Determination of Available Energy Values of Lard at Different Oxidation Levels: Postprint

Authors: LI Lilang, Zhang Cheng, Guan Wutai, Deng Zixiao, Cheng Lin

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Abstract

This study aimed to determine the digestible energy (DE) and metabolizable energy (ME) values of lard with varying degrees of oxidation to provide baseline data for its application in animal feed. Eight Duroc × Landrace × Large White crossbred barrows with an average body weight of (36.38±1.03) kg were selected and assigned to four dietary treatments according to a replicated 4×4 Latin square design: a basal diet, a basal diet supplemented with 10% fresh lard with a peroxide value (POV) of 0.44 mmol/kg (FL group), a basal diet supplemented with 10% oxidized lard with a POV of 29.64 mmol/kg (OL1 group), and a basal diet supplemented with 10% oxidized lard with a POV of 55.79 mmol/kg (OL2 group). The experiment consisted of four periods, each lasting 10 days with a 5-day preliminary period followed by a 5-day collection period. The results showed that compared with the FL group, the DE values of lard in the OL1 and OL2 groups decreased by 4.62% ($P>0.05$) and 9.45% ($P<0.01$), respectively; ME values decreased by 3.80% ($P>0.05$) and 9.63% ($P<0.01$), respectively; apparent energy digestibility decreased by 4.06% ($P>0.05$) and 7.91% ($P<0.05$), respectively; and apparent energy metabolic rate decreased by 3.23% ($P>0.05$) and 8.12% ($P<0.05$), respectively. It was concluded that oxidation of lard reduces its DE and ME values and decreases the apparent digestibility and metabolic rate of energy, with greater oxidation levels resulting in larger reductions in these parameters.

Full Text

Determination of Effective Energy Values of Lard at Different Oxidation Levels

LI Lilang, ZHANG Cheng, GUAN Wutai*, DENG Zixiao, CHENG Lin

(Scau-Unioil Feeding Oil & Fat Research Center, College of Animal Science, South China Agricultural University, Guangzhou 510642, China)

Abstract: This experiment was conducted to determine the digestible energy (DE) and metabolizable energy (ME) values of lard at different oxidation levels to provide fundamental data for its application in feed. Eight Yorkshire×Landrace×Duroc crossbred barrows with an average body weight of (36.38±1.03) kg were used in a repeated 4×4 Latin square design. Four diets were prepared: a basal diet, a basal diet supplemented with 10% fresh lard with a peroxide value (POV) of 0.44 mmol/kg (FL group), a basal diet supplemented with 10% oxidized lard with a POV of 29.64 mmol/kg (OL1 group), and a basal diet supplemented with 10% oxidized lard with a POV of 55.79 mmol/kg (OL2 group). The experiment consisted of 4 periods, each lasting 10 days (5 days for adaptation and 5 days for sample collection). The results showed that compared with the fresh lard in the FL group, the DE values of lard in the OL1 and OL2 groups decreased by 4.62% ($P>0.05$) and 9.45% ($P<0.01$), respectively; ME values decreased by 3.80% ($P>0.05$) and 9.63% ($P<0.01$), respectively; apparent energy digestibility decreased by 4.06% ($P>0.05$) and 7.91% ($P<0.05$), respectively; and apparent energy metabolic rate decreased by 3.23% ($P>0.05$) and 8.12% ($P<0.05$), respectively. In conclusion, oxidation reduces the DE and ME values of lard and decreases its apparent energy digestibility and metabolic rate, with higher oxidation degrees resulting in greater reductions in these indices.

Keywords: lard; oxidized lard; digestible energy; metabolizable energy

*Corresponding author, professor, E-mail: wtguan@scau.edu.cn

Fats and oils are widely used in animal feed; however, they are highly susceptible to oxidation during storage, generating various primary and secondary oxidation products. When ingested by animals, these oxidation products disrupt normal physiological and biochemical functions, jeopardize health, and impair growth, causing significant losses to the livestock industry. Consequently, the hazards of lipid oxidation have attracted considerable attention from animal nutrition researchers. Numerous studies have reported on the adverse effects of oxidized fats on animals and their impact on the effective energy values of compound feeds. Research has demonstrated that adding oxidized oils to swine diets reduces antioxidant enzyme activity, inhibits lymphocyte proliferation, alters cellular morphology, and activates lipid catabolism pathways, leading to oxidative metabolic imbalance, compromised immune regulation, intestinal and hepatic lesions, and reduced fat and protein deposition, which ultimately decreases nutrient absorption and impairs growth performance. However, other studies have found no significant effects of oxidized oils on intestinal barrier function, immune response characteristics, or growth performance in pigs, suggesting that these effects may depend on the degree of oxidation.

Yuan et al. found that adding 5% oxidized fish oil to weaned piglet diets decreased dietary metabolizable energy (ME) values. Rosero et al. observed that adding 6% oxidized soybean oil to weaned piglet diets significantly reduced ap-

parent energy digestibility, which exhibited a linear relationship with the degree of oil oxidation. How does oxidation affect the intrinsic effective energy values of fats? Liu et al. added 10% vegetable oils and animal fats from different sources and oxidation levels to weaned piglet diets and found that while DE values differed among fat sources, ME values showed no significant differences, and the oxidation degree did not affect the effective energy values. Lard is widely available in China, but both fresh lard and lard mixed into diets may oxidize due to improper storage or prolonged storage time, affecting the expected energy concentration in feed formulations. How the DE and ME values of oxidized lard change remains unclear, and research data are scarce. Therefore, this study used growing pigs as experimental animals, added lard at different oxidation levels to diets, and determined the effective energy values before and after oxidation using biological methods. This approach not only helps understand the changes in effective energy values caused by lipid oxidation but also provides scientific basis and practical reference for lard storage and its application in diets.

1.1 Experimental Materials

The three batches of fresh lard used in this experiment were provided by Guangzhou Baker Unioil Feed Oil & Fat Co., Ltd., containing no antioxidants. Oxidized lard was prepared in our laboratory.

1.2 Preparation of Oxidized Lard

The preparation of oxidized lard followed the method of Andrews et al. with appropriate modifications. Specifically, ferrous ions (Fe^2) at 30 mg/kg, cupric ions (Cu^2) at 15 mg/kg, hydrogen peroxide (H O) at 600 mg/kg, and 0.3% water were added to fresh lard. After thorough mixing, air was continuously bubbled through the mixture, which was stirred and oxidized at (60±1)°C to produce two types of oxidized lard with target POV values of 30 and 60 mmol/kg, respectively. The actual measured POV values served as the preparation control standard. After each preparation, the oxidized lard was stored at -20°C for later use. Fresh lard (original samples) from three batches was used to prepare the two types of oxidized lard with target POV values of 30 and 60 mmol/kg, with three separate preparations conducted. Each preparation yielded the same amount of oxidized lard, and samples were collected to determine POV and related indices. Finally, the three batches of oxidized lard were mixed in equal proportions to form the oil samples for the digestion and metabolism trial and stored at -20°C for diet preparation.

1.3 Experimental Animals and Diets

Eight Yorkshire×Landrace×Duroc crossbred barrows with similar body weight, genetic background, and parity, averaging (36.38±1.03) kg, were selected and fed four diets: a basal diet, a basal diet supplemented with 10% fresh lard (FL group), a basal diet supplemented with 10% oxidized lard with a target POV of 30 mmol/kg (OL1 group), and a basal diet supplemented with 10%

oxidized lard with a target POV of 60 mmol/kg (OL2 group). The basal diet was a conventional corn-soybean meal type formulated according to NRC (1998) nutrient requirements for 20–50 kg growing pigs, with composition and nutrient levels shown in Table 1 . Experimental diets were prepared by mixing 10% fresh or oxidized lard into the basal diet. Each diet was prepared in three separate batches, sampled for index determination, and then mixed in equal proportions for feeding in the digestion and metabolism trial.

1.4 Experimental Design and Management

The experiment employed a repeated 4×4 Latin square design using the total fecal and urine collection technique for digestion and metabolism trials. The trial was conducted in the Digestion and Metabolism Laboratory of the Department of Animal Nutrition at South China Agricultural University. The experiment consisted of 4 periods, each lasting 10 days (5 days for adaptation and 5 days for sample collection). Pigs were individually housed in metabolism cages. During the 10-day adaptation period, pigs were fed the basal diet, and ad libitum feed intake was recorded. Daily feed allowance during adaptation and collection periods was set at 85% of ad libitum intake. Pigs were fed twice daily at 08:00 and 16:00, with free access to water. All diets were fed as powder. During each period, two pigs were assigned to each group. Samples from each pig were collected and analyzed independently, with the measured values used as individual data points for statistical analysis.

1.5 Sample Collection

Before the experiment, samples of the four diets were collected using bagged feed sampling methods and stored in sealed bags. From day 6 of each period, feces were collected using the total collection method, with 25% of the daily fecal collection sampled using the quartering method and stored in sealed bags. For urine collection, 50 mL of 10% sulfuric acid solution was added to collection containers before sampling. Daily urine volume was measured, and 5% of the urine was proportionally sampled and stored in plastic sealed bottles. All samples were stored at -20°C for later analysis.

1.6 Analytical Methods

1.6.1 Lipid Oxidation Indices The POV, acid value (AV), thiobarbituric acid reactive substances (TBARS) content, iodine value (IV), and saponification value (SV) of lard were determined before mixing into diets. After lard was mixed into diets, crude fat was extracted using the Soxhlet method to determine POV, AV, TBARS content, IV, and SV. Detection methods for POV, AV, IV, and SV followed GB/T 5538-2005, GB/T 5530-2005, GB/T 5532-2008, and GB/T 5534-2008, respectively. TBARS content was determined according to Huang Weikun. All indices were measured in three independent replicates.

1.6.2 Routine Component Analysis of Diets, Feces, and Urine Diet samples were ground to pass through a 40-mesh sieve before determining dry matter content and gross energy (GE) values. Fecal samples were mixed uniformly after collection, inactivated at 105°C for 10–15 minutes, dried to constant weight at 65°C, equilibrated at room temperature for 24 hours, weighed, ground to pass through a 40-mesh sieve, and then analyzed for dry matter content and GE values. Urine sample processing followed Kerr et al.: 2 mL of urine was added to a crucible containing 0.5 g of quantitative filter paper, dried at 50°C for 24 hours, and then analyzed for GE value. Dry matter content was determined according to GB/T 6435-2006. GE values were measured using an IKA C200 bomb calorimeter (rapid dynamic, 23°C) calibrated with benzoic acid.

1.7 Calculation Formulas

Diet DE and ME values were calculated as follows:

Diet DE value (MJ/kg) = [Dietary GE value (MJ/d) -Fecal GE value (MJ/d)] / Average daily feed intake (kg/d)

Diet ME value (MJ/kg) = [Dietary GE value (MJ/d) -Fecal GE value (MJ/d) -Urinary GE value (MJ/d)] / Average daily feed intake (kg/d)

All indices were expressed on a dry matter basis.

Lard DE and ME values and apparent energy digestibility and metabolic rate were calculated as follows:

Lard DE value (MJ/kg) = {Experimental diet DE value (MJ/kg) -Basal diet DE value (MJ/kg) × [100 -Proportion of lard in experimental diet (%)]} / Proportion of lard in experimental diet (%)

Lard ME value (MJ/kg) = {Experimental diet ME value (MJ/kg) -Basal diet ME value (MJ/kg) × [100 -Proportion of lard in experimental diet (%)]} / Proportion of lard in experimental diet (%)

Apparent energy digestibility of lard (%) = [Lard DE value (MJ/kg) / Lard GE value (MJ/kg)] × 100

Apparent energy metabolic rate of lard (%) = [Lard ME value (MJ/kg) / Lard GE value (MJ/kg)] × 100

All indices were expressed on a dry matter basis.

1.8 Statistical Analysis

Lard oxidation characteristic data were analyzed using SPSS 17.0 software for variance analysis. Data from the digestion and metabolism trial were analyzed using the General Linear Model (GLM) module in SPSS 17.0 with the model:

$Yijk = \mu + Pi + Bj + Tk + Eijk$

Where Yijk is the dependent variable value for pigs under different diets; μ is the overall mean; Pi is the period effect (i = 1, 2, 3, 4); Bj is the random effect of pigs (j = 1, 2, 3, 4, 5, 6, 7, 8); Tk is the dietary treatment effect (k = 1, 2, 3, 4); and Eijk is the residual error. Multiple comparisons among groups were performed using the LSD method, with $P < 0.05$ considered statistically significant and $P < 0.01$ considered highly significant. Results are presented as means ± standard error.

2.1 Oxidation Characteristics of Experimental Lard

The oxidation indices of experimental lard before and after mixing into diets are shown in Table 2 . Before mixing, the POV of lard in the FL group (fresh lard) was 0.44 mmol/kg, while the POV values of lard in the OL1 and OL2 groups (oxidized lard with target POV values of 30 and 60 mmol/kg) were 29.64 and 55.79 mmol/kg, respectively, which were extremely significantly higher than fresh lard ($P < 0.01$). Additionally, compared with the FL group, the AV and TBARS content of lard in the OL1 and OL2 groups increased extremely significantly ($P < 0.01$), IV decreased extremely significantly ($P < 0.01$), and SV in the OL2 group was significantly higher than in the FL group ($P < 0.05$). After mixing into diets, the POV, AV, TBARS content, and SV of crude fat in the OL1 and OL2 groups were extremely significantly higher than in the FL group ($P < 0.01$), while IV was extremely significantly lower ($P < 0.01$).

2.2 Average Apparent Energy Values of Experimental Groups

As shown in Table 3 , the average daily DE and ME intake (i.e., dietary DE and ME values) per pig decreased with increasing lard oxidation degree, though the differences were not significant ($P > 0.05$). The proportions of DE intake to GE intake (i.e., dietary GE values) in the FL, OL1, and OL2 groups were 90.04%, 89.66%, and 89.03%, respectively. The proportions of ME intake to GE intake were 87.19%, 86.68%, and 85.98%, respectively. The proportions of ME intake to DE intake were 96.84%, 96.67%, and 96.58%, respectively.

2.3 DE and ME Values of Diets and Lard

As shown in Table 4 , the DE and ME values and apparent energy digestibility and metabolic rate of both diets and lard decreased gradually with increasing lard oxidation degree. Compared with the FL group diet, the DE values of diets in the OL1 and OL2 groups decreased by 1.13% ($P > 0.05$) and 2.21% ($P < 0.01$), respectively, while ME values decreased by 0.85% ($P > 0.05$) and 2.22% ($P < 0.01$), respectively. Compared with fresh lard in the FL group, the DE values of lard in the OL1 and OL2 groups decreased by 4.62% ($P > 0.05$) and 9.45% ($P < 0.01$), respectively; ME values decreased by 3.80% ($P > 0.05$) and 9.63% ($P < 0.01$), respectively; apparent energy digestibility decreased by 4.06%

($P > 0.05$) and 7.91% ($P < 0.05$), respectively; and apparent energy metabolic rate decreased by 3.23% ($P > 0.05$) and 8.12% ($P < 0.05$), respectively.

3.1 Oxidation Characteristics of Experimental Lard

The heating oxidation process of lard involves complex changes that generate a series of primary oxidation products. These oxidation products are unstable and easily decompose during heating and storage, forming stimulating metabolites. To estimate lard oxidation products, this experiment determined POV, AV, TBARS content, IV, and SV in both lard and dietary crude fat. The results showed that whether before or after mixing into diets, POV, AV, TBARS, and SV increased gradually, while IV decreased gradually with increasing lard oxidation degree. POV and TBARS content are indicators for measuring primary and secondary lipid oxidation products, respectively, with higher values indicating greater oxidation degree. Before mixing into diets, the POV values of lard in the OL1 and OL2 groups were 67.36 and 127.80 times that of fresh lard, respectively. After mixing into diets, the POV values of crude fat in the OL1 and OL2 groups were 4.86 and 9.23 times that of the FL group, respectively. Before mixing, the TBARS content of lard in the OL1 and OL2 groups were 9.74 and 14.59 times that of fresh lard, respectively. After mixing, the TBARS content of crude fat in the OL1 and OL2 groups were 3.66 and 4.87 times that of the FL group, respectively. These results indicate that large amounts of polar components formed during lard oxidation and that substantial secondary lipid peroxidation products formed under metal ion catalysis. Therefore, it can be confirmed that the oxidized lard used in this experiment contained high concentrations of both primary and secondary lipid peroxidation products.

3.2 Effects of Oxidation on DE and ME Values of Lard

Oxidized fats in diets can cause oxidative stress in animals, reduce immune function, destroy biological membrane integrity, affect the antioxidant system, accelerate tissue damage in the cardiovascular system, and cause nutrient digestion and absorption disorders. Liu et al. found that adding 15% oxidized soybean oil to mouse diets decreased fat apparent digestibility by 4.96%. Yuan et al. reported that compared with fresh fish oil, 3% oxidized fish oil significantly decreased crude fat and dry matter digestibility in weaned piglet diets by 35.18% and 13.05%, respectively, and extremely significantly decreased nitrogen apparent digestibility and utilization by 21.91% and 30.55%, respectively. This study employed the total fecal and urine collection technique to conduct digestion and metabolism trials in growing pigs and used the difference method to evaluate changes in DE and ME values of lard at different oxidation degrees. The results demonstrated that with increasing lard oxidation degree, the DE and ME values and apparent energy digestibility and metabolic rate of diets formulated with the lard decreased gradually. The DE and ME values and apparent energy digestibility and metabolic rate of the lard itself also decreased to varying degrees. These findings indicate that lard oxidation not only reduces

the effective energy value of the lard itself but also decreases the effective energy value and energy utilization efficiency of complete diets formulated with the oxidized lard. This study suggests that oil quality should be controlled during production, storage, transportation, and application to avoid oxidative rancidity. Feed manufacturers should detect the oxidation degree of oils when purchasing and using them and may even adjust feed formulations based on POV values to achieve expected energy concentrations and desirable feeding performance. In conclusion, oxidation reduces the DE and ME values of lard and decreases its apparent energy digestibility and metabolic rate, with higher oxidation degrees resulting in greater reductions in these indices.

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