Postprint: Rumen Degradation Characteristics of Six Feed Ingredients and Small Intestinal Digestibility of Rumen Undegraded Protein

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Abstract

This experiment aimed to investigate the rumen degradation characteristics and intestinal digestibility (Idg) of rumen undegradable protein (RUP) of six dairy cow feed ingredients in the Xinjiang region: corn silage, cottonseed hulls, alfalfa meal, alfalfa hay, grape seed meal, and tomato paste residue. Three lactating Holstein dairy cows fitted with permanent rumen fistulas were used to determine the rumen degradation characteristics of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF), as well as the Idg of RUP and intestinally digestible crude protein (IDCP) content of these feed ingredients using the nylon bag technique and a modified three-step in vitro method. The results showed that: 1) Alfalfa meal and corn silage had the highest DM effective degradability, which was significantly higher than that of alfalfa hay and tomato paste residue ($P<0.05$), while grape seed meal and cottonseed hulls had the lowest values, which were significantly lower than those of the other ingredients ($P<0.05$). The CP effective degradability ranked as tomato paste residue > alfalfa meal > corn silage > alfalfa hay > cottonseed hulls > grape seed meal, with significant differences among all ingredients ($P<0.05$). The NDF effective degradability ranked as corn silage > alfalfa meal > cottonseed hulls > alfalfa hay > tomato paste residue > grape seed meal, with significant differences among all feed ingredients ($P<0.05$). The ADF effective degradability ranked as corn silage > cottonseed hulls > alfalfa hay > tomato paste residue > alfalfa meal > grape seed meal, with significant differences among all feed ingredients ($P<0.05$). 2) Alfalfa meal had the highest Idg of RUP and IDCP content; its Idg was significantly higher than those of alfalfa hay, corn silage, tomato paste residue, grape seed meal, and cottonseed hulls in descending order ($P<0.05$). The IDCP content showed no significant difference with alfalfa hay ($P>0.05$), but was significantly higher than those of corn silage, tomato paste residue, grape seed meal, and cottonseed hulls in descending order

(P<0.05). In conclusion, different feed ingredients possess distinct rumen degradation characteristics, and the efficiency of IDCP entering the small intestine also varies. Corn silage exhibited relatively high effective degradability of DM, NDF, and ADF in the rumen, alfalfa meal showed higher Idg of RUP, and both alfalfa meal and alfalfa hay had relatively high IDCP content.

Full Text

Ruminal Degradation Characteristics and Small Intestinal Digestibility of Rumen Undegraded Protein of Six Feed Ingredients

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Abstract: This experiment was conducted to investigate the ruminal degradation characteristics and small intestinal digestibility of rumen undegraded protein (RUP) of six feed ingredients for dairy cows commonly used in Xinjiang: corn silage, cottonseed hulls, alfalfa meal, alfalfa hay, grape seed meal, and tomato sauce residue. Three lactating Holstein cows fitted with permanent rumen fistulas were selected to determine the ruminal degradation characteristics of dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF), as well as the intestinal digestibility (Idg) of RUP and intestinal digestible crude protein (IDCP) content using the nylon bag technique and a modified three-step in vitro method. The results showed that: 1) Alfalfa meal and corn silage exhibited the highest DM effective degradability, significantly greater than that of alfalfa hay and tomato sauce residue (P<0.05), while grape seed meal and cottonseed hulls showed the lowest values, significantly lower than other ingredients (P<0.05). CP effective degradability followed the order: tomato sauce residue > alfalfa meal > corn silage > alfalfa hay > cottonseed hulls > grape seed meal, with significant differences among all ingredients (P<0.05). NDF effective degradability ranked as: corn silage > alfalfa meal > cottonseed hulls > alfalfa hay > tomato sauce residue > grape seed meal, with significant differences between groups (P<0.05). ADF effective degradability showed the pattern: corn silage > cottonseed hulls > alfalfa hay > tomato sauce residue > alfalfa meal > grape seed meal, with significant differences among all groups (P<0.05). 2) Alfalfa meal demonstrated the highest Idg of RUP and IDCP content; its Idg was significantly higher than the progressively decreasing values observed for alfalfa hay, corn silage, tomato sauce residue, grape seed meal, and cottonseed hulls (P<0.05). The IDCP content of alfalfa meal did not differ significantly from alfalfa hay (P>0.05) but was signif-

icantly higher than the progressively decreasing values for corn silage, tomato sauce residue, grape seed meal, and cottonseed hulls ($P<0.05$). In conclusion, different feed ingredients exhibit distinct ruminal degradation characteristics and vary in their efficiency of IDCP production. Corn silage shows high effective degradability of DM, NDF, and ADF in the rumen, alfalfa meal demonstrates high intestinal digestibility of RUP, and both alfalfa meal and alfalfa hay possess relatively high IDCP content.

Keywords: feed ingredient; ruminal degradability; small intestinal digestibility of rumen undegraded protein

Introduction

Xinjiang' s animal husbandry industry has developed rapidly, establishing the region as a major livestock production area in China. However, compared with the advanced state of animal husbandry, feed resource scarcity has become a limiting factor for further development. Corn silage, alfalfa meal, and alfalfa hay are commonly used feed ingredients in dairy farming, while cottonseed hulls, grape seed meal, and tomato sauce residue represent unconventional feed materials that are abundantly available in Xinjiang. Exploring the nutritional value of these ingredients to enable their effective utilization in livestock production can not only make full use of agricultural by-products, reducing waste and pollution, but also address feed resource shortages to achieve increased production efficiency and optimal resource utilization.

The degradation characteristics of feed nutrients in the rumen constitute an important indicator for evaluating the nutritional value of dairy feed. Previous studies by Diao et al. and Yao et al. have published rumen degradation rates of common feeds, providing valuable guidance for practical production. Additionally, protein content and utilization efficiency are crucial metrics for assessing feed nutritional value. In modern protein evaluation systems, beyond rumen degradability, the small intestinal digestibility of rumen undegraded protein (RUP) serves as a key indicator for measuring the supply of absorbable protein in the small intestine. Currently, domestic reports on the small intestinal digestibility (Idg) of RUP for dairy feeds are scarce, and China's *Feeding Standard of Dairy Cattle* (NY/T 34-2004) lacks relevant data on duodenal digestibility of feed RUP, without considering variations in feed sources and processing methods. This study employed the nylon bag technique to investigate the ruminal degradation patterns of nutrients in six feed ingredients for dairy cows and used a modified three-step in vitro method to examine the Idg of RUP, aiming to provide a theoretical basis for the rational utilization of these feed resources.

1.1 Experimental Animals and Diet

Three lactating Holstein cows of similar body weight, identical lactation days, and good health, fitted with permanent rumen fistulas, were selected from the Beijing Zhongdi Dairy Cattle Science and Technology Park Experimental Base.

The feeding trial was conducted at the same facility. The basal diet was formulated according to NRC (2001) nutrient requirements for dairy cattle, with a concentrate-to-forage ratio of 40:60. The composition and nutrient levels are presented in Table 1 . Cows were fed three times daily at 07:30, 13:00, and 19:30, with free access to water.

1.2 Experimental Materials

Six feed ingredients were evaluated: grape seed meal, corn silage, tomato sauce residue, cottonseed hulls, alfalfa meal, and alfalfa hay. Grape seed meal, corn silage, tomato sauce residue, and cottonseed hulls were collected from Shihezi, Xinjiang, while alfalfa meal and alfalfa hay were obtained from Tacheng, Xinjiang. Alfalfa meal contained more alfalfa leaves than alfalfa hay. Samples were dried at 65°C to produce air-dried samples, passed through a 40-mesh sieve, and stored for analysis. The nutrient composition of the six feed ingredients is shown in Table 2 .

1.3.1 Nylon Bag Technique for Determining Rumen Fermentation Characteristics

Nylon cloth with a pore size of 50 m was cut into 17 cm × 13 cm rectangles, folded in half, and sewn with double-stitched polyester thread to create 8 cm × 12 cm nylon bags. The bottom and corners of each bag were rounded, and loose edges were sealed with a soldering iron. Bags were dried at 65°C to constant weight (48 h) before use. Approximately 2.5 g of sample was placed into a pre-weighed nylon bag. Four replicates were prepared for each feed ingredient per fistulated cow (total of 3 cows). Two hours after morning feeding, all sample-containing bags were simultaneously placed into the rumen ventral sac and retrieved at 2, 6, 12, 24, 48, and 72 h. Retrieved bags were rinsed with tap water until the rinse water ran clear, then dried at 65°C to constant weight (48 h). Bags for the 0 h time point were not incubated in the rumen but were rinsed and dried similarly to serve as blank controls.

1.3.2 Modified Three-Step In Vitro Method for Determining Idg of RUP

Following the principles and methods of Gargallo et al., the modified three-step in vitro procedure (MTSP) was employed. Five grams of feed ingredient were weighed into nylon bags and incubated in the rumen for 12 h (four replicates per feed ingredient per cow, total of 3 cows). After removal, bags were washed until clean, then placed in 0.1% methylcellulose solution and shaken at 37°C for 30 min. Following this treatment, bags were removed, thoroughly washed, and dried at 55°C to constant weight (48 h) to obtain rumen-degraded residue samples for CP content determination.

One gram of residue was then weighed into a 5 cm × 10 cm nylon bag, sealed, and placed in a Daisy II incubator bottle (maximum capacity of 30 bags per bottle;

six replicates of the same feed ingredient were placed in each bottle). Each bottle contained 2 L of hydrochloric acid solution (pH = 1.9) with 1 g/L pepsin (P-7000, Sigma), preheated before use. Bottles were incubated in an ANKOM Daisy II in vitro fermentation incubator at 39°C with rotation for 1 h. After incubation, bags were removed, washed, and transferred to 2 L of preheated phosphate buffer (0.5 mol/L) containing 3 g/L trypsin (P-7545, Sigma) and 50 g/L thymol. Bottles were returned to the incubator and rotated at 39°C for 24 h. Finally, bags were removed, washed until clean, and dried at 55°C to constant weight (48 h) to obtain post-intestinal digestion residue samples for CP content determination.

1.4 Measurement Indicators

Sample contents of dry matter (DM), crude protein (CP), and crude ash were determined according to GB 6435-86, GB/T 6432-94, and GB 6438-92 methods, respectively. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were measured using the method of Van Soest et al.

1.5.1 Rumen Degradability

The formula for calculating nutrient degradability at different time points was: Degradability of nutrient at time point (%) = 100 × (nutrient content before degradation - nutrient content after degradation) / nutrient content before degradation.

1.5.2 Rumen Degradation Parameters

Rumen degradation parameters of feed ingredients were calculated using the exponential model of Ørskov et al.:
$P = a + b(1 - e^{(-ct)})$

Where: t = retention time of feed ingredient in rumen (h); P = degradability of nutrient at time t (%); a = rapidly degradable fraction (%); b = slowly degradable fraction (%); c = degradation rate of b (%/h).

Values for a, b, and c were calculated using the least squares method, and effective degradability was calculated as:
$ED = a + bc/(c + k)$

Where: ED = effective degradability (%); k = outflow rate of feed ingredient (%/h). In this experiment, k values were 0.0253 for forages and crop straws, 0.0399 for bran products, and 0.0800 for meals and grains.

1.5.3 Idg of RUP and Intestinal Digestible Crude Protein (IDCP)

Idg and IDCP were calculated using the following formulas:
$Idg\ (\%) = 100 \times (CP_{12}h - CPi) / CP_{12}h$
$IDCP\ (g/kg) = RDP \times 0.85 \times 0.7 + RUP \times Idg$

Where: $CP_{12}h$ = CP content in residue after 12 h rumen fermentation (g/kg); CPi = CP content in residue after simulated intestinal digestion (g/kg); RDP = rumen degradable protein content (g/kg).

1.6 Statistical Analysis

Data were organized using Excel and analyzed using the ANOVA procedure in SAS 9.2 software. Differences were considered significant at $P < 0.05$.

2.1.1 DM Rumen Degradation Characteristics

As shown in Table 3 , DM rumen degradability of feed ingredients increased over time, with substantial variation in degradation parameters among ingredients at each time point. Corn silage exhibited the highest degradability at 2 h (33.81%), while alfalfa meal showed the highest degradability at 6 h (48.62%). At 12, 24, and 48 h, alfalfa meal and corn silage had identical degradability values that were significantly higher than other ingredients ($P < 0.05$). At 72 h, corn silage and alfalfa meal maintained higher degradability, significantly exceeding the progressively lower values for alfalfa hay, tomato sauce residue, cottonseed hulls, and grape seed meal ($P < 0.05$), with no significant difference between corn silage and alfalfa meal ($P > 0.05$).

Analysis of DM rumen degradation parameters revealed differences in rapidly degradable fractions among the six ingredients. Alfalfa meal had the highest rapidly degradable fraction, followed by corn silage, though the difference was not significant ($P < 0.05$). Cottonseed hulls showed the lowest rapidly degradable fraction at 9.05%. The slowly degradable fraction of cottonseed hulls was significantly higher than other ingredients ($P < 0.05$), which is unfavorable for animal utilization, while grape seed meal had the lowest value at 19.49%. Effective degradability of alfalfa meal and corn silage was significantly higher than other ingredients ($P < 0.05$), with tomato sauce residue and alfalfa hay showing intermediate values that did not differ significantly ($P > 0.05$). Grape seed meal and cottonseed hulls exhibited lower effective degradability without significant difference between them ($P > 0.05$).

2.1.2 CP Rumen Degradation Characteristics

As presented in Table 4 , CP rumen degradability of feed ingredients increased with time. Tomato sauce residue showed the highest CP degradability at 2 h ($P < 0.05$). Alfalfa meal demonstrated significantly higher CP degradability than all other ingredients at 12, 24, 48, and 72 h ($P < 0.05$), indicating superior protein quality. Cottonseed hulls consistently exhibited the lowest CP degradability at all time points ($P < 0.05$), with only 34.90% degradation at 72 h, reflecting low nutritional value of its protein content.

Corn silage had the highest rapidly degradable fraction of CP, significantly exceeding the progressively lower values for tomato sauce residue, alfalfa hay, alfalfa meal, grape seed meal, and cottonseed hulls ($P < 0.05$). Alfalfa meal

possessed the highest slowly degradable fraction at 59.62%, significantly greater than grape seed meal ($P < 0.05$), while tomato sauce residue had the lowest at 17.61%. Tomato sauce residue and alfalfa meal showed higher effective degradability, with grape seed meal exhibiting the lowest value at 39.57%. Significant differences in CP effective degradability were observed among all six feed ingredients ($P < 0.05$).

2.1.3 NDF Rumen Degradation Characteristics

Table 5 shows that corn silage maintained significantly higher NDF rumen degradability than other ingredients at all time points ($P < 0.05$), with 64.12% degradability at 12 h, indicating that NDF degradation of corn silage occurred primarily within the first 12 h. Except for the lowest values observed for tomato sauce residue at 2 and 6 h, grape seed meal consistently showed the lowest NDF degradability at all other time points.

The rapidly degradable fraction of NDF in corn silage was significantly higher than other ingredients ($P < 0.05$) at 44.34%, while tomato sauce residue had the lowest value at 12.14%, with significant differences among all ingredients ($P < 0.05$). Alfalfa hay exhibited the highest slowly degradable fraction at 45.90%, whereas grape seed meal had the lowest at only 9.60%. Corn silage achieved the highest NDF effective degradability at 68.60%, followed by alfalfa meal, cottonseed hulls, alfalfa hay, tomato sauce residue, and grape seed meal at only 25.19%.

2.1.4 ADF Rumen Degradation Characteristics

As shown in Table 6 , corn silage demonstrated significantly higher ADF rumen degradability than other ingredients at all time points ($P < 0.05$), reaching 66.04% at 72 h. Cottonseed hulls ranked second in ADF degradability at 2, 6, and 12 h, with values ranging from 22.25% to 38.56% for cottonseed hulls and 11.11% to 22.47% for grape seed meal. Alfalfa meal showed the lowest ADF degradability at 2 h ($P < 0.05$), while alfalfa hay was lowest at 6 and 12 h ($P < 0.05$). Grape seed meal had the lowest ADF degradability at 24, 48, and 72 h, with only 22.47% degradation at 72 h.

The rapidly degradable fraction of ADF in cottonseed hulls was significantly higher than other ingredients ($P < 0.05$), followed by corn silage, while alfalfa meal had the lowest value at only 1.66%. Corn silage exhibited significantly higher ADF effective degradability than other ingredients ($P < 0.05$). Grape seed meal had the lowest ADF effective degradability at 18.33%, while other ingredients ranged around 30%.

2.2 Idg of RUP and IDCP of Six Feed Ingredients

Table 7 reveals that both Idg of RUP and IDCP content varied among feed ingredients. Alfalfa meal showed significantly higher Idg of RUP and IDCP than other ingredients ($P < 0.05$). Alfalfa hay and corn silage ranked second in

Idg of RUP, with alfalfa hay' s IDCP not differing significantly from alfalfa meal ($P > 0.05$). Grape seed meal and cottonseed hulls exhibited low Idg of RUP and IDCP without significant differences between them ($P > 0.05$). These results indicate that among the six feed ingredients, alfalfa meal, alfalfa hay, and corn silage provided greater amounts of CP to the small intestine.

Discussion

3.1 Nutrient Composition of Six Feed Ingredients

This study found that alfalfa meal contained relatively high CP content and low levels of NDF, ADF, and EE, whereas alfalfa hay contained less CP but higher fiber content (NDF, ADF) with similar EE content. This difference likely arises because alfalfa meal consists primarily of alfalfa leaves, while alfalfa hay contains more stems. The DM and CP contents of grape seed meal measured in this study were similar to values reported by Liu (DM 95.61%, CP 12.20%). Du et al. reported that adding 1% to 4% grape seed meal to dairy cow diets could improve production performance. The CP, NDF, and ADF contents of cottonseed hulls in this study were similar to values reported by Yuan (CP 6.20%, NDF 81.31%, ADF 65.13%). Tomato sauce residue contained relatively high CP, NDF, and ADF contents, slightly higher than values reported by Liu, possibly due to differences in origin and other factors. Jia reported corn silage CP, NDF, and ADF contents of 10.32%, 48.65%, and 21.58%, respectively; our measured CP content was lower while NDF and ADF contents were higher. Variations in feed nutritional value are related to geographic environment, cultivation methods, and processing/storage conditions. Based on preliminary assessment of routine nutrient contents, tomato sauce residue, alfalfa meal, and alfalfa hay appear to have higher feeding value than grape seed meal, corn silage, and cottonseed hulls.

3.2 Rumen Degradation Patterns of Six Feed Ingredients

DM rumen degradability positively correlates with DM intake; higher DM rumen degradability within a certain timeframe corresponds to greater DM intake in dairy cows. This study found no significant differences in DM rumen degradability between alfalfa meal and corn silage at various time points, with both exceeding other ingredients, indicating easy digestibility in the rumen with effective degradability of 60.64% and 60.54%, respectively. Yu et al. and Liu reported corn silage DM effective degradability of 53.07% and 64.70%, respectively, demonstrating that feed origin and animal breed affect results. Alfalfa hay DM rumen degradability increased steadily after 24 h, reaching 60.04% at 72 h, indicating that DM digestion of alfalfa hay concentrated in the first 24 h. Alfalfa meal showed higher DM rumen degradability than alfalfa hay at all time points, confirming greater digestibility. This aligns with Leng et al., who reported higher DM rumen degradability for alfalfa meal than alfalfa hay. The 72 h DM rumen degradability of tomato sauce residue, grape seed meal, and cottonseed hulls was below 60%, though tomato sauce residue exceeded the other

two at all time points. Their DM effective degradability values were 44.86%, 29.94%, and 28.28%, respectively, consistent with Liu' s report of decreasing effective degradability in this order.

Multiple factors influence CP rumen degradability, including retention time, fermentation difficulty, and inherent feed characteristics. Tomato sauce residue showed the highest CP effective degradability at 77.30%, followed by alfalfa meal at 71.34%, likely related to their high original CP content. Leng et al. suggested that high CP content in forages facilitates degradation. Corn silage CP rumen degradability exceeded alfalfa hay at all time points except 24 h, with effective degradability of 66.02%, significantly higher than alfalfa hay. Grape seed meal showed 72.92% CP degradability at 72 h but only 39.57% effective degradability. Cottonseed hulls consistently exhibited the lowest CP rumen degradability, with slow changes before 12 h, possibly due to increased lignin content with plant maturation and aging, resulting in slower degradation.

Fiber degradability represents an important indicator for evaluating feed nutritional value. Crude fiber, the main component of plant cell walls comprising cellulose, hemicellulose, lignin, and pectin, is the most difficult fraction for ruminants to digest and absorb. Limited and inconsistent data exist on fiber degradation. This study found corn silage had the highest NDF and ADF rumen degradability and effective degradability, significantly exceeding alfalfa-based feeds, contrary to Xia et al. but consistent with Liu' s findings showing no significant difference between alfalfa and corn silage. Variations in origin, variety, harvest time, and processing methods contribute to these differences. Cottonseed hulls showed high initial degradability that increased slowly thereafter, indicating degradation occurred primarily within the first 2 h. Tomato sauce residue fiber degradation started low but became relatively high at 72 h, suggesting degradation mainly occurred after 24 h. Grape seed meal consistently showed low NDF and ADF rumen degradability, consistent with Liu' s report. Degradation dynamic parameters indicate that corn silage and alfalfa meal have superior nutritional quality, while grape seed meal' s low NDF and ADF rumen degradability suggests poor digestibility for ruminants.

3.3 Idg of RUP and IDCP Content of Six Feed Ingredients

The small intestine demonstrates good absorption capacity for feed protein that bypasses rumen degradation, making RUP an excellent source of intestinally digestible protein. This study' s Idg and IDCP values were estimated using models from *Nutrient Requirements and Feed Composition of Dairy Cows*. Alfalfa meal and alfalfa hay, with relatively high CP content, showed correspondingly high Idg of RUP, consistent with Chen et al.' s conclusion that high-protein, low-fiber feeds are readily digested and utilized in the small intestine. However, tomato sauce residue was an exception: despite having the same original CP content as alfalfa meal and higher NDF and ADF contents than both alfalfa products, its Idg of RUP was much lower, possibly because most protein was degraded in the rumen, leaving the remaining fraction bound to lignin and resistant to intestinal

digestion. Corn silage Idg of RUP was 57.18%, within the range of 54.85% to 64.93% reported by Li et al. Cottonseed hulls showed only 19.34% Idg of RUP, likely related to its high crude fiber content. Limited domestic research exists on grape seed meal, with international studies focusing on oligomeric proanthocyanidins. Although this study found grape seed meal provided limited CP to the small intestine, its low cost and rich content of oligomeric proanthocyanidins —which exhibit significant antibacterial and anti-mutagenic effects—make it a valuable feed ingredient for nutritional supplementation and disease prevention.

Taghizadeh et al. suggested that to maintain total digestive tract digestibility in ruminants, low rumen degradability should be compensated by relatively high intestinal digestibility. Approximately 40% of dietary protein should be digested and absorbed in the small intestine to meet tissue metabolic requirements. Excessive rumen protein degradation may result in insufficient protein reaching the small intestine to meet nutritional needs. Chalupa et al. emphasized that dietary protein should minimize rumen degradation to provide adequate amino acids for intestinal digestion and utilization. RUP represents a crucial indicator for evaluating ruminant protein requirements and feed protein content, based on theories of protein digestion and absorption mechanisms, cellular metabolism, and rumen degradation characteristics. This study demonstrates that alfalfa meal, alfalfa hay, and corn silage have high IDCP content, providing better protein nutrition for ruminants.

Different feed ingredients exhibit distinct rumen degradation characteristics and vary in their efficiency of IDCP production. Corn silage shows high effective degradability of DM, CP, NDF, and ADF in the rumen, alfalfa meal demonstrates high intestinal digestibility of RUP, and both alfalfa meal and alfalfa hay possess relatively high IDCP content.

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