Comparative Study on the Nutritional Value of Solid-State Fermented Rapeseed Meal and Rapeseed Meal for Growing Meat Rabbits (Postprint)

Authors: Tian Gang, Wang Lecheng, Yu Bing, Chen Hang, Luo Yuheng, Chen Daiwen, Liu Shuliang

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

Abstract

This study aimed to compare the nutritional value of solid-state fermented rapeseed meal (SFRSM) and rapeseed meal (RSM) in growing meat rabbits through digestibility trials, providing fundamental data for rabbit feed ingredient databases. Based on the chemical composition analysis of SFRSM and RSM, thirty-six healthy 42-day-old French Ira commercial rabbits with identical genetic background were randomly allocated into 3 groups (12 rabbits per group) according to similar body weight, housed individually in metabolic cages with separate feces and urine collection systems, and fed one basal diet and two experimental diets (85% basal diet + 15% SFRSM or RSM) to conduct an in vivo digestibility trial. The experimental period lasted 11 days (7-day adaptation period and 4-day collection period). The results showed: 1) Compared with RSM, SFRSM exhibited significantly reduced contents of crude fiber (CF), nitrogen-free extract (NFE), acid detergent fiber (ADF), and isothiocyanate (ITC) ($P<0.01$), while other conventional nutrients [except neutral detergent fiber (NDF)] and small peptide contents were significantly increased ($P<0.05$ or $P<0.01$); the contents of various amino acids were enhanced to varying degrees, with arginine (Arg), aspartic acid (Asp), glutamic acid (Glu), histidine (His), isoleucine (Ile), phenylalanine (Phe), proline (Pro), and serine (Ser) reaching extremely significant levels ($P<0.01$). 2) Compared with RSM, the digestible energy (DE) value of SFRSM (14.97 MJ/kg DM) increased by 34.3% ($P<0.01$); the apparent total tract digestibility of gross energy (GE), crude protein (CP), ADF, NDF, calcium (Ca), and phosphorus (P) also showed improvements ($P>0.05$); the apparent total tract digestibility of various amino acids increased to different extents, with Asp, cysteine (Cys), glycine (Gly), Ile, tyrosine (Tyr), and valine (Val) reaching significant or extremely significant levels ($P<0.05$ or $P<0.01$). The results indicated that, overall, SFRSM possessed higher nutritional value than RSM for growing meat rabbits.

Full Text

Comparison of Nutritional Value between Solid-State Fermented Rapeseed Meal and Rapeseed Meal for Growing Rabbits

TIAN Gang[1], WANG Lecheng[1]*, YU Bing[1], CHEN Hang[1], LUO Yuheng[1], CHEN Daiwen[1], LIU Shuliang[2]

[1]Key Laboratory for Animal Disease-Resistance Nutrition of Ministry of Education, Animal Nutrition Institute, Sichuan Agricultural University, Chengdu 611130, China
[2]College of Food Science, Sichuan Agricultural University, Ya' an 625014, China

Abstract

This study aimed to compare the nutritional value of solid-state fermented rapeseed meal (SFRSM) and rapeseed meal (RSM) for growing meat rabbits through digestion trials, thereby providing fundamental data for rabbit feed ingredient databases. Following the determination of chemical composition in SFRSM and RSM, thirty-six healthy French Ira commercial rabbits at 42 days of age with identical genetic backgrounds were randomly allocated into three groups (12 rabbits per group) based on similar body weights. The animals were housed individually in metabolic cages that allowed separate collection of feces and urine, with each rabbit receiving one of three diets: a basal diet or one of two experimental diets (85% basal diet + 15% SFRSM or RSM). The in vivo digestion trial lasted 11 days, comprising a 7-day preliminary period and a 4-day collection period. The results demonstrated: (1) Compared with RSM, SFRSM exhibited significantly reduced contents of crude fiber (CF), nitrogen-free extract (NFE), acid detergent fiber (ADF), and isothiocyanate (ITC) ($P<0.01$), while showing significantly increased contents of other conventional nutrients (except neutral detergent fiber) and small peptides ($P<0.05$ or $P<0.01$). Amino acid contents were also elevated to varying degrees, with arginine (Arg), aspartic acid (Asp), glutamic acid (Glu), histidine (His), isoleucine (Ile), phenylalanine (Phe), proline (Pro), and serine (Ser) reaching extremely significant levels ($P<0.01$). (2) The digestible energy (DE) value of SFRSM (14.97 MJ/kg DM) was 34.3% higher than that of RSM ($P<0.01$). Although differences in apparent total tract digestibility of gross energy (GE), crude protein (CP), ADF, NDF, calcium (Ca), and phosphorus (P) between the two meals were not statistically significant ($P>0.05$), SFRSM showed numerical improvements. Furthermore, the apparent total tract digestibility of amino acids in SFRSM increased comprehensively, with Asp, cysteine (Cys), glycine (Gly), Ile, tyrosine (Tyr), and valine (Val) achieving significant or extremely significant differences ($P<0.05$ or $P<0.01$). These findings indicate that the overall nutritional value of SFRSM surpasses that of RSM for growing meat rabbits.

Keywords: solid-state fermented rapeseed meal; rapeseed meal; growing rab-

bits; chemical composition; apparent total tract digestibility

Introduction

Protein constitutes a vital component of rabbit diets and is typically supplied by soybean meal [1]. However, the increasing scarcity of protein feed resources and the continuously rising prices of high-quality protein feeds such as soybean meal have severely constrained the healthy and sustainable development of animal husbandry in China, including the rabbit industry [2]. Consequently, developing novel protein feed sources and evaluating their nutritional value holds significant practical importance and broad application prospects. Rapeseed meal (RSM), the world' s second-largest protein meal by production volume [3] with an annual output of approximately 7 million tons in China [4], represents a promising alternative. Although RSM contains high protein content with relatively balanced amino acid composition, it also contains various antinutritional factors including glucosinolates (GS) and their degradation products [such as isothiocyanates (ITC), oxazolidinethiones (OZT), and nitriles], α-galactosides, phytic acid, and phenolic compounds (e.g., tannins), which limit its application in non-ruminant diets [5-7].

Numerous technologies have been employed domestically and internationally to improve the nutritional and feeding value of RSM, among which solid-state fermentation demonstrates distinct advantages [8]. The product obtained through appropriate microbial solid-state fermentation of RSM is designated as solid-state fermented rapeseed meal (SFRSM). Current literature contains extensive reports on the chemical composition of SFRSM and RSM, with consistent findings that SFRSM exhibits substantially increased content and variety of beneficial components such as crude protein (CP), small peptides (SP), and free amino acids (FAA), alongside significantly reduced content and variety of antinutritional factors and fiber [9-20]. However, comparative studies investigating the digestive utilization of nutrients in SFRSM and RSM remain scarce and have been limited to in vitro experiments [10,15-16,18], rats [9], pigs [13,21-22], and chickens [14,19], with no reports available for rabbits. Therefore, this study was designed to analyze the chemical composition of SFRSM and RSM, determine and compare their apparent total tract digestibility of nutrients and DE values using growing meat rabbits as a model, thereby supplementing data for rabbit feed ingredient databases and providing references for SFRSM application in rabbit production.

Materials and Methods

1.1 Experimental Design

A single-factor experimental design was employed to determine the apparent total tract digestibility of gross energy and nutrients using the substitution

method. Thirty-six healthy French Ira commercial rabbits at 42 days of age with identical genetic backgrounds were randomly divided into three groups (12 rabbits per group) based on similar body weights. The experimental animals were housed individually in metabolic cages (60 cm × 60 cm × 45 cm) that allowed separate collection of feces and urine, with each rabbit receiving one of three different diets (one basal diet and two experimental diets).

1.2 Experimental Diets

The basal diet was formulated according to the nutritional requirements for growing rabbits recommended by De Blas et al. [1], with its composition and nutrient levels presented in . The two experimental diets were prepared by mixing 85% basal diet with 15% RSM or SFRSM. All diets were pelleted to a diameter of 3.0 mm. The RSM used was conventional rapeseed meal purchased from Mianyang Youxian Grain and Oil Purchase and Sale Company in Sichuan Province. The SFRSM was prepared through solid-state fermentation of RSM using the method described by Tian et al. [23], with the fermentation inoculum comprising *Pediococcus pentosaceus* (ATCC33316), *Candida guilliermondii* (ATCC6260), and *Rhodococcus rhodochrous* (ATCC13808).

1.3 Animal Management

The digestion trial was conducted at the research base of the Animal Nutrition Institute of Sichuan Agricultural University following the method described by Perez et al. [24]. The trial lasted 11 days, including a 7-day preliminary period and a 4-day collection period. Prior to the experiment, the rabbit house was fumigated and disinfected with formaldehyde and potassium permanganate (2:1) for 7 days. During the preliminary period, each group received their respective diets, fed twice daily (09:00 and 18:00) with ad libitum access to feed and water, while feed intake and health status were closely monitored. During the collection period, feed consumption was accurately weighed daily, with residual and wasted feed (due to rabbits' digging behavior) accounted for. Starting at 08:00 each day, all fecal samples from each rabbit were collected and weighed for four consecutive days. The ambient temperature was maintained at (17.0 ± 0.8)°C with relative humidity of (68.25 ± 5.78)%, under natural lighting and ventilation conditions.

1.4 Sample Collection and Analysis

1.4.1 Sample Collection and Chemical Analysis Fresh feces were cleaned of rabbit hair, sprayed with 10% hydrochloric acid for nitrogen fixation, and stored at -18°C. At the end of the trial, fecal samples from each rabbit collected over the 4 days were mixed, weighed, dried at 65°C, reweighed, and stored at -18°C for subsequent analysis. Chemical composition analysis of SFRSM, RSM, basal diet, experimental diets, and dried fecal samples was conducted according to the methods described by Zhang [25]. All samples were ground to pass through a 40-mesh sieve before quartering. The contents of gross energy

(GE), dry matter (DM), CP, crude fiber (CF), ADF, neutral detergent fiber (NDF), nitrogen-free extract (NFE), ether extract (EE), crude ash, calcium (Ca), total phosphorus (TP), amino acids (AA), SP, and ITC were determined at the laboratory of the Animal Nutrition Institute of Sichuan Agricultural University.

1.4.2 Calculation Formula The apparent total tract digestibility of nutrients (including GE) in SFRSM and RSM was calculated using the following formula [26]:

Apparent total tract digestibility of nutrients in test ingredient (%) = (DT - DB) / F

Where DT represents the digestibility of nutrients in the test diet, DB represents the digestibility of nutrients in the basal diet, and F represents the proportion of the test ingredient in the test diet.

Results

2.1 Comparison of Chemical Composition and ITC Content between SFRSM and RSM

As shown in , compared with RSM, SFRSM exhibited significantly reduced contents of crude fiber (CF), nitrogen-free extract (NFE), acid detergent fiber (ADF), and isothiocyanate (ITC) ($P<0.01$), while demonstrating significantly increased contents of other conventional nutrients [except neutral detergent fiber (NDF)] and small peptides ($P<0.05$ or $P<0.01$). Amino acid contents were also elevated to varying degrees, with arginine (Arg), aspartic acid (Asp), glutamic acid (Glu), histidine (His), isoleucine (Ile), phenylalanine (Phe), proline (Pro), and serine (Ser) reaching extremely significant levels ($P<0.01$).

As presented in , the total amino acid content of SFRSM was 11.9% higher than that of RSM ($P>0.05$), with individual amino acid contents also exceeding those of RSM to varying extents. Notably, Arg, Asp, Glu, His, Ile, Phe, Pro, and Ser showed extremely significant differences ($P<0.01$).

2.2 Comparison of Apparent Total Tract Digestibility and DE Value between SFRSM and RSM for Growing Rabbits

According to , the DE value of SFRSM was 34.3% higher than that of RSM ($P<0.01$). Although no significant differences were observed between the two meals in apparent total tract digestibility of gross energy and conventional nutrients ($P>0.05$), SFRSM showed numerical improvements in DM, GE, CP, ADF, NDF, Ca, and TP digestibility.

As indicated in , the apparent total tract digestibility of total amino acids and individual amino acids (except lysine) in SFRSM exceeded those in RSM to varying degrees. Particularly, Asp, cysteine (Cys), glycine (Gly), isoleucine

(Ile), tyrosine (Tyr), and valine (Val) demonstrated significant or extremely significant differences ($P<0.05$ or $P<0.01$).

Discussion

3.1 Comparison of Nutrient and ITC Content between SFRSM and RSM

Chemical composition provides a preliminary reflection of feed nutritional value. This study revealed that compared with RSM, SFRSM exhibited significantly reduced ITC, CF, ADF, and NFE contents, along with significantly increased GE, CP, SP, EE, ash, total amino acids, Arg, Asp, Glu, His, Ile, Phe, Pro, and Ser contents, meeting China' s national standard for low-ITC feed-grade RSM [27]. These findings indicate enhanced nutritional value of SFRSM relative to RSM.

The SFRSM in this study was produced through mixed-culture solid-state fermentation of RSM using *Candida guilliermondii*, *Pediococcus pentosaceus*, and *Rhodococcus rhodochrous*. The observed differences in chemical composition between SFRSM and RSM align with most reports on SFRSM produced by mixed fermentation with yeasts and lactic acid bacteria [14-18,20]. For instance, Pan [16] reported that SFRSM produced by mixed fermentation of *Pichia guilliermondii* and *Geotrichum candidum* reduced ITC+OZT content by 88.9% and increased CP content by 13.39%. Wang [18] documented that SFRSM from mixed fermentation of *Pediococcus pentosaceus* and *Bacillus subtilis* decreased glucosinolate content while increasing CP, SP, and amino acid contents. Hu [14] found that SFRSM from mixed fermentation of *Candida utilis*, *Bacillus subtilis*, and *Enterococcus faecium* significantly increased CP, EE, true protein, water-soluble protein, and SP contents, improved various amino acid contents, significantly reduced CF content, and nearly completely degraded ITC. Similar results have been reported by other researchers [15,17,19,20].

The substantial differences in chemical composition between SFRSM and RSM in this study may be attributed to several mechanisms during solid-state fermentation. Microorganisms secrete extracellular enzymes (such as endocellulases and xylanases) that act on RSM cell walls [10,28-29], disrupting surface structures (reduced particle size, irregular shape, rough and porous surfaces) [12,29]. Other extracellular enzymes (including proteases, amylases, hemicellulases, xylanases, and carboxylesterases) [10,14-15,29] further degrade glucosinolates, ITC, starch, protein, cellulose, and hemicellulose into other substances (such as SP and FAA). Additionally, microorganisms synthesize new substances like microbial protein and lipids during growth by utilizing degradation products and external nutrients (e.g., carbon and nitrogen sources from the culture medium) [9-20]. The production of these high-energy, high-protein substances likely represents the primary reason for the significantly higher GE and CP contents in SFRSM compared to RSM.

3.2 Comparison of Apparent Total Tract Digestibility and DE Value between SFRSM and RSM

Nutrient digestibility and DE value more accurately reflect feed nutritional value and represent commonly used indicators for evaluating feedstuffs in rabbits. This study found that although most nutrient apparent total tract digestibility values (except Asp, Cys, Gly, Ile, Tyr, and Val) in SFRSM did not differ significantly from RSM for growing rabbits, the majority of nutrients (except CF, ash, and Lys) showed numerical improvements. These findings are not entirely consistent with similar reports in pigs [13,21-22], chickens [14,19], and in vitro studies [10,15-16,18].

The DE value of RSM measured in this study for growing rabbits was 11.15 MJ/kg DM, which aligns closely with existing reports [1,30]. The DE value of SFRSM (14.97 MJ/kg DM) was significantly higher than that of RSM, consistent with reports in pigs [21-22] and chickens [14,19], but with a greater magnitude of increase (+34.3%). For instance, in growing pigs, Fu et al. [22] reported that *Aspergillus niger*-fermented rapeseed cake showed significantly higher apparent total tract digestibility of GE, CF, ADF, and NDF, apparent and ileal digestibility of CP, nitrogen apparent and true digestibility and utilization, apparent and standardized ileal digestibility of most amino acids, and DE value compared with rapeseed cake. Shi et al. [21] found that SFRSM fermented by *Aspergillus niger* exhibited no significant differences in CP and most amino acid digestibility compared with RSM, though showing improving trends, while DE and metabolizable energy (ME) values and phosphorus digestibility were significantly higher. In finishing pigs, Gao [13] reported that RSM fermented by lactic acid bacteria and *Bacillus subtilis* showed significantly higher DM apparent total tract digestibility and CP apparent and true digestibility. In adult roosters, Hu [14] documented that SFRSM produced by mixed fermentation of *Candida utilis*, *Bacillus subtilis*, and *Enterococcus faecium* exhibited significantly higher apparent metabolizable energy (AME), true metabolizable energy (TME), apparent and true utilization rates of CP, CF, EE, total amino acids, and total essential amino acids compared with RSM. Wu et al. [19] reported that fermented RSM showed significantly higher TME and phosphorus utilization than RSM, with no significant differences in true amino acid digestibility, though improvements were observed for certain amino acids (Cys, His, Leu, Lys, Phe, Thr, Tyr, and Val).

The reasons for the slightly higher apparent total tract digestibility of most nutrients and significantly higher digestibility of several amino acids (Asp, Cys, Gly, Ile, Tyr, and Val) and DE value in SFRSM may include: (1) Enzymes produced during solid-state fermentation disrupted RSM surface structures [10,12,28-29], increasing the contact area between substrates and digestive enzymes (including endogenous rabbit digestive enzymes, rabbit gut microbial enzymes, and/or residual extracellular enzymes from fermentation microorganisms [10,14-15,29]); (2) Fermentation degraded detrimental components (CF, ADF, GS, ITC) [9-20] while generating beneficial components (organic acids, probiotics) [15,18],

thereby promoting rabbit health and digestive function. Additionally, the numerically lower DM apparent total tract digestibility of SFRSM compared with RSM may be attributed to the high fiber content in rabbit diets and feces (hard feces), which have large volume and poor homogeneity after grinding, potentially causing sampling errors.

In conclusion, from the perspectives of chemical composition and nutrient digestibility, the nutritional value of SFRSM is generally superior to that of RSM for growing meat rabbits.

References

[1] DE BLAS C, WISEMAN J. Nutrition of rabbit[M]. 2nd ed. Wallingford: CABI Publishing, 2010.

[2] National Feed Industry Office. China's feed industry development has weathered storms to see rainbows[J]. China Animal Husbandry Bulletin, 2009, 45(4): 1-3.

[3] LOMASCOLO A, UZAN-BOUKHRIS E, SIGOILLOT J C, et al. Rapeseed and sunflower meal: a review on biotechnology status and challenges[J]. Applied Microbiology and Biotechnology, 2012, 95(5): 1105-1114.

[4] JIN Hu, LI Kunpeng, HUANG Fenghong, et al. Research progress on biotransformation and high-value utilization of rapeseed meal[J]. Chinese Journal of Oil Crop Sciences, 2014, 36(4): 545-550.

[5] BELL J M. Nutrients and toxicants in rapeseed meal: a review[J]. Journal of Animal Science, 1984, 58(4): 996-1010.

[6] CLANDININ D R, ROBBLEE A R. Rapeseed meal in animal nutrition: . Nonruminant animals[J]. Journal of the American Oil Chemists' Society, 1981, 58(6): 682-686.

[7] LI Jianfan, GAO Zhenchuan, JIANG Yunxia, et al. Nutritional components and antinutritional factors of Chinese rapeseed meal[J]. Acta Veterinaria et Zootechnica Sinica, 1995, 26(3): 193-199.

[8] JIN Jing, XU Zhihong, WEI Zhencheng, et al. Research progress on antinutritional factors in rapeseed meal and their removal methods[J]. China Oils and Fats, 2009, 34(7): 18-21.

[9] BAU H M, VILLAUME C, LIN C F, et al. Effect of a solid-state fermentation using *Rhizopus oligosporus* sp. T-3 on elimination of antinutritional substances and modification of biochemical constituents of defatted rapeseed meal[J]. Journal of the Science of Food and Agriculture, 1994, 65(3): 315-322.

[10] SHI C Y, HE J, YU J, et al. Solid state fermentation of rapeseed cake with *Aspergillus niger* for degrading glucosinolates and upgrading nutritional value[J]. Journal of Animal Science and Biotechnology, 2015, 6: 13.

[11] VIG A P, WALIA A. Beneficial effects of *Rhizopus oligosporus* fermentation on reduction of glucosinolates, fibre and phytic acid in rapeseed (*Brassica napus*) meal[J]. Bioresource Technology, 2001, 78(3): 309-312.

[12] FU Min, HE Jun, YU Bing, et al. Effects of mixed-culture solid-state fermentation on nutritional value and antinutritional factor content of rapeseed cake[J]. Chinese Journal of Animal Nutrition, 2013, 25(7): 1579-1586.

[13] GAO Dongyu. Study on anaerobic solid-state fermentation of rapeseed meal[D]. Master' s thesis. Hefei: Anhui Agricultural University, 2010.

[14] HU Yongna. Evaluation of nutritional value of microbial fermented rapeseed meal and its effects on growth and development of broilers[D]. Master' s thesis. Ya' an: Sichuan Agricultural University, 2012.

[15] NI Guangyuan. Study on biological improvement technology of rapeseed meal for feed use[D]. Master's thesis. Wuhan: Huazhong Agricultural University, 2009.

[16] PAN Lei. Effects of different oil extraction processes on nutritional value of rapeseed cake and study on its microbial detoxification[D]. Master' s thesis. Hefei: Anhui Agricultural University, 2009.

[17] QIU Liangwei, GU Yongjian, SHA Wenfeng, et al. Study on solid-state fermentation of rapeseed meal by compound microorganisms[J]. Journal of Anhui Agricultural Sciences, 2015, 43(3): 209-211.

[18] WANG Gang. Preliminary study on improving rapeseed meal quality through microbial fermentation[D]. Master' s thesis. Wuxi: Jiangnan University, 2011.

[19] WU Dong, QIAN Kun, ZHOU Fen, et al. Nutritional value evaluation of conventional and fermented rapeseed meal as chicken feed[J]. Journal of Anhui Agricultural Sciences, 2012, 40(9): 5263-5264.

[20] ZHU Shaohua, QU Lu, LI Xiaoding, et al. Study on solid-state stepwise fermentation process of rapeseed meal[J]. China Oils and Fats, 2014, 39(7): 37-41.

[21] SHI C, HE J, YU J, et al. Amino acid, phosphorus, and energy digestibility of *Aspergillus niger* fermented rapeseed meal in growing pigs[J]. Journal of Animal Science, 2015, 93(6): 2916-2925.

[22] FU Min, HE Jun, YU Bing, et al. Nutritional value evaluation of fermented rapeseed cake in growing pigs[J]. Chinese Journal of Animal Nutrition, 2014, 26(7): 1916-1924.

[23] TIAN Gang, YU Bing, CHEN Daiwen, et al. A microbial combination solid-state fermentation method for reducing isothiocyanate content in rapeseed meal: China, CN103704466B[P]. 2014-12-17.

[24] PEREZ J M, LEBAS F, GIDENNE T, et al. European reference method for in vivo determination of diet digestibility in rabbits[J]. World Rabbit Science, 1995, 13: 41-43.

[25] ZHANG Liying. Feed analysis and feed quality detection technology[M]. 3rd ed. Beijing: China Agricultural University Press, 2007.

[26] SONG Zhongqi, GAN Youmin, TIAN Gang, et al. Nutritional value evaluation of Italian ryegrass in growing meat rabbits[J]. Acta Prataculturae Sinica, 2014, 23(5): 352-358.

[27] General Administration of Quality Supervision, Inspection and Quarantine of the People' s Republic of China, Standardization Administration of China. GB/T 23736-2009 Rapeseed meal for feed use[S]. Beijing: Standards Press of China, 2009.

[28] KUDANGA T, MWENJE E. Extracellular cellulase production by tropical isolates of *Aureobasidium pullulans*[J]. Canadian Journal of Microbiology, 2005, 51(9): 773-776.

[29] SHI C Y, HE J, YU J, et al. Physicochemical properties analysis and secretome of *Aspergillus niger* in fermented rapeseed meal[J]. PLoS One, 2016, 11(4): e0153230.

[30] SAUVANT D, PEREZ J M, TRAN G. Tables of composition and nutritional value of feed materials: pigs, poultry, cattle, sheep, goats, rabbits, horses, fish[M]. Paris: Wageningen Academic Publishers, 2004.

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