

Determination of Available Energy Values of Lard at Different Oxidation Levels (Postprint)

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Abstract

This experiment was conducted to determine the digestible energy (DE) and metabolizable energy (ME) values of lard with varying degrees of oxidation, thereby providing baseline data for its utilization in feed formulations. Eight Duroc × Landrace × Yorkshire crossbred barrows with an average body weight of (36.38 ± 1.03) kg were utilized in a replicated 4×4 Latin square design and fed four diets: a basal diet, a test diet containing 10% fresh lard with a peroxide value (POV) of 0.44 mmol/kg added to the basal diet (FL group), a test diet containing 10% oxidized lard with a POV of 29.64 mmol/kg added to the basal diet (OL1 group), and a test diet containing 10% oxidized lard with a POV of 55.79 mmol/kg added to the basal diet (OL2 group). The experiment comprised 4 periods, each lasting 10 d, with a 5-d preliminary period followed by a 5-d collection period. The results demonstrated that, compared with the 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 metabolizability 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 apparent metabolizability of energy, with higher oxidation levels resulting in greater reductions in these indices.

Full Text

Determination of Effective Energy Values of Lard at Different Oxidation Degrees

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Abstract: This experiment was conducted to determine the digestible energy (DE) and metabolizable energy (ME) values of lard at different oxidation degrees, providing fundamental data for its application in feed. Eight Duroc \times Landrace \times Yorkshire 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 (peroxide value [POV] of 0.44 mmol/kg) (FL group), a basal diet supplemented with 10% oxidized lard (POV of 29.64 mmol/kg) (OL1 group), and a basal diet supplemented with 10% oxidized lard (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. It was concluded that oxidation reduces the DE and ME values of lard and decreases its apparent energy digestibility and metabolic rate, with higher oxidation degrees causing greater reductions in these indices.

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

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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, impair growth performance, and cause economic losses to the livestock industry [1]. Consequently, the hazards of fat 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 value of compound feed. Research has demonstrated that adding oxidized fats to pig diets reduces antioxidant enzyme activity [2-3], inhibits lymphocyte proliferation [4], alters cellular morphology [5], and activates lipolytic metabolic pathways [6], leading to imbalanced oxidative metabolism, 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 reported that dietary oxidized fats exert no significant effects on intestinal barrier function, immune response characteristics, or growth performance in pigs, suggesting that these effects may depend on the degree of oxidation [7-8].

Yuan et al. [9] found that adding 5% oxidized fish oil to weaned piglet diets reduced dietary metabolizable energy (ME) values. Rosero et al. [10] observed

that adding 6% oxidized soybean oil to weaned piglet diets significantly reduced dietary apparent energy digestibility, which exhibited a linear relationship with the degree of fat oxidation. How does oxidation affect the intrinsic effective energy value of fats? Liu et al. [11] added 10% vegetable oils and animal fats from different sources and with varying oxidation degrees to weaned piglet diets and found that DE values differed among fat sources, but ME values showed no significant differences, concluding that oxidation degree did not affect effective energy values. Lard is widely available in China, but fresh lard may oxidize due to improper storage or prolonged storage time before or after mixing into feed, affecting the expected energy concentration in feed formulations. How oxidation affects the DE and ME values of lard remains unclear, and research data are scarce. Therefore, this study used growing pigs as experimental animals, added lard at different oxidation degrees to diets, and determined the effective energy values before and after oxidation using biological methods. This not only helps understand the changes in effective energy values caused by fat oxidation but also provides scientific basis and practical reference for lard storage and its application in feed.

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. [12] with appropriate modifications. Specifically, ferrous ions (Fe^{2+}) at 30 mg/kg, cupric ions (Cu^{2+}) at 15 mg/kg, hydrogen peroxide (H_2O_2) 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 $(60 \pm 1)^\circ\text{C}$ 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. Each preparation batch was stored at -20°C after completion. Fresh lard from three batches was used to prepare the two types of oxidized lard (target POV of 30 and 60 mmol/kg) in three separate preparations. Equal amounts of oxidized lard from the three preparations were thoroughly mixed 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 Duroc \times Landrace \times Yorkshire 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 diet formulated according to the NRC (1998) nutrient requirements for 20-50 kg growing pigs. Its composition and nutrient levels are 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 analysis, and the three batches were then mixed in equal proportions for feeding during the digestion and metabolism trial.

1.4 Experimental Design and Management

The experiment followed 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, they were fed the basal diet, and ad libitum feed intake was recorded. Daily feed allowance during the 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, and samples from each pig were collected and analyzed independently, with the measured values used as independent data for statistical analysis.

1.5 Sample Collection

Before the experiment began, 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, and 25% of the daily fecal collection was sampled using the quartering method and stored in sealed bags. For urine collection, 50 mL of 10% sulfuric acid solution was added to the collection container before collection. Daily urine volume was measured, and 5% of the urine was proportionally sampled and stored in plastic bottles. All samples were stored at $-20\text{ }^{\circ}\text{C}$ for subsequent analysis.

1.6.1 Oil Peroxidation Indices

The POV, acid value (AV), thiobarbituric acid reactive substances (TBARS) content, iodine value (IV), and saponification value (SV) of lard in each group were measured before mixing into diets. After mixing into diets, crude fat was extracted from the diets using the Soxhlet method [13], and the POV, AV, TBARS content, IV, and SV of the extracted crude fat were measured. The 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 measured according to Huang [14]. All indices were determined in three independent measurements.

1.6.2 Routine Component Analysis of Diets, Feces, and Urine

Diet samples were ground and passed 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 min, dried to constant weight at 65 °C, equilibrated at room temperature for 24 h, weighed, ground, and passed through a 40-mesh sieve for dry matter content and GE determination. Urine sample processing followed Kerr et al. [15]: 2 mL of urine was added to a crucible containing 0.5 g of quantitative filter paper, dried at 50 °C for 24 h, and then analyzed for GE. 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) and calibrated with benzoic acid as the standard.

1.7 Calculation Formulas

The DE and ME values of diets were calculated as follows:

$$\text{Dietary DE value (MJ/kg)} = [\text{Dietary GE value (MJ/d)} - \text{Fecal GE value (MJ/d)}] / \text{Average daily feed intake (kg/d)}$$

$$\text{Dietary ME value (MJ/kg)} = [\text{Dietary GE value (MJ/d)} - \text{Fecal GE value (MJ/d)} - \text{Urinary GE value (MJ/d)}] / \text{Average daily feed intake (kg/d)}$$

All indices were expressed on a dry matter basis.

The DE and ME values of lard and its apparent energy digestibility and metabolic rate were calculated as follows:

$$\text{Lard DE value (MJ/kg)} = \{ \text{Dietary DE value of test diet (MJ/kg)} - \text{Dietary DE value of basal diet (MJ/kg)} \times [100 - \text{Proportion of lard in test diet (\%)}] \} / \text{Proportion of lard in test diet (\%)}$$

$$\text{Lard ME value (MJ/kg)} = \{ \text{Dietary ME value of test diet (MJ/kg)} - \text{Dietary ME value of basal diet (MJ/kg)} \times [100 - \text{Proportion of lard in test diet (\%)}] \} / \text{Proportion of lard in test diet (\%)}$$

$$\text{Apparent digestibility of energy for lard (\%)} = [\text{Lard DE value (MJ/kg)} / \text{Lard GE value (MJ/kg)}] \times 100$$

$$\text{Apparent metabolic rate of energy for lard (\%)} = [\text{Lard ME value (MJ/kg)} / \text{Lard GE value (MJ/kg)}] \times 100$$

All indices were expressed on a dry matter basis.

1.8 Statistical Analysis

Data on the oxidation characteristics of lard were analyzed using SPSS 17.0 software. Data from the digestion and metabolism trial in growing pigs were analyzed using the General Linear Model (GLM) module in SPSS 17.0 according to the model:

$$Y_{ijk} = \mu + P_i + B_j + T_k + E_{ijk}$$

Where Y_{ijk} is the dependent variable value for pigs under different diets; μ is the overall mean; P_i is the period effect ($i = 1, 2, 3, 4$); B_j is the random effect of pig ($j = 1, 2, 3, 4, 5, 6, 7, 8$); T_k is the dietary treatment effect ($k = 1, 2, 3, 4$); and E_{ijk} 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 expressed as means \pm standard error.

2.1 Oxidation Characteristics of Experimental Lard

The peroxidation indices of 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 that of 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 that 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 those in the FL group ($P < 0.01$), while IV was extremely significantly lower ($P < 0.01$).

2.2 Average Apparent Energy Values of Each Group

As shown in Table 3, the average daily DE and ME intake (i.e., dietary DE and ME values) per pig tended to decrease with increasing lard oxidation degree, but the differences were not significant ($P > 0.05$). The proportions of DE intake to GE intake (i.e., dietary GE value) 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 of diets and lard, as well as apparent energy digestibility and metabolic rate, gradually decreased with increasing lard oxidation degree. Compared with the diet in the FL group, 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 the 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

Lard undergoes complex changes during thermal oxidation, producing a series of primary oxidation products that are unstable and easily decompose during heating and storage [16], forming irritating metabolites. To estimate the oxidation products of lard, this experiment measured the POV, AV, TBARS content, IV, and SV of lard and crude fat in experimental diets. The results showed that both before and after mixing into diets, POV, AV, TBARS, and SV increased gradually, while IV decreased 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 [17]. 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 were formed during lard oxidation and that substantial secondary lipid peroxidation products were generated under metal ion catalysis [18]. 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 and rancid fats in diets can cause oxidative stress in animals [19], reduce immune function [20], destroy biological membrane integrity [21-22], impair the antioxidant system [23-24], accelerate tissue damage in the cardiovascular system [25], and cause nutrient digestion and absorption disorders [26-27]. Liu et al. [28] found that adding 15% oxidized soybean oil to mouse diets decreased fat apparent digestibility by 4.96%. Yuan et al. [2] reported that compared with fresh fish oil, 3% oxidized fish oil significantly reduced the digestibility of crude fat and dry matter in weaned piglet diets by 35.18% and 13.05%, respectively, and extremely significantly reduced nitrogen apparent digestibility and utilization by 21.91% and 30.55%, respectively.

Using the total fecal and urine collection technique, this study conducted digestion and metabolism trials in growing pigs and evaluated changes in DE and ME values of lard at different oxidation degrees using the difference method. The results demonstrated that with increasing lard oxidation degree, the DE and ME values of diets formulated with the lard, as well as apparent energy digestibility and metabolic rate, gradually decreased. The DE and ME values

of lard itself, along with its apparent energy digestibility and metabolic rate, also decreased to varying degrees.

Therefore, 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. These findings suggest that oil quality should be controlled during production, storage, transportation, and application to avoid oxidation and rancidity. Feed manufacturers should measure the oxidation degree of fats when purchasing and using them, and may even adjust feed formulations based on POV values to achieve expected energy concentrations and satisfactory animal 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 causing greater reductions in these indices.

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