Postprint: Development of a Prediction Model for Metabolizable Energy of Commonly Used Protein Feed Ingredients for Meat Sheep Based on Nutrient Composition and Digestible Nutrients

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Abstract

This study employed the difference method to analyze the effects of nutrient composition and digestible nutrients in commonly used protein feed ingredients for meat sheep on effective energy values, and to establish a prediction model for metabolizable energy (ME) of protein feed ingredients based on nutrient composition and digestible nutrients. Thirty-six 22-month-old Dorper × Small-tailed Han F1 crossbred castrated meat sheep with a body weight of (52.6$±$1.4) kg were selected and allocated to 6 groups using a completely randomized block design, comprising 1 basal diet group and 5 experimental diet groups. Digestion and metabolism trials and respiration metabolism trials were conducted, and the difference method was utilized to calculate the digestible energy (DE) and ME of 5 protein feed ingredients. The correlations between DE and ME of protein feed ingredients and their nutrient composition [dry matter (DM), organic matter (OM), gross energy (GE), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF)] and digestible nutrients [digestible dry matter (DDM), digestible organic matter (DOM), digestible crude protein (DCP), digestible ether extract (DEE), digestible neutral detergent fiber (DNDF), digestible acid detergent fiber (DADF)] were analyzed. The results demonstrated that OM content, DDM, DOM, and DCP in feed ingredients were all extremely significantly positively correlated with DE and ME ($P<0.01$). Furthermore, DADF was extremely significantly negatively correlated with DE ($P<0.01$) and significantly negatively correlated with ME ($P<0.05$). The equation for predicting ME based on nutrient composition in feed ingredients was: ME (MJ/kg) =-82.855+2.391OM (%) +1.802EE (%) -6.21GE

(MJ/kg) -0.121ADF (%) (R2=0.910, n=30, P < 0.01) . The equation for predicting ME based on digestible nutrients in feed ingredients was: ME (MJ/kg) =-5.564+30.526DOM (%) +55.402DEE (%) (R2=0.841, n=30, P < 0.01). The equation for predicting ME based on both digestible nutrients and DE in feed ingredients was: ME=-5.787+1.126DE (MJ/kg) +20.769DEE (%) (R2=0.879, n=30, P < 0.01) . In summary, in this study, there were significant correlations between some nutrient composition and digestible nutrients in protein feed ingredients and ME, and the ME of protein feed ingredients for meat sheep could be effectively predicted using nutrient composition and digestible nutrients.

Full Text

Establishment of Prediction Model of Metabolizable Energy of Protein Feedstuffs for Mutton Sheep Using Nutrient Contents and Digestible Nutrients of Feedstuffs

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Abstract: This experiment aimed to investigate the effects of nutrient contents and digestible nutrients in common protein feedstuffs for mutton sheep on effective energy values using the substitution method, and to establish prediction models for metabolizable energy (ME) of protein feedstuffs based on these parameters. Thirty-six castrated Dorper×thin-tailed Han F1 crossbred rams, aged 22 months with a body weight of
(52.6\$±1.4)$kg, wererandomlyassignedto6groupsaccordingtoacompletelyrandomizedblockdesign, includingon$
$metabolismtrialsandrespiration-metabolismtrialswereconductedincombinationwiththesubstitutionmethod$
0.01)$.Additionally, DADFshowedanextremelysignificantnegativecorrelationwithDE(P <$
0.01)$andasignificantnegativecorrelationwithME(P < 0.05).ThepredictionequationforMEusingnutrientco$
$ME(MJ/kg) = -82.855+2.391OM(^{\{2\}= 0.910, n = 30, P < 0.01).Thepredictionequationusingdigestiblenutrientswas: ME(M}$
0.841, $n = 30, P < 0.01).ThepredictionequationusingdigestiblenutrientsandDEwas:$
$ME = -5.787+1.126DE(MJ/kg)+20.769DEE(^{2}=0.879, n=30, P<0.01).
In conclusion, certain nutrient contents and digestible nutrients of protein feedstuffs showed significant correlations with ME in this experiment, and can be effectively used to predict the ME of protein feedstuffs for mutton sheep.

Keywords: mutton sheep; metabolizable energy; digestible energy; protein feedstuffs; digestible nutrients; prediction model

Introduction

China is a major sheep-raising country, ranking first in the world in both inventory and mutton production [1]. In recent years, Chinese researchers have conducted extensive studies on efficient and healthy mutton sheep production, improving traditional feeding practices from aspects of nutritional requirements, disease prevention, and feeding modes, gradually transitioning from grazing with supplementary feeding to complete indoor feeding and intensive management [2-5]. From the perspective of nutritional requirements, the needs of mutton sheep are not only closely related to feedstuff composition and nutritional levels but also affected by the digestion process of feed in animals and the actual utilization of feed. Currently, for mutton sheep, energy and protein feedstuffs can only be measured for gross energy (GE), while digestible energy (DE) and metabolizable energy (ME) cannot be directly measured. Moreover, the recommended DE and ME values for feedstuffs in current domestic and international feed value tables are mostly derived from theoretical calculations or in vitro experiments [6]. Therefore, establishing a set of prediction models for effective energy values of feedstuffs is particularly important.

Studies in monogastric animals have shown that ME prediction models can be accurately established using nutrient contents of feedstuffs as predictors through the substitution method [7-9]. Our research team has conducted extensive studies on energy metabolism in mutton sheep. Liu et al. [10] investigated the correlation between dietary nutrient contents and effective energy values in mutton sheep and found that dietary ME could be predicted using nutrient contents, allowing the prediction of feed utilization in animals through routine laboratory analysis after mixed feed preparation. Zhao et al. [11] and Zhao et al. [12] found no significant differences when using direct and substitution methods to determine ME of single forage and single concentrate feeds, indicating that the optimal substitution proportion was 20% for Leymus chinensis and 30% for single concentrate feeds when using the substitution method. However, further research revealed that predicting effective energy values using mixed diets alone might yield inaccurate results due to large differences in diet types. Additionally, large variations in energy and protein levels among different concentrate feeds could affect the accuracy of prediction models. Therefore, further research is needed to investigate the accuracy of predicting effective energy values using feedstuffs. By classifying concentrate feeds into energy feeds and protein feeds to explore prediction models, more practical models can be established. Pan et al. [13] confirmed that classifying feedstuffs could improve the accuracy and application value of prediction models. Therefore, this experiment classified and selected feedstuffs, focusing on common protein feedstuffs for mutton sheep to study their metabolic patterns in animals, establish correlations between nutrient contents or digestible nutrients and effective energy values, and further develop ME prediction models to achieve accurate prediction of ME in protein feedstuffs for mutton sheep, providing a basis for nutritional value evaluation of feedstuffs, rational utilization of feed resources, and formulation of feeding standards for mutton sheep in China.

Materials and Methods

1.1 Experimental Time and Location

The experiment was conducted from December 2015 to January 2016 at the Nankou Pilot Base of the Chinese Academy of Agricultural Sciences.

1.2 Experimental Design and Animals

Thirty-six castrated Dorper×thin-tailed Han F1 crossbred rams aged 22 months with a body weight of (52.6$±$1.4) kg were selected and randomly assigned to 6 treatments according to a completely randomized block design, including one basal diet treatment and five experimental diet treatments. Each treatment had 6 sheep. The experiment lasted for 16 days, with an 8-day preliminary period followed by an 8-day fecal and urine collection period. During the last 3 days of the collection period, a respiration-metabolism trial was conducted, with the first 24 hours for animal adaptation to the respiration chamber to ensure normal status, and the subsequent 48 hours for actual measurement of methane production [14].

1.3 Experimental Diets

The basal diet consisted of Leymus chinensis, corn, soybean meal, and premix, prepared using the same batch of raw materials to ensure consistency. Based on our previous research on different substitution proportions of concentrate feeds, when the substitution proportion of a single concentrate feed was 30%, the resulting ME value was closest to the actual measured value [12]. Therefore, in this experiment, the experimental diets were formulated by replacing 30% of the basal diet (only replacing Leymus chinensis, corn, and soybean meal in the basal diet) with soybean meal, rapeseed meal, cottonseed meal, peanut meal, and corn distillers dried grains with solubles (DDGS), respectively. The composition and nutrient levels of the diets are shown in Table 1 .

1.4 Feeding Management

Before the experiment, sheep were dewormed with ivermectin, weighed before morning feeding, and adapted to metabolism cages. Due to significant differences in raw materials among diets, ad libitum feed intake would vary among groups. Therefore, a pre-feeding trial was conducted before the experiment, and the feeding amount of the group with the lowest intake was set as the feeding amount for all groups during the experimental period [10].

After completing the diet transition during the preliminary period, digestion-metabolism and respiration-metabolism trials were initiated. Sheep were fed at

08:00 and 18:00 daily, with 600 g provided each time and free access to water. Feces and urine were collected using the total collection method. Daily fecal output was weighed and recorded for each sheep, with 10% sampled and mixed over 5 days, then stored at -20°C for analysis. Urine was collected in plastic buckets containing 100 mL of 10% H_2SO_4 to prevent uric acid precipitation during storage, diluted to 5 L, thoroughly mixed, filtered through gauze, and 30 mL sampled daily. The 5-day urine samples from each sheep were mixed and stored at -20°C for determination of urine energy (UE) [15].

1.5 Measurement Indicators and Methods

Representative diet and fecal samples were collected during the experiment. Nutrient contents were determined according to the methods in *Feed Analysis and Feed Quality Detection Technology* (3rd edition) [16]. Gross energy (GE) was measured using a PARR-6400 automatic oxygen bomb calorimeter. Crude protein (CP) content was determined using a KDY-9830 automatic Kjeldahl nitrogen analyzer. Ether extract (EE) content was measured using an ANKOMXT15i automatic fat analyzer. Additionally, crude ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca), and phosphorus (P) contents were determined.

For UE determination, the following method was used: the energy value of five quantitative filter papers was measured to calculate the average energy value of filter paper. Ten milliliters of urine were dropped onto filter paper in multiple portions, dried at 65°C, and the energy value was measured to obtain the total energy value of filter paper and urine. The UE was obtained by subtracting the filter paper energy value from this total.

Methane production was measured using a sealed respiration chamber connected to a SABLE open-circuit gas measurement system, an LGR gas analyzer (purchased from Sable Systems International, North Las Vegas, USA), and a supporting computer program. After the experimental animals adapted to the respiration chamber on day 1, continuous 24-hour gas measurement was initiated, with methane production in the respiration chamber measured every 0.5 hours. A total of 48 methane production data points were obtained for each experimental animal, and the average was taken as the daily methane production per animal.

1.6 Calculation Formulas

The apparent digestibility of nutrients in diets and test feedstuffs was calculated according to Adeola [17]:

Apparent digestibility of a nutrient in diet (%) = 100 × (total diet intake × nutrient content in diet - fecal output × nutrient content in feces) / (total diet intake × nutrient content in diet)

Apparent digestibility of a nutrient in test feedstuff (%) = 100 × [apparent digestibility of the nutrient in experimental diet - (100 - substitution percentage of test feedstuff for basal diet) × apparent digestibility of the nutrient in basal diet] / substitution percentage of test feedstuff for basal diet

The calculation formula for determining effective energy values of test feedstuffs using the substitution method [18] was:

Effective energy value of test feedstuff (including DE and ME) = 100 × [effective energy value of experimental diet - (100 - substitution percentage of test feedstuff for basal diet) × effective energy value of basal diet] / substitution percentage of test feedstuff for basal diet

DE of diet (MJ/kg) = GE - fecal energy (FE)
ME of diet (MJ/kg) = GE - [FE + UE + methane energy (CH_4E)]
UE (MJ/kg) = total energy value of filter paper and urine - energy value of filter paper
CH_4E (kJ) = methane production (L) × 39.54 kJ/L [19]

1.7 Data Processing

Experimental data were initially processed using Excel 2013. Correlation analysis among nutrient contents, digestible nutrients, and energy values of feedstuffs was performed using the Correlate procedure in SAS 9.2 statistical software. Regression analysis was performed using the Regression procedure to establish prediction equations for ME.

Results and Analysis

2.1 Nutrient Contents of Five Protein Feedstuffs

The nutrient contents of five protein feedstuffs are shown in Table 2 .

2.2 Apparent Digestibility of Nutrients and Effective Energy Values of Five Protein Feedstuffs

Through digestion-metabolism and respiration-metabolism trials, the apparent digestibility of nutrients in diets, along with FE, UE, CH_4-E, DE, and ME were measured. Using the classical substitution method formula, the apparent digestibility of nutrients, DE, and ME of the five protein feedstuffs were obtained (Table 3). Significant differences were observed among the five protein feedstuffs in apparent digestibility of nutrients, DE, and ME ($P<0.05$).

2.3 Correlation Between Nutrient Contents and DE or ME

Correlation analysis was conducted between nutrient contents of protein feedstuffs and DE and ME obtained through the substitution method (Table 4).

The results showed that OM content had an extremely significant positive correlation with ME ($P<0.01$).

The correlation results (Table 4) were introduced into linear regression analysis to establish prediction equations between nutrient contents and DE and ME (Table 5). The results indicated that DE and ME could be predicted using nutrient contents of protein feedstuffs. The study also found that OM was the best predictor for both DE and ME in prediction models composed of different protein feed sources. The coefficient of determination (R^2) improved to varying degrees when OM was combined with other nutrient contents.

Using the ternary equation ME = -77.310 + 2.347OM + 1.886EE - 6.424GE and the quaternary equation ME = -82.855 + 2.391OM + 1.802EE - 6.21GE - 0.121ADF derived from the correlation between ME and nutrient contents, ME values were predicted and compared with substitution values obtained under the experimental conditions. The results are shown in Table 6 . The deviations between predicted values from the ternary equation and substitution values for soybean meal, rapeseed meal, cottonseed meal, peanut meal, and corn DDGS were -2.85%, 2.68%, 1.13%, -1.42%, and -0.21%, respectively. The deviations from the quaternary equation were -0.18%, -0.19%, -0.14%, -0.10%, and -0.12%, respectively. Compared with measured substitution values, the quaternary equation showed lower overall deviation and predicted values closer to the measured substitution values.

2.4 Correlation Between Digestible Nutrients and DE or ME

Using nutrient contents and their corresponding apparent digestibility rates, digestible dry matter (DDM), digestible crude protein (DCP), digestible organic matter (DOM), digestible ether extract (DEE), digestible acid detergent fiber (DADF), and digestible neutral detergent fiber (DNDF) were calculated and correlated with DE and ME obtained through the substitution method. Table 7 shows that DDM, DOM, and DCP had extremely significant positive correlations with both DE and ME ($P<0.01$). Additionally, DADF had an extremely significant negative correlation with DE ($P<0.01$) and a significant negative correlation with ME ($P<0.05$).

The correlation results between digestible nutrients and DE or ME (Table 7) were introduced into linear regression analysis to establish prediction equations (Table 8). The results showed that DE could be accurately predicted by combining DOM, DEE, DCP, and DADF, with R^2 reaching 0.934. ME could be accurately predicted by combining DOM and DEE, with R^2 reaching 0.841. Additionally, ME could also be accurately predicted using DE and DEE, with R^2 reaching 0.879 (Table 9), indicating that combining DE with multiple digestible nutrients yielded the best prediction results, and the fitted equation had good reference value.

Discussion

3.1 Effects of Nutrient Contents on Apparent Digestibility

The digestibility of nutrients in feed can serve as an important parameter for evaluating nutritional value, and differences in nutrient contents among feedstuffs directly affect their digestion and utilization in animals. Nutrient digestibility is also influenced by animal species, feed composition, processing methods, and feeding levels. Under the conditions of this experiment, all parts of the diets except the substituted portion of the basal diet were consistent. Using the substitution method to calculate the apparent digestibility of nutrients in each feedstuff revealed significant differences among different feedstuffs. First, soybean meal, as the main protein source in ruminant diets, had higher apparent digestibility of CP than other feedstuffs, indicating that soybean meal protein is more easily absorbed by animals. Although cottonseed meal and peanut meal had higher CP contents than soybean meal, their apparent digestibility was lower, possibly because soybean meal contains more easily degradable small molecular proteins and polypeptides than cottonseed meal and peanut meal. Second, the apparent digestibility of NDF and ADF in rapeseed meal was higher than in other feedstuffs, possibly due to the poor palatability of rapeseed meal diets, which caused experimental sheep to complete feed intake through multiple meals. Since crude fiber degradation mainly depends on rumen fiber-degrading bacteria, more complete digestion by these bacteria led to higher NDF and ADF digestibility. Tafaj et al. [20] also showed that nutrient digestibility is closely related to feed intake; reduced feed intake improves nutrient digestibility, and multiple daily meals can improve nutrient digestibility compared with single meals.

3.2 Establishment of DE Prediction Model Using Nutrient Contents

Protein feed (CP content >20%) has been a hot research topic as one of the primary essential elements in animal nutrition. Many researchers have attempted to explore the digestion and metabolism patterns of protein feed in the rumen and small intestine by studying the optimal protein requirement of animals, and have found differences between simple dietary nutrient levels and actual animal requirements [21]. Currently, ME is widely used internationally to evaluate dietary metabolic utilization in mutton sheep [6]. For ruminants, nutrient contents or digestible nutrients are mostly used to predict effective energy values [22-23]. Since predictors can be obtained through routine laboratory analysis and then used in calculation formulas to estimate effective energy, this ensures the objectivity and rationality of prediction models, which is significant for both diet formulation in production and research on nutritional requirements of mutton sheep.

China has many types of protein source feedstuffs for ruminants, making it impossible to feed each protein feedstuff to mutton sheep through digestion-metabolism and respiration-metabolism trials to evaluate actual utilization and calculate ME. Therefore, it is necessary to study the correlation between nutrient contents of protein feedstuffs and ME to estimate the digestion of protein feedstuffs in mutton sheep through prediction models. Under the conditions of this experiment, both DE and ME had extremely significant positive correlations with OM content, consistent with previous studies on the correlation between effective energy values and dietary nutrient contents [10] and between effective energy values of roughage and nutrient contents [24]. This indicates that OM content can serve as a predictor for effective energy values in diets, roughage, and protein feedstuffs.

Currently, reports on prediction models are mostly found in monogastric animals [25-26] and dairy cows [23], with limited and inconsistent research on ME prediction models for mutton sheep. Zhao et al. [24] used the substitution method to estimate ME of roughage for mutton sheep and found significant correlations between nutrient contents and ME, allowing the establishment of ME prediction models using nutrient contents. They proposed ternary and quinary equations: $\mathrm{ME} = -31.002 - 0.097\mathrm{NDF} + 0.474\mathrm{OM} + 0.154\mathrm{CP}$ ($R^2=0.953$) and $\mathrm{ME} = 6.943 - 0.101\mathrm{NDF} + 0.704\mathrm{GE} - 0.101\mathrm{ADF} + 0.138\mathrm{OM} + 0.032\mathrm{CP}$ ($R^2=0.994$). Comparing the best predictors and combined factors, different feed types yielded different best predictors, but the R^2 values were higher than those of mixed diet ME prediction models [10], and both studies found that predicted values approached substitution values as the number of predictors increased. Zhao et al. [27] used the same method to estimate ME of concentrate feeds for mutton sheep and found no significant correlation between nutrient contents and ME, making it impossible to establish ME prediction models. Combined with the results of this experiment, the main reason is that the concentrate feed category is broad, with differences between energy feeds and protein feeds that may lead to low R^2 values or failure to establish prediction models when combined. Pan et al. [13] established prediction models for ME requirements recommended by NRC (2012) for pigs using nutrient contents and found that using conventional nutrient contents as independent variables made it difficult to establish ME prediction models applicable to all feed types, but classification of feedstuffs enabled model construction. The successful establishment of ME prediction models using nutrient contents in this experiment also demonstrates the feasibility of classification-based construction.

The prediction models for effective energy values using nutrient contents in this experiment showed that the best predictor was OM, which differs from the best predictor for energy feeds (CP) [28], suggesting that different feed types yield different best predictors in prediction models. This indirectly verifies the feasibility of predicting ME after feed classification proposed by Pan et al. [13]. The study also found that combining OM with other nutrient indices improved R^2 values, reaching a maximum of 0.910. Compared with prediction models from other national feeding standards, NRC (2001) [29] proposed that the efficiency of converting DE to ME for fat is nearly 100%, allowing the establishment of ME prediction models using EE and DE: $\mathrm{ME} = (1.01\mathrm{DE} - 0.45) + 0.046 \times (\mathrm{EE} - 3)$, where EE refers to total dietary EE content (above 3%). This model targets complete diets, while this experiment demonstrated that EE in protein feedstuffs can still serve as a predictor for ME. The deeper significance of this experiment is using feedstuffs as the research tool and establishing ME prediction models for feedstuffs as the research objective, making the models more practical.

3.3 Establishment of ME Prediction Model Using Digestible Nutrients

Studies have shown that dietary digestible nutrients can be used to predict ME in ruminants [24]. This experiment used the substitution method combined with in vivo methods to explain the actual metabolic patterns of each protein feedstuff in mutton sheep and obtained DE, ME, and digestible nutrients to establish prediction models for effective energy values. The results showed that compared with nutrient contents, the ME prediction model using digestible nutrients had higher R^2 values, indicating that digestible nutrients can predict ME more accurately. This may be due to several reasons: first, digestible nutrients were obtained through the substitution method, representing actual digestion and utilization by animals, while nutrient contents are measured values from laboratory analysis; second, some protein feedstuffs contain anti-nutritional factors, and using nutrient contents alone to predict ME may cause deviations from actual ME, leading to nutrient waste or insufficient nutrient intake that inhibits full expression of animal growth performance.

This experiment found that DOM was the best predictor for ME, consistent with Alderman [30] who also recommended using DOM as a predictor. The study also found that combining DOM with other factors improved R^2 values, reaching a maximum of 0.841, demonstrating the strong feasibility and high accuracy of ME prediction models using digestible nutrients. Additionally, DE can be accurately determined through digestion-metabolism trials, while ME measurement requires gas analysis equipment not available at all research facilities. Therefore, establishing ME prediction models using DE can be widely applied.

This study showed that combining DE with digestible nutrients yielded the highest R^2 value of 0.879, indicating higher prediction accuracy. The average ME/DE ratio of measured effective energy values in this experiment was 0.81, slightly lower than the recommended value of ME = 0.82DE by NRC (2007) [31]. This difference is because NRC (2007) provides recommended values that may differ from actual conditions, while this experiment used measured values. Additionally, different anti-nutritional factors in various protein feedstuffs cause differences in digestion and metabolism rates, leading to deviations from recommended values.

Conclusions

1. ME of protein feedstuffs can be predicted using nutrient contents, with prediction accuracy improving as the number of predictors increases. The

prediction equation is: ME (MJ/kg) = -82.855 + 2.391OM + 1.802EE - 6.21GE - 0.121ADF (R^2=0.910, n=30, $P<0.01$).

2. ME of protein feedstuffs can be predicted using digestible nutrients. The prediction equation is: ME = -5.564 + 30.526DOM + 55.402DEE (R^2=0.841, n=30, $P<0.01$). Additionally, ME can be predicted using digestible nutrients and DE: ME (MJ/kg) = -5.787 + 1.126DE + 20.769DEE (R^2=0.879, n=30, $P<0.01$).

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