# Estimation of Metabolic Energy of Concentrate Feed for Meat Sheep Using the Difference Method: Postprint

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## Abstract

This experiment aimed to establish a metabolic energy estimation model for concentrate feed in meat sheep using the substitution method. Sixty-six 18-month-old Dorper × Small-tailed Han F1 crossbred castrated meat sheep with a body weight of (49.6$±$1.3) kg were selected and divided into 11 groups using a completely randomized block design, including 1 basal diet group and 10 experimental diet groups, with 6 sheep per group. Through digestion-metabolism trials (lasting 8 d) and gas metabolism trials (lasting 3 d) combined with the substitution method, the digestible energy and metabolic energy of 10 concentrate feeds were calculated, and models between concentrate feed metabolic energy and its proximate nutrients or digestible nutrients were established. The results showed that: 1) The gross energy and acid detergent fiber content of the 10 concentrate feeds were significantly correlated with digestible energy ($P<0.05$), and organic matter content was extremely significantly correlated with digestible energy ($P<0.01$); however, there was no correlation between proximate nutrients of concentrate feeds and metabolic energy ($P>0.05$). 2) The digestible nutrients of the 10 concentrate feeds were extremely significantly correlated with metabolic energy ($P<0.01$), and the established prediction equations were: ME=-1.907+1.344DE+1.321DDM-5.347DOM-2.093DADF (R2=0.845, n=60, $P<0.01$); ME=-2.105+1.349DE-6.577DOM (R2=0.842, n=60, $P<0.01$). [ME is metabolic energy (MJ/kg), DE is digestible energy (MJ/kg), DDM is digestible dry matter (%), DOM is digestible organic matter (%), DADF is digestible acid detergent fiber (%).] In conclusion, under the conditions of this experiment, proximate nutrients of concentrate feeds could not be used to predict their metabolic energy, while metabolic energy could be accurately predicted through digestible nutrients of concentrate feeds.

# Establishment of Prediction Model of Metabolizable Energy of Concentrate for Mutton Sheep by Substitution Method

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**Abstract:** This experiment was conducted to establish a prediction model for the metabolizable energy of concentrate feeds for mutton sheep using the substitution method. Sixty-six castrated Dorper × thin-tailed Han F1 crossbred rams (49.6 ± 1.3 kg) at 18 months of age were randomly assigned to 11 groups, including one basal diet group and ten experimental diet groups, with six sheep per group. Digestion and metabolism trials (8 days) and gas metabolism trials (3 days) were combined with the substitution method to calculate the digestible energy and metabolizable energy of ten concentrate feeds, and to establish models relating the metabolizable energy of concentrates to their proximate nutrients or digestible nutrients. The results showed that: 1) Gross energy, acid detergent fiber content, and organic matter content of the ten concentrate feeds were significantly correlated with digestible energy ($P < 0.05$ for the former two, $P < 0.01$ for organic matter), while proximate nutrients showed no correlation with metabolizable energy ($P > 0.05$). 2) Digestible nutrients were extremely significantly correlated with metabolizable energy ($P < 0.01$), with the following prediction equations:

$ME = -1.907 + 1.344DE + 1.321DDM - 5.347DOM - 2.093DADF$ ($R^2 = 0.845$, $n = 60$, $P < 0.01$)
$ME = -2.105 + 1.349DE - 6.577DOM$ ($R^2 = 0.842$, $n = 60$, $P < 0.01$)

[ME = metabolizable energy (MJ/kg), DE = digestible energy (MJ/kg), DDM = digestible dry matter (%), DOM = digestible organic matter (%), DADF = digestible acid detergent fiber (%).] In conclusion, under the conditions of this experiment, proximate nutrients of concentrate feeds cannot be used to predict their metabolizable energy, whereas metabolizable energy can be accurately predicted using digestible nutrients.

**Keywords:** mutton sheep; concentrate; digestible energy; metabolizable energy; digestible nutrients; prediction model

## Introduction

The nutritional value of feed for animals depends not only on its nutrient content but also on the efficiency of chemical digestion and biological transformation of these nutrients in livestock. Only by considering both aspects can we comprehensively, thoroughly, and objectively evaluate feed nutritional value. Currently, metabolizable energy (ME) or net energy (NE) systems are predominantly used to assess energy requirements in ruminants, necessitating knowledge of raw material ME values for diet formulation. However, ME values of feed ingredients are generally difficult to determine directly, and the ME values for concentrate feeds in domestic and international feeding standards are typically simple estimates lacking validation studies. Consequently, practical application may result in dietary energy levels that either fail to meet animal requirements or exceed them, causing resource waste. Therefore, accurately predicting the ME of feed ingredients is crucial for scientifically formulating diets to meet the nutritional needs of mutton sheep.

Studies in pigs have demonstrated that ME prediction models can be established with reasonable accuracy using proximate nutrients as predictors. In sheep, Liu et al. developed a prediction model for ME in compound feeds using proximate nutrients, enabling prediction of feed utilization through simple laboratory analysis after feed production. However, the actual utilization of individual ingredients remains unknown. Concentrate ingredients are critical cost factors in mutton sheep production. This study selected ten commonly used concentrate feeds in China and determined their ME values through digestion and metabolism trials combined with respirometry and the substitution method. We established correlations between ME and proximate or digestible nutrients to enable accurate prediction of ME in commonly used concentrate feeds, providing a reference for feed nutritional value evaluation and the establishment of Chinese mutton sheep feeding standards.

### 1.1 Experimental Period and Location

The experiment was conducted from July to September 2015 at the Nankou Pilot Base of the Chinese Academy of Agricultural Sciences.

### 1.2 Experimental Design and Animals

Based on preliminary trials, sixty-six castrated Dorper × thin-tailed Han F1 crossbred rams (49.6 ± 1.3 kg) at 18 months of age were randomly assigned to 11 groups using a completely randomized block design, comprising one basal diet group and ten experimental diet groups (six sheep per group). The experimental period lasted 16 days, including an 8-day preliminary period followed by an 8-day formal digestion and metabolism trial. During the final 3 days of the formal period, gas metabolism trials were conducted concurrently, with the first 24 hours for adaptation to the respiration chambers to ensure normal animal status, followed by 48 hours of actual methane production measurement.

### 1.3 Experimental Diets

The basal diet consisted of Chinese wildrye, corn, soybean meal, and premix. All diets were formulated using the same batch of raw materials to ensure ingredient consistency. Based on preliminary gradient replacement trials with different concentrate proportions, a 30% replacement rate of a single concentrate for energy-supplying ingredients in the basal diet yielded ME values closest to actual measured values. Therefore, in this experiment, diets were reformulated by replacing 30% of the energy-supplying ingredients (Chinese wildrye, corn, and soybean meal) in the basal diet with oat, barley, wheat, corn, sorghum, soybean meal, rapeseed meal, cottonseed meal, peanut meal, or dried distillers grains with solubles (DDGS), respectively. Diet composition and nutrient levels are presented in Table 1 .

### 1.4 Animal Management

Prior to the experiment, sheep were dewormed with ivermectin, weighed before morning feeding, and adapted to metabolism cages. Due to substantial differences in ingredients among experimental diets, ad libitum feed intake varied among groups. Therefore, a pre-feeding trial was conducted, and the feeding level of the group with the lowest intake was adopted for all groups during the experimental period. Following diet transition during the preliminary period, the digestion and metabolism trial commenced. Sheep were fed at 08:00 and 18:00 daily, receiving 600 g at each feeding, with free access to water. Total feces and urine were collected using the total collection method. Daily fecal output was weighed and recorded, with 10% sampled and pooled for each sheep over 5 days and stored frozen. 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 sampled daily (30 mL). Urine samples from each sheep over 5 days were pooled and stored at -20°C for urinary energy determination.

### 1.5 Measurements and Methods

Feed and fecal samples were analyzed for gross energy (GE), organic matter (OM), dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), and acid detergent fiber (ADF) according to "Feed Analysis and Feed Quality Detection Technology." Energy was determined using a Parr-6400 oxygen bomb calorimeter. For urinary energy determination, five quantitative filter papers were measured for energy content to calculate an average value. Ten milliliters of urine were pipetted onto filter paper in multiple aliquons, dried at 65°C, and measured for energy content to obtain the combined energy value of filter paper and urine. Methane production was measured using an LGR gas analyzer (SABLE Systems, USA) connected to semi-open respiration chambers. After 24 hours of adaptation, continuous 48-hour gas measurement was conducted, with methane concentration in the chambers measured every

0.5 hours, yielding 96 data points per animal that were averaged to determine daily methane production.

Apparent total tract digestibility of nutrients in diets and concentrates was calculated using Adeloa’ s formulas:

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

Apparent total tract digestibility of a nutrient in concentrate (%) = 100 × [nutrient digestibility in diet - (1 - X) × nutrient digestibility in basal diet] / X

where X is the percentage of test concentrate replacing energy-supplying ingredients in the basal diet.

Energy values of concentrates were calculated using the substitution method:

Energy value = [energy value of test diet - (1 - X) × energy value of basal diet] / X

where X is the replacement percentage, and energy value includes both digestible energy and metabolizable energy.

$DE = GE - FE$
$CH_4E = CH_4P \times 39.54 / 1{,}000$
$ME = GE - (FE + UE + CH_4E)$
UE = energy value of filter paper with urine - energy value of filter paper

where DE = digestible energy (MJ/kg), GE = gross energy (MJ/kg), FE = fecal energy (MJ/kg), $CH_4E$ = methane energy (MJ/kg), $CH_4P$ = methane production (L/kg), ME = metabolizable energy (MJ/kg), and UE = urinary energy (MJ/kg).

### 1.6 Data Processing

Experimental data were initially processed using Excel. Correlation analysis among proximate nutrients, digestible nutrients, and energy values was performed using the CORR procedure, and regression analysis was conducted using the REG procedure in SAS 9.2 statistical software to establish prediction equations.

## Results

### 2.1 Digestible and Metabolizable Energy of Concentrates

Digestion and metabolism trials combined with respirometry and the substitution method revealed that wheat had the highest digestible energy at 14.11 MJ/kg, while rapeseed meal had the lowest at 8.98 MJ/kg. Peanut meal had the highest metabolizable energy (12.18 MJ/kg) and rapeseed meal the lowest (6.65 MJ/kg) (Table 2 ).

### 2.2 Correlation Analysis Between Proximate Nutrients and Energy Values

Correlation analysis between proximate nutrients and energy values determined by the substitution method showed no correlation between metabolizable energy and proximate nutrients of concentrates ($P > 0.05$). Digestible energy was significantly correlated with acid detergent fiber content ($P < 0.05$) and extremely significantly correlated with organic matter content ($P < 0.01$) (Table 3 ).

### 2.3 Correlation Analysis Between Digestible Nutrients and Metabolizable Energy

Correlation analysis between digestible nutrients and energy values indicated that metabolizable energy was extremely significantly correlated with digestible energy, digestible dry matter (DDM), and digestible organic matter (DOM) ($P < 0.01$) (Table 4 ).

### 2.4 Establishment of Prediction Equations for Metabolizable Energy

When digestible energy was used as the sole predictor of metabolizable energy, the coefficient of determination was 0.770. When both DOM and digestible energy were used to predict metabolizable energy, the $R^2$ reached 0.842 (Table 5 ).

## Discussion

### 3.1 Relationship Between Proximate Nutrients and Metabolizable Energy of Concentrates

The "Chinese Feed Composition and Nutritional Value Tables" provide proximate nutrients and digestible energy values for various concentrates commonly used in sheep feeding. Preliminary analysis of the relationship between proximate nutrients and digestible energy of several concentrate ingredients revealed significant correlations between crude protein, acid detergent fiber, and ether extract contents with metabolizable energy. However, crude protein and dry matter showed negative correlations with digestible energy. Since the digestible energy values provided in the tables are estimated rather than measured, the relationship between digestible energy and feed proximate nutrients requires experimental validation. Although metabolizable energy is more accurate than digestible energy for ruminants, its determination is impractical for most production settings. Therefore, practically determining animal metabolizable energy and establishing prediction models using feed proximate nutrients is realistic for production conditions. Additionally, nutrient composition varies among individual feeds or mixed feeds, leading to differences in digestibility that directly affect effective energy values. Practical, rapid, and realistic prediction models are widely applied internationally. In monogastric animals, He (2004) determined effective energy values of bran, distillers' grains, and oilseed meals for pig feeds, while Liu (2014) established net energy prediction equations for commonly used pig feeds using the substitution method. Other researchers have developed prediction models for effective energy values of DDGS, rapeseed, and cottonseed products using proximate nutrients. Various methods exist for determining ruminant feed metabolizable energy, including in vivo, in vitro, semi-in vivo, and prediction methods. This study employed the in vivo method combined with the substitution method to measure digestible and metabolizable energy of diets containing ten different concentrates, attempting to establish prediction equations for digestible and metabolizable energy of concentrates using proximate nutrients. However, our results showed no correlation between proximate nutrients and metabolizable energy of concentrates, preventing the development of prediction equations. Several factors may explain this outcome. First, each feed ingredient contains different truly utilizable nutrients and may contain different anti-nutritional factors, requiring different optimal predictors for each ingredient, making it difficult to predict metabolizable energy using only proximate nutrients. Second, other dietary ingredients may have interacted with the test concentrates, affecting correlations between proximate nutrients and energy values. Third, due to the unique physiological characteristics of ruminants, laboratory proximate analysis categorizes nutrients through physical methods, but ruminants utilize not the original nutrients but those produced through microbial fermentation, making it more difficult than in monogastric animals to directly link proximate nutrients to effective energy.

Researchers have successfully established prediction models for compound feeds in ruminants. Compared to our study, their success likely resulted from simpler feed composition and stable nutrient structure types, leading to consistent digestion and fermentation processes that were more likely to yield results. Future attempts to develop ingredient ME prediction models should consider grouping ingredients with similar nutritional structures, which may produce satisfactory outcomes.

### 3.2 Establishing Metabolizable Energy Prediction Models Using Digestible Nutrients

Numerous researchers have established prediction models for effective energy using proximate nutrients in monogastric animals or compound feeds for ruminants. While these models are simple and rapid, feeds with identical nutrient compositions may differ in structure, ultimately leading to variations in nutrient digestibility. For example, certain feeds contain specific anti-nutritional factors that affect digestibility of particular nutrients, reducing prediction accuracy when using proximate nutrients as predictors. Digestible nutrients represent the nutrients truly utilized by animals, and incorporating digestible nutrients into prediction equations significantly improves accuracy. Studies have shown that digestible nutrients in feeds are significantly correlated with metabolizable energy and can serve as predictors. Early prediction equations for digestible nutrients primarily consisted of crude fiber and other digestible nutrients, but with methodological improvements, crude fiber has gradually been replaced by detergent fiber, increasing equation accuracy and complexity. Metabolizable energy represents a refined measure of animal metabolism derived from digestible energy. In practice, metabolizable energy is often predicted by multiplying digestible energy by a coefficient of 0.82, and some researchers have established simple, practical prediction equations for metabolizable energy from digestible energy in ruminants. In this study, using digestible nutrients and digestible energy to predict feed metabolizable energy revealed that equation accuracy gradually increased with more predictors, but improvements became minimal beyond three factors. In practical applications, both accuracy and practicality must be considered, making the prediction equation using digestible energy and organic matter as predictors the most practical.

## Conclusions

For mutton sheep, acid detergent fiber content of concentrates was significantly correlated with digestible energy, while organic matter content was extremely significantly correlated with digestible energy. However, proximate nutrients of concentrates showed no significant correlation with metabolizable energy.

The prediction equations for metabolizable energy established using digestible nutrients were:

$\mathrm{ME} = -1.907 + 1.344\mathrm{DE} + 1.321\mathrm{DDM} - 5.347\mathrm{DOM} - 2.093\mathrm{DADF}$ ($R^2 = 0.845$, $n = 60$, $P < 0.01$)
$\mathrm{ME} = -2.105 + 1.349\mathrm{DE} - 6.577\mathrm{DOM}$ ($R^2 = 0.842$, $n = 60$, $P < 0.01$)

[ME = metabolizable energy (MJ/kg), DE = digestible energy (MJ/kg), DDM = digestible dry matter (%), DOM = digestible organic matter (%), DADF = digestible acid detergent fiber (%).]

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