Effects of Dietary Fat Type on Relative Expression of the Glyceraldehyde-3-Phosphate Dehydrogenase Gene in Different Tissues of Broiler Chickens: Postprint

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Abstract

This experiment aimed to investigate the effects of oil types on the relative expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene in different tissues of broiler chickens. A total of 240 1-day-old Cobb broiler pullets were selected and randomly divided into 8 groups (4 single-oil groups, with 5.00% linseed oil, corn oil, sesame oil, and lard added to the diet, respectively; 4 mixed-oil groups, with 2.50% lard + 2.50% corn oil, 2.50% lard + 2.50% sesame oil, 2.50% linseed oil + 2.50% corn oil, and 2.50% linseed oil + 2.50% sesame oil added to the diet, respectively), with 6 replicates per group and 5 chickens per replicate. The experimental period was 42 days. The results showed that: 1) Tissue and the interaction between oil type and tissue had significant effects on the relative expression of GAPDH gene in tissues of 42-day-old broilers ($P<0.05$), while oil type alone had no significant effect on the relative expression of GAPDH gene in tissues of 42-day-old broilers ($P>0.05$). The relative expression of GAPDH gene in breast muscle of 42-day-old broilers was significantly higher than that in liver and abdominal fat ($P<0.05$), being 37.50–89.50 times that of liver and 129.54–190.64 times that of abdominal fat, while the relative expression of GAPDH gene showed no significant difference between liver and abdominal fat ($P>0.05$); the relative expression of GAPDH gene in breast muscle of the corn oil group was significantly higher than that of the lard group ($P<0.05$). 2) The relative expression of GAPDH gene in liver of 21-day-old broilers was significantly or extremely significantly higher than that of 42-day-old broilers ($P<0.05$ or $P<0.01$). 3) Oil combination and the interaction between oil combination and age had no significant effects on the relative expression of GAPDH gene in liver of broilers ($P>0.05$), but age had a significant effect on the relative expression of GAPDH gene in liver of broilers

(P<0.05). It can be seen that the effect of oil type on the relative expression of GAPDH gene in broilers showed tissue-specific differences, and corn oil could increase the relative expression of GAPDH gene in breast muscle. The relative expression of GAPDH gene in breast muscle of 42-day-old broilers was significantly higher than that in liver and abdominal fat, and the relative expression of GAPDH gene in liver of 21-day-old broilers was significantly higher than that of 42-day-old broilers.

Full Text

Effects of Oil Types on Glyceraldehyde-3-Phosphate Dehydrogenase Gene Relative Expression Level in Different Tissues of Broilers

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Abstract: This experiment was conducted to investigate the effects of oil types on the relative expression level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene in different tissues of broilers. A total of 240 one-day-old female Cobb broiler chicks were randomly assigned to 8 groups: four single oil groups fed diets supplemented with 5.00% linseed oil, corn oil, sesame oil, or lard oil; and four mixed oil groups fed diets supplemented with 2.50% lard oil + 2.50% corn oil, 2.50% lard oil + 2.50% sesame oil, 2.50% linseed oil + 2.50% corn oil, or 2.50% linseed oil + 2.50% sesame oil. Each group consisted of 6 replicates with 5 broilers per replicate. The experimental period lasted 42 days. The results showed that: (1) Tissue type and the interaction between oil type and tissue significantly affected the relative expression level of GAPDH gene in 42-day-old broiler tissues (P<0.05), whereas oil type alone had no significant effect (P>0.05). The relative expression level of GAPDH gene in pectoralis muscle of 42-day-old broilers was significantly higher than that in liver and abdominal fat (P<0.05), being 37.50–89.50 times that of liver and 129.54–190.64 times that of abdominal fat, while the difference between liver and abdominal fat was not significant (P>0.05). The corn oil group exhibited significantly higher GAPDH gene expression in pectoralis muscle compared to the lard oil group (P<0.05). (2) The relative expression level of GAPDH gene in liver of 21-day-old broilers was significantly or extremely significantly higher than that of 42-day-old broilers (P<0.05 or P<0.01). (3) Neither oil combination nor the interaction between oil combination and age significantly affected the relative expression level of GAPDH gene in broiler liver (P>0.05), though age itself had a significant effect (P<0.05). In conclusion, the effect of oil type on GAPDH gene relative expression level in broilers varies among tissues, with corn oil enhancing GAPDH gene expression in pectoralis muscle. The relative expression level of GAPDH

gene in pectoralis muscle of 42-day-old broilers is significantly higher than in liver and abdominal fat, and the relative expression level in liver of 21-day-old broilers is significantly higher than that of 42-day-old broilers.

Keywords: broilers; oil; GAPDH; gene expression

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a key metabolic enzyme in animal energy metabolism pathways, composed of 4 subunits with molecular mass of 30–40 ku each, yielding a total molecular mass of 146 ku [1]. Early research on GAPDH primarily focused on its role in glycolysis, where it functions as one of the key enzymes [2-4] and is distributed across cell membranes, cytoplasm, and nuclei. As research progressed, GAPDH was found to possess biological activities unrelated to glycolysis, including involvement in membrane trafficking and fusion on biological membranes [5], cell protection and microtubule polymerization catalysis in the cytoplasm [6], tRNA export and DNA repair in the nucleus [7], and participation in apoptosis and age-related neurological diseases [8]. Studies have also demonstrated that GAPDH exhibits phosphotransferase/kinase activity [9]. The GAPDH gene features highly conserved sequences across species and is widely present in numerous organisms, expressing at high levels in almost all tissues. In molecular biology research methods, the GAPDH gene is commonly used as a housekeeping gene and serves as a standard internal reference for gene expression studies at both RNA and protein levels.

Corn oil and sesame oil, rich in linoleic acid and oleic acid, represent n-6 fatty acids [10-11]. Linseed oil, with an n-6/n-3 polyunsaturated fatty acid (PUFA) ratio of approximately 0.34:1.00, is far lower than other oils and represents n-3 fatty acids [12-13]. Lard is an animal oil rich in monounsaturated fatty acids, with oleic acid content up to 48.70%. Previous studies have shown that appropriate oil supplementation in broiler diets can meet the energy requirements for rapid growth, improve palatability, and enhance feed utilization efficiency [14]. Oil types affect animal lipid metabolism and fatty acid synthase (FAS) mRNA expression [15]. However, systematic reports on the effects of oil types on GAPDH gene expression in broilers are lacking. This study primarily investigated the effects of single oils and their combinations on GAPDH gene expression levels in different tissues of broilers at different ages.

1 Materials and Methods

1.1 Experimental Animals and Design

This experiment utilized 240 one-day-old female Cobb broiler chicks randomly divided into 8 groups, with 6 replicates per group and 5 broilers per replicate. Broilers were raised in cages (5 birds per cage) with ad libitum access to feed and water, following conventional immunization programs. Diets were formulated according to NRC (1994) broiler nutrition requirements and divided into two

phases: 1–3 weeks and 4–6 weeks of age. The experimental period lasted 42 days. The four single oil groups received diets supplemented with 5% linseed oil, corn oil, sesame oil, or lard oil, with diet composition and nutrient levels shown in Table 1 . The four mixed oil groups comprised two lard combination groups (2.5% lard oil + 2.5% corn oil, 2.5% lard oil + 2.5% sesame oil) and two vegetable oil combination groups (2.5% linseed oil + 2.5% corn oil, 2.5% linseed oil + 2.5% sesame oil), with diet composition and nutrient levels shown in Table 2 .

1.2 Sample Collection

At 21 and 42 days of age, one broiler from each replicate per group was randomly selected for slaughter. Liver, pectoralis muscle, and abdominal fat were collected aseptically. All samples were washed with diethylpyrocarbonate (DEPC) water, snap-frozen in liquid nitrogen, and stored at -80°C.

1.3 Total RNA Extraction and Reverse Transcription

Total RNA from tissue samples was extracted following the instructions of TaKaRa' s Trizol reagent kit (using 50–100 mg tissue). RNA integrity was analyzed using a NanoDrop 2000 UV spectrophotometer (Thermo Scientific, Germany). Reverse transcription was performed according to the kit instructions (PrimeScript® RT Reagent Kit with gDNA Eraser, TaKaRa, Dalian), which removes residual genomic DNA from extracted RNA. The reaction mixture (containing 2.0 µg total RNA) was prepared on ice in a 20 µL system. After brief centrifugation, the mixture was placed in a PCR instrument for reaction at 37°C for 15 min and 85°C for 5 s, then stored at -20°C.

1.4 Primer Design

Based on the GAPDH gene sequence of red junglefowl (GenBank accession number: NM_{204305}.1), primers for fluorescent quantitative PCR were designed using Oligo 6.0 software and synthesized by Shanghai Bioengineering Technology Service Co., Ltd. The PCR amplification fragment length was 98 bp. The fluorescent quantitative PCR primers are shown in Table 3 .

1.5 Fluorescent Quantitative PCR Reaction Conditions

Following the method described by Zhang et al. [16], probe-based fluorescent quantitative PCR was used for gene expression analysis. The 25.0 µL reaction system included 1.0 µL cDNA (from 0.1 µL total RNA), 0.5 µL forward primer (12.5 µmol/L), 0.5 µL reverse primer (12.5 µmol/L), 2.5 µL TaqMan™ probe, 0.5 µL RoxII, 3.5 µL Mg^{2+} (25 mmol/L), 12.5 µL 2×Mix (containing 1.5 mmol/L Mg^{2+}), and 4.0 µL ultrapure water. Reaction conditions were: pre-denaturation at 95°C for 2 min; 40 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 1 min. Each sample was run in triplicate. Purified PCR products were quantified using a NanoDrop 2000 UV spectrophotometer and serially diluted

(10^4, 10^5, 10^6, 10^7, 10^8, 10^9, and 10^{10}) to establish standard curves for each plate. Negative controls were included on each plate. Following the method reported by Bustin [17], this study used a micro-quantifier to determine RNA concentration, with equal amounts of RNA and cDNA added for each sample normalization.

1.6 Data Statistics and Analysis

First, GAPDH copy numbers for each sample were converted to relative expression levels. The relative expression level of GAPDH gene = sample copy number / mean copy number of the lowest group at 42 days of age (expression value of 5.00% sesame oil group in abdominal fat tissue). Experimental data were analyzed using the ANOVA (GLM) procedure in SAS 8.1 statistical software for two-way ANOVA to examine the effects of oil type and tissue, oil type and age, and oil combination and age on GAPDH gene relative expression levels in broilers. $P<0.05$ was considered significant and $P<0.01$ extremely significant.

2 Results

2.1 Effects of Single Oils on GAPDH Gene Relative Expression Level in Different Tissues of 42-Day-Old Broilers

As shown in Table 4 , tissue type and the interaction between oil type and tissue significantly affected GAPDH gene relative expression levels in different tissues of 42-day-old broilers ($P<0.05$), while oil type alone had no significant effect ($P>0.05$). GAPDH gene relative expression levels in different tissues of broilers followed the pattern: pectoralis muscle > liver > abdominal fat. In all single oil groups, pectoralis muscle GAPDH gene relative expression levels were significantly higher than those in liver and abdominal fat ($P<0.05$), being 37.50–89.50 times that of liver and 129.54–190.64 times that of abdominal fat, while expression levels between liver and abdominal fat showed no significant difference ($P>0.05$). In pectoralis muscle, the corn oil group exhibited significantly higher GAPDH gene expression than the lard oil group ($P<0.05$), whereas no significant differences were observed among oil type groups in liver and abdominal fat ($P>0.05$). These results indicate that GAPDH gene relative expression in broilers exhibits distinct spatiotemporal expression characteristics and is significantly influenced by tissue type.

2.2 Effects of Single Oil Types on GAPDH Gene Relative Expression Level in Liver of Broilers at Different Ages

As shown in Table 5 , age significantly affected GAPDH gene relative expression level in broiler liver ($P<0.05$), while oil type and the interaction between oil type and age had no significant effects ($P>0.05$). The relative expression levels of GAPDH gene in liver of 21-day-old broilers in linseed oil, corn oil, sesame oil, and lard oil groups were significantly or extremely significantly higher than those of 42-day-old broilers ($P<0.05$ or $P<0.01$), being 2.46–4.15 times that of

42-day-old broilers. However, no significant differences were observed among oil types at either 21 or 42 days of age (P>0.05). These results demonstrate that GAPDH gene relative expression level in broiler liver is significantly affected by age, while the effect of oil type on GAPDH gene expression exhibits tissue specificity.

2.3 Effects of Mixed Oils on GAPDH Gene Relative Expression Level in Liver of Broilers at Different Ages

As shown in Table 6 and Table 7 , neither oil combination nor the interaction between age and oil combination significantly affected GAPDH gene relative expression level in broiler liver (P>0.05), though age remained a significant factor (P<0.05). Similar to single oils, the relative expression levels of GAPDH gene in liver of 21-day-old broilers were significantly or extremely significantly higher than those of 42-day-old broilers (P<0.05 or P<0.01), being 2.46–3.57 times that of 42-day-old broilers.

3 Discussion

This study found that GAPDH gene relative expression levels in broilers differed significantly among tissues, with pectoralis muscle being the predominant expression site. In broiler liver, GAPDH gene relative expression levels also varied significantly with age, indicating distinct spatiotemporal expression characteristics. Barber et al. [18] reported that human GAPDH gene expression also showed significant variation among tissues, with the highest relative expression in skeletal muscle and the lowest in mammary gland, differing by 15-fold. Lowe et al. [19] demonstrated that both GAPDH gene and protein levels in fast-twitch skeletal muscle of aged rats (37 months) were significantly lower than in young rats (9 months), with no significant difference in slow-twitch muscle, indicating that glycolytic capacity in fast-twitch muscle declines with age. Slagboom et al. [20] found that GAPDH gene relative expression in spleen of 36-month-old female rats was significantly higher than in 24-month-old rats, while no age-related changes were observed in liver and brain. Mozdziak et al. [21] reported that GAPDH gene relative expression in chicken pectoralis muscle at 7 days of age was significantly higher than in younger chicks. In this experiment, GAPDH gene relative expression in liver of 21-day-old broilers was significantly or extremely significantly higher than that of 42-day-old broilers, suggesting that glycolytic capacity in broiler liver declines with age. The effect of age on GAPDH gene relative expression varies depending on species, tissue, or muscle type.

Hanke et al. [22] found that low glucose concentrations could directly inhibit GAPDH promoter activity during rabbit skeletal muscle cell culture, thereby reducing its transcription level and enzyme activity. Mozdziak et al. [21] reported that GAPDH gene relative expression in pectoralis muscle of 3-day-old broilers was significantly higher in fed groups than in fasted groups, indicating that nutritional status can alter GAPDH gene transcription levels. This

study found that 5% corn oil upregulated GAPDH gene relative expression in pectoralis muscle, while oil types and combinations had no significant effect on GAPDH gene relative expression in liver of 42-day-old broilers, suggesting that oils influence GAPDH transcription levels in a tissue- and type-specific manner.

In recent years, increasing researchers have found that GAPDH exhibits instability when used as an internal reference gene [23-24], with its expression levels regulated by various factors at transcriptional or post-transcriptional levels, and both gene and protein levels changing in response to different stimuli [20]. Therefore, caution should be exercised when selecting GAPDH as an internal reference gene in molecular biology research.

4 Conclusions

1. The effect of oil type on GAPDH gene relative expression level in broilers varies among tissues, with corn oil enhancing GAPDH gene expression in pectoralis muscle.
2. The relative expression level of GAPDH gene in pectoralis muscle of 42-day-old broilers is significantly higher than in liver and abdominal fat.
3. The relative expression level of GAPDH gene in liver of 21-day-old broilers is significantly or extremely significantly higher than that of 42-day-old broilers.

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