Comparative Study on Determination of Metabolizable Energy of Leymus chinensis for Meat Sheep by Direct and Substitution Methods: Postprint

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Abstract

This experiment aimed to compare the direct and substitution methods for determining the metabolic energy of Leymus chinensis for meat sheep. Thirty crossbred meat wethers with good body condition and an average body weight of approximately 45.00 kg were selected and randomly assigned to 5 treatments using a randomized block design, with 6 replicates per treatment and 1 sheep per replicate. The treatments consisted of a direct-method whole Leymus chinensis diet, a substitution-method basal diet (concentrate-to-forage ratio of 1:1), and three experimental diets in which the basal diet was substituted with 60%, 40%, and 20% Leymus chinensis, respectively. All diets were fed at 600 g per meal at 08:00 and 18:00. Each experimental period lasted 19 days, including a 10-day preliminary period and a 9-day formal collection period. The results showed that the apparent digestibility of dry matter, organic matter, gross energy, and crude protein followed the order: basal diet group > 20% group > 40% group > 60% group > whole Leymus chinensis diet group ($P<0.05$). Digestible energy and metabolic energy followed the same trend: basal diet group > 20% group > 40% group > 60% group > whole Leymus chinensis diet group ($P<0.05$). The ratio of metabolic energy to digestible energy did not differ significantly among the dietary groups ($P>0.05$). The direct and substitution methods did not significantly affect the apparent digestibility of crude protein and neutral detergent fiber in Leymus chinensis as a feed ingredient ($P>0.05$). In the direct method, the apparent digestibility of dry matter, organic matter, and gross energy of Leymus chinensis in the whole Leymus chinensis diet group was significantly higher than that in the 60% group ($P<0.05$), but did not differ significantly from the 40% and 20% groups ($P>0.05$). The digestible energy and metabolic energy of Leymus chinensis in the whole Leymus chinensis diet

group were significantly higher than those in the 60% group (P<0.05), with no significant differences compared to the 40% and 20% groups (P>0.05). In conclusion, different substitution ratios significantly affected the results when using the direct and substitution methods to determine the metabolic energy of Leymus chinensis, and the optimal substitution ratio was 20% when employing the substitution method.

Full Text

A Comparison of Direct and Substitution Methods for Determining the Metabolizable Energy of Leymus chinensis in Mutton Sheep

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Abstract

This experiment aimed to compare the metabolizable energy (ME) of Leymus chinensis in mutton sheep determined by direct and substitution methods. Thirty crossbred mutton wethers with good body condition and an average weight of 45.00 kg were randomly allocated to five treatments using a randomized block design, with six replicates per treatment and one sheep per replicate. The treatments consisted of a full Leymus chinensis diet for the direct method, a basal diet (concentrate-to-forage ratio of 1:1) for the substitution method, and three experimental diets in which the basal diet was replaced by Leymus chinensis at 60%, 40%, and 20% levels. All diets were fed at 600 g per meal at 08:00 and 18:00 daily. Each experimental period lasted 19 days, comprising a 10-day preliminary period and a 9-day collection period. The results showed that the apparent digestibility of dry matter (DM), organic matter (OM), gross energy (GE), and crude protein (CP) followed the pattern: basal diet group > 20% group > 40% group > 60% group > full Leymus chinensis diet group (P<0.05). Digestible energy (DE) and ME exhibited the same trend: basal diet group > 20% group > 40% group > 60% group > full Leymus chinensis diet group (P<0.05). The ratio of ME to DE did not differ significantly among groups (P>0.05). The direct and substitution methods showed no significant difference in the apparent digestibility of CP and neutral detergent fiber (NDF) of Leymus chinensis (P>0.05). In the direct method, the apparent digestibility of DM, OM, and GE of Leymus chinensis in the full Leymus chinensis diet group

was significantly higher than in the 60% substitution group (P<0.05), but did not differ significantly from the 40% and 20% groups (P>0.05). The DE and ME of Leymus chinensis in the full Leymus chinensis diet group were significantly higher than in the 60% group (P<0.05), with no significant differences compared to the 40% and 20% groups (P>0.05). In conclusion, different substitution proportions significantly affect the results when determining the ME of Leymus chinensis using the substitution method, with the optimal substitution proportion being 20%.

Keywords: direct method; substitution method; metabolizable energy estimation; mutton sheep; Leymus chinensis

Introduction

China is a major sheep-producing country but not yet a strong one in terms of production efficiency. As intensive feeding systems for mutton sheep continue to develop, feed formulation has become a key technology. Nutritional requirements and feed nutritional values constitute essential baseline data for animal diet formulation. Roughage represents an important nutritional source for ruminants, and its rational and efficient utilization offers a scientific pathway toward sustainable development of China' s animal husbandry. The foundation of feed nutritional value assessment lies in the determination and calculation of metabolizable energy (ME). Therefore, it is necessary to identify an accurate and objective method for evaluating the ME of single roughage ingredients, which is crucial for establishing a nutritional database for ruminant feeds, optimizing diet formulations, and reducing feeding costs.

Research reports from various countries on effective energy evaluation methods for ruminant feeds exhibit different characteristics, particularly regarding the operability and repeatability of measurement methods, which often fail to meet the requirements for accurate assessment of feed ingredient effective energy values. The direct method and substitution method within in vivo approaches are classical methods for evaluating ingredient energy values. Previous studies have reported that the substitution method yields more accurate measurements for rabbit feed ingredients, while the ME of single feedstuffs for broiler chicks, adult chickens, and laying hens is suitably determined using the substitution method. Similarly, the net energy and digestible energy (DE) of pig feed ingredients have been measured using the substitution method.

Currently, most assessments of roughage nutritional levels in ruminants employ in vitro or semi-in vivo methods to evaluate nutrient content and degradation rates, with fewer studies investigating nutrient availability. Existing reports on effective energy values of single roughage ingredients primarily rely on mathematical formula calculations or in vitro estimations. However, the most accurate and intuitive method for evaluating feed nutritional levels is animal experimentation. Both in vivo and semi-in vivo methods require experimental animals, but semi-in vivo methods are unsuitable for evaluating the ME of single roughage

ingredients due to inherent limitations. In contrast, in vivo evaluation of single roughage ingredient ME has rarely been reported in ruminants. Given the complex rumen structure of mutton sheep and the characteristics of roughage itself, it is necessary to investigate the application of direct and substitution methods for evaluating single roughage energy values in ruminants. This experiment used Leymus chinensis as the test ingredient and crossbred mutton wethers weighing 45.0 kg as experimental animals to determine DE, ME, and apparent digestibility of various nutrients through both direct and substitution methods, thereby examining the effects of these two methods on Leymus chinensis ME and providing methodological data for evaluating single roughage energy values in mutton sheep.

Materials and Methods

Experimental Animals and Design

Digestion and metabolism trials and gas metabolism measurements were conducted at the Nankou Pilot Base of the Chinese Academy of Agricultural Sciences, while sample analyses were performed at the Livestock Nutrition and Feed Laboratory of the Feed Research Institute. Thirty crossbred mutton wethers (Dorper × Small-tailed Han sheep F1 generation) with good body condition and an initial weight of (45.00$±$1.96) kg were divided into five treatments with six replicates per treatment and one sheep per replicate. Each sheep was individually housed in stainless steel pens (3.2 m × 0.8 m).

Experimental Diets and Preparation

Diets for both direct and substitution methods were formulated according to NRC (2007) recommendations for 40–50 kg adult rams at 1.3 times maintenance requirements. The direct method employed a full Leymus chinensis diet, while the substitution method utilized a basal diet and three experimental diets in which Leymus chinensis replaced the basal diet at 60%, 40%, and 20% levels, designated as the 60%, 40%, and 20% groups, respectively. All experimental diets were processed into pelleted feed (pellet diameter 4.5 mm, length 10 mm). Diet composition and nutrient levels are presented in Table 1 .

Experimental Procedure

The experimental period lasted 19 days, consisting of a 10-day preliminary period and a 9-day collection period. The collection period included 3 days for gas metabolism measurement and 6 days for digestion and metabolism trials. Prior to the preliminary period, maintenance feed intake was determined by feeding the basal diet to achieve zero daily weight gain. Experimental sheep were limit-fed (1,200 g/d of each diet) with 600 g provided at 08:00 and 18:00 daily, with free access to water throughout the day.

Digestion and metabolism trials employed metabolic cages designed and manu-

factured by the Feed Research Institute, featuring automatic separation of feces and urine. Total collection method was used to collect feces and urine. Daily fecal output was weighed and recorded, with 10% sampled and mixed from each sheep over the 6-day collection period and stored frozen. Urine was collected in plastic buckets containing 100 mL of 10% H_2SO_4, diluted to 5 L to prevent uric acid precipitation during storage. The diluted urine was thoroughly mixed, filtered through gauze, and 20 mL sampled daily. Urine samples from each sheep were pooled over the 6-day period and stored at -20 °C.

Gas metabolism trials utilized the Sable open-circuit respiratory gas metabolism system connected to an LGR gas analyzer to measure methane production. The system accommodated six gas metabolism chambers simultaneously. Experimental sheep were moved into the chambers in five batches, with six sheep from the same treatment measured per batch. After a 24-hour adaptation period, methane emissions (from respiration, digestion, and body surface) were measured over the subsequent 48 hours for ME calculation. At the end of the digestion trial, fecal samples from each sheep were mixed and dried in a 65 °C oven for 48 hours, equilibrated for 48 hours, and weighed to determine initial moisture content. Fecal samples were then ground through a 40-mesh sieve to prepare analytical samples.

Measurements and Analyses

Nutrient Digestibility Dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), ash, and gross energy (GE) in diets, ingredients, and feces, as well as calcium (Ca) and phosphorus (P) content in ingredients, were determined according to "Feed Analysis and Feed Quality Detection Technology" [**?**].

Methane Production Measurement The Sable open-circuit respiratory calorimetry system was used. After weighing, experimental sheep were moved into gas metabolism chambers and methane production was measured over 48 hours following adaptation (L).

Metabolizable Energy Determination Urine energy was determined by measuring the energy value of five quantitative filter papers to calculate the average filter paper energy value. Ten milliliters of urine were applied dropwise to filter paper multiple times, dried at 65 °C, and measured using an IKA C2000 bomb calorimeter to obtain the combined energy value of filter paper and urine. Urine energy was calculated as: Urine energy = (Filter paper + urine energy value) - (Filter paper energy value). Methane energy (CH_4-E, kJ) = Methane production (L) × 39.54 (kJ/L) [**?**]. ME was calculated as: ME = GE - Fecal energy - Urine energy - CH_4-E.

Calculation Formulas

Apparent total tract digestibility of nutrients in diets and ingredients was calculated using Adeola' s formula [**?**]:

Apparent total tract digestibility of dietary nutrients (%) = 100 × (Nutrient intake - Nutrient content in feces) / Nutrient intake.

Apparent total tract digestibility of ingredient nutrients (%) = 100 × [Apparent total tract digestibility of nutrients in experimental diet - (1-X) × Apparent total tract digestibility of nutrients in basal diet] / X.

Where: X is the proportion of test ingredient replacing the basal diet (%).

The substitution method formula for determining ingredient energy values followed Liu Dewen [**?**] and Tao Chunwei [**?**]:

Energy value = 100 × [Energy value of experimental diet - (1-X) × Energy value of basal diet] / X.

Where: X is the proportion of test ingredient replacing the basal diet; Energy value (MJ/kg) can be DE or ME.

Statistical Analysis

Experimental data were analyzed using the ANOVA procedure in SAS 9.2 statistical software, with Duncan' s multiple range test for post-hoc comparisons. Differences were considered significant at $P < 0.05$.

Results

Nutrient Levels of Leymus chinensis

The nutrient levels of Leymus chinensis are presented in Table 2 .

Apparent Nutrient Digestibility of Diets Determined by Direct and Substitution Methods

As shown in Table 3 , apparent total tract digestibility of DM, OM, CP, and GE followed the pattern: basal diet group > 20% group > 40% group > 60% group > full Leymus chinensis diet group, with significant differences among groups ($P < 0.05$). However, apparent total tract digestibility of NDF did not differ significantly among groups ($P > 0.05$).

Energy Values of Diets Determined by Direct and Substitution Methods

Table 4 shows that fecal energy, CH_4-E, DE, and ME differed significantly among diets ($P < 0.05$), whereas urine energy did not ($P > 0.05$). Fecal energy followed the pattern: full Leymus chinensis diet group (10.11 MJ/d) > 60% group (9.53 MJ/d) > 40% group (8.63 MJ/d) > 20% group (7.64 MJ/d) > basal diet group

(7.05 MJ/d), with significant differences among groups ($P < 0.05$). CH_4-E was significantly higher in the basal diet group (1.58 MJ/d) and 40% group (1.57 MJ/d) compared to the 20% group (1.40 MJ/d), 60% group (1.35 MJ/d), and full Leymus chinensis diet group (1.29 MJ/d) ($P < 0.05$). DE and ME followed the pattern: basal diet group (12.27 and 9.66 MJ/kg DM) > 20% group (11.54 and 9.13 MJ/kg DM) > 40% group (10.78 and 8.44 MJ/kg DM) > 60% group (9.70 and 7.65 MJ/kg DM) > full Leymus chinensis diet group (8.76 and 6.81 MJ/kg DM), with significant differences among groups ($P < 0.05$).

Apparent Nutrient Digestibility and Energy Values of Leymus chinensis Determined by Direct and Substitution Methods

As shown in Table 5 , apparent total tract digestibility of CP and NDF did not differ significantly among groups ($P > 0.05$). In the direct method, apparent digestibility of DM, OM, and GE of Leymus chinensis in the full Leymus chinensis diet group was significantly higher than in the 60% substitution group ($P < 0.05$), but did not differ significantly from the 40% and 20% groups ($P > 0.05$), with the 20% group being closest to the full Leymus chinensis diet group. The DE of Leymus chinensis in the full Leymus chinensis diet group (8.95 MJ/kg DM) was significantly higher than in the 60% group (7.98 MJ/kg DM) ($P < 0.05$), with no significant differences compared to the 40% group (8.56 MJ/kg DM) and 20% group (8.62 MJ/kg DM) ($P > 0.05$), with the 20% group being closest to the full Leymus chinensis diet group. The ME of Leymus chinensis in the full Leymus chinensis diet group (6.96 MJ/kg DM) differed significantly from the 60% group (6.31 MJ/kg DM) ($P < 0.05$), but showed no significant differences compared to the 40% group (6.61 MJ/kg DM) and 20% group (7.01 MJ/kg DM) ($P > 0.05$), with the 20% group being closest to the full Leymus chinensis diet group. Additionally, the ME:DE ratio did not differ significantly between the two methods ($P > 0.05$). These findings suggest that when using the substitution method to calculate ME of Leymus chinensis, a substitution proportion of 20% is optimal.

Discussion

Comparison of Energy Values Among Full Leymus chinensis Diet, Basal Diet, and Substitution Diets

Feed energy serves as the source of nutrients for livestock, with energy losses occurring during ingestion, digestion, absorption, and metabolism. Fecal energy represents the largest portion of feed energy loss, being highest in the full Leymus chinensis diet group, followed by the 60%, 40%, 20%, and basal diet groups. The basal diet exhibited higher DE and ME than the full Leymus chinensis diet, 60%, 40%, and 20% groups, primarily due to differences in feed ingredient composition. Variations in nutrient digestibility among feed ingredients lead to differences in effective energy. Feed ingredient composition is the main factor affecting feed energy values, with both readily digestible protein and poorly digestible cellulose exerting substantial influence. Feed fiber content

shows a strong negative correlation with ME. In this experiment, NDF content was highest in the full Leymus chinensis diet group, followed by the 60%, 40%, 20%, and basal diet groups, while DE and ME increased progressively in the opposite order. These results align with Liu Jie' s findings.

Significant differences in CH_4-E were observed among the five diets. Numerous factors influence CH_4-E. In this study, CH_4-E ranked from highest to lowest as: basal diet group, 40% group, 20% group, 60% group, and full Leymus chinensis diet group. CH_4-E accounted for 11.23% to 13.38% of DE. Tamminga reported that typically 8% to 12% of DE is lost as methane in dairy cow rumen. Zhao Yiguang found CH_4-E accounted for 10.76% to 12.27% of DE in mutton sheep. Despite similar diet composition and concentrate-to-forage ratios, our results differ from these studies, possibly due to variations in raw material nutritional values and because Zhao Yiguang used a respiratory headbox that only measured methane expelled through eructation from the mouth and nose, failing to capture real-time methane emissions from the rectum. In contrast, our study employed respiratory metabolism chambers for continuous 48-hour monitoring.

Comparison of Apparent Nutrient Digestibility Among Full Leymus chinensis Diet, Basal Diet, and Substitution Diets

Apparent nutrient digestibility of diets reflects nutrient utilization and physiological status in sheep. Digestibility can be affected by diet composition and formulation, feeding level, physiological stage, experimental animals, and environmental conditions. The significant differences in DM, OM, GE, and CP apparent digestibility among the five diets can be attributed to several factors. First, NDF content significantly affects apparent total tract digestibility of DM, OM, CP, and NDF, with the full Leymus chinensis diet group containing the most dietary NDF, followed by the 60%, 40%, and 20% groups. Valdés et al. confirmed that DM apparent digestibility decreases with increasing dietary roughage proportion. In this experiment, as the Leymus chinensis substitution proportion increased and dietary roughage proportion rose, the basal diet group showed progressively higher DM apparent digestibility than the 60%, 40%, 20%, and full Leymus chinensis diet groups. Second, apparent digestibility of DM, OM, GE, and CP correlates significantly positive with their concentrations in feed, with CP having the greatest impact on apparent digestibility. Our results are consistent with this pattern. Liu Jie reported slightly higher digestibility in diets with similar nutritional levels. Therefore, the DE and ME values obtained for the basal diet and substitution diets in this experiment were measured under normal physiological digestion and metabolism conditions, making them objective and suitable for substitution method calculations.

Effects of Direct and Substitution Methods on Apparent Nutrient Digestibility and Energy Values of Leymus chinensis

This experiment demonstrated that the direct and substitution methods showed no significant differences in apparent total tract digestibility of CP and NDF

of Leymus chinensis. However, apparent digestibility of DM, OM, and GE of Leymus chinensis in the direct method was significantly higher than in the 60% substitution group, with no significant differences compared to the 40% and 20% groups, among which the 20% group was closest to the full Leymus chinensis diet. The DE of Leymus chinensis in the full Leymus chinensis diet group was significantly higher than in the 60% group, with no significant differences compared to the 40% and 20% groups, with the 20% group being closest to the full Leymus chinensis diet group. The ME of Leymus chinensis in the full Leymus chinensis diet group differed significantly from the 60% group, with no significant differences compared to the 40% and 20% groups, among which the 20% group was closest to the full Leymus chinensis diet group. Additionally, the energy conversion efficiency from DE to ME (ME:DE) showed no significant differences between the two methods. These findings indicate that when using the substitution method to calculate ME of Leymus chinensis, a substitution proportion of 20% is appropriate.

The direct and substitution methods are classical approaches for measuring single feedstuffs. For sheep, roughage can be fed alone to effectively avoid nutrient interactions among ingredients. Using the test ingredient as the sole energy source allows direct calculation of the difference between ingested GE and energy lost through digestion and metabolism, though this method has limited application due to constraints from ingredient palatability and nutritional composition. In this study, Leymus chinensis contained 8.87% moisture, 87.62% OM, 7.64% CP, 1.94% EE, 64.87% NDF, 40.24% ADF, 0.19% P, 0.46% Ca, and 16.29 MJ/kg GE. Processing Leymus chinensis into pelleted feed improved palatability and ensured adequate intake.

Few studies have investigated different methods for determining feed ingredient energy values, with relatively more research conducted on monogastric animals such as poultry and pigs. Bolarinwa and Adeola reported no significant differences between direct and regression methods for determining wheat energy values in pigs. Liu Dewen explored the effects of direct and substitution methods on determining corn net energy in growing pigs, finding no significant differences in DE, ME, net energy, and retained net energy among diets with 8.38% CP in the direct method corn diet, 17.19% CP in the basal diet, and 13.76% CP in the substituted diet. Nie Dawa applied interpolation to investigate appropriate substitution proportions for determining corn ME in 28-day-old broilers, finding no significant differences in apparent metabolizable energy (AME) values across substitution proportions (20% to 70%), except for the 80% substitution group. In our study, Leymus chinensis substitution proportions of 60%, 40%, and 20% yielded no significant differences in ME among groups except for the 60% group, suggesting that substitution proportions of 20% or 40% are suitable for determining Leymus chinensis ME using the substitution method.

The substitution method requires formulating a basal diet that meets animal nutritional requirements, yet no fixed substitution proportion has been established, making it difficult to maintain consistent protein and fiber levels in experimen-

tal diets. The objective of this study was to determine the optimal substitution proportion for Leymus chinensis. Our substitution method created experimental diets with varying nutritional levels by replacing different proportions of the basal diet' s energy components (Leymus chinensis, corn, soybean meal) with Leymus chinensis. The results showed that the 40% and 20% groups yielded ME values not significantly different from the full Leymus chinensis group, while the 60% group showed significantly different and lower ME values, indicating that excessive substitution proportions negatively affect ME, possibly due to altered protein and fiber ratios. Villamide reviewed direct, substitution, and multiple regression methods for determining rabbit feed energy values, concluding that the substitution method achieved highest accuracy at 20% to 30% substitution proportions. In mutton sheep research, previous studies have used semi-in vivo and in vitro methods to evaluate nutrient content and availability. Tao Chunwei used the substitution method with a basal diet of approximately 8:2 concentrate-to-forage ratio, replacing energy components with test feeds (Leymus chinensis, corn straw, alfalfa, silage) at 65% of the basal diet, resulting in new diets with CP contents of 9.18%, 8.21%, 6.19%, and 15.29%. The determined DE and ME of Leymus chinensis (9.56$\pm$0.14*and*7.63$\pm$$0.51 MJ/kg) did not differ significantly from in vitro values. In contrast, our experiment measured DE and ME of Leymus chinensis as 8.95 and 6.96 MJ/kg DM. Given the significant differences between direct and substitution methods, our study found that direct method measurements were closest to values obtained from the 20% substitution group. While the two methods showed some differences in nutrient digestibility, the direct method values for DM, OM, and GE digestibility did not differ significantly from the 40% and 20% groups, with the 20% group providing the closest ME value to the direct method. Therefore, a substitution proportion of 20% is recommended when using the substitution method to determine Leymus chinensis ME.

Conclusion

When determining the ME of Leymus chinensis, different substitution proportions significantly affect the results obtained by the substitution method. The optimal substitution proportion for determining Leymus chinensis ME using the substitution method is 20%.

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