Effect of n-6/n-3 PUFA ratio on body fat deposition, tissues fatty acid composition and key genes expression of liver lipid metabolism in silver foxes (Vulpes vulpes fulva) during the winter fur-growth period

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Objective: The proportion of n-6/n-3 polyunsaturated fatty acid (PUFA) plays an important role in regulating lipid metabolism. This study aimed to investigate the effects of dietary n-6/n-3 PUFA ratios on body fat deposition, tissues fatty acid composition, and gene expression of liver lipid metabolism of silver foxes during the winter fur-growth period.

Methods: Forty-eight age-matched male silver foxes with similar body weights were randomly divided into four dietary groups for 47 days, which were fed n-6/n-3 PUFA ratio with 3, 18, 41, and 136 experimental diets, respectively.

Results: Dietary n-6/n-3 PUFA ratio did not significantly influence fat deposition parameters except for hepatic fat content. The variation trend of the fatty acid composition of liver, intramuscular fat, and subcutaneous fat in silver fox was directly related to dietary fatty acid content (p < 0.05). With the dietary n-6/n-3 PUFA ratio increasing, the expression of liver fatty acid synthase (FAS) mRNA and peroxisome proliferator-activated receptor (PPAR) mRNA exhibited the trend of first decreasing and then increasing (p < 0.05), whereas L-fatty acid binding protein (L-FABP) mRNA expression showed a gradual increasing trend (p < 0.05).

Conclusion: In summary, silver foxes fed an n-6/n-3 PUFA ratio 18:1 diet (supplementing with 9.38% corn oil and 4.62% soybean oil) was more conducive to lipid decomposition, PUFA transport, and utilization of tissues, thereby meeting it for supplying energy and withstanding the cold.

Keywords
n-6/n-3PUFA, silver fox, body fat deposition, tissues fatty acid composition, genes expression of lipid metabolism
Introduction

Polyunsaturated fatty acids (PUFAs), especially the n-3 and n-6 series PUFAs, play an important role in the body's lipid metabolism, gene expression regulation, and fatty acid composition of animal products (1, 2). Because n-6 and n-3 series PUFAs cannot be converted into each other and have to be taken in through food, the balancing of the n-6/n-3 PUFA ratio has attracted much attention lately in improving inflammation and decreasing the risk of metabolic diseases (3–6). Dietary fatty acid composition clearly influenced the body fat composition of fur animals (7–9). The previous studies have shown that n-3 and n-6 PUFA in diets regulate the lipid deposition and oxidation in human and animals, thereby affecting the composition of fatty acids in tissues (10–13). Extensive investigations into gene expression as it relates to lipid metabolism have been conducted in swine, chickens, and geese, among other animals, and have mainly focused on lipid deposition and product quality regulation, among other factors (14–17). The silver fox was a precious fur animal and could utilize a higher content fat diet, but its body could keep healthy and have less ability for body fat deposition, which is rather different from other monogastric animals (18). However, how to utilize and metabolize fatty acids in silver fox have not been investigated. Thus, in this study, we aimed to examine the effect of the dietary n-6/n-3 PUFA ratio on the tissues fatty acid composition, body fat deposition, and expression of key genes of liver lipid metabolism of the silver fox during the winter fur growth period to provide basic data to understand the silver fox's lipid metabolism mechanism.

Materials and methods

Ethics approval and consent to participate

All animals used in the study were treated following the guidelines established by the Council of China Animal Welfare. Protocols of the experiments were approved by the Animal Ethics Committee of the Chinese Academy of Agricultural Sciences (CAAS).

Animals, experimental design, and diets

The experiment was performed at the fur animal breeding base. Forty-eight 157-day-old healthy male silver foxes with an average weight of 5,450 ± 140 g at the fur growth stage were randomly divided into four groups (12 replicates per group, one silver fox per replicate). Each group was provided diets of different lipid compositions, and the feeds had the same ingredients except for the composition and ratio of the lipids. The diet of Group I was supplemented with 12% fish oil and 2% soybean oil, yielding an n-6/n-3 of 3.00; the diet of Group II was supplemented with 9.38% corn oil and 4.62% soybean oil, yielding an n-6/n-3 of 18.03; the diet of Group III was supplemented with 12% corn oil and 2% soybean oil, yielding an n-6/n-3 of 40.83; and the diet of Group IV was supplemented with 1.5% fish oil and 12.5% corn oil, yielding an n-6/n-3 of 136.36.

Each of the experimental animals was raised separately in a cage. The experiment was initiated on October 13th, and completed on December 1st. The pre-feeding period was 7 days, and the formal experimental period was 40 days, during which the animals were fed twice a day (at 8:00 am and 15:00 pm), with free access to water. The ingredients, nutrient levels, and fatty acid compositions of the feeds of the different groups are shown in Tables 1, 2.

Sample collection

At the end of the experimental period, eight silver foxes from every group were randomly selected and were euthanized. The liver and subcutaneous fats of silver foxes were weighed. 2 g liver sample was taken, rinsed blood stains with normal saline, put into the frozen storage tube, put into liquid nitrogen for more than 10 min, and then moved into −80°C refrigerator storage for gene detection. Samples collected from partly liver were dried to constant weight at 65 degrees and fat content was determined by soxhlet extraction. The animals were sampled for liver, medial thigh muscle, and subcutaneous belly fat 50 g, respectively, which were cleaned by normal saline and stored frozen (−20°C) for fatty acid analysis.

Chemical analyses

Samples of feed were analyzed for DM, CP, EE, Ca, P, and AA according to AOAC (19) methods. Amino acids were determined by hydrolyzing samples with 6 mol/L HCl for 24 h at 110°C (20) and analyzed using an Amino Acid Analyzer (Hitachi L-8800; Hitachi, Ltd., Tokyo, Japan). Methionine and cysteine were determined as methionine sulfone and cysteic acid after cold performic acid oxidation overnight and hydrolyzed. The GE concentration was measured using an adiabatic bomb calorimeter (C2000, Calorimeter; IKA Company; Germany). Fatty acids were pretreated using the methyl esterification method and analyzed with Gas Chromatography–Mass Spectrometry (GC–MS; Agilent 7890A-7000B, USA) referring to the standard method (21).
**TABLE 1** Feed ingredients and chemical composition of experimental diets (air-dry basis) (%).

| Items         | Groups (n-6/n-3PUFA ratio) |
|---------------|----------------------------|
|               | I (3:1)                    |
|               | II (18:1)                  |
|               | III (41:1)                 |
|               | IV (136:1)                 |
| Ingredients   |                           |
| Extruded corn | 32.75                      |
| Soybean meal  | 12.00                      |
| Corn protein meal | 8.00   |
| DDGS          | 1.55                       |
| Fish meal     | 16.00                      |
| Meat meal     | 10.00                      |
| Lysine        | 0.80                       |
| Methionine    | 0.40                       |
| Premix        | 1.00                       |
| Fish oil      | 12.00                      |
| Corn oil      | 9.38                       |
| Soybean oil   | 2.00                       |
| CaHPO₄         | 3.00                       |
| NaCl          | 0.50                       |
| Total         | 100.00                     |
| Chemical composition | 19.04        |
| Gross energy, MJ/kg | 29.76        |
| Crude protein | 15.00                      |
| Ether extract | 41.43                      |
| Crude carbohydrate | 2.36         |
| Lysine        | 1.43                       |
| Methionine    | 1.14                       |
| Calcium       | 1.61                       |
| Phosphorous   | 1.03                       |

**Fatty acids**

| C12:0 | C14:0 | C14:1 | C15:0 | C15:1 | C16:0 | C16:1 | C17:0 | C17:1 | C18:0 |
|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  |
| 0.00  | 0.01  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  |
| 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  |
| 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  | 0.00  |

**TABLE 2** Fatty acid compositions of the experimental diets (%).

- **SFA saturated fatty acid(s)**
- **MUFA monounsaturated fatty acid(s)**
- **PUFA polyunsaturated fatty acid(s)**

**Gene expression analysis of liver lipid metabolism**

The relative amounts of expression of fatty acid synthase (FAS) mRNA, peroxisome proliferator-activated receptor (PPAR) mRNA and fatty acid binding protein (FABP) were determined through real-time quantitative PCR assay (SYBR)
Green dye method, Trans-Start kit) using β-actin as the reference gene. The primers were synthesized by the Shanghai Biological Engineering Co., Ltd (Table 3). The PCR reaction (20 µl) comprised the following reagents: 10 µl 2 x Trans Start Top Green qPCR SuperMix, 0.4 µl of each of the two primers (10 µmol/L), 0.4 µl Passive Reference Dye (50×), 7.8 µl of RNAse-free dH₂O and 1 µl of cDNA. The PCR cycling program was as follows: 95°C for 1 min, followed by 40 cycles of 95°C for 5 s and annealing (with specific annealing temperatures) shown in Table 3 for 5 s. The melting curve was used to determine the specificity of amplicons, using the following program: from 65 to 95°C, an assay was conducted at increments of 0.5°C until reaching 95°C, for a total of 61 cycles.

Statistical analyses

Experimental data were exported to EXCEL2003 and collated, and statistical analyses were performed using the GLM program of SAS V8 software; multiple comparisons were performed using Duncan’s test, and p < 0.05 and p > 0.05 were used to determine whether findings were significant or insignificant, respectively. The test measurements were presented in the form of means ± standard deviations.

Results

Effects of the dietary n-6/n-3 PUFA ratio on body fat deposition traits of silver foxes

The dietary n-6/n-3 PUFA ratio exerted a significant impact on the hepatic fat content of the silver foxes (p < 0.05, Table 4), and the hepatic fat content of the silver foxes of Groups I, III, and IV was significantly higher than that of Group II (p < 0.05), whereas the hepatic fat content among the silver foxes of Groups I, III, and IV showed no significant differences (p > 0.05). The dietary n-6/n-3 PUFA ratio did not show any significant effect on the hepatic somatic index, liver fat percentage, subcutaneous fat weight, and subcutaneous fat percentage (p > 0.05), but of which in Group II was relatively lower than that of the other groups, except for hepatic somatic index.

| Genes | Primer sequence (5’ – 3’) | Gene bank No. | Product size /bp |
|-------|--------------------------|---------------|------------------|
| β-actin | F: TGCCCATCTATGAGGGGTATG R: CCTTGATGTCAAGCAGATT | XM_041749381 | 153 |
| FAS | F: GATACCTGTGGTTTGGTCRC R: CAGCGATGCAGATGTAT | XM_026014782 | 183 |
| PPAR | F: AAAGAGCCTAAGGGAGCC R: GCAAAATGATAGCACGGACCA | XM_041757116 | 345 |
| FABP | F: ACAGACTTGTGCCTTGG R: GAATGTGCAGAATGG | NM_001287051 | 185 |
In the same row, values with different superscript mean significant difference (P < 0.05).

TABLE 4 Effects of dietary n-6/n-3 PUFA ratio on body fat deposition traits of silver fox during the winter fur-growing period %.

| Items                        | Groups (n-6/n-3PUFA ratio) | P-value |
|------------------------------|----------------------------|---------|
|                              | I (3:1)                    | II (18:1) | III (41:1) | IV (136:1) |
| Hepatic somatic index        | 2.78 ± 0.36                | 3.27 ± 0.32 | 2.93 ± 0.26 | 2.78 ± 0.48 | 0.0938 |
| Hepatic fat content          | 7.73 ± 0.78a               | 5.50 ± 0.90b | 7.78 ± 1.53a | 7.36 ± 1.72a | 0.0481 |
| Liver fat percentage         | 1.67 ± 0.58                | 1.56 ± 0.22 | 2.21 ± 0.42 | 2.17 ± 0.57 | 0.0703 |
| Subcutaneous fat weight/g    | 310.40 ± 54.03             | 278.17 ± 61.76 | 324.00 ± 47.35 | 301.83 ± 63.20 | 0.576  |
| Subcutaneous fat percentage  | 4.40 ± 0.91                | 4.36 ± 0.90 | 4.70 ± 0.80 | 4.94 ± 0.84 | 0.620  |

TABLE 5 Effects of dietary n-6/n-3 PUFA ratio on fatty acid profiles of liver of silver fox during the winter fur-growing period (proportion of total fatty acid) %.

| Items           | Groups (n-6/n-3PUFA ratio) | P-value |
|-----------------|----------------------------|---------|
|                 | I (3:1)                    | II (18:1) | III (41:1) | IV (136:1) |
| C10:0           | 0.24 ± 0.14                | ND       | ND         | 0.42 ± 0.03 | 0.1876 |
| C12:0           | 0.38 ± 0.20b               | 1.04 ± 0.34a | 0.85 ± 0.13a | 0.71 ± 0.13ab | 0.0015 |
| C14:0           | 0.76 ± 0.21                | 0.81 ± 0.19 | 1.00 ± 0.48 | 0.90 ± 0.23 | 0.5392 |
| C16:0           | 15.55 ± 2.48               | 15.58 ± 1.93 | 13.43 ± 1.95 | 17.57 ± 3.66 | 0.0589 |
| C16:1           | 2.02 ± 0.51ab              | 1.58 ± 0.63b | 1.56 ± 0.82b | 2.50 ± 0.55b | 0.0447 |
| C17:0           | 0.69 ± 0.10                | 0.95 ± 0.20 | 0.92 ± 0.21 | 0.76 ± 0.16 | 0.0567 |
| C18:0           | 43.03 ± 5.37a              | 41.69 ± 2.83ab | 37.8 ± 4.23bc | 36.35 ± 3.02a | 0.0191 |
| C18:1n9c        | 9.29 ± 2.75                | 12.13 ± 2.28 | 10.60 ± 4.80 | 13.77 ± 3.15 | 0.1346 |
| C18:2n6c        | 13.91 ± 2.21c              | 18.34 ± 2.13b | 21.73 ± 1.62a | 18.48 ± 2.32b | <0.0001 |
| C20:0           | 0.35 ± 0.09                | ND       | ND         | 0.42 ± 0.09 | 0.3292 |
| C20:3n3         | 0.49 ± 0.16                | ND       | ND         | 0.47 ± 0.24 | 0.8980 |
| C20:4n6         | 0.38 ± 0.13                | ND       | ND         | 0.36 ± 0.00 | 0.8124 |
| C22:6n3         | 5.31 ± 1.78b               | 6.81 ± 1.83b | 10.01 ± 2.94a | 5.30 ± 2.16b | 0.0039 |
| SFA             | 5.78 ± 2.36a               | 1.76 ± 0.47b | 3.21 ± 0.64a | 2.83 ± 1.05b | 0.0006 |
| MUFA            | 60.14 ± 5.40a              | 59.60 ± 3.95a | 53.02 ± 4.26a | 57.18 ± 3.31ab | 0.0237 |
| PUFA            | 11.30 ± 3.22b              | 13.71 ± 2.86ab | 10.28 ± 3.98b | 16.27 ± 3.55a | 0.0222 |
| N-6             | 27.77 ± 6.81b              | 26.70 ± 4.35b | 34.71 ± 5.45a | 28.46 ± 4.41b | 0.0477 |
| N-3             | 19.54 ± 3.85c              | 25.15 ± 3.75b | 31.88 ± 4.28b | 22.50 ± 4.01b | <0.0001 |
|                 | 3.08 ± 0.65ab              | 1.86 ± 0.56bc | 3.30 ± 0.66ab | 2.11 ± 0.82b | 0.0102 |

In the same row, values with different superscript mean significant difference (P < 0.05).

but no significant difference from Group IV (p > 0.05). There was no significant difference in subcutaneous fat MUFA among Group II, III, and IV (p > 0.05). Subcutaneous fat PUFAs of Group I were significantly lower than those of the other Groups (p < 0.05), but no significant difference was found among Group II, III, and IV (p > 0.05). Subcutaneous fat N-3 PUFAs of Group I were highly significant higher than those of the other groups (p < 0.05). Subcutaneous fat N-3 PUFAs of Group II were highly significant lower than that of Group IV (p < 0.05), but no significant difference from Group III (p > 0.05), and that of Group III was significantly lower than that of Group IV (p < 0.05).

Effects of the dietary n-6/n-3 PUFA ratio on the genes expression of key enzymes of lipid metabolism in the livers of silver foxes

The dietary n-6/n-3 PUFA ratio exerted a significant impact on the relative expression level of FAS mRNA (p < 0.05, Figure 1). FAS mRNA expression in Group I was significantly higher than those of Group II and III (p < 0.05), and no significant difference was found between Group I and IV (p > 0.05). FAS mRNA expression in Group II was significantly lower than that of Group IV (p < 0.05), and no
TABLE 6 Effects of dietary n-6/n-3 PUFA ratio on fatty acid profiles of intramuscular fat of silver fox during the winter fur-growing period (proportion of total fatty acid) %.

| Items | Groups (n-6/n-3PUFA ratio) | P-value |
|-------|---------------------------|---------|
|       | I (3:1)                   | II (18:1)| III (41:1) | IV (136:1) |
| C12:0 | 0.11 ± 0.03               | 0.07 ± 0.01| 0.09 ± 0.03| 0.08 ± 0.02| 0.3098 |
| C14:0 | 2.83 ± 0.29$^{a}$         | 1.59 ± 0.32$^{b}$| 1.66 ± 0.26$^{b}$| 1.84 ± 0.34$^{b}$| <0.0001 |
| C14:1 | 0.28 ± 0.04$^{a}$         | 0.18 ± 0.08$^{b}$| 0.18 ± 0.08$^{b}$| 0.19 ± 0.05$^{b}$| 0.0242 |
| C15:0 | 0.17 ± 0.03$^{b}$         | 0.09 ± 0.02$^{b}$| 0.10 ± 0.01$^{b}$| 0.12 ± 0.01$^{b}$| <0.0001 |
| C16:0 | 22.90 ± 0.79$^{a}$        | 17.15 ± 1.76$^{a}$| 17.40 ± 1.32$^{b}$| 17.31 ± 1.61$^{b}$| <0.0001 |
| C16:1 | 9.17 ± 1.39$^{a}$         | 4.72 ± 1.22$^{b}$| 5.19 ± 1.84$^{a}$| 5.68 ± 1.33$^{a}$| <0.0001 |
| C17:0 | 0.22 ± 0.03               | 0.22 ± 0.09| 0.19 ± 0.05| 0.17 ± 0.02| 0.1970 |
| C18:0 | 4.97 ± 0.42$^{a}$         | 4.12 ± 0.65$^{b}$| 4.21 ± 0.80$^{b}$| 4.12 ± 0.48$^{b}$| 0.0399 |
| C18:1n9c | 37.46 ± 1.71            | 36.22 ± 2.73| 35.78 ± 2.12| 38.94 ± 2.33| 0.0644 |
| C18:1n9t | ND                     | 0.09 ± 0.03| 0.09 ± 0.04| 0.11 ± 0.03| 0.6245 |
| C18:2n6c | 19.51 ± 1.15$^{c}$      | 34.31 ± 2.96$^{a}$| 33.04 ± 3.33$^{ab}$| 29.63 ± 4.59$^{b}$| <0.0001 |
| C18:3n3 | 0.73 ± 0.11$^{b}$         | 0.47 ± 0.06$^{c}$| 0.99 ± 0.23$^{a}$| 0.60 ± 0.23$^{bc}$| 0.0002 |
| C20:0 | ND                       | 0.13 ± 0.05| 0.11 ± 0.05| 0.12 ± 0.04| 0.9155 |
| C20:1 | 0.22 ± 0.03$^{a}$         | 0.15 ± 0.03$^{bc}$| 0.14 ± 0.03$^{c}$| 0.18 ± 0.02$^{b}$| 0.0008 |
| C20:4n6 | 0.66 ± 0.10$^{b}$        | 0.66 ± 0.09$^{a}$| 0.53 ± 0.13$^{ab}$| 0.48 ± 0.16$^{b}$| 0.0349 |
| C20:5n3 | 0.69 ± 0.14$^{b}$         | ND       | 0.16 ± 0.04$^{c}$| 0.16 ± 0.05$^{b}$| <0.0001 |
| C22:6n3 | 0.44 ± 0.04$^{a}$        | 0.14 ± 0.05$^{c}$| 0.18 ± 0.04$^{bc}$| 0.22 ± 0.04$^{b}$| <0.0001 |
| SFA   | 31.10 ± 1.06$^{a}$        | 23.15 ± 2.62$^{b}$| 23.76 ± 1.52$^{b}$| 23.75 ± 1.69$^{b}$| <0.0001 |
| MUFA  | 47.22 ± 2.41$^{a}$        | 41.25 ± 2.93$^{b}$| 41.34 ± 3.40$^{b}$| 45.04 ± 3.40$^{b}$| 0.0031 |
| PUFA  | 21.67 ± 1.82$^{c}$        | 35.50 ± 2.98$^{a}$| 34.90 ± 3.33$^{ab}$| 31.21 ± 4.95$^{b}$| <0.0001 |
| N-6   | 20.08 ± 1.27$^{c}$        | 34.96 ± 2.93$^{a}$| 33.60 ± 3.34$^{ab}$| 30.11 ± 4.60$^{b}$| <0.0001 |
| N-3   | 1.86 ± 0.24$^{a}$         | 0.54 ± 0.07$^{c}$| 1.30 ± 0.26$^{b}$| 1.10 ± 0.39$^{b}$| <0.0001 |

In the same row, values with different superscript mean significant difference (P < 0.05).

The dietary n-6/n-3 PUFA ratio exerted a significant effect on the relative expression level of PPAR mRNA (p < 0.05, Figure 2). With an increasing dietary n-6/n-3 PUFA ratio, the relative expression level of PPAR mRNA showed a trend of first declining and then rising, and the relative expression level of PPAR mRNA of Group I was highly significantly higher than those of Groups II, III, and IV (p < 0.05). Furthermore, the relative expression level of PPAR mRNA of Group III and IV was obviously significantly higher than that of Group II (p < 0.05), whereas that of Group III was no significant difference from that of Group IV (p > 0.05).

The dietary n-6/n-3 PUFA ratio had obviously significant effect on the relative expression level of L-FABP mRNA (p < 0.05, Figure 3). With the increasing dietary n-6/n-3 PUFA ratio, the relative expression level of L-FABP mRNA showed a gradual increasing trend. The relative expression level of L-FABP mRNA of Group I was highly significantly lower than those of Group II, III, and IV (p < 0.05). L-FABP mRNA expression did not have a significant difference (p > 0.05) among Group II, III, and IV.

**Discussion**

Effects of the dietary n-6/n-3 PUFA ratio on the body fat deposition

When the n-6/n-3 PUFA ratio was 18 or 41, the hepatic somatic index of the arctic fox was lower than that of the other n-6/n-3 PUFA ratio (3 or 136); however, the other body deposition indexes were not influenced by the different ratios of n-6/n-3 PUFAs (22). In the present study, hepatic fat content of silver fox fed the diet containing an n-6/n-3 PUFA ratio of 18 was significantly lower than that of the other n-6/n-3 PUFA ratio, whereas body fat deposition indexes were not significantly affected by the dietary n-6/n-3 PUFA ratio in silver fox. This indicated that canine with different genera had similar body fat composition and variation fed the same diet composition. Fish research literatures (23, 24) reported that...
TABLE 7 Effects of dietary n-6/n-3 PUFA ratio on fatty acid profiles of subcutaneous fat of silver fox during the winter fur-growing period (proportion of total fatty acid) %.

| Items  | Groups (n-6/n-3 PUFA ratio) | P-value |
|--------|----------------------------|---------|
|        | I (3:1)                    | II (18:1)       | III (41:1)       | IV (136:1)       |        |
| C12:0  | 2.01 ± 0.82<sup>a</sup>    | 0.08 ± 0.02<sup>b</sup> | 0.09 ± 0.01<sup>b</sup> | 0.08 ± 0.02<sup>b</sup> | <0.0001 |
| C14:0  | 2.92 ± 0.66<sup>a</sup>    | 1.29 ± 0.18<sup>b</sup> | 1.44 ± 0.34<sup>b</sup> | 1.57 ± 0.17<sup>b</sup> | <0.0001 |
| C14:1  | 0.18 ± 0.01                | 0.15 ± 0.05 | 0.15 ± 0.04 | 0.17 ± 0.03 | 0.3869  |
| C15:0  | 0.12 ± 0.01                | 0.11 ± 0.02 | 0.11 ± 0.01 | 0.11 ± 0.01 | 0.4802  |
| C16:0  | 18.36 ± 0.83<sup>a</sup>   | 14.02 ± 0.70<sup>b</sup> | 13.87 ± 1.85<sup>b</sup> | 15.12 ± 1.64<sup>b</sup> | 0.0002  |
| C16:1  | 6.08 ± 0.33<sup>a</sup>    | 3.75 ± 0.45<sup>b</sup> | 4.14 ± 1.43<sup>b</sup> | 4.89 ± 0.99<sup>ab</sup> | 0.0039  |
| C17:0  | 0.17 ± 0.02                | 0.15 ± 0.007 | 0.16 ± 0.03 | 0.16 ± 0.03 | 0.5212  |
| C18:0  | 4.49 ± 1.08<sup>a</sup>    | 2.85 ± 0.35<sup>b</sup> | 3.42 ± 0.65<sup>b</sup> | 2.99 ± 0.51<sup>b</sup> | 0.0068  |
| C18:1n9c | 37.09 ± 1.09             | 35.25 ± 2.78 | 34.25 ± 2.13 | 36.55 ± 1.10 | 0.1313  |
| C18:2n6c | 26.16 ± 2.01<sup>b</sup>  | 40.91 ± 2.36<sup>a</sup> | 40.06 ± 4.62<sup>a</sup> | 36.82 ± 3.10<sup>a</sup> | <0.0001 |
| C20:0  | 0.16 ± 0.04                | 0.23 ± 0.05 | 0.20 ± 0.07 | 0.16 ± 0.02 | 0.0728  |
| C20:1  | 0.95 ± 0.07                | 0.85 ± 0.05 | 1.69 ± 0.05 | 0.90 ± 0.07 | 0.0001  |
| C20:2n6 | 0.17 ± 0.02<sup>a</sup>   | 0.14 ± 0.03<sup>b</sup> | 0.12 ± 0.03<sup>b</sup> | 0.13 ± 0.03<sup>b</sup> | 0.0329  |
| C20:4n6 | 0.13 ± 0.02                | 0.15 ± 0.04 | 0.12 ± 0.01 | 0.12 ± 0.05 | 0.6629  |
| C20:5n3 | 0.24 ± 0.05                | ND         | ND         | 0.15 ± 0.06 | 0.1113  |
| C22:6n3 | 0.23 ± 0.04<sup>a</sup>   | 0.11 ± 0.04<sup>b</sup> | 0.12 ± 0.05<sup>b</sup> | 0.19 ± 0.06<sup>b</sup> | 0.0034  |
| SFA    | 28.41 ± 3.00<sup>a</sup>   | 18.72 ± 0.77<sup>b</sup> | 19.28 ± 1.77<sup>b</sup> | 20.91 ± 1.98<sup>b</sup> | <0.0001 |
| MUFA   | 44.29 ± 1.42<sup>a</sup>   | 39.99 ± 2.56<sup>b</sup> | 40.04 ± 3.18<sup>b</sup> | 42.51 ± 1.26<sup>ab</sup> | 0.0164  |
| PUFA   | 26.93 ± 2.06<sup>b</sup>   | 41.28 ± 2.39<sup>a</sup> | 40.46 ± 4.59<sup>a</sup> | 37.31 ± 3.04<sup>a</sup> | <0.0001 |
| N-6    | 26.47 ± 2.03<sup>b</sup>   | 41.29 ± 2.39<sup>a</sup> | 40.30 ± 4.65<sup>a</sup> | 37.05 ± 3.08<sup>a</sup> | <0.0001 |
| N-3    | 0.47 ± 0.09<sup>a</sup>    | 0.11 ± 0.04<sup>i</sup> | 0.17 ± 0.11<sup>c</sup> | 0.31 ± 0.09<sup>b</sup> | <0.0001 |

In the same row, values with different superscript mean significant difference (P < 0.05).

FIGURE 1
Effects of dietary n-6/n-3 PUFA ratio on liver FAS mRNA relative expression in silver fox during the winter fur-growth period. Data are presented as the mean ± SD. a, b, c means values with different letters are significantly different (p < 0.05).
body fat deposition parameters were not affected by the ratio of n-6/n-3 PUFA, the results of this experiment were consistent with these literatures. The reason that lower hepatic fat content of silver fox fed n-6/n-3 PUFA ratio of 18 might increase the...
transport of long-chain fatty acids in the liver and reduce the formation of cholesterol and triglycerides in the liver by the expression of lower FAS mRNA and higher L-FABP in liver in the present study.

Effects of the dietary n-6/n-3 PUFA ratio on the tissues fatty acids composition and contents

The dietary fatty acid composition clearly influences the body fat composition of fur animals (7–9). There are differences in the composition of fatty acids in different tissues of fur animals (10, 25). Body tissues’ fatty acid composition in pig and fish was greatly influenced by the feed fatty acid composition and to some extent can reflect the fatty acid composition in feed (3, 26, 27). In this study, we found that the variation trends of the fatty acid composition of liver, intramuscular fat, and subcutaneous fat in silver fox were directly related to dietary fatty acid content, which was consistent with the previous literatures. The content change of SFA, MUFA, and PUFA in liver, intramuscular, and subcutaneous fat of silver fox was consistent with the arctic fox (22, 28). From subcutaneous fat, intramuscular fat to liver in silver fox, SFA content showed a gradual increase trend, whereas UFA (MUFA plus PUFA) content showed a gradual decrease trend, which was consistent with the result (8). These indicated that as compared to SFA, the UFA under its intramuscular and subcutaneous fat was more conducive to oxidating and decomposing to supply the silver fox’s energy need. The selective deposition of fat in body tissues was an inherited morphological characteristic in adaptive evolution (29).

Effects of the dietary n-6/n-3 PUFA ratio on the expression of lipid metabolism-related genes in the livers

An elevated FAS expression level significantly increases the deposition of triglycerides in the body, leading to obesity (30). PUFAs significantly inhibited the activity of fatty acid synthesis in rat liver, and also proved that n-3 PUFAs are more effective than n-6 PUFAs in inhibiting the transcription of FAS gene (31, 32). The previous studies show with the dietary n-6/n-3 PUFA ratio increasing, the expression of FAS genes related to liver fat synthesis decreased first and then increased in Lateolabrax maculatus (27). In this study, we found that the hepatic level of FAS mRNA expression exhibited the trend of first decreasing and then increasing with the n-6/n-3 PUFA increasing, and the relative expression level of FAS mRNA was the highest in Group I for silver foxes, which was consistent with the previous literatures. This may be due to lower PUFA content in Group I diet compared to the other three Groups. From Group II to IV, n-3 PUFA content gradually decreased, but PUFA content was similar, which might result in the change of FAS mRNA expression. When dietary n-6/n-3 PUFA was 18, FAS mRNA expression was the lowest, which is consistent with the result of the liver fat content, indicating that proper n-6/n-3 PUFA ratio was beneficial to lipid metabolism, thereby keeping the healthy stage of silver fox.

Liver PPAR regulates the transport of fatty acids to mitochondria by inducing the expression of liver-specific carnitine palmitoyltransferase to stimulate the β-oxidation process and reduce the synthesis of fatty acids and triglycerides (33). PUFA inhibited the expression of related genes in the process of fat synthesis and promotes the process of fat oxidation by acting on liver PPAR (34). The lowering of the dietary n-6:n-3 PUFA ratio might stimulate PPAR target gene expressions (35–37). In this study, the results showed that liver PPAR mRNA expression exhibited the trend of first decreasing and then increasing with increasing n-6/n-3 PUFA ratio. PPAR mRNA expression in Group I was the highest probably due to lower n-6 PUFA and higher n-3PUFA contents, which was consistent with the literature. PPAR mRNA expression change was basically the same with the dietary PUFA content from Group II to IV, which was consistent with the previous literatures, and confirmed that PUFAs could effectively activate PPARs (38).

The previous studies found that knocking out the L-FABP gene can induce liver cholesterol and triglyceride accumulation (39) and L-FABP has a high affinity for long-chain (C14) fatty acids, which had an important role in absorbing and transferring fatty acids (40–42). In this study, L-FABP mRNA expression showed a gradual increasing trend from Group I to IV with the n-6/n-3 PUFA ratio increasing, which indicated higher PUFA content increasing the expression of L-FABP (28, 43).

Conclusion

In summary, silver foxes fed an n-6/n-3 PUFA ratio 18:1 diet (supplementing with 9.38% corn oil and 4.62% soybean oil) was more conducive to improving the expression of lipolysis genes, facilitating the lipid decomposition, transporting, and utilizing fatty acids, thereby to meeting the physiological needs of silver foxes for supplying energy and withstanding the cold during the winter fur-growth period.

Data availability statement

The original contributions presented in the study are included in the article-supplementary material, further inquiries can be directed to the corresponding author/s.
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