Effects of Different Dietary Fat Ratios on Growth Performance, Serum Biochemical Indices, and Intestinal Morphology in Male Silver Foxes during the Winter Fur Period Postprint

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Abstract

This study aimed to investigate the effects of different dietary oil ratios on growth performance, serum biochemical indices, and intestinal morphological structure of male silver foxes during the winter fur period. Forty-eight healthy male silver foxes at 157 days of age with an average body weight of (5,450±140) g were randomly allocated to 4 groups, with 12 replicates per group and 1 fox per replicate. The four experimental diets contained different oil ratios but the same total oil inclusion level of 14%: Group I diet comprised 12.00% fish oil and 2.00% soybean oil, Group II diet comprised 9.38% corn oil and 4.62% soybean oil, Group III diet comprised 12.00% corn oil and 2.00% soybean oil, and Group IV diet comprised 1.50% fish oil and 12.50% corn oil. The preliminary period lasted 7 days, and the formal experimental period lasted 40 days. The results showed that: 1) Different dietary oil ratios had a significant effect on body length of silver foxes ($P<0.05$), but no significant effects on final body weight, average daily gain, average daily feed intake, feed-to-gain ratio, or fresh pelt length ($P>0.05$). Body length in Group II was significantly higher than that in Group IV ($P<0.05$), with no significant differences from Groups I and III ($P>0.05$). 2) Different dietary oil ratios had significant or extremely significant effects on serum triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) concentrations ($P<0.05$ or $P<0.01$), but no significant effects on serum cholesterol (CHO), high-density lipoprotein cholesterol (HDL-C), or glucose (GLU) concentrations ($P>0.05$). Serum TG in Group II was significantly lower than that in Groups I, III, and IV ($P<0.05$), while serum LDL-C concentration in Group I was extremely significantly higher than that in Groups II, III, and IV ($P<0.01$). 3) Different dietary oil ratios had a significant effect on serum globulin (GLOB) concentration ($P<0.05$), but no significant effects on serum total protein (TP), albumin (ALB), or urea nitrogen (UN) concen-

trations (P>0.05). Serum GLOB concentration in Group II was significantly higher than that in Groups III and IV (P<0.05), with no significant difference from Group I (P>0.05). 4) Different dietary oil ratios had a significant effect on serum complement 4 (C4) concentration (P<0.05), but no significant effects on serum immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin G (IgG), complement 3 (C3), interleukin-2 (IL-2), or tumor necrosis factor (TNF) concentrations (P>0.05). Serum C4 concentration in Group III was significantly lower than that in Groups I, II, and IV (P<0.05), with no significant differences among Groups I, II, and IV (P>0.05). 5) Different dietary oil ratios extremely significantly affected intestinal villus height, crypt depth, and villus height/crypt depth ratio (P<0.01). Villus height in Group II was extremely significantly higher than that in Groups I, III, and IV (P<0.01), while that in Groups III and IV was extremely significantly higher than that in Group I (P<0.01); crypt depth in Group I was extremely significantly higher than that in Groups II, III, and IV (P<0.01), with no significant differences among Groups II, III, and IV (P>0.05); the villus height/crypt depth ratio in Group II was extremely significantly higher than that in Group I (P<0.01), significantly higher than that in Group IV (P<0.05), with no significant difference from Group III (P>0.05). Based on the comprehensive results of this experiment, when dietary oil sources consisted of 9.38% corn oil and 4.62% soybean oil, the concentrations of TG and LDL-C in serum were reduced, the concentration of GLOB in serum was increased, and intestinal morphological structure was improved, thereby promoting the increase in body length of male silver foxes during the winter fur period.

Full Text

Effects of Dietary Different Oil Ratios on Growth Performance, Serum Biochemical Parameters and Intestinal Morphology of Male Silver Foxes during Winter-Furring Period

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Abstract

This experiment was conducted to investigate the effects of different dietary oil ratios on growth performance, serum biochemical parameters, and intestinal morphology in male silver foxes during the winter-furring period. Forty-eight healthy male silver foxes aged 157 days with an average body weight of (5,450±140) g were randomly allocated into four groups, each comprising 12 replicates with one fox per replicate. The four experimental diets contained 14% oil at varying ratios: Group I received 12.00% fish oil and 2.00% soybean

oil; Group II received 9.38% corn oil and 4.62% soybean oil; Group III received 12.00% corn oil and 2.00% soybean oil; and Group IV received 1.50% fish oil and 12.50% corn oil. The study consisted of a 7-day adaptation period followed by a 40-day experimental period. The results revealed: (1) Dietary oil ratios significantly affected body length ($P<0.05$) but had no significant effects on final weight, average daily gain, average daily feed intake, feed-to-gain ratio, or fresh fur length ($P>0.05$). Group II exhibited significantly greater body length than Group IV ($P<0.05$), while showing no significant differences from Groups I and III ($P>0.05$). (2) Dietary oil ratios significantly or extremely significantly influenced serum triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) concentrations ($P<0.05$ or $P<0.01$), but did not significantly affect serum cholesterol (CHO), high-density lipoprotein cholesterol (HDL-C), or glucose (GLU) levels ($P>0.05$). Group II had significantly lower serum TG than Groups I, III, and IV ($P<0.05$), while Group I showed extremely significantly higher serum LDL-C than Groups II, III, and IV ($P<0.01$). (3) Dietary oil ratios significantly affected serum globulin (GLOB) content ($P<0.05$) but had no significant effects on serum total protein (TP), albumin (ALB), or urea nitrogen (UN) levels ($P>0.05$). Group II demonstrated significantly higher serum GLOB than Groups III and IV ($P<0.05$), with no significant difference from Group I ($P>0.05$). (4) Dietary oil ratios significantly influenced serum complement 4 (C4) content ($P<0.05$) but did not significantly affect serum immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin G (IgG), complement 3 (C3), interleukin-2 (IL-2), or tumor necrosis factor (TNF) levels ($P>0.05$). Group III exhibited significantly lower serum C4 than Groups I, II, and IV ($P<0.05$), while no significant differences were observed among Groups I, II, and IV ($P>0.05$). (5) Dietary oil ratios extremely significantly affected intestinal villous height, crypt depth, and villous height-to-crypt depth ratio ($P<0.01$). Group II showed extremely significantly greater villous height than Groups I, III, and IV ($P<0.01$), while Groups III and IV were extremely significantly higher than Group I ($P<0.01$). Group I displayed extremely significantly greater crypt depth than Groups II, III, and IV ($P<0.01$), with no significant differences among the latter three groups ($P>0.05$). The villous height-to-crypt depth ratio in Group II was extremely significantly higher than Group I ($P<0.01$) and significantly higher than Group IV ($P<0.05$), but did not differ significantly from Group III ($P>0.05$). Based on these findings, a dietary oil combination of 9.38% corn oil and 4.62% soybean oil reduced serum TG and LDL-C concentrations, increased serum GLOB content, and improved intestinal morphology, thereby promoting increased body length in male silver foxes during the winter-furring period.

Keywords: oil ratio; silver fox; growth performance; serum biochemical parameters; intestinal morphology

Introduction

Appropriate mixing of different dietary oils at specific ratios can produce complementary fatty acid effects and satisfy animals' requirements for various fatty acids, particularly essential fatty acids, thereby enhancing animal production performance. Polyunsaturated fatty acids (PUFAs), especially n-3 and n-6 PUFAs, are essential fatty acids that play crucial roles in regulating lipid metabolism, immune function, and intestinal microflora, as well as improving intestinal mucosal barrier function [1-5]. Since mammals lack n-3 desaturase enzymes, n-3 and n-6 PUFAs cannot be interconverted in vivo and must be obtained through diet [6-7]. In livestock and poultry production, fatty acid ratios are typically adjusted through oil supplementation. Research has demonstrated that diets with appropriate n-6/n-3 PUFA ratios can regulate lipid metabolism and improve growth performance in arctic foxes during the winter-furring period [8-9]. Studies have also reported that diets with different n-6/n-3 PUFA ratios significantly affect lipid metabolism and immune function in Yangzhou geese [10], and that appropriate n-6/n-3 PUFA ratios can improve blood glucose and lipid health while maintaining favorable immune status in chickens [11-12]. Additionally, n-3 PUFA-rich oils have been shown to increase beneficial intestinal bacteria in rats, enhancing intestinal mucosal barrier function by improving gut microflora and increasing mucosal thickness [4].

Silver foxes (*Vulpes vulpes*), originally from northern North America and eastern Siberia, belong to the Canidae family and Carnivora order, representing one of the world' s valuable fur-bearing animals. Although silver foxes and arctic foxes both belong to Canidae, they are different genera. Studies have revealed significant differences in fat deposition and metabolism patterns between these two fox genera [13]. However, no research has been reported on the effects of different dietary oil ratios on fatty acid utilization, lipid metabolism, immune function, or intestinal morphology in silver foxes. Therefore, this experiment was designed to investigate the effects of different dietary oil ratios on growth performance, serum biochemical parameters, and intestinal morphology in male silver foxes during the winter-furring period, aiming to provide a theoretical basis for silver fox production and fatty acid metabolism research.

1.1 Experimental Animals

The experimental silver foxes were local Finnish strain silver foxes, a regional breed developed through years of breeding from Finnish stock.

1.2 Experimental Design and Diets

Forty-eight healthy male silver foxes aged 157 days with an average body weight of (5,450±140) g were randomly divided into four groups, each consisting of 12 replicates with one fox per replicate. The four groups were fed experimental diets with different oil compositions. All diets contained 14% oil, with Group

I receiving 12.00% fish oil and 2.00% soybean oil, Group II receiving 9.38% corn oil and 4.62% soybean oil, Group III receiving 12.00% corn oil and 2.00% soybean oil, and Group IV receiving 1.50% fish oil and 12.50% corn oil. The composition and nutrient levels of the experimental diets are presented in Table 1 , and the fatty acid composition is shown in Table 2 .

1.3 Feeding Management

The experiment was conducted at the Fur Animal Experimental Base of the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences. Animals were housed individually in cages. The trial ran from October 13, 2014, to December 1, 2014, including a 7-day adaptation period and a 40-day experimental period. Feed was provided twice daily at 08:00 and 15:00, and water was available ad libitum.

1.4.1 Nutrient Analysis of Diets

Dietary crude protein content was determined using the Kjeldahl method according to GB/T 6432–1994. Crude fat content was measured using the Soxhlet extraction method according to GB/T 6433–1994. Crude ash content was determined by incineration at 550°C according to GB/T 6438–1992. Calcium content was measured using EDTA complexometric titration according to GB/T 6436–1992. Phosphorus content was determined according to GB/T 6437–1992. Lysine and methionine contents were analyzed using acid extraction and an automatic amino acid analyzer (HITACHI L-8900, Japan) according to GB/T 18246-2000. Dietary fatty acids were methylated and analyzed using gas chromatography-mass spectrometry (Agilent 7890A-7000B) with external standard method according to GB/T 21514-2008, following the procedure described by Chen et al. [14].

1.4.2 Growth Performance Measurement

Daily feed intake was recorded throughout the experimental period to calculate average daily feed intake. Initial and final body weights were measured to determine average daily gain. Feed-to-gain ratio was calculated based on average daily gain and average daily feed intake. At the end of the experiment, all 12 foxes in each group were slaughtered for measurement of body length and fresh fur length. Body length was measured as the distance from nose tip to tail base with the fox placed on a horizontal surface. Fresh fur length was measured after stretching the pelt on a board to ensure complete extension, recording the distance from nose tip to tail base.

1.4.4 Serum Sample Collection and Biochemical Analysis

At the end of the feeding trial, eight healthy foxes were randomly selected from each group and weighed. Blood samples (5 mL) were collected via cardiac puncture into procoagulant tubes and centrifuged at 4,000 r/min for 8

min at 4°C. Serum was separated and stored at -80°C for subsequent analysis. Serum total protein (TP), albumin (ALB), triglyceride (TG), cholesterol (CHO), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and glucose (GLU) concentrations were measured using an automatic biochemical analyzer (Selectra E, Netherlands) with reagent kits from Zhongsheng Beikong Biotechnology Co., Ltd. Serum urea nitrogen (UN) content was determined using the urease method with a UV spectrophotometer and reagent kits from Nanjing Jiancheng Bioengineering Institute. Serum immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) concentrations were measured by enzyme-linked immunosorbent assay. Complement 3 (C3) and complement 4 (C4) concentrations were determined by immunoturbidimetry. Interleukin-2 (IL-2) and tumor necrosis factor (TNF) concentrations were measured by chemiluminescence using Roche reagent kits. Serum globulin (GLOB) content was calculated as the difference between serum TP and ALB concentrations.

1.4.4 Intestinal Tissue Collection and Morphological Analysis

After slaughter, the jejunum of each fox was longitudinally opened, and a 1 cm × 1 cm tissue sample was collected. After rinsing with physiological saline, samples were fixed in 10% formalin solution. Fixed specimens were processed through dehydration, clearing, paraffin infiltration, embedding, trimming, sectioning, and routine hematoxylin-eosin (HE) staining to prepare 4–6 m thick paraffin slices [15]. Qualified sections were examined under a microscope (Leica DM 1000, Germany) at 100× magnification. Multiple non-consecutive fields were observed, and typical fields were photographed. Villous height and crypt depth were measured using Toupview software, and the villous height-to-crypt depth ratio (V/C) was calculated. Six non-adjacent sections were analyzed per animal, with five measurements taken per section.

1.5 Data Processing and Statistical Analysis

Experimental data were organized using Excel 2010 and analyzed using the General Linear Model (GLM) procedure in SAS 8.0 software. Duncan' s multiple range test was used for post-hoc comparisons. Differences were considered extremely significant at $P<0.01$, significant at $P<0.05$, and not significant at $P>0.05$. Results are expressed as "mean ± standard deviation."

2.1 Effects of Dietary Oil Ratios on Growth Performance

As shown in Table 3 , dietary oil ratios had no significant effects on final body weight, average daily feed intake, average daily gain, feed-to-gain ratio, or fresh fur length ($P>0.05$) in male silver foxes during the winter-furring period. However, body length was significantly affected ($P<0.05$). Specifically, body length

in Group II was significantly greater than in Group IV ($P<0.05$), while no significant differences were observed among the other groups ($P>0.05$).

2.2 Effects of Dietary Oil Ratios on Serum Glucose and Lipid Metabolism

Table 4 shows that dietary oil ratios extremely significantly affected serum LDL-C content ($P<0.01$) and significantly affected serum TG content ($P<0.05$), but had no significant effects on serum CHO, HDL-C, or GLU levels ($P>0.05$). Group II exhibited significantly lower serum TG than Groups I, III, and IV ($P<0.05$), while Groups I, III, and IV did not differ significantly from each other ($P>0.05$). Group I showed extremely significantly higher serum LDL-C than Groups II, III, and IV ($P<0.01$), with no significant differences among the latter three groups ($P>0.05$).

2.3 Effects of Dietary Oil Ratios on Serum Protein Metabolism

As presented in Table 5 , dietary oil ratios significantly affected serum GLOB content ($P<0.05$) but had no significant effects on serum TP, ALB, or UN levels ($P>0.05$). Serum GLOB content in Group II was significantly higher than in Groups III and IV ($P<0.05$), though it did not differ significantly from Group I ($P>0.05$).

2.4 Effects of Dietary Oil Ratios on Serum Immune Parameters

Table 6 indicates that dietary oil ratios significantly affected serum C4 content ($P<0.05$) but had no significant effects on serum IgA, IgM, IgG, C3, IL-2, or TNF levels ($P>0.05$). Serum C4 content in Group III was significantly lower than in Groups I, II, and IV ($P<0.05$), while no significant differences were observed among Groups I, II, and IV ($P>0.05$).

2.5 Effects of Dietary Oil Ratios on Intestinal Morphology

As shown in Table 7 , dietary oil ratios extremely significantly affected intestinal villous height, crypt depth, and villous height-to-crypt depth ratio ($P<0.01$). Villous height in Group II was extremely significantly greater than in Groups I, III, and IV ($P<0.01$), while Groups III and IV were extremely significantly higher than Group I ($P<0.01$). Crypt depth in Group I was extremely significantly greater than in Groups II, III, and IV ($P<0.01$), with no significant differences among the latter three groups ($P>0.05$). The villous height-to-crypt depth ratio in Group II was extremely significantly higher than in Group I ($P<0.01$) and significantly higher than in Group IV ($P<0.05$), but did not differ significantly from Group III ($P>0.05$).

3.1 Effects on Growth Performance

Previous studies have shown that dietary oil ratios affect animal production performance by influencing metabolic processes and improving dietary digestibility [16]. Research has reported that different oil ratios had no significant effects on production performance in Yangzhou geese [17]. Supplementing laying hen diets with oils differing in fatty acid saturation and double bond position did not significantly affect feed intake, laying rate, egg weight, or body weight [18]. However, dietary oil ratios extremely significantly affected growth performance in arctic foxes during the winter-furring period, with the combination of 1.5% fish oil and 12.5% corn oil yielding better growth performance [9]. In contrast, the current study found that dietary oil ratios had no significant effects on body weight or fresh fur length but significantly affected body length in male silver foxes during the winter-furring period. These discrepancies may be attributed to the fact that although silver foxes and arctic foxes both belong to Canidae, they are different genera. During the winter-furring period, foxes primarily promote fur growth and accumulate fat to withstand cold temperatures. The differences in fatty acid utilization and deposition between these two fox genera [13] may be the main reason for the divergent results regarding growth performance, though the specific mechanisms require further investigation.

3.2 Effects on Serum Glucose and Lipid Metabolism

Oil type and ratio determine the intake of n-6 and n-3 PUFAs, which serve as precursors for eicosanoid synthesis. The composition of PUFAs in phospholipids is associated with chronic diseases such as coronary heart disease, hypertension, and cancer [19-20], prompting increased attention to dietary oil types in human nutrition. In animal production, extensive research has been conducted on oil source selection and ratio application to obtain high-quality animal products. Studies have shown that n-3 PUFA-rich oils can effectively reduce plasma CHO, TG, and LDL-C concentrations while increasing HDL-C levels [21-23]. Appropriate n-6/n-3 PUFA ratios achieved through different oil combinations can lower blood lipid levels when fed long-term [8,10,24]. The current results demonstrate that the diet containing 9.38% corn oil and 4.62% soybean oil (n-6/n-3 PUFA ratio of 18) significantly reduced serum TG content compared to other oil combinations and also resulted in relatively lower serum LDL-C levels, though no significant differences were observed in serum TC, HDL-C, or GLU levels among groups. These findings align with previous research, indicating that appropriate oil ratios (balanced n-6/n-3 PUFA ratios) are more conducive to maintaining normal lipid metabolism [25]. In this study, the diet containing 12.00% fish oil and 2.00% soybean oil (Group I) yielded slightly higher serum TG, TC, LDL-C, and GLU levels than other groups, consistent with previous findings in arctic foxes [8]. The higher fish oil content in this diet resulted in relatively higher saturated fatty acid (SFA) content, which may be the primary cause of elevated blood lipid levels in silver foxes.

3.3 Effects on Serum Protein Metabolism

Under normal conditions, animals maintain serum protein balance to preserve physiological function. Serum TP serves as a protein source for tissue repair and energy provision, representing to some extent the dietary protein level and protein digestion/absorption efficiency. Yu et al. [26] demonstrated that serum TP and ALB levels increase with elevated dietary protein levels. In the current study, dietary protein levels were consistent across groups, which may explain the lack of significant differences in serum TP, ALB, and UN levels. However, Group II (9.38% corn oil and 4.62% soybean oil) showed significantly higher serum GLOB content than Groups III (12.00% corn oil and 2.00% soybean oil) and IV (1.5% fish oil and 12.5% corn oil), though it did not differ significantly from Group I (12.00% fish oil and 2.00% soybean oil). Serum GLOB content correlates with immune function, suggesting that diets with relatively lower n-6/n-3 PUFA ratios favor better immune status in silver foxes, consistent with findings by Yu [10] that lower n-6/n-3 PUFA ratios maintain animals in a favorable immune state.

3.4 Effects on Serum Immune Parameters

Research has shown that appropriate intake of n-3 PUFA-rich oils, such as fish oil, can enhance immune function. n-3 PUFAs are major structural components of cell membrane phospholipids that modulate immune receptor and molecular expression on cell membranes, thereby influencing cellular immune responses [3]. n-3 and n-6 PUFAs compete in immune mechanisms; n-3 PUFAs promote immune function, while n-6 PUFAs metabolize into eicosanoids that exert provasoconstrictive, platelet-aggregating, and chemotactic effects, producing certain immunosuppressive actions [27-29]. Different dietary oil ratios can regulate the n-6/n-3 PUFA ratio and thus influence immune responses [7], with imbalanced n-6/n-3 PUFA supply potentially acting as an immunosuppressant [30]. Previous studies have shown that dietary n-6/n-3 PUFA ratios significantly affect serum C4 content but not IgA, IgM, IgG, C3, IL-2, or TNF levels in arctic foxes [8]. The current results align with these findings. From Groups I to IV, dietary n-6/n-3 PUFA ratios gradually increased while n-3 PUFA content decreased and n-6 PUFA content increased. The competitive effects of n-3 and n-6 PUFAs for desaturase enzymes may be the primary reason for the lack of significant changes in serum immune parameters. However, when feeding the diet containing 12.00% corn oil and 2.00% soybean oil (n-6/n-3 PUFA ratio of 41), serum C4 content was significantly lower than with other diets, indicating that the high n-6 PUFA and low n-3 PUFA combination produced more pronounced immunosuppressive effects on serum C4 than other oil combinations, though the specific mechanisms require further investigation.

3.5 Effects on Intestinal Morphology

Silver foxes belong to the Carnivora order and have relatively short intestines, only 3–4 times their body length, resulting in short digestion time. The small

intestine serves as the primary site for nutrient digestion and absorption, with villous height, crypt depth, and their ratio serving as key indicators of intestinal digestive function [31]. Villous height correlates with cell number, and only mature cells possess nutrient absorption capacity. Greater villous height indicates more mature cells and stronger nutrient absorption capability. Crypt depth reflects cell generation rate; shallower crypts indicate increased cell maturation and enhanced secretory function [32]. The villous height-to-crypt depth ratio comprehensively reflects small intestinal function, with higher ratios indicating greater nutrient absorption capacity [15]. Studies have shown that n-3 PUFAs can increase beneficial intestinal bacteria, improve gut microflora, and increase mucosal thickness to enhance intestinal barrier function [4]. Reducing the n-6/n-3 PUFA ratio in mice has been reported to affect hypothalamic feeding-related gene expression and intestinal autophagy, favoring increased probiotic numbers [33]. The current results demonstrate that feeding the diet containing 9.38% corn oil and 4.62% soybean oil (n-6/n-3 PUFA ratio of 18) resulted in the greatest villous height, shallowest crypt depth, and highest villous height-to-crypt depth ratio in silver foxes, indicating that this oil combination improved intestinal morphology and enhanced nutrient digestion and absorption capacity.

4 Conclusion

Based on the comprehensive results of this experiment, a dietary oil source comprising 9.38% corn oil and 4.62% soybean oil reduced serum TG and LDL-C concentrations, increased serum GLOB content, improved intestinal morphology, and consequently promoted increased body length in male silver foxes during the winter-furring period.

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