

## Near-Infrared Reflectance Spectroscopy for Evaluating Nutritional Value of Cottonseed Meal and Metabolic Energy in Adult Roosters: Postprint

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**Date:** 2017-10-10T00:00:00+00:00

### Abstract

This study aimed to investigate the feasibility of using near-infrared reflectance spectroscopy technology to determine the conventional nutritional component contents and rooster metabolic energy of cottonseed meal. Seventy-six cottonseed meal samples from different origins, years, and processing methods were collected nationwide to determine their conventional nutritional component contents, and the apparent metabolic energy and true metabolic energy were determined through rooster force-feeding trials. Samples were randomly selected for calibration set (n=56) and external validation set (n=20) to establish near-infrared calibration models. The results showed that: 1) The nutritional components and rooster metabolic energy of cottonseed meal from different sources varied considerably, with coefficients of variation ranging from 2.52% to 84.75%. The coefficients of variation for moisture, crude fat, crude fiber, apparent metabolic energy, and true metabolic energy exceeded 10%; those for crude protein, crude ash, and gross energy were 9.58%, 9.81%, and 2.52%, respectively. 2) The calibration coefficients of determination for moisture, crude protein, crude fat, crude fiber, crude ash, and gross energy ranged from 0.923 5 to 0.975 8, the cross-validation coefficients of determination ranged from 0.824 7 to 0.930 3, and the external validation coefficients of determination ranged from 0.879 to 0.896; the calibration coefficients of determination for apparent metabolic energy and true metabolic energy were 0.969 0 and 0.926 8, respectively, the cross-validation coefficients of determination were 0.917 0 and 0.905 7, respectively, and the external validation coefficients of determination were 0.911 and 0.892, respectively. Therefore, the calibration equations for conventional nutritional components and metabolic energy can both be used for routine analysis.

## Full Text

# Determination of Nutrient Value and Metabolizable Energy in Cottonseed Meal for Roosters Using Near-Infrared Reflectance Spectroscopy

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## Abstract

This experiment aimed to explore the feasibility of using near-infrared reflectance spectroscopy (NIRS) to determine conventional nutrient contents and metabolizable energy in cottonseed meal for roosters. Seventy-six cottonseed meal samples with different origins, production years, and processing methods were collected nationwide. Conventional nutrient contents were analyzed, and apparent metabolizable energy (AME) and true metabolizable energy (TME) were measured through force-feeding experiments with roosters. Samples were randomly divided into a calibration set (n=56) and an external validation set (n=20) for NIRS model development. The results showed that: (1) Nutrients and metabolizable energy varied considerably among cottonseed meal samples from different sources, with coefficients of variation ranging from 2.52% to 84.75%. Coefficients of variation for moisture, ether extract, crude fiber, AME, and TME exceeded 10%, while those for crude protein, ash, and gross energy were 9.58%, 9.81%, and 2.52%, respectively. (2) For conventional nutrients, the coefficient of determination for calibration (RSQ<sub>cal</sub>), cross-validation (1-VR), and external validation (RSQ<sub>v</sub>) ranged from 0.9235 to 0.9758, 0.8247 to 0.9303, and 0.879 to 0.896, respectively. For AME and TME, RSQ<sub>cal</sub> values were 0.9690 and 0.9268, 1-VR values were 0.9170 and 0.9057, and RSQ<sub>v</sub> values were 0.911 and 0.892, respectively. Therefore, the calibration equations for both conventional nutrients and metabolizable energy can be used for routine analysis.

**Keywords:** near-infrared reflectance spectroscopy; cottonseed meal; conventional nutrients; metabolizable energy

## Introduction

China is the world's largest cottonseed producer, possessing substantial plant protein resources[1], with annual cottonseed production exceeding 40 million tons in recent years. Cottonseed meal is a byproduct obtained after cottonseed undergoes delinting, dehulling, and kernel-shell separation, followed by pre-pressing and solvent extraction or direct solvent extraction for oil, or by solvent extraction from cottonseed cake. Rich in protein, cottonseed meal represents the second most important plant protein source globally after soybean[2].

Currently, livestock feed formulation in practice relies primarily on nutrient requirements for cottonseed meal based on values recommended by NRC (2012)[3] and the *Tables of Feed Composition and Nutritive Value in China (25th Edition, 2014)*. These standards provide average values for conventional nutrients in cottonseed meal. However, factors such as origin, variety, processing method, storage conditions, and planting year cause considerable variation in the nutrient composition of cottonseed meal from different sources. To utilize cottonseed meal resources more effectively in practical animal diet formulation, it is necessary to test the nutrient content of each batch from different sources before designing formulas, and to formulate diets based on measured values. This approach improves raw material utilization efficiency and achieves precise diet formulation[4-8].

Near-infrared reflectance spectroscopy (NIRS) technology can determine specific component contents, identify adulteration, and trace origins based on the near-infrared spectral characteristics of samples. This technology offers advantages including rapid and efficient measurement, low cost, non-pollution, and simultaneous multi-component detection, and has been widely applied for rapid prediction of nutrient composition in animal feed ingredients and diets[9]. This study used cottonseed meal as the research object, collecting samples nationwide to determine conventional nutrient contents and measuring apparent metabolizable energy (AME) and true metabolizable energy (TME) through force-feeding experiments with roosters to evaluate nutritional value and variation. Simultaneously, NIRS data of cottonseed meal samples were collected using a FOSS near-infrared spectrometer to construct correlation models between quantitative NIRS data and conventional nutrient indicators and metabolizable energy, serving quality control and real-time monitoring in the feed industry.

## Materials and Methods

### 1.1 Sample Collection and Preparation

A total of 76 cottonseed meal samples with normal color and odor were collected from different growing regions in China, including Xinjiang, Shandong, Beijing, Shaanxi, and Hubei, as experimental materials. Sample sources are shown in Table 1. Each sample was ground using a low-temperature grinder to pass through a 40-mesh sieve (0.42 mm), then reduced using the quartering method. The reduced sample of approximately 500 g was divided into four portions for

laboratory chemical analysis, NIRS scanning, rooster force-feeding, and sample retention. Samples were sealed in self-sealing bags and stored in a  $-18\text{ }^{\circ}\text{C}$  freezer. Before conventional nutrient analysis and NIRS scanning, samples were removed from the freezer and equilibrated at room temperature for approximately 1 hour.

## 1.2 Analytical Methods

**1.2.1 Conventional Nutrient Determination** Moisture content was determined using an intelligent temperature controller (Jinghong XMTD-8222) according to GB/T 6435–2006. Crude protein content was determined using an automatic Kjeldahl nitrogen analyzer (FOSS-8400) according to GB/T 6432–1994. Ether extract content was determined using an automatic Soxhlet extraction system (FOSS Soxtec<sup>TM</sup>-2050) according to GB/T 10359–2008. Crude fiber content was determined using an automatic fiber analysis system (FOSS-2010) according to GB/T 6433–2006. Ash content was determined using an intelligent box-type resistance furnace (Tianjin Zhonghuan SX2-5-12) according to GB/T 6438–1992. Gross energy was determined using an automatic oxygen bomb calorimeter (Parr6300, USA). All nutrient contents were expressed on an air-dry basis. Each sample was analyzed twice on average, and the mean value was used as the reference value for NIRS analysis, with strict adherence to national standard chemical methods.

**1.2.2 Experimental Animals and Management** A single-factor caged randomized block design was adopted. Seventy-two healthy 28-week-old Hy-Line Brown roosters with body weight of  $(2.5\pm 0.3)$  kg were selected and divided into 12 groups with 6 replicates per group. Each group of roosters was used to determine the AME and TME of one type of cottonseed meal. The experiment was completed in 7 periods, with one group reserved as an endogenous group in each period. A 15-day recovery period was provided between every two periods, during which birds had free access to feed and water with adequate commercial layer feed. Before the experiment, feathers around the cloaca of roosters were clipped, and a bottle cap with a hole was sutured. Birds were allowed to adapt for 2 weeks, and fecal collection bags were prepared 3 days before the formal test.

**1.2.3 Force-Feeding Procedure** The excreta collection method was used. After a 12-hour fasting period with water but no feed, roosters were force-fed 30 g of air-dry cottonseed meal each. Fecal collection bags were immediately attached to collect excreta for 36 hours. The endogenous group served as the control, with all operations identical except for force-feeding.

**1.2.4 Excreta Processing** After collection, excreta were poured into corresponding petri dishes, and 10 mL of 10% hydrochloric acid was added. Samples were placed in a  $105\text{ }^{\circ}\text{C}$  oven for 15 minutes to inactivate enzymes and microorganisms, then adjusted to  $65\text{ }^{\circ}\text{C}$  and dried continuously for 72 hours. After

laboratory moisture equilibration for 24 hours, samples were weighed, ground, and passed through a 40-mesh sieve (0.42 mm).

**1.2.5 Metabolizable Energy Determination** Dry matter in air-dry feed samples, gross energy in feed samples, and gross energy in excreta were determined. Dry matter was determined according to GB/T 6435–2006.

**1.2.6 Calculation Methods** Apparent metabolizable energy = (gross energy intake - gross energy in excreta) / total dry matter intake; True metabolizable energy = apparent metabolizable energy + endogenous excreta energy.

**1.2.7 NIRS Parameters and Spectral Collection** A FOSS XDS near-infrared spectrometer was used for spectral collection of cottonseed meal. The scanning range was 400–2,650 nm, resolution 8 cm<sup>-1</sup>, scanning times 64, wavelength interval 2 nm. Each sample was loaded and scanned twice, with the average taken and spectral data recorded in log(1/R) format. The instrument was equipped with a mobile sample cell and standard cover.

**1.2.8 Model Development** The instrument's built-in chemometric software WinISI4 was used for spectral data analysis. Samples were randomly divided into a calibration set (n=56) and a validation set (n=20) at a 3:1 ratio for calibration model establishment and external validation. Modified partial least squares (MPLS) was used to establish calibration models. Before calibration, six scattering correction methods (no scattering, standard normal variate combined with detrending, standard normal variate, detrending, standard multiplicative scatter correction, and weighted multiplicative scatter correction) and three derivative treatments (0,0,1,1; 1,4,4,1; 2,4,4,1) were combined, totaling 18 methods, to preprocess raw spectra, improve signal-to-noise ratio, and optimize model prediction performance. The four values in the three derivative treatments represent derivative order, derivative data gap, first smoothing point number, and second smoothing point number, respectively.

**1.2.9 Model Validation** Global distance (GH) and T-test were used for two rounds of outlier removal, with GH  $> 10$  as the criterion for removing spectral outliers and  $T > 2.5$  for removing chemical outliers. Internal validation of models used cross-validation. Parameters for evaluating calibration effectiveness included coefficient of determination for calibration ( $1 - VR$ ), and standard error of cross-validation (SECV). The model with the highest  $RSQ_{cal}$  and lowest SECV was suitable for routine analysis.

## Results

### 2.1 Conventional Nutrient Content and Variation in Cottonseed Meal

As shown in Table 1, Table 2, and Table 5, the cottonseed meal samples collected in this study originated from different years, growing regions, and processing methods, with wide distribution ranges and large variations in conventional

nutrient and metabolizable energy contents, demonstrating strong representativeness for NIRS sampling. Cottonseed meal samples were basically categorized as yellow, brown, red, and black. Samples from Shandong were yellowish, especially the dephenolized cottonseed meal from Dezhou; most from Xinjiang were brownish-red, with some having less cotton lint and higher protein content; other samples were mostly brown, with a few red samples possibly due to excessive heating during processing causing gossypol denaturation; no black cottonseed meal samples were collected in this study.

Feed production enterprises primarily evaluate cottonseed meal quality based on protein content. In this study, the sample with the lowest protein and highest crude fiber content came from Hebei, containing more cotton lint and hull components; the sample with the highest protein content came from Xinjiang, with less cotton lint and hull components.

As shown in Table 2 and Table 5, compared with data from the *Tables of Feed Composition and Nutritive Value in China (25th Edition, 2014)*, cottonseed meal showed large variations in ether extract, crude fiber, and true metabolizable energy, while average contents of crude protein, ash, and apparent metabolizable energy fell within the ranges for cottonseed meal in the Tables. The coefficient of variation for conventional nutrients and metabolizable energy in tested cottonseed meal ranged from 2.52% to 84.75%. Coefficients of variation increased in the order: gross energy, crude protein, ash, moisture, true metabolizable energy, apparent metabolizable energy, crude fiber, and ether extract, with moisture, ether extract, crude fiber, apparent metabolizable energy, and true metabolizable energy all exceeding 10%.

Comparisons among Table 2, Table 3, Table 4, and Table 5 show that the full set, calibration set, and validation set of cottonseed meal had similar ranges, means, and coefficients of variation for all indicators, and conventional nutrient contents in external validation fell within the calibration set range, indicating similar sample distributions across the three sets, which is beneficial for calibration model establishment and external validation analysis.

**Table 1** Different sources of cottonseed meal samples

Items	Different factors	Number of samples
Area		
Year		
Processing method		

**Table 2** Nutrient contents and variation ranges of all cottonseed meal (air-dry basis, n=76)

Items	Amplitude of variation	Standard deviation	Coefficient of variation	Cottonseed meal
Moisture	5.38~12.06			43.5~47
CP	34.23~54.67			0.25~6.21
EE	2.84~13.45			10.2~10.5
CF	5.31~9.24			6.0~6.6
Ash	16.94~19.29			
GE/(MJ/kg)				

*Cottonseed meal data were from Tables of Feed Composition and Nutritive Value in China, 25th ed., 2014. The same as below.*

**Table 3** Nutrient contents and variation ranges of calibration set of cottonseed meal (air-dry basis, n=56)

Items	Amplitude of variation	Standard deviation	Coefficient of variation	Cottonseed meal
Moisture	5.38~12.06			43.5~47
CP	34.23~54.67			0.25~6.21
EE	2.84~13.45			10.2~10.5
CF	5.31~9.24			6.0~6.6
Ash	16.94~19.29			
GE/(MJ/kg)				

**Table 4** Nutrient contents and variation ranges of validation set of cottonseed meal (air-dry basis, n=20)

Items	Amplitude of variation	Standard deviation	Coefficient of variation	Cottonseed meal
Moisture	5.73~11.13			43.5~47
CP	38.84~53.05			0.26~3.74
EE	3.02~11.84			10.2~10.5
CF	5.74~7.61			6.0~6.6
Ash	17.01~18.60			
GE/(MJ/kg)				

**Table 5** Metabolizable energy and variation ranges of cottonseed meal (DM basis)

Statistical parameter	AME	TME
	All (n=76)	Calibration set (n=56)

Statistical parameter	AME	TME
Amplitude of variation	4.63~11.90	4.63~11.90
Standard deviation		
Mean		
Coefficient of variation/%		
Cottonseed	1.86~8.49	

## 2.2 Establishment and Validation of Optimal Calibration Models

The original near-infrared spectra of all cottonseed meal samples are shown in Figure 1 [Figure 1: see original paper], with wavelength on the horizontal axis and absorbance on the vertical axis. The original spectra showed obvious absorption peaks in the near-infrared region, but the peaks were relatively broad with severe baseline drift (vertical offset). As shown in Figure 2 [Figure 2: see original paper], after second derivative processing, sample absorption peaks were enhanced, spectral differences became more pronounced, and baseline drift was corrected. The organic matter in the 76 cottonseed meal samples contained hydrogen-containing groups such as C-H, O-H, N-H, and S-H. Different contents of these components affected absorption peak sizes, and spectra among samples did not completely overlap, allowing determination of chemical composition and content based on spectral differences[12].

The study used modified partial least squares combined with different spectral preprocessing methods to establish calibration models. Calibration, cross-validation, and external validation results for optimal models are shown in Table 6, Table 7, and Table 8, respectively. For conventional nutrients, RSQcal values for moisture, crude protein, ether extract, crude fiber, ash, and gross energy ranged from 0.9235 to 0.9758, and 1-VR values ranged from 0.8247 to 0.9303, achieving good calibration performance. RSQv values for moisture, crude protein, ether extract, crude fiber, ash, and gross energy ranged from 0.879 to 0.896. For apparent metabolizable energy and true metabolizable energy, RSQcal values were 0.9690 and 0.9268, 1-VR values were 0.9170 and 0.9057, and RSQv values were 0.911 and 0.892.

This study selected the 400-2,650 nm wavelength range for modeling. In the optimal calibration models, wavelengths for moisture, crude protein, ether extract, crude fiber, ash, gross energy, apparent metabolizable energy, and true metabolizable energy ranged from 408 to 2,492.8 nm.

**Table 6** Optimal calibration models for common nutrients of cottonseed meal from different sources (air-dry basis)

Items	Spectral data preprocessing	Calibration sample number	Main factor number	SEC	RSQ <sub>cal</sub>	Relative deviation
Moisture	Detrending				0.9068	-0.2624
CP	Standard multiplicative scatter correction				0.9303	-0.1410
EE	Standard normal variate				0.9016	-0.3826
CF	Standard normal variate				0.9225	-0.0120
Ash	Standard multiplicative scatter correction				0.8655	-0.1036
GE	2,4,4,1					

**Table 7** Optimal calibration models of common nutrients for external validation (air-dry basis)

Items	Validation sample number	SEP	RSQ <sub>v</sub>	Relative deviation
Moisture				-0.065
CP				-0.213
EE				-0.057
CF				-0.001
Ash				
GE/(MJ/kg)				

**Table 8** Optimal calibration models of metabolizable energy for internal cross-validation and external validation (DM basis)

Items	Spectral data preprocessing	Calibration set	Validation set
	Sample number	SEC	RSQ <sub>cal</sub>
AME	Weighted multiplicative scatter correction		
TME			

Figure 2 [Figure 2: see original paper] shows the correlation between measured values and NIRS-predicted values for conventional nutrients and metabolizable energy in the validation set of cottonseed meal. In the figure, the vertical

axis represents chemically analyzed values and the horizontal axis represents NIRS-predicted values. As shown, predictions for moisture, crude protein, ether extract, crude fiber, ash, gross energy, and metabolizable energy in validation samples all fell near the regression line and within warning limits.

## Discussion

Cottonseed meal shows large variations in nutrient composition due to differences in variety, origin, year, and processing method[4]. Different growing environments, including variations in rainfall, temperature, sunshine duration, and soil conditions, affect cottonseed plumpness and thus cottonseed meal quality. Studies have shown that cottonseed quality varies significantly due to variety and growing environment, with growth period length affecting maturity; better cottonseed maturity results in higher protein content in cottonseed meal[13].

In this study, cottonseed meal from different years was stored long-term at 4 °C without heating or mold occurrence, with minimal changes in dry matter and crude protein content during the experimental period and almost no change in mineral content. However, due to different oil extraction processes, ether extract and crude protein contents varied considerably among cottonseed meal samples in this study, consistent with previous research[14]. Additionally, cottonseed quality and processing methods significantly affect cottonseed meal quality[13]. High-temperature pressing severely damages protein in cottonseed meal and results in low oil yield, but provides high digestible energy when used as a feed ingredient. Low-temperature pressing tends to result in high free gossypol content; while extrusion-extraction successfully solves the high gossypol problem in cottonseed meal produced by traditional pressing, it affects protein and amino acid digestibility[15]. Pre-pressing and extraction produces cottonseed meal with high oil yield, appearing as brownish-yellow powder[5].

In cottonseed processing, cottonseed protein refers to products with crude protein content above 50% produced from cottonseed or cottonseed meal, representing a general term for high-protein cottonseed processed products[2]. This study included 13 cottonseed protein samples, mostly from Xinjiang. The study found that cottonseed meal with high protein content had lower cotton lint, hull, and crude fiber contents, while low-protein cottonseed meal had higher contents, consistent with findings by Hou et al.[16]. Fiber components in cottonseed meal are highly negatively correlated with available energy values[17]. Compared with reports by Wang et al.[18] and Zhang et al.[19], this study found larger variation ranges for moisture, crude protein, ether extract, and ash contents in cottonseed meal, but lower crude fiber content. This may be due to differences in dehulling and delinting degrees during cottonseed meal production, as well as different processing methods (pressing, pre-pressing extraction, and extraction)[5,20]; specific influencing factors require further investigation.

In addition to chemical information from samples, NIRS contains irrelevant information and noise such as electrical noise, sample background, and stray

light[10]; therefore, spectral preprocessing is necessary. As shown in Figure 1, after second derivative processing, sample absorption peaks were enhanced, spectral differences became more pronounced, and baseline drift was corrected. Research results show that among three common NIRS modeling methods, calibration models established by modified partial least squares regression (MPLS) demonstrate better prediction performance. Xiao et al.[21] and Shu et al.[22] used MPLS, partial least squares regression (PLS), and principal component regression (PCR) to establish calibration models for amylose content in rice seeds and milled rice flour, finding that MPLS regression equations had significantly smaller calibration and validation standard errors and higher determination coefficients than the other two methods.

This study also used MPLS combined with different spectral preprocessing methods to establish calibration models for conventional nutrients in cottonseed meal, achieving  $RSQ_{cal}$  values of 0.9235-0.9758 and 1-VR values of 0.8247-0.9303, demonstrating good calibration performance. Currently, well-known foreign NIRS manufacturers include FOSS, Bruker, Thermo, and ABB, while domestic manufacturers include Focused Photonics and Neil Optoelectronics. Taking FOSS as an example, commercial NIRS instruments for determining crude protein and moisture in wheat and corn have correlation coefficients greater than 0.95 in their 配套 models, which were developed based on nearly 20,000 corresponding samples. However, in practical applications, enterprises generally do not share public databases, making the technology relatively closed.

Domestic Focused Photonics Inc. has collaborated with the Chinese Academy of Agricultural Sciences and COFCO Group to develop nutritional value models for soybean, rapeseed meal, and soybean meal, with prediction model correlation coefficients for moisture, crude protein, and crude fiber in soybean meal all greater than 0.9, already applied in large enterprises such as COFCO Group and Yihai Group. Currently, no enterprise-level NIRS calibration models for cottonseed meal nutritional indicators have been found. The calibration model database for cottonseed meal in this study will continue to be updated and expanded for enterprise application.

In this study,  $RSQ_v$  values for moisture, crude protein, ether extract, crude fiber, ash, and gross energy ranged from 0.879 to 0.896, with ideal external validation results, demonstrating that rapid analysis of conventional nutrient contents in cottonseed meal using NIRS technology is feasible, providing technical reference for rapid analysis of feed ingredients. In cottonseed nutritional quality trait analysis, calibration models have been successfully constructed for components including cottonseed protein, oil content, moisture, and gossypol content[23-25]. NIRS and chemical methods showed relatively large errors in determining ether extract in cottonseed meal, but no significant differences in crude protein, ash, and moisture determination results[26].

Li Jing[27] collected 60 cottonseed meal samples and established calibration models for predicting chemical composition using Fourier transform NIRS. Calibration models for moisture, crude protein, ether extract, neutral detergent

fiber, acid detergent fiber, and ash could be used for quantitative analysis, with determination coefficients of 0.8521-0.9972. However, the model for predicting ether extract performed poorly, possibly due to small sample size and low content. Nevertheless, prediction models for moisture, crude protein, ether extract, crude fiber, and ash could be used for routine analysis, consistent with this study's results. In this study, the RSQ<sub>v</sub> for the ether extract model was 0.892, suitable for routine analysis.

In NIRS detection technology, relevant component content should not be lower than 0.1%, and component content ranges should be as wide as possible[28]. High component content, uniform sample distribution, and large variation are beneficial for model establishment. In this study, samples came from a wide range of sources with large differences in component contents. Except for ether extract, all other components were detected at levels greater than 1%; except for gross energy, variation in all other components approached or exceeded 10%, all beneficial for model establishment. The external validation correlation coefficient for crude fiber was the lowest in this study, with RSQ<sub>v</sub> of 0.879, and calibration correlation coefficients for other indicators also did not exceed 0.9, possibly due to the relatively small number of cottonseed meal samples. Increasing the number of calibration samples could improve calibration effectiveness[23].

In the process of establishing calibration models, the number of samples in the set and the content range of sample components play critical roles. Meanwhile, sample representativeness and laboratory chemical analysis errors are major factors affecting NIRS analysis accuracy. Therefore, when establishing calibration, sample components should be accurately determined in strict accordance with error requirements of standard methods[29].

Currently, no research reports on NIRS prediction models for metabolizable energy of cottonseed meal in roosters have been found. Wu et al.[30] used the excreta collection-force feeding method to determine metabolizable energy values of 29 cottonseed meal samples, with apparent metabolizable energy of 7.89-12.02 MJ/kg (mean 10.25 MJ/kg) and true metabolizable energy of 9.74-13.87 MJ/kg (mean 12.12 MJ/kg), both higher than values measured in this study, possibly due to larger sample size and wider range in this study. The metabolizable energy of cottonseed meal for roosters is determined by interactions among conventional nutrient contents, making its spectral information more complex.

Chen Liye[31] established a NIRS prediction model for apparent metabolizable energy in broiler chickens using 70 flaxseed meal samples, with RSQ<sub>v</sub> of 0.964. Li Juntao[28] established a NIRS prediction model for apparent metabolizable energy in pigs using wheat and corn samples, also obtaining results suitable for routine analysis. Losada et al.[32] used NIRS to evaluate nitrogen-corrected apparent metabolizable energy of energy feed for roosters, with RSQ<sub>v</sub> of 0.952, demonstrating that NIRS can be used for feed metabolizable energy evaluation. Currently, the application evaluation standard in the feed industry is generally a relative analysis error  $\leq 2.5$ , *meaning models with RSQ<sub>v</sub>  $\geq 0.84$  can be used for*

practical detection[25]. The cottonseed meal samples in this study covered as much as possible the range of unknown samples to be analyzed in the future, with uniform distribution within this range and strong representativeness. RSQv values for all indicators exceeded 0.84, therefore all indicator models can be used for routine analysis.

## Conclusion

1. Nutrients and metabolizable energy in cottonseed meal from different sources varied considerably, with coefficients of variation ranging from 2.52% to 84.75%. Coefficients of variation for moisture, ether extract, crude fiber, apparent metabolizable energy, and true metabolizable energy all exceeded 10%.
2. The NIRS models for cottonseed meal achieved RSQcal values of 0.9235-0.9758 and 1-VR values of 0.8247-0.9303 for moisture, crude protein, ether extract, crude fiber, ash, and gross energy, with RSQv values of 0.879-0.896. For apparent metabolizable energy and true metabolizable energy, RSQcal values were 0.9690 and 0.9268, 1-VR values were 0.9170 and 0.9057, and RSQv values were 0.911 and 0.892. The calibration performance was satisfactory, and the calibration equations can be used for routine analysis.

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## Figure Captions

**Fig. 1** The original picture of cottonseed meal samples and second derivative processing 2,4,4,1 (n=76)

**Fig. 2** The correlation picture between analyzed value of common nutrients & metabolizable energy in cottonseed meal and predicted value by near infrared spectrum

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

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