

## Effects of Dietary n-6/n-3 Polyunsaturated Fatty Acid Ratio on Growth Performance and Hepatic Expression of Fatty Acid Metabolism-Related Protein Genes in Arctic Foxes during the Winter Fur Period (Postprint)

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### Abstract

This study aimed to investigate the effects of dietary n-6/n-3 polyunsaturated fatty acid (PUFA) ratio on growth performance, liver fatty acid composition, and the gene expression of liver-type fatty acid binding protein (L-FABP) and fatty acid transport protein (FATP) in Arctic foxes during the winter fur period. Forty-eight healthy male Arctic foxes at 157 days of age with an average body weight of  $(5658 \pm 47)$  g were selected and randomly divided into 4 groups, with 12 replicates per group and 1 fox per replicate. Group I was fed a diet supplemented with 12.00% fish oil and 2.00% soybean oil, with an n-6/n-3 PUFA ratio of 3.00; Group II was fed a diet supplemented with 9.38% corn oil and 4.62% soybean oil, with an n-6/n-3 PUFA ratio of 18.03; Group III was fed a diet supplemented with 12.00% corn oil and 2.00% soybean oil, with an n-6/n-3 PUFA ratio of 40.83; Group IV was fed a diet supplemented with 1.50% fish oil and 12.50% corn oil, with an n-6/n-3 PUFA ratio of 136.36. All groups had consistent feed ingredients except for the composition and proportion of oils. The pre-trial period was 7 days, and the formal trial period was 40 days. The results showed that: 1) Dietary n-6/n-3 PUFA ratio had an extremely significant effect on average daily gain (ADG), average daily feed intake (ADFI), and feed to gain ratio (F/G) of Arctic foxes during the winter fur period ( $P < 0.01$ ). ADG of groups I and IV was extremely significantly higher than that of groups II and III ( $P < 0.01$ ), ADFI of groups I, II, and IV was extremely significantly higher than that of group III ( $P < 0.01$ ), and F/G of group IV was extremely significantly lower than that of groups II and III ( $P < 0.01$ ). 2) Dietary n-6/n-3 PUFA ratio had a significant or extremely significant effect on the contents of monounsaturated fatty acids (MUFA), PUFA, n-3 PUFA, and n-6 PUFA in

the liver of Arctic foxes during the winter fur period ( $P < 0.05$  or  $P < 0.01$ ), but had no significant effect on the content of saturated fatty acids (SFA) ( $P > 0.05$ ). The hepatic n-3 PUFA content of groups I and IV was extremely significantly higher than that of groups II and III ( $P < 0.01$ ), and the hepatic n-6 PUFA content of groups II and III was extremely significantly higher than that of groups I and IV ( $P < 0.01$ ). 3) Dietary n-6/n-3 PUFA ratio had no significant effect on the relative mRNA expression level of hepatic L-FABP ( $P > 0.05$ ), but had an extremely significant effect on the relative mRNA expression level of hepatic FATP ( $P < 0.01$ ). The relative mRNA expression level of hepatic FATP in groups I and IV was extremely significantly higher than that in groups II and III ( $P < 0.01$ ). In conclusion, dietary supplementation with a mixed oil of 1.50% fish oil and 12.50% corn oil, i.e., a dietary n-6/n-3 PUFA ratio of 136.36, upregulated the expression of the FATP gene in the liver, increased the transport and utilization efficiency of long-chain fatty acids in the liver, and promoted the growth of Arctic foxes during the winter fur period.

## Full Text

### Effects of Dietary n-6/n-3 Polyunsaturated Fatty Acids Ratio on Growth Performance and Hepatic Fatty Acid Metabolism-Related Protein Gene Expression in Arctic Foxes During the Winter Fur-Growing Period

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## Abstract

This study investigated the effects of dietary n-6/n-3 polyunsaturated fatty acids (PUFA) ratio on growth performance, liver fatty acid composition, and the expression of liver-type fatty acid binding protein (L-FABP) and fatty acid transport protein (FATP) genes in Arctic foxes during the winter fur-growing period. Forty-eight healthy male Arctic foxes at 157 days of age with an average body weight of  $(5,658 \pm 47)$  g were randomly allocated into four groups with 12 replicates per group and one fox per replicate. Group I received a diet containing 12.00% fish oil and 2.00% soybean oil (n-6/n-3 PUFA ratio = 3.00); Group II received 9.38% corn oil and 4.62% soybean oil (n-6/n-3 PUFA ratio = 18.03); Group III received 12.00% corn oil and 2.00% soybean oil (n-6/n-3 PUFA ratio = 40.83); and Group IV received 1.50% fish oil and 12.50% corn oil (n-6/n-3 PUFA ratio = 136.36). All diets were identical except for oil composition and proportion. The experiment consisted of a 7-day adaptation period followed by a 40-day trial period. The results showed that: (1) Dietary n-6/n-3

PUFA ratio significantly affected average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) ( $P < 0.01$ ). ADG in Groups I and IV was significantly higher than in Groups II and III ( $P < 0.01$ ). ADFI in Groups I, II, and IV was significantly higher than in Group III ( $P < 0.01$ ). The F/G in Group IV was significantly lower than in Groups II and III ( $P < 0.01$ ). (2) Dietary n-6/n-3 PUFA ratio significantly or extremely significantly affected liver monounsaturated fatty acids (MUFA), PUFA, n-3 PUFA, and n-6 PUFA contents ( $P < 0.05$  or  $P < 0.01$ ), but had no significant effect on saturated fatty acids (SFA) ( $P > 0.05$ ). Liver n-3 PUFA content in Groups I and IV was significantly higher than in Groups II and III ( $P < 0.01$ ), while liver n-6 PUFA content in Groups II and III was significantly higher than in Groups I and IV ( $P < 0.01$ ). (3) Dietary n-6/n-3 PUFA ratio did not significantly affect liver L-FABP mRNA expression ( $P > 0.05$ ), but significantly affected liver FATP mRNA expression ( $P < 0.01$ ). FATP mRNA expression in Groups I and IV was significantly higher than in Groups II and III ( $P < 0.01$ ). In conclusion, supplementation with 1.50% fish oil and 12.50% corn oil (n-6/n-3 PUFA ratio = 136.36) upregulated hepatic FATP gene expression, enhanced hepatic long-chain fatty acid transport and utilization efficiency, and promoted growth in Arctic foxes during the winter fur-growing period.

**Keywords:** n-6/n-3 PUFA; Arctic fox; liver; fatty acids; liver-type fatty acid binding protein; fatty acid transport protein

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Polyunsaturated fatty acids (PUFA), particularly n-3 and n-6 PUFA, play crucial roles in lipid metabolism, gene expression regulation, immune function, and the fatty acid composition of livestock products. Since n-6 and n-3 PUFA cannot be interconverted *in vivo* and must be obtained through diet, the balance of n-6/n-3 PUFA ratio has become a critical concern. As essential fatty acids, studies have shown that dietary supplementation with n-6 and n-3 PUFA not only meets essential fatty acid requirements but also that an appropriate n-6/n-3 PUFA ratio maintains physiological functions, regulates lipid metabolism, and promotes healthy growth in livestock. The fatty acid composition of fur-bearing animals corresponds to dietary fatty acid composition, with variations among different tissues. Liver-type fatty acid binding protein (L-FABP) and fatty acid transport protein (FATP) are two proteins involved in fatty acid transport. L-FABP is an important member of the fatty acid binding protein family (FABPs), while FATP belongs to the fatty acid transport protein superfamily (FATPs). Both proteins exhibit high affinity for long-chain fatty acids and play vital roles in fatty acid uptake and transport during lipid metabolism. The Arctic fox (*Alopex lagopus*), a carnivorous canid native to northern Asia, Europe, North America, and Arctic regions, is a valuable fur-bearing animal. Arctic foxes differ from livestock in fat tolerance, and research on their fatty acid utilization, transport, and deposition patterns remains limited. Therefore, this study aimed to investigate the effects of dietary n-6/n-3 PUFA ratio on growth performance, liver fatty acid composition, and L-FABP and FATP gene expression in Arctic

foxes during the winter fur-growing period to provide a theoretical basis for Arctic fox production and fat metabolism research.

### 1.1 Experimental Animals

The experimental Arctic foxes were local Finnish strain foxes, derived from Finnish breeding stock after years of local propagation.

### 1.2 Experimental Design and Diets

Forty-eight healthy male Arctic foxes at 157 days of age with an average body weight of  $(5,658 \pm 47)$  g were randomly divided into four groups with 12 replicates per group and one fox per replicate. Experimental diets were formulated using extruded corn, soybean meal, corn protein meal, dried distillers grains with solubles (DDGS), fish meal, meat meal, oils, and nutritional additives including minerals and vitamins. Dietary fatty acid requirements followed FEDIAF (European Pet Food Industry Federation, 2011) guidelines. Fatty acid ratios were adjusted by varying dietary oil composition, with all other ingredients remaining consistent across groups. Group I received 12.00% fish oil and 2.00% soybean oil (n-6/n-3 PUFA ratio = 3.00); Group II received 9.38% corn oil and 4.62% soybean oil (n-6/n-3 PUFA ratio = 18.03); Group III received 12.00% corn oil and 2.00% soybean oil (n-6/n-3 PUFA ratio = 40.83); and Group IV received 1.50% fish oil and 12.50% corn oil (n-6/n-3 PUFA ratio = 136.36). Diet composition and nutrient levels are presented in Table 1, and fatty acid composition is shown in Table 2.

### 1.3 Animal Management

The experiment was conducted at the Fur Animal Research Base of the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, from October 13 to December 1, 2014, comprising a 7-day adaptation period and a 40-day trial period. Foxes were housed individually in cages and fed once daily at 08:00.

### 1.4 Sample Collection and Analysis

At the end of the trial, seven foxes were randomly selected from each group, euthanized via intracardiac injection of 5 mL succinylcholine, and immediately dissected. Approximately 2 g of liver tissue from the same hepatic lobule region was collected, rinsed with physiological saline, placed in cryovials, snap-frozen in liquid nitrogen for over 10 minutes, and stored at  $-80^{\circ}\text{C}$ . An additional ~50 g liver sample was collected, rinsed, placed in sealed bags, and stored at  $-20^{\circ}\text{C}$  for fatty acid composition analysis.

**1.4.1 Dietary Nutrient Analysis** Dry matter, crude protein, crude fat, crude ash, calcium, and phosphorus contents were determined. Dry matter was measured by oven drying at  $105^{\circ}\text{C}$  (GB/T 6435–2006), crude protein by

Kjeldahl method (GB/T 6432–1994), crude fat by Soxhlet extraction (GB/T 6433–1994), crude ash by combustion at 550°C (GB/T 6438–1992), calcium by EDTA complexometric titration (GB/T 6436–1992), phosphorus by ammonium vanadomolybdate colorimetry (GB/T 6437–1992), and amino acids by automatic amino acid analyzer (HITACHI L-8900, Japan).

**1.4.2 Growth Performance Calculations** Average daily feed intake (g/d) = total feed intake / trial days;  
Average daily gain (g/d) = (final weight - initial weight) / trial days;  
Feed-to-gain ratio = average daily feed intake / average daily gain.

**1.4.3 Fatty Acid Analysis** Fatty acid methyl esterification followed GB/T 21514-2008, with external standard method for quantification. Gas chromatography-mass spectrometry (Agilent 7890A-7000B) was used with a DB-5MS column (30 m × 250 μm × 0.25 μm). Temperature program: initial 55°C for 2 min, ramped at 5°C/min to 200°C (held 1 min), then 2°C/min to 230°C (held 3 min), then 5°C/min to 270°C (held 10 min). Injector temperature: 250°C; carrier gas: helium (99.999%) at 1.0 mL/min; injection volume: 1 μL; split ratio: 10:1. MS conditions: EI source at 230°C, 70 eV electron energy, interface temperature 250°C, scan range 50–500 m/z.

#### **1.4.4 Hepatic L-FABP and FATP mRNA Expression Analysis** **1.4.4.1 Total RNA Extraction and cDNA Synthesis**

Liver samples were ground in liquid nitrogen and collected in 1.5 mL RNase-free Eppendorf tubes. Total RNA was extracted using RNAiso Reagent (TaKaRa) following manufacturer instructions. RNA integrity was verified by gel electrophoresis, and purity was assessed by OD<sub>260</sub>/280 ratio. Reverse transcription was performed using a TaKaRa kit, with cDNA stored at -20°C.

##### **1.4.4.2 Quantitative Real-Time PCR**

L-FABP and FATP mRNA expression was measured by SYBR Green real-time PCR (Trans-Start kit) using β-actin as the reference gene. Primer sequences are listed in Table 3 (synthesized by Shanghai Sangon Biotech). The 20 μL reaction contained: 2×TransStart Top Green qPCR SuperMix 10 μL, forward and reverse primers (10 μmol/L) each 0.4 μL, Passive Reference Dye (50×) 0.4 μL, RNase-free dH<sub>2</sub>O 7.8 μL, and cDNA 1 μL. Cycling: pre-denaturation at 95°C for 1 min; 40 cycles of 95°C for 5 s and annealing for 25 s (temperatures in Table 3). Melting curve analysis (65–95°C, 0.5°C increments) verified product specificity.

## **1.5 Statistical Analysis**

Data were processed in Excel 2003 and analyzed using SPSS 9.13 GLM procedure. Duncan's multiple comparison test was applied, with P < 0.01 indicating extremely significant differences, P < 0.05 significant differences, and P > 0.05 non-significant differences. Results are expressed as mean ± standard deviation.

### 2.1 Effects of Dietary n-6/n-3 PUFA Ratio on Growth Performance

As shown in Table 4, dietary n-6/n-3 PUFA ratio significantly affected ADG, ADFI, and F/G ( $P < 0.01$ ). ADG in Groups I and IV was significantly higher than in Groups II and III ( $P < 0.01$ ), with no significant difference between Groups I and IV or between Groups II and III ( $P > 0.05$ ). ADFI in Groups I, II, and IV was significantly higher than in Group III ( $P < 0.01$ ), with no significant differences among Groups I, II, and IV ( $P > 0.05$ ). The F/G in Group IV was significantly lower than in Groups II and III ( $P < 0.01$ ), with no significant difference compared to Group I ( $P > 0.05$ ).

### 2.2 Effects of Dietary n-6/n-3 PUFA Ratio on Liver Fatty Acid Composition

Table 5 shows that dietary n-6/n-3 PUFA ratio significantly or extremely significantly affected liver MUFA, PUFA, n-3 PUFA, and n-6 PUFA contents ( $P < 0.05$  or  $P < 0.01$ ), but had no significant effect on SFA ( $P > 0.05$ ). Liver MUFA content in Groups I and IV was significantly higher than in Group III ( $P < 0.05$ ). Liver PUFA content in Group II was significantly higher than in Groups I and IV ( $P < 0.05$ ). Liver n-3 PUFA content in Groups I and IV was significantly higher than in Groups II and III ( $P < 0.01$ ), with none detected in Group II. Liver n-6 PUFA content in Groups II and III was significantly higher than in Groups I and IV ( $P < 0.01$ ).

### 2.3 Effects of Dietary n-6/n-3 PUFA Ratio on Hepatic L-FABP and FATP Gene Expression

Figure 1 [Figure 1: see original paper] shows that hepatic L-FABP mRNA expression increased then decreased with rising dietary n-6/n-3 PUFA ratio, peaking in Group III, but differences among groups were not significant ( $P > 0.05$ ). Figure 2 [Figure 2: see original paper] shows that hepatic FATP mRNA expression decreased then increased with rising dietary n-6/n-3 PUFA ratio, with Groups I and IV being significantly higher than Groups II and III ( $P < 0.01$ ), but no significant differences between Groups I and IV or between Groups II and III ( $P > 0.05$ ).

### 3.1 Effects on Growth Performance

Fish oil is rich in n-3 PUFA and has been shown to promote growth in poultry while enhancing immunity. Guo (2011) reported that fish oil replacing corn oil improved intestinal immune response and feed efficiency in piglets. In this study, Groups IV and I showed higher ADFI and ADG and lower F/G than Groups II and III, indicating that mixed animal and plant oils outperformed plant oil blends alone. This aligns with findings in broilers that mixed oil sources provide complementary fatty acid profiles, improving fat digestion and utilization. Although Groups III and IV had similar oil proportions, Group IV showed superior

performance, likely due to differences in n-6/n-3 PUFA ratio. Studies demonstrate that appropriate n-6/n-3 PUFA ratios improve production performance by enhancing immune function and metabolic efficiency in weaned piglets.

### 3.2 Effects on Liver Fatty Acid Composition

Liver fatty acids in Arctic foxes consisted of approximately 62% SFA, 12% MUFA, and 26% PUFA, indicating predominant saturation, consistent with Rouvinen et al. (1989). Dietary fatty acid composition influences tissue fatty acid profiles. In this study, liver MUFA, PUFA, n-3 PUFA, and n-6 PUFA generally mirrored dietary patterns: MUFA and n-3 PUFA decreased then increased, while PUFA and n-6 PUFA increased then decreased with rising n-6/n-3 PUFA ratio, consistent with Gudbjarnason and Oskarsdottir (1977). Groups I and IV, containing fish oil, showed highest liver n-3 PUFA deposition. Fish oil reduces  $\Delta$ -6 desaturase, elongase, and  $\Delta$ -5 desaturase activities involved in n-6 PUFA synthesis. Groups II, III, and IV, rich in n-6 PUFA, showed higher liver n-6 PUFA deposition than Group I.

### 3.3 Effects on L-FABP and FATP Gene Expression

In vitro studies show L-FABP and FATP have high affinity for long-chain (>C14) fatty acids and regulate fatty acid uptake and transport. L-FABP preferentially binds unsaturated fatty acids. Group III showed highest L-FABP mRNA expression, possibly due to relatively higher dietary unsaturated fatty acids promoting gene expression. Groups I and IV exhibited significantly higher FATP mRNA expression than Groups II and III, indicating more efficient hepatic fatty acid transport and utilization, which correlated with improved growth performance. Further research is needed to elucidate the regulatory mechanisms of FABPs and FATPs in Arctic fox lipid metabolism.

### Conclusion

In conclusion, dietary supplementation with 1.50% fish oil and 12.50% corn oil (n-6/n-3 PUFA ratio = 136.36) upregulated hepatic FATP gene expression, enhanced long-chain fatty acid transport and utilization efficiency, and promoted growth in Arctic foxes during the winter fur-growing period.

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