradation rates increasing with extended rumen retention time and eventually reaching equilibrium.

Studies have shown that when nutritional levels are similar, altering the concentrate-to-forage ratio inevitably changes dietary composition, which can affect rumen microbial populations and consequently influence nutrient degradation rates [28-29]. Bo et al. [30] reported in nylon bag tests that vinegar residue had lower DM digestibility than white liquor residue, related to its relatively higher NDF level. In this experiment, as GSBR level increased sequentially, concentrate proportion decreased correspondingly, and degradation rates of DM, OM, and CP gradually declined and then stabilized or slightly increased, consistent with previous research [31]. The upward trend in degradation rate in TMR5 may be related to insufficient nylon bag rinsing in the nylon bag test [32]. Crude protein in roughages primarily exists in cell contents, and its degradation rate depends on plant cell wall fiber structure. Therefore, fiber degradation in diets significantly impacts CP degradation rate. Liu et al. [33] used the nylon bag method to determine rumen DM and CP degradation rates of common roughages for sheep and found that relatively high dietary crude fiber level could reduce rumen CP degradation rate, consistent with our findings.

Effects of Different GSBR Levels on RENB

MCP is the primary source of metabolizable protein for the small intestine. Rumen MCP synthesis is closely related to the utilization efficiency of RDP and FOM. MCP synthesis rate is highest when energy provided by FOM balances RDP degradation quantity in the rumen. In this experiment, all five TMRs showed ruminal energy-nitrogen negative balance, indicating RDP excess and low nitrogen utilization efficiency in these TMRs, with FOM deficiency becoming a limiting factor for metabolic energy. Imbalanced energy and RDP proportions in the rumen caused negative associative effects among feedstuffs. Chen et al. [34] found that the efficiency of RDP conversion to microbial nitrogen was affected by dietary protein degradation rate, degradation speed, and the balance between RDP and microbially available energy. In this experiment, dietary protein degradation rate decreased with increasing GSBR level, reducing the efficiency of RDP conversion to microbial nitrogen and causing RENB values to decline with higher TMR GSBR levels. FOM supplementation should be provided to further improve RDP utilization efficiency and achieve FOM-RDP balance to maximize MCP synthesis.

Conclusion

1. As GSBP level in TMR increased, rumen digesta outflow rate increased linearly, causing TMR nutrient effective degradability to initially decrease and then stabilize or slightly increase.
2. GSBP levels exceeding 12.5% of TMR significantly reduced DM effective degradability, MCP, and FOM, affecting MCP synthesis.
3. The optimal GSBP level in TMR for fattening sheep ranges from 8.33% to 12.5%.

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