Effects of Dietary Fat Level on Energy Metabolism, Serum Biochemical Indices, Hepatic Fatty Acid Composition, and Liver-Type Fatty Acid Binding Protein Gene Expression in Silver Foxes during the Winter Fur Period: Postprint

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Abstract

This study aimed to investigate the effects of dietary fat level on energy metabolism, serum biochemical indices, liver fatty acid composition, and liver-type fatty acid binding protein (L-FABP) gene expression in silver foxes during the winter fur period. Fifty healthy male silver foxes at 145 days of age with similar body weight were randomly allocated to 5 groups (10 replicates per group, 1 fox per replicate) and fed experimental diets containing 10%, 12%, 16%, 20%, and 24% mixed oil (chicken oil:soybean oil=1:1). The corresponding dietary fat levels were 12.85% (Group A), 14.71% (Group B), 18.72% (Group C), 22.23% (Group D), and 26.11% (Group E). The pre-trial period lasted 15 days, and the formal trial period lasted 75 days. The results showed: 1) Dietary fat level significantly or extremely significantly affected dry matter intake, metabolizable energy intake, average daily gain, and metabolizable energy to gain ratio ($P<0.05$ or $P<0.01$) in silver foxes during the winter fur period, but had no significant effect on gross energy digestibility ($P>0.05$). 2) With increasing dietary fat level, serum triglyceride (TG) and glucose (GLU) levels in silver foxes during the winter fur period showed an increasing trend, with serum TG level in Group E being significantly higher than that in Groups A and B ($P<0.05$), and serum GLU level in Group A being significantly lower than those in all other groups ($P<0.05$). Dietary fat level had no significant effect on serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) levels, or alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities ($P>0.05$). 3) Dietary fat level had a significant or extremely significant effect on the proportions of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), and saturated fatty acids (SFA) in the liver of silver foxes during the winter fur period ($P<0.05$ or $P<0.01$). As dietary fat level increased, the proportions of PUFA and MUFA in the liver increased, while the proportion of SFA decreased. 4) With increasing dietary fat level, the relative L-FABP mRNA expression level in the liver of silver foxes during the winter fur period generally showed an increasing trend, with Group E being extremely significantly higher than Groups A, B, and C ($P<0.01$). It was concluded that excessively high dietary fat level is not conducive to full utilization of dietary nutrients by silver foxes during the winter fur period; increasing dietary fat level can increase serum TG and GLU levels in silver foxes during the winter fur period while promoting deposition of unsaturated fatty acids in the liver; increasing dietary fat level can upregulate L-FABP gene expression in the liver of silver foxes during the winter fur period, thereby promoting fatty acid transport and alleviating liver damage caused by high-fat diets.

Full Text

Effects of Dietary Fat Level on Energy Metabolism, Serum Biochemical Indices, Liver Fatty Acid Composition and Liver-Type Fatty Acid Binding Protein Gene Expression in Silver Foxes During the Winter Fur-Growing Period

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Abstract

This experiment was conducted to investigate the effects of dietary fat level on energy metabolism, serum biochemical indices, liver fatty acid composition, and liver-type fatty acid binding protein (L-FABP) gene expression in silver foxes during the winter fur-growing period. Fifty healthy male silver foxes at 145 days of age with similar body weight were randomly allocated into five groups (10 replicates per group, one fox per replicate) and fed experimental diets containing 10%, 12%, 16%, 20%, and 24% mixed oil (chicken oil:soybean oil = 1:1), corresponding to dietary fat levels of 12.85% (Group A), 14.71% (Group B), 18.72% (Group C), 22.23% (Group D), and 26.11% (Group E), respectively. The experiment consisted of a 15-day preliminary period followed by a 75-day formal trial period. The results showed that: 1) Dietary fat level significantly or extremely significantly affected daily dry matter intake, daily metabolizable energy intake, average daily gain, and metabolizable energy to gain ratio in winter fur-growing silver foxes ($P<0.05$ or $P<0.01$), while having no significant effect on gross energy digestibility (P>0.05). 2) Serum triglyceride (TG) and glucose (GLU) levels exhibited increasing trends with elevated dietary fat levels, with Group E showing significantly higher TG levels than Groups A and B (P<0.05), and Group A displaying significantly lower GLU levels than all other groups (P<0.05). Dietary fat level had no significant influence on serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) levels, or alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (P>0.05). 3) Dietary fat level significantly or extremely significantly affected the proportions of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), and saturated fatty acids (SFA) in the liver (P<0.05 or P<0.01). As dietary fat level increased, the proportions of PUFA and MUFA in the liver increased while the proportion of SFA decreased. 4) The relative expression level of L-FABP mRNA in the liver generally showed an increasing trend with rising dietary fat levels, with Group E being extremely significantly higher than Groups A, B, and C (P<0.01). These findings indicate that excessively high dietary fat levels are detrimental to nutrient utilization in winter fur-growing silver foxes. Elevating dietary fat level can increase serum TG and GLU levels while promoting the deposition of unsaturated fatty acids in the liver. Moreover, increased dietary fat upregulates hepatic L-FABP gene expression, thereby facilitating fatty acid transport and mitigating liver damage induced by high-fat diets.

Keywords: silver foxes; fat level; serum biochemical indices; liver; fatty acid composition; liver-type fatty acid binding protein

Introduction

Fat represents the most economical and efficient energy source, and its inclusion in diets helps increase energy density while reducing feed costs. Numerous studies have demonstrated that high-fat diets can promote growth in fur-bearing animals. Compared with reports by Hansen et al., the body weight of silver foxes reported in NJF (2012) increased by 60%, with increased body fat deposition being one of the primary contributing factors. However, excessive fat intake and substantial body fat deposition are detrimental to liver health in fur-bearing animals. Liver-type fatty acid binding protein (L-FABP) is an important member of the fatty acid binding protein family (FABPs) that exhibits high affinity for long-chain fatty acids and plays a crucial role in the absorption and transport of dietary fats within the body. Fur-bearing animals have higher dietary fat requirements than other monogastric animals, which is closely related to their unique fat metabolism characteristics. This study aims to investigate the effects of dietary fat level on energy metabolism, serum biochemical indices, liver fatty acid composition, and L-FABP gene expression in silver foxes during the winter fur-growing period, thereby providing a reference for research on fatty acid nutrition and fat metabolism in silver foxes.

Materials and Methods

1.1 Experimental Design and Animal Management

Fifty healthy male silver foxes at 145 days of age with similar body weight were randomly divided into five groups (10 replicates per group, one fox per replicate). The foxes were fed experimental diets containing 10%, 12%, 16%, 20%, and 24% mixed oil (chicken oil:soybean oil = 1:1), corresponding to dietary fat levels of 12.85% (Group A), 14.71% (Group B), 18.72% (Group C), 22.23% (Group D), and 26.11% (Group E), respectively. All experimental silver foxes were housed individually in outdoor cages (100 cm × 80 cm × 80 cm) under natural lighting conditions. They were fed twice daily (morning and evening) with ad libitum access to feed and water. The experiment consisted of a 15-day preliminary period followed by a 75-day formal trial period.

1.2 Experimental Diets

The experimental diets were formulated using extruded corn, soybean meal, Peruvian fish meal, meat and bone meal, corn gluten meal, dried distillers grains with solubles (DDGS), chicken oil, soybean oil, and other ingredients, supplemented with mineral and vitamin premixes. The composition and nutrient levels of the experimental diets are presented in Table 1 , and the fatty acid composition is shown in Table 2 .

Table 1 Composition and nutrient levels of experimental diets (air-dry basis)

Table 2 Fatty acid composition of experimental diets

1.3 Sample Collection

On day 65 of the experiment, blood samples (5 mL) were collected from the lateral saphenous vein of eight foxes per group before morning feeding using disposable vacuum coagulation-promoting tubes. Samples were immediately inverted 5–8 times, allowed to stand at room temperature for 30 minutes, then centrifuged at 4,500 r/min for 7 minutes. The serum was harvested and stored at -80°C for subsequent analysis. At the end of the experiment, eight foxes per group were randomly selected and euthanized by intracardiac injection of 5 mL succinylcholine. The liver was rapidly dissected; approximately 2 g of liver tissue from the same hepatic lobule was rinsed with physiological saline, placed in a cryovial, immediately immersed in liquid nitrogen for over 10 minutes, and then transferred to -80°C storage. An additional approximately 50 g of liver tissue was rinsed with physiological saline, placed in a self-sealing bag, and stored at -20°C for fatty acid composition analysis.

1.4 Analytical Methods

1.4.1 Feed Nutrient Analysis Crude protein content in experimental diets was determined by the Kjeldahl method according to GB/T 6432–1994. Crude fat content was measured using a Soxhlet extractor according to GB/T 6433–1994. Ash content was determined by high-temperature incineration according to GB/T 6438–1992. Calcium content was measured by EDTA complexometric titration according to GB/T 6436–1992. Phosphorus content was determined by ammonium vanadomolybdate colorimetry according to GB/T 6437–1992. Amino acid content was analyzed using an automatic amino acid analyzer (HITACHI L-8900, Japan).

1.4.2 Serum Biochemical Indices Serum biochemical indices were measured using a VetTest automatic biochemical analyzer (Netherlands) with assay kits purchased from Zhongsheng Beikong Biotechnology Co., Ltd. Specific assay procedures followed the kit instructions.

1.4.3 Liver Fatty Acid Composition Analysis Fatty acid composition was determined by gas chromatography-mass spectrometry (Agilent 7890A-7000B). Chromatographic conditions: DB-5MS column (30 m × 250 m × 0.25 m); initial oven temperature 55°C held for 2 minutes, increased to 200°C at 5°C/min and held for 1 minute, then increased to 230°C at 2°C/min and held for 3 minutes, finally increased to 270°C at 5°C/min and held for 10 minutes; injector temperature 250°C; helium carrier gas (99.999%) at 1.0 mL/min; injection volume 1 L; split ratio 10:1. Mass spectrometry conditions: electron impact (EI) ion source; ion source temperature 230°C; electron energy 70 eV; interface temperature 250°C; scan range m/z 50–500.

1.4.4 Liver L-FABP mRNA Expression Analysis 1.4.4.1 Total RNA Extraction and cDNA Synthesis

Liver samples were ground into powder in liquid nitrogen and collected in 1.5 mL RNase-free Eppendorf tubes. Total RNA was extracted using RNAiso Reagent (TaKaRa) according to the manufacturer's protocol. RNA integrity was assessed by gel electrophoresis, and purity was determined by measuring absorbance at 260 and 280 nm. Reverse transcription was performed using a commercial kit (TaKaRa), and cDNA products were stored at -20°C.

1.4.4.2 L-FABP mRNA Relative Expression Quantification

L-FABP mRNA relative expression was determined by real-time PCR using SYBR® Green dye (TransStart kit) with β-actin as the internal reference gene. Primer sequences are listed in Table 3 and were synthesized by Shanghai Sangon Biotech Co., Ltd. The 20 L PCR reaction mixture contained: 2×TransStart Top Green qPCR SuperMix 10 L, forward primer (10 mol/L) 0.4 L, reverse primer (10 mol/L) 0.4 L, Passive Reference Dye (50×) 0.4 L, RNase-free dH2O 7.8 L, and cDNA 1 L. Thermal cycling conditions: initial denaturation at 95°C for 1 minute, followed by 40 cycles of 95°C for 5 seconds and annealing for 25 seconds (specific annealing temperatures in Table 3). A melting curve analysis (65–95°C, increment 0.5°C) was performed to confirm product specificity.

1.5 Statistical Analysis

Data are expressed as means ± standard deviation. Statistical analysis was performed using SAS 8.0 software with the GLM procedure. Differences among means were compared using Duncan' s multiple range test. $P<0.01$ was considered extremely significant, $P<0.05$ significant, and $P>0.05$ not significant.

Table 3 Primer sequences and parameters for real-time PCR

Results

2.1 Effects of Dietary Fat Level on Energy Metabolism

As shown in Table 4 , dietary fat level significantly or extremely significantly affected daily dry matter intake, daily metabolizable energy intake, average daily gain, and metabolizable energy to gain ratio in winter fur-growing silver foxes ($P<0.05$ or $P<0.01$). Daily dry matter intake decreased with increasing dietary fat level, with Group C being extremely significantly lower than all other groups ($P<0.01$). Groups B and C showed significantly higher daily metabolizable energy intake than Groups A, D, and E ($P<0.05$). Average daily gain in Groups B, C, and D was extremely significantly higher than in Groups A and E ($P<0.01$), while Group A was extremely significantly higher than Group E ($P<0.01$). Group E exhibited a significantly higher metabolizable energy to gain ratio than all other groups ($P<0.05$). Dietary fat level had no significant effect on gross energy digestibility ($P>0.05$).

Table 4 Effects of dietary fat level on energy metabolism of silver foxes during the winter fur-growing period

In the same row, values with different small letter superscripts indicate significant difference (P<0.05), different capital letter superscripts indicate extremely significant difference (P<0.01), and same or no letters indicate no significant difference (P>0.05). The same applies below.

2.2 Effects of Dietary Fat Level on Serum Biochemical Indices

As presented in Table 5 , serum triglyceride (TG) levels showed an increasing trend with elevated dietary fat levels, with Group E being significantly higher than Groups A and B ($P<0.05$). Group A had the lowest serum glucose (GLU) level, which was significantly lower than all other groups ($P<0.05$). Dietary fat level had no significant effects on serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) levels, or alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities ($P>0.05$).

Table 5 Effects of dietary fat level on serum biochemical indices of silver foxes during the winter fur-growing period

2.3 Effects of Dietary Fat Level on Liver Fatty Acid Composition

As shown in Table 6 , dietary fat level significantly or extremely significantly affected the proportions of polyunsaturated fatty acids (PUFA) such as C18:2n-6 and C18:3n-3, monounsaturated fatty acids (MUFA) such as C18:1n-9, and saturated fatty acids (SFA) such as C18:0 in the liver ($P<0.05$ or $P<0.01$). With increasing dietary fat level, the proportions of PUFA and MUFA in the liver increased while the proportion of SFA decreased.

Table 6 Effects of dietary fat level on fatty acid composition of silver foxes during the winter fur-growing period (percentage of total fatty acid)

2.4 Effects of Dietary Fat Level on Hepatic L-FABP mRNA Expression

As illustrated in Figure 1 [Figure 1: see original paper], the relative expression level of L-FABP mRNA in the liver generally increased with dietary fat level, with Group E being extremely significantly higher than Groups A, B, and C ($P<0.01$).

Figure 1 Effects of dietary fat level on relative expression level of L-FABP mRNA in liver of silver foxes during the winter fur-growing period

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Discussion

3.1 Effects of Dietary Fat Level on Energy Metabolism

Energy in diets is stored within nutrients, with fat being the most effective energy source, and its inclusion level significantly affects dietary energy concentration. The present results showed that daily dry matter intake of winter fur-growing silver foxes gradually decreased with increasing dietary fat level, which is consistent with findings in blue foxes. Yang reported that increasing dietary energy level significantly increased gross energy digestibility in male mink. However, this study found that increasing dietary fat level did not cause significant changes in gross energy digestibility in silver foxes. Although dietary metabolizable energy increased with fat level, daily metabolizable energy intake showed a trend of initial increase followed by stabilization, possibly reflecting the animals' ability to adjust intake based on energy demand. The significantly higher metabolizable energy to gain ratio in the 24% mixed oil group (Group E) indicates that excessively high fat levels are not conducive to optimal nutrient utilization for tissue synthesis in silver foxes.

3.2 Effects of Dietary Fat Level on Serum Biochemical Indices

Blood lipids encompass all lipid substances in blood, including TG, TC, phospholipids, and free fatty acids. TG represents the primary form of energy storage in mammals, and previous studies have shown that increasing dietary energy level can elevate serum TG levels in dogs. Van de Ligt et al. reported that serum GLU levels in growing pigs increased with dietary energy level. The current results align with these findings, showing that serum TG and GLU levels in silver foxes increased with dietary fat level, indicating that high-fat diets promote fat synthesis. Additionally, serum TC, HDL-C, and LDL-C levels showed increasing trends with dietary fat level, though differences among groups were not significant, likely because serum TC level is less responsive to dietary changes. Serum ALT and AST activities are important indicators for evaluating liver health in human disease diagnosis. The absence of significant differences in these enzyme activities among groups suggests that silver foxes possess a certain tolerance to dietary fat.

3.3 Effects of Dietary Fat Level on Liver Fatty Acid Composition

The liver fatty acid composition observed in this study is similar to that reported for other fur-bearing animals such as European bears, blue foxes, and mink. The proportion of PUFA in tissues is determined by multiple factors including synthesis rate, interconversion, and oxidative decomposition. In this study, the proportions of C18:1n-9, C18:2n-6, and C18:3n-3 in silver fox liver increased with dietary fat level, which can be attributed to the high proportions of these fatty acids in the dietary oils. As dietary fat level increased, the intake of C18:1n-9 and C18:2n-6 increased, leading to greater deposition in the liver. Hepatic fat synthesis is primarily catalyzed by fatty acid synthase in the cytoplasm, with the main products being saturated fatty acids C16:0 and C18:0. Ahlstrøm et al. found that increasing dietary fat level (using fish oil as the fat source) significantly decreased the proportion of C18:0 in blue fox liver. Similar results were obtained in this study, where the proportion of C18:0 in silver fox liver significantly decreased with increasing dietary fat level, possibly due to increased PUFA intake inhibiting fatty acid synthase expression. Previous studies have shown that high-fat diets can cause insulin resistance in mice, leading to substantial fat deposition in the liver. Whether high-fat diets induce insulin resistance in silver foxes requires further investigation.

3.4 Effects of Dietary Fat Level on Hepatic L-FABP Gene Expression

FABPs exhibit high affinity for long-chain fatty acids (>14C), making L-FABP gene expression a valuable indicator of hepatic fat metabolism. Previous research has demonstrated that L-FABP expression is regulated by dietary fats. Matzinger et al. reported that FABP expression is higher in tissues with active fat metabolism such as liver, small intestine, and adipose tissue. This study showed that hepatic L-FABP mRNA expression in silver foxes generally increased with dietary fat level. The upregulation of L-FABP mRNA expression likely results from increased intake and utilization of fatty acids. When silver foxes consumed high-fat diets, the elevated levels of long-chain fatty acids absorbed from the intestine and transported to the liver stimulated L-FABP expression to enhance fatty acid transport and reduce hepatic fat accumulation. As research on FABP genes progresses, their potential for diagnosing fat metabolism disorders has attracted increasing attention. Whether high-fat diets cause fat metabolism diseases in silver foxes warrants further study.

Conclusion

Under the conditions of this experiment: (1) Increasing dietary fat level decreased daily dry matter intake in winter fur-growing silver foxes, while daily metabolizable energy intake showed an initial increase followed by a decrease, indicating that excessively high fat levels are not conducive to optimal nutrient utilization. (2) Elevating dietary fat level increased serum TG and GLU levels and promoted the deposition of unsaturated fatty acids in the liver. (3) Increasing dietary fat level upregulated hepatic L-FABP gene expression, thereby promoting fatty acid transport and alleviating liver damage caused by high-fat diets.

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