Postprint of a Study on Prediction Models for Nutrient Digestibility and Metabolizable Energy in Meat Sheep Diets

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Date: 2017-11-07T00:00:00+00:00

Abstract

This experiment aimed to establish prediction models for nutrient digestibility and metabolizable energy (ME) in meat sheep diets. Sixty-six healthy F1 hybrid meat wethers (Dorper × Small-tailed Han) with a body weight of (45.0$±$2.0) kg were selected and randomly divided into 11 treatments, with 6 replicates per treatment and 1 sheep per replicate. Using a randomized block design, the nutrient contents of 11 diets with different roughage compositions were determined, and the nutrient digestibility, digestible energy (DE), and ME of these 11 diets were measured through metabolism trials and gas metabolism trials. Based on the analysis of dietary nutrient contents, digestible nutrients, DE, and ME, the best predictors were selected and prediction equations were established. The results showed that the digestibility of dietary dry matter (DM), organic matter (OM), crude protein (CP), and gross energy (GE) was significantly or highly significantly positively correlated with the contents of CP, GE, and OM in the diets ($P<0.05$ or $P<0.01$), and significantly negatively correlated with neutral detergent fiber (NDF) content ($P<0.05$). The digestibility of dietary NDF was significantly or highly significantly negatively correlated with the contents of DM, OM, CP, and GE in the diets ($P<0.05$ or $P<0.01$), and highly significantly positively correlated with NDF content ($P<0.01$). The best equation for estimating ME using dietary nutrient contents was ME=-49.593+0.594OM-0.107NDF (R2=0.949, $P<0.01$). It was concluded that dietary nutrient digestibility and ME were strongly correlated with nutrient contents, and that dietary nutrient digestibility and ME could be reasonably estimated through nutrient contents.

Full Text

A Study on the Prediction Model of Dietary Nutrient Digestibility and Metabolizable Energy of Mutton Sheep

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Abstract: This study aimed to establish prediction models for dietary nutrient digestibility and metabolizable energy (ME) in mutton sheep. Sixty-six Dorper × Small-tailed Han F1 crossbred mutton wethers with good body condition and initial body weight of (45.0$±2.0) kg were randomly allocated to 11 treatments, with 6 replicates per treatment and 1
0.05 or P < 0.01), but significantly negatively correlated with neutral detergent fiber (NDF) content (P <
0.05). Dietary NDF digestibility was significantly or extremely significantly negatively correlated with dietary D
0.05 or P < 0.01), but extremely significantly positively correlated with NDF content (P <
0.01). The optimal equation for predicting ME from dietary nutrient contents was ME =
−49.593 + 0.594 OM − 0.107 NDF (R^{2}$ = 0.949, P<0.01). It was concluded that dietary nutrient digestibility and ME have strong correlations with nutrient contents, and can be reasonably predicted using dietary nutrient contents.

Keywords: mutton sheep; digestibility; nutrient; metabolizable energy; prediction model

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Feed nutritional value evaluation requires not only determination of nutrient contents but also assessment of nutrient digestion and absorption efficiency and nutritional effects on animals. Feed energy value is a crucial factor affecting diet cost and feeding efficacy. Due to limitations in energy evaluation methods, input costs, result accuracy, and reproducibility, it is impractical to measure all forages, agricultural by-products, and concentrate feeds individually [1-2]. Therefore, establishing a simple and accurate method to evaluate dietary effective energy values is important for rational utilization of ruminant feed resources and scientific diet formulation. Currently, when evaluating feed energy utilization efficiency in monogastric animals such as pigs, chickens, and ducks, digestible energy and metabolizable energy are primarily measured, with measured values typically obtained directly through animal trials. Prediction models for effective energy have been established based on feed nutrient contents [3-5]. However, few studies have investigated in vivo measurement methods for ruminant feed energy in China in recent years, and reported equations for estimating roughage energy values are mostly based on calculated values or parameters obtained through in vitro methods [6-7]. Theoretically, in vivo digestion and metabolism trials yield the most authentic and objective results for determining feed effective energy values. Liu et al. [8] used diets with identical ingredient composition but varying neutral detergent fiber (NDF) levels covering all possible concentrate-to-forage ratios that meat sheep might consume in production. Through animal feeding trials, they measured nutrient digestibility and effective energy values in vivo and established equations to estimate dietary nutrient digestibility and effective energy values from nutrient contents. However, the ingredient composition in that trial was single, which may not be applicable to diets with other ingredient compositions. This study selected 10 commonly used roughage ingredients for mutton sheep. By measuring the nutrient contents of diets with different roughage compositions and combining material metabolism trials with gas metabolism trials, we obtained in vivo measured values of digestion parameters and metabolizable energy (ME) for different nutrients in meat sheep. The objective was to explore whether dietary nutrient digestibility and effective energy values can be objectively and accurately estimated using dietary nutrient content and other indicators, thereby providing references for rational utilization of feed resources and scientific diet formulation for mutton sheep.

1.1 Experimental Animals and Design

Animal feeding trials and gas metabolism trials were conducted at the Nankou Pilot Base of the Chinese Academy of Agricultural Sciences, while sample analysis was performed in the Laboratory of Animal Nutrition and Feed, Feed Research Institute, Chinese Academy of Agricultural Sciences. Sixty-six Dorper × Small-tailed Han F1 crossbred mutton wethers with good body condition and initial body weight of (45.0$±$2.0) kg were selected and randomly divided into 11 treatments, with 6 replicates per treatment and 1 sheep per replicate. Each sheep was individually housed in stainless steel pens (3.2 m × 0.8 m × 1.0 m).

1.2 Experimental Diets and Preparation

A basal diet (BD) was formulated according to the NRC (2007) [9] feeding standard for adult meat sheep at 1.3 times maintenance requirement (40–50 kg). Then, 10 experimental diets were prepared by substituting 20% [10] (this proportion was determined by our research team) of the main ingredients (corn + soybean meal + Chinese leymus) in the basal diet with 10 different ingredients, namely Chinese leymus diet (LC), alfalfa diet (AH), whole-plant corn silage diet (WPCS), corn stalk silage diet (CSC), sweet potato vine diet (SPV), peanut vine diet (PV), corn stalk diet (CS), soybean stalk diet (SS), wheat straw diet (WS), and rice straw diet (RS). Diet composition and nutrient levels are shown in Table 1 .

1.3 Experimental Methods and Procedures

The experimental period lasted 19 days, consisting of a 10-day preliminary period and a 9-day formal trial period, including 3 days for gas metabolism trial and 6 days for material metabolism trial. At the end of the trial, feces collected from each sheep were mixed and placed in a 65°C oven for 48 hours, then rehydrated for 48 hours and weighed to calculate initial moisture content. The fecal samples were then ground to pass through a 40-mesh sieve to prepare analytical samples for subsequent analysis.

1.3.1 Material Metabolism Trial Prior to the preliminary period, the maintenance feed intake at zero daily weight gain was determined by feeding the basal diet. Experimental sheep were fed using a restricted feeding method (1,200 g/d of each diet), with 600 g fed at 08:00 and 18:00 daily, and free access to water throughout the day. The material metabolism trial utilized metabolic cages designed and manufactured by the Feed Research Institute of the Chinese Academy of Agricultural Sciences, equipped with automatic separation devices for feces and urine. The total collection method was used to collect feces and urine. Daily fecal excretion was weighed and recorded, and 10% was sampled. Fecal samples from each sheep over 6 days were mixed and stored at -20°C. Urine was collected in plastic buckets containing 100 mL of 10% (v/v) H_2SO_4, diluted to 5 L (to prevent uric acid precipitation during storage), thoroughly mixed, filtered through gauze, and 20 mL was sampled daily. Urine samples from each sheep over 6 days were mixed and stored at -20°C.

1.3.2 Gas Metabolism Trial The gas metabolism trial used a closed-circuit respiration chamber system (Sable) with an LGR gas analyzer to measure methane production. Methane concentration entering and exiting the chamber and the gas volume flowing through the chamber within a certain time period were detected using an infrared methane analyzer to calculate actual methane emissions, carbon dioxide production, and oxygen consumption of the animals during that period. The system was connected to 6 closed respiration chambers, enabling continuous and uninterrupted measurement and recording of the respiratory status of 6 animals simultaneously. During the trial, experimental sheep were moved into the chambers in 11 batches, with 6 sheep from the same treatment measured per batch. After a 24-hour adaptation period in the chamber, methane emissions (including methane from respiratory tract, digestive tract, and body surface) were measured for the subsequent 48 hours and used to calculate dietary ME.

1.4 Calculations

1.4.1 Nutrient Digestibility The contents of dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), and gross energy (GE) in diets and fecal samples were determined according to "Feed Analysis and Feed Quality Detection Technology" [11]. Calculation methods for diet and ingredient nutrient digestibility followed formulas provided by Adeola [13] and Liu [14]:

Diet nutrient digestibility (%) = [Nutrient intake (g) -Nutrient content in feces

(g)] / Nutrient intake (g)
Diet digestible nutrient content (%) = [Feed nutrient content (%) × Diet nutrient digestibility (%)] / 100

1.4.2 Energy Values Three quantitative filter papers were weighed and recorded as m_1, then their energy values were measured through repeated determinations to calculate the average energy value of the filter paper. Another three filter papers were weighed and recorded as m_2, then 10 mL of urine was dropped onto these three filter papers in multiple aliquots, dried at 65°C, cooled, and weighed again as m_3. The total energy value of filter paper and urine was determined using a Parr 6400 oxygen bomb calorimeter.

Urine energy (UE, MJ) = Total energy value of filter paper and urine (MJ/g) × m_3 (g) -Average energy value of filter paper (MJ/g) × m_2 (g)
Methane energy (CH_4-E, MJ) = Methane production (L) × 39.54 (kJ/L) / 1,000 [12]
Digestible energy (DE) = GE (MJ) -Fecal energy (FE) (MJ)
ME (MJ) = GE (MJ) -FE (MJ) -UE (MJ) -CH_4-E (MJ)

1.5 Statistical Analysis

Experimental data were preliminarily processed using Excel 2003, and correlation analysis of dietary nutrient contents, nutrient digestibility, and energy values was performed using the Correlate procedure in SAS 9.2 statistical software. Regression analysis was conducted using the Regression procedure to establish prediction equations.

2.1 Nutrient Digestibility of Diets with Different Roughage Compositions

As shown in Table 2 , diets with different roughage compositions exhibited significant differences in DM, OM, CP, and GE digestibility ($P<0.05$). Specifically, DM, OM, CP, and GE digestibility of the alfalfa diet were significantly higher than those of corn stalk, Chinese leymus, soybean stalk, wheat straw, and rice straw diets ($P<0.05$). However, changes in dietary roughage did not significantly affect NDF digestibility ($P>0.05$).

2.2 Correlation Between Dietary Nutrient Digestibility and Nutrient Contents

Correlation analysis was performed between DM, OM, CP, GE, and NDF digestibility of diets with different roughage compositions and their respective nutrient contents in the diets. Correlation coefficients between nutrient digestibility and nutrient contents are presented in Table 3 .

Table 3 shows that dietary DM digestibility was significantly or extremely significantly positively correlated with dietary CP, GE, and OM contents ($P<0.05$

or P<0.01), but significantly negatively correlated with NDF content (P<0.05). Dietary OM digestibility was extremely significantly positively correlated with dietary OM, CP, and GE contents (P<0.01), but significantly negatively correlated with NDF content (P<0.05). Dietary CP and GE digestibility were extremely significantly positively correlated with dietary OM, CP, and GE contents (P<0.01), but extremely significantly negatively correlated with NDF content (P<0.01). Dietary DM, OM, CP, and GE digestibility were not significantly correlated with dietary DM content (P>0.05). In contrast, dietary NDF digestibility was significantly or extremely significantly negatively correlated with dietary DM, OM, CP, and GE contents (P<0.05 or P<0.01), but extremely significantly positively correlated with NDF content (P<0.01).

To further predict dietary nutrient digestibility from nutrient contents, stepwise regression was performed based on correlation analysis results to establish equations for predicting nutrient digestibility from dietary nutrient contents (Table 4). The results indicated that the optimal variable for DM, OM, GE, and CP digestibility was CP content, while the optimal single variable for predicting NDF digestibility was NDF content.

2.3 Energy Composition of Different Diets

Energy metabolism of mutton sheep fed different diets is shown in Table 5. Feed ingredient changes had significant or extremely significant effects on dietary DE, ME, FE, and ME as a percentage of GE (P<0.05 or P<0.01), but no significant effects on CH_4-E and UE (P>0.05).

2.4 Relationship Between Dietary Energy Values and Nutrient Contents

As shown in Table 6, dietary OM, CP, and GE contents were significantly or extremely significantly negatively correlated with FE (P<0.05 or P<0.01), but significantly or extremely significantly positively correlated with UE, CH_4-E, DE, and ME (P<0.05 or P<0.01). Dietary NDF content was extremely significantly positively correlated with FE (P<0.01), but significantly or extremely significantly negatively correlated with UE, CH_4-E, DE, and ME (P<0.05 or P<0.01).

To further predict feed energy content from routine nutrient composition, stepwise regression analysis was performed between dietary energy values and nutrient contents based on correlation analysis results. The prediction equations for dietary energy values using nutrient contents are presented in Table 7 .

2.5 Correlation Between Dietary DE and ME with Digestible Nutrients

Correlation analysis was performed between dietary digestible nutrients and DE and ME, with correlation coefficients shown in Table 8 .

Table 8 indicates that DE and ME were extremely significantly positively correlated with digestible OM and digestible protein (P<0.01), but significantly or extremely significantly negatively correlated with digestible NDF (P<0.05 or P<0.01). Based on these results, stepwise analysis was conducted between digestible nutrients and DE and ME to establish equations for predicting dietary DE and ME from digestible nutrients (Table 9). The results showed that the single variables for estimating DE and ME using dietary digestible nutrients both included digestible OM and digestible protein. As the number of prediction factors in the equations increased, the coefficient of determination (R^2) also increased.

Since measuring ME in practical production requires determination of CH_4-E produced by animals, which is often difficult to measure in vivo, a prediction equation for estimating ME from DE was established based on all measured DE and ME values from this trial, as shown in Table 10 .

3.1 Relationship Between Dietary Nutrient Digestibility and Nutrient Contents

Diets provide all nutrients for maintenance, growth, and reproduction in mutton sheep and generally consist of both concentrate and roughage. After entering the digestive tract of sheep, part of the diet is decomposed and absorbed through mechanical (chewing, gastrointestinal motility) and chemical (digestive juices, enzymes) actions, while the undigested residue is eventually excreted as feces. Dietary nutrient digestibility is an important indicator reflecting nutrient utilization and physiological status in sheep. Numerous studies have demonstrated significant correlations between dietary nutrient digestibility and nutrient contents [15-20]. Jiang [21] collected six feed ingredients from five major regions in China and found using nylon bag and in vitro gas production methods that feed DM degradability was highly positively correlated with CP content and 24-hour gas production, but negatively correlated with crude ash and NDF content. Deng et al. [22] reported that in vitro DM digestibility of feeds was extremely significantly negatively correlated with NDF content and significantly positively correlated with CP content, and that regression equations could be used to predict in vitro DM digestibility of roughages from feed nutrient composition. In this study, 10 commonly used roughage ingredients for mutton sheep were selected to form new diets by substituting 20% of the basal diet, with a concentrate-to-roughage ratio of 4:6 and dietary NDF content ranging from 44.99% to 51.45%. Nutrient digestibility of 11 diets was measured in vivo through digestion and metabolism trials. Theoretically, when establishing prediction models, more replicates yield higher equation accuracy. Each treatment had 6 experimental animals. Throughout the trial, controllable factors such as experimental environment and sheep conditions were strictly controlled to ensure stable and consistent physiological status with minimal individual differences among experimental sheep, resulting in relatively small data variation and objective, accurate results. Analysis revealed that dietary nutrient digestibility could be accurately predicted from dietary nutrient contents, with CP being the optimal variable for predicting DM, OM, GE, and CP digestibility, while NDF was the optimal single variable for predicting NDF digestibility. Liu [23] studied the digestibility of 12 diets with varying concentrate-to-roughage ratios (0:100 to 88:12) in mutton sheep. That research used diets with identical ingredient composition but different proportions, with Chinese leymus content ranging from 97.81% to 11.66% and NDF content ranging from 17.03% to 51.73%. The study demonstrated correlations between dietary nutrient digestibility and proximate nutrient contents. Our results are consistent with their findings regarding the pattern of positive and negative correlations, and the use of more diverse dietary ingredients in this study further demonstrates the accuracy of using dietary nutrient contents to predict nutrient digestibility.

3.2 Relationship Between Dietary Energy Values and Nutrient Contents

Energy metabolism in animals follows the law of energy conservation, which determines energy utilization efficiency and dietary effective energy values to ultimately meet animal requirements. After consuming diets, proteins, carbohydrates, and fats in the feed undergo a series of digestive and metabolic processes in ruminants. Different digestibility leads to different effective energy values. Given the diversity of diets consumed by ruminants, the special nature of their rumen and gastrointestinal tract, and the complexity of energy determination, most energy evaluation systems estimate dietary energy values using easily obtainable routine nutrient content indicators. Research on predicting dietary energy values from nutrient contents began in the 1930s, and after Vansoest proposed introducing ADF and NDF contents into prediction equations, many researchers have made more accurate explorations of prediction factors. This approach is currently widely applied in pigs [24], poultry [3, 25-26], and in vitro studies [27-28]. Two major factors in dietary nutrient contents significantly affect dietary energy values: substances with high digestibility in diets, such as protein, and nutrients with low digestibility like NDF. Numerous studies have demonstrated a significant negative correlation between dietary fiber content and effective energy values, indicating that introducing other highly correlated factors into equations yields better results than models based primarily on NDF content [8, 29-31]. In this study, dietary DE and ME were significantly correlated with dietary OM, CP, GE, and NDF contents, with R^2 values above 0.786. Compared with single-prediction-factor equations, binary and ternary equations showed improved R^2 values, indicating higher prediction accuracy. Considering factors such as speed, simplicity, and accuracy, easily obtainable variables should be selected for energy value estimation in production practice.

3.3 Relationship Between Dietary Energy Values and Digestible Nutrients

Although predicting dietary energy values from nutrient contents is simple and rapid, it has limitations for feeds containing anti-nutritional factors that directly affect nutrient digestibility. In such cases, researchers have used dietary digestion parameters as prediction factors to establish energy value estimation equations. Some widely applied feeding standard systems, such as AFRC (1993), use digestible OM as a prediction factor for ME estimation equations [33]. This study used a wide variety of roughage ingredients. Based on correlation analysis between digestible nutrients and dietary energy values, the regression equation ME = -0.127 + 0.015DOM (where DOM is digestible OM) was obtained with R^2 = 0.671, and ME = 5.694 + 0.033DP (where DP is digestible protein) with R^2 = 0.833. Liu [23] reported ME = -0.438 + 0.014DOM (R^2 = 0.936) and ME = 6.823 + 0.027DP (R^2 = 0.870). Although the R^2 values in our study are relatively lower, they still demonstrate the objectivity and validity of the prediction equations. Yan et al. [33] reported that dietary ME was significantly positively correlated with GE, DE, percentage of digestible OM in DM, and DM, OM, GE, CP, and NDF digestibility in their study on prediction equations for ME of ryegrass silage for sheep. Our study also analyzed the correlation between dietary energy values and nutrient digestibility (Table 11), with results consistent with Yan et al. [33]. The results show that dietary DE and ME had significant or extremely significant correlations with DM, OM, CP, GE, and NDF digestibility. Additionally, this study established a model for estimating ME from DE: ME = 0.132 + 0.796DE, which is very close to ME = 0.82DE from NRC (2007) when the constant is added, despite slight deviation in the DE coefficient. This comparison with NRC (2007) further validates the objectivity and accuracy of the equations established in this trial.

When using digestible nutrients to estimate dietary energy values, the same pattern holds as with using dietary nutrient contents: equations with multiple prediction factors typically have higher R^2 than those with single factors. However, using digestible nutrients to estimate dietary energy values requires substantial manpower and financial resources, and animal digestion and metabolism trials have long cycles that cannot guarantee reproducibility and accuracy of results. Therefore, despite the high accuracy of prediction models using digestible nutrients as factors, dietary nutrient contents remain more ideal prediction factors from a practical standpoint.

In conclusion, dietary nutrient digestibility has strong correlations with nutrient contents and can be predicted using dietary OM, CP, GE, and NDF contents. Dietary ME has strong correlations with DM, OM, CP, GE, and NDF contents, as well as with digestible OM, CP, and NDF contents, and with DM, OM, CP, GE, and NDF digestibility. Therefore, dietary ME can be predicted using nutrient contents and digestion parameters.

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