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Ruminal Effective Degradation Rate of Total Mixed Rations Containing Different Levels of Grape Seed Wine Residue in Fattening Sheep: Postprint

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Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment was conducted to evaluate the effective degradability in the rumen of total mixed rations (TMR) containing different levels of grape seed wine residue in fattening sheep. Three Kazakh castrated rams fitted with permanent rumen fistulas, with an average body weight of (35.0±3.7) kg, were selected as experimental animals. A total of five periods were conducted, with each period using TMR containing one of five different grape seed wine residue levels [0 (TMR1), 4.17% (TMR2), 8.33% (TMR3), 12.50% (TMR4), and 16.67% (TMR5)] for rumen infusion trials, conducted sequentially from low to high grape seed wine residue levels. Each period lasted 15 days, with days 1–7 as the preliminary period, days 8–12 as the continuous infusion period, and days 13–15 as the sampling period. Simultaneously, nylon bag experiments were conducted on days 13–15 of each period. Ytterbium acetate (Yb-ac) was used as a digesta marker to plot concentration decay curves, and nonlinear regression was employed to fit the outflow rate; degradation kinetic parameters of dry matter (DM), organic matter (OM), and crude protein (CP) were evaluated, and rumen effective degradability was calculated. The results showed a significant positive linear correlation between grape seed wine residue level and rumen digesta outflow rate (r=0.607 0, P<0.05); the effective degradability of DM, OM, and CP all initially decreased and then stabilized or slightly increased with increasing TMR grape seed wine residue level, with both TMR4 and TMR5 groups being significantly lower than the TMR1 group (P<0.05). All five TMRs were of the rumen energy-nitrogen negative balance type, and rumen energy-nitrogen balance (RENB) values showed a linear decreasing trend with increasing grape seed wine residue level (P=0.005 5). In conclusion, the appropriate level of grape seed wine residue in TMR for fattening sheep is 8.33%–12.5%.

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Full Text

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

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Abstract

This experiment aimed to evaluate the effective rumen degradability of total mixed rations (TMR) containing different levels of grape seed brewing residue (GSBR) in fattening sheep. Three Kazakh wethers (average body weight 35.0 ± 3.7 kg) fitted with permanent rumen cannulas were used as experimental animals. Five experimental periods were conducted, each using one of five TMRs with varying GSBR levels [0% (TMR1), 4.17% (TMR2), 8.33% (TMR3), 12.50% (TMR4), and 16.67% (TMR5)], administered sequentially from lowest to highest GSBR content. Each period lasted 15 days, comprising a 7-day preliminary period (days 1–7), a 5-day continuous infusion period (days 8–12), and a 3-day sampling period (days 13–15). Nylon bag trials were simultaneously conducted on days 13–15 of each period. Ytterbium acetate (Yb-ac) served as a digesta marker, with concentration decay curves plotted and outflow rates fitted using nonlinear regression. Degradation kinetic parameters for dry matter (DM), organic matter (OM), and crude protein (CP) were evaluated to calculate ruminal effective degradability. The results demonstrated a significant 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, with both TMR4 and TMR5 groups showing significantly lower values than TMR1 ($P < 0.05$). All five TMRs exhibited negative ruminal energy-nitrogen balance, with RENB values decreasing linearly as GSBR level increased ($P = 0.0055$). In conclusion, the appropriate GSBR inclusion 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
CLC number: S826

Introduction

With the development of animal husbandry, feed resources have become generally scarce despite being largely utilized. Grape is one of the most widely cultivated fruits worldwide, with annual production in China reaching approximately 5 million tons, predominantly in the Xinjiang Uygur Autonomous Region. Grape fruits are primarily used for wine production (80%), fresh consumption

(13%), and juice processing (7%). Grape seeds, a byproduct of grape industrial processing, account for 4–7% of fresh fruit dry matter (DM). Based on a 5% DM content, global grape seed byproduct availability is estimated at 3.8 million tons annually, with China' s share reaching approximately 485,000 tons, indicating substantial potential for utilization.

Numerous studies have investigated the development and application of grape pomace and grape seed products both domestically and internationally. Several reports have documented the use 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 an optimal inclusion level of approximately 10% in dairy concentrate feed. Zhang et al. [3] replaced corn with different levels of grape seed powder in diets, concluding that substituting 7% of corn in basal diets yielded optimal weight gain and economic benefits in adult ewes. The GSBR used in this study was a byproduct from white spirit production using grape seeds, provided by Xinjiang Western Animal Husbandry Company in Shihezi City. Our research group previously evaluated its nutritional value, finding non-fibrous carbohydrate content of only 2.64% and unavailable fiber content reaching 34.18%, resulting in relatively low available energy values. The crude protein (CP) content was 14.2%, with most protein existing as bound protein (58.17% of CP) after partial solubilization and rumen degradation, leading to low amino acid (AA) digestibility in the small intestine [4]. These findings indicate that GSBR 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 rates 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 and provide experimental basis for GSBR utilization in fattening sheep production.

1.1 Grape Seed Brewing Residue

The GSBR used in this experiment was provided by Xinjiang Western Animal Husbandry Company in Shihezi City, representing the residue remaining after grape seeds underwent "solid-state alcohol fermentation—comprehensive extraction tank—extraction, fractionation, and chromatographic purification" processing for white spirit production. The nutrient composition is presented in Table 1 .

1.2 Experimental TMRs

Based on nutrient requirements for fattening sheep recommended by NRC (1985), five isocaloric and isonitrogenous TMRs were formulated with different GSBR levels [0% (TMR1), 4.17% (TMR2), 8.33% (TMR3), 12.50% (TMR4), and 16.67% (TMR5)]. 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. Following isolation quarantine and ivermectin injection for deworming, permanent rumen cannulas were surgically installed. 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 fed TMR1 ad libitum, with daily voluntary DM intake exceeding 1,400 g per lamb. To avoid feed residue, 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

Five periods were conducted using the three wethers, with each period employing rumen infusion tests using one of the five TMRs with different GSBR levels, administered sequentially from low to high GSBR content. Each period lasted 15 days, including a 7-day preliminary period (days 1–7), a 5-day continuous infusion period (days 8–12), and a 3-day sampling period (days 13–15). Nylon bag trials were simultaneously performed on days 13–15 of each period.

1.5.1 Rumen Infusion Test

During the continuous infusion period, ytterbium acetate tetrahydrate [99.99% purity, purchased from Saen Chemical Technology (Shanghai) Co., Ltd.] served as a ruminal digesta marker. A Yb-ac solution was prepared at an infusion rate of 100 mg Yb/d in 500 mL volume (1 mL sampled for Yb concentration measurement) and continuously infused 24 h/d via peristaltic pump through the rumen cannula. During the sampling period, 50 mL of rumen digesta was collected at 0, 2, 4, 8, 12, 24, 36, 48, 60, and 72 h post-infusion cessation. Samples were dried at 60°C, ground, and analyzed for Yb concentration using inductively coupled plasma atomic emission spectrometry (ICP-AES). Concentration decay curves were plotted, and outflow rates were fitted using nonlinear regression (NLIN).

1.5.2 Nylon Bag Test

The nylon bag technique was employed to determine degradation kinetic parameters of DM, organic matter (OM), and CP for the five TMRs in fattening sheep. Nylon bags measuring 5 cm × 8 cm with 40–50 m pore size were used, with each bag containing 2.5 g of the test TMR sample, sealed with nylon thread and attached to an iron chain. Each chain held nine bags, which were placed in the rumen ventral sac before morning feeding on day 13 of each period, with the terminal nylon thread fixed to wool using a clip to prevent loss. Bags were retrieved from the rumen of three sheep at 2, 4, 8, 12, 24, 36, 48, 60, and 72 h post-incubation, rinsed with tap water, and stored at -20°C. After retrieval of all bags, they were repeatedly rinsed under running water until the rinse water became clear. Zero-hour bags were not placed in the rumen but were washed

and stored following the same procedure. DM, OM, and CP contents in 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 represents Yb concentration in rumen contents at time t (mg/kg), C_0 is Yb concentration at time zero (mg/kg), t is time after infusion cessation (h), k is rumen digesta outflow rate (%/h), and e is the base of natural logarithms. Parameters were estimated by fitting the Yb concentration decline curve using the DUD method in the NLIN procedure of SAS 8.01 software [6].

1.6.2 Rumen Degradation Kinetic Parameters

Degradation kinetic parameters of DM, OM, and CP for TMRs were fitted using the Marquardt method in the NLIN procedure of SAS 8.01 software [6]. Effective degradability 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 is effective degradability (%), a is the rapidly degradable fraction (%), b is the slowly degradable fraction (%), K_d is the degradation rate of the slowly degradable fraction (%/h), and K_p is rumen digesta outflow rate (%/h). The same formula applies below.

Effective residence time of DM in the rumen was calculated as:

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

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) yields 23.2 g microbial nitrogen or 145 g microbial crude protein (MCP). Based on RENB theory and assuming a 0.9 conversion efficiency from rumen degradable protein (RDP) to MCP, RENB values were calculated as:

RENB value (g/kg) = MCP estimated from FOM−MCP estimated from RDP[8]

MCP conversion rate was calculated as:

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

1.7 Data Processing and Statistical Analysis

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

Results and Analysis

All experimental sheep remained healthy throughout the trial, consumed the prescribed TMR quantities with minimal residue, and achieved an average final body weight of 39.0 ± 2.3 kg.

2.1 Rumen Digesta Outflow Rate

Yb concentration in rumen digesta decreased over time, approaching zero after 48 h (Figure 1 [Figure 1: see original paper]). A significant positive linear relationship existed between GSBR 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 GSBR level, 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 significant positive linear correlation 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. Similarly, ADF level exhibited a significant positive linear relationship with outflow rate ($r = 0.6050$, $P < 0.05$), modeled as $y = 0.13058x$, $R^2 = 0.9871$.

2.2 Dynamic Degradation Rates of DM, OM, and CP in Rumen

As shown in Table 4 , DM degradation rates for all five TMRs increased significantly with extended rumen retention time ($P < 0.01$). GSBR level exerted a highly significant effect on DM degradation rate ($P < 0.01$), which decreased initially and then increased with rising GSBR levels. The mean value across all time points was lowest for TMR4 (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 for all five TMRs also increased significantly with prolonged rumen retention time ($P < 0.01$). Mean OM degradation rates for TMR4 and TMR5 were significantly lower than 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 for all five TMRs increased significantly with extended 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 among TMR groups ($P > 0.05$).

2.3 Degradation Kinetic Parameters of DM, OM, and CP in Rumen

Table 7 indicates that rapidly and slowly degradable fractions and degradation rates of DM, OM, and CP did not differ significantly among TMR groups ($P > 0.05$). However, effective residence time of DM in the rumen was significantly higher for TMR1 than 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 all other groups ($P < 0.05$).

As shown in Table 8 , effective degradability of DM decreased linearly with increasing GSBR level ($P = 0.011$), while OM effective degradability showed a highly significant linear decline ($P = 0.002$), and CP effective degradability exhibited a significant quadratic decrease ($P = 0.020$). 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.

2.4 Rumen Energy-Nitrogen Balance

Table 9 demonstrates that FOM, RDP, and RENB values decreased linearly with increasing GSBR level ($P < 0.001$, $P = 0.006$, $P = 0.005$, respectively). All TMR groups exhibited negative RENB values, indicating RDP excess and FOM deficiency that increased with GSBR level, requiring FOM supplementation to improve RDP utilization efficiency. MCP conversion rates calculated from regression formulas were higher for TMR1 and TMR2 groups.

Discussion

Du et al. [9] reported that incorporating 2–4% grape seed meal in dairy cow diets had no adverse effects on production performance or health status. Sun et al. [10] found that adding 8% grape pomace to basal diets optimized weight gain in adult ewes. Zhang et al. [3] observed that replacing 7% of corn with grape seed powder in diets promoted weight gain and improved economic benefits in adult ewes. In the present study, GSBR supplementation in TMR did not

significantly affect feed intake in fattening sheep, likely due to the restricted feeding regimen employed.

3.1 Effects of GSBR Level on Rumen Digesta Outflow Rate

Rumen digesta outflow rate in fattening sheep represents the percentage of solid or liquid digesta volume flowing from the rumen per unit time relative to total rumen content volume, expressed as %/h. Outflow rate is a critical parameter for evaluating effective degradability of DM and CP in the rumen, making accurate assessment under specific physiological, feeding, and environmental conditions essential for evaluating feed nutritional value and formulating scientifically balanced diets. Increased outflow rate reduces the extent of roughage digestion in the rumen and overall digestive tract digestibility in ruminants, while decreased outflow rate enhances digestion. Moreover, passage rate is a dynamic value influenced by numerous factors including animal breed [11–12], feed intake [13–14], dietary concentrate-to-forage ratio and NDF level [15], and feeding level [17–18].

Under isocaloric and isonitrogenous conditions across the five TMRs, NDF level significantly affected rumen digesta outflow rate in this study, contrasting with Wang et al. [19] who reported that dietary fiber level did not significantly affect digesta outflow rate at the same digestive site in sheep consuming similar energy and nitrogen levels. This discrepancy may be attributed to differences in fiber sources among TMRs, as fiber level [20–21], fiber quality [22–23], and fiber length [24–25] all influence fiber utilization, potentially leading to divergent results. Chase et al. [26] observed that digesta passage rate decreased linearly with declining dietary fiber level, from 3.90%/h to 3.68%/h, consistent with our findings.

3.2 Effects of GSBR Level on Rumen Degradation Kinetic Parameters of DM, OM, and CP

Li et al. [27] reported that dietary degradability increased with prolonged rumen microbial action time, a result similarly observed in this study where DM, OM, and CP degradation rates increased with extended rumen retention time before eventually stabilizing.

Research indicates that altering the concentrate-to-forage ratio while maintaining similar nutritional levels inevitably changes dietary composition, affecting rumen microbial populations and consequently nutrient degradability [28–29]. Bo et al. [30] found in nylon bag studies that vinegar residue exhibited lower DM digestibility than distiller' s grains, attributed to its relatively higher NDF content. In this experiment, as GSBR level increased sequentially, concentrate proportion decreased correspondingly, causing DM, OM, and CP degradation rates to decline initially before stabilizing or increasing slightly, consistent with previous findings [31]. The increased degradation rate observed in TMR5 may be related to insufficient nylon bag washing during the procedure [32]. Crude feed

proteins primarily exist within cell contents, with degradation rates dependent on plant cell wall fiber structure; thus, dietary fiber degradation significantly impacts CP degradability. Liu et al. [33] measured rumen DM and CP degradability of common roughages for sheep using the nylon bag method, finding that relatively high dietary crude fiber levels may reduce rumen CP degradability, consistent with our results.

3.3 Effects of GSBR Level on RENB

Microbial crude protein (MCP) represents the primary source of metabolizable protein for the small intestine, with its synthesis in the rumen closely related to the utilization efficiency of RDP and FOM. Maximum MCP synthesis occurs when energy supplied by FOM balances RDP degradation quantity. In this study, all five TMRs exhibited negative ruminal energy-nitrogen balance, indicating RDP excess and low nitrogen utilization efficiency, with metabolizable energy becoming the limiting factor. The imbalance between ruminal energy and RDP proportions created negative associative effects among feed components. Chen et al. [34] reported that the efficiency of RDP conversion to microbial nitrogen is influenced by dietary protein degradability, degradation rate, and the balance between RDP and microbially available energy. In this experiment, increasing GSBR levels reduced dietary protein degradability, consequently decreasing the efficiency of RDP conversion to microbial nitrogen and causing RENB values to decline with higher GSBR levels. Supplementing FOM would be necessary to improve RDP utilization efficiency and achieve FOM-RDP balance for maximizing MCP synthesis.

Conclusion

1. Increasing GSBR level in TMR caused a linear increase in rumen digesta outflow rate, leading to an initial decline followed by stabilization or slight increase in ruminal effective degradability of TMR nutrients.
2. GSBR levels exceeding 12.5% of TMR significantly reduced DM effective degradability, MCP, and FOM, thereby impairing MCP synthesis.
3. The appropriate GSBR inclusion level in TMR for fattening sheep ranges from 8.33% to 12.5%.

Acknowledgments

We sincerely thank Dr. Li Fengming and Dr. Li Hao from the College of Animal Science and Technology, Xinjiang Agricultural University, for their assistance and support during the experiment.

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