Effects of the Dietary n-6/n-3 Polyunsaturated Fatty Acid Ratio on Growth Performance, Nutrient Digestibility, and Nitrogen Metabolism in Growing Male Blue Foxes[1]

Authors: Zhong Wei, Luo Jing, Zhang Ting, Sun Weili, Liu Hanlu, Sun Xuyang, Guangyu Li

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Abstract

This experiment aimed to investigate the effects of dietary n-6/n-3 polyunsaturated fatty acid (PUFA) ratio on growth performance, nutrient digestibility, and nitrogen metabolism in growing male blue foxes. Forty-eight healthy male blue foxes at 102 days of age with an average body weight of (2,370$±$24) g were selected and randomly divided into 4 groups, with 12 replicates per group and 1 fox per replicate. The diets of each group were identical in all other ingredients except for the oil composition. Group I diet was supplemented with fish oil and soybean oil, with an n-6/n-3 ratio of 3.00; Group II diet was supplemented with fish oil and corn oil, with an n-6/n-3 ratio of 6.03; Group III diet was supplemented with fish oil and corn oil, with an n-6/n-3 ratio of 9.01; Group IV diet was supplemented with corn oil and soybean oil, with an n-6/n-3 ratio of 18.04. The experiment consisted of a 7-day preliminary period and a 46-day experimental period. The results showed: 1) The body weight at 125 days of age in Groups III and IV was significantly higher than that in Group II ($P<0.05$); the average daily gain from 102 to 125 days of age in Groups I, III, and IV was highly significantly higher than that in Group II ($P<0.01$); the feed-to-gain ratio from 102 to 125 days of age in Groups I, III, and IV was significantly lower than that in Group II ($P<0.05$). Dietary n-6/n-3 PUFA ratio had no significant effect on growth performance from 126 to 147 days of age ($P>0.05$). 2) The dry matter intake in Groups I and III was significantly higher than that in Group II ($P<0.05$); the dry matter digestibility in Groups I, III, and IV was significantly higher than that in Group II ($P<0.05$); the crude protein digestibility in Groups III and IV was highly significantly higher than that in Group II ($P<0.01$); the gross energy digestibility in Groups III and IV was significantly higher than

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that in Group II (P<0.05); the crude fat digestibility in Group III was significantly higher than that in Group II (P<0.05). Dietary n-6/n-3 PUFA ratio had no significant effect on carbohydrate digestibility (P>0.05). 3) The nitrogen intake in Groups I and III was highly significantly higher than that in Groups II and IV (P<0.01); the fecal nitrogen content in Group IV was significantly lower than that in Groups I and II (P<0.05). Dietary n-6/n-3 PUFA ratio had no significant effect on urinary nitrogen content, nitrogen retention, net protein utilization, and protein biological value (P>0.05). In conclusion, when the dietary n-6/n-3 PUFA ratio was 9.01 or 18.04, blue foxes could achieve better growth performance; however, considering feed cost and storage stability, the group with mixed corn oil and soybean oil was superior.

Full Text

Effects of the Dietary n-6/n-3 Polyunsaturated Fatty Acid Ratio on Growth Performance, Nutrient Digestibility, and Nitrogen Metabolism in Growing Male Blue Foxes[2]

Zhong Wei, Luo Jing, Zhang Ting, Sun Weili, Liu Halü, Sun Xuyang, Li Guangyu*

(Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences; Jilin Provincial Co-constructed Laboratory of Molecular Biology for Special Economic Animals, Changchun 130112, China)

Abstract: This experiment was conducted to investigate the effects of the dietary n-6/n-3 polyunsaturated fatty acid ratio on growth performance, nutrient digestibility, and nitrogen metabolism in growing male blue foxes. Forty-eight healthy male blue foxes, 102 days of age and with an average body weight of (2 370$±$24) g, were selected and randomly divided into 4 groups, with 12 replicates per group and 1 blue fox per replicate. Except for the oil composition, the ingredients of the diets in all groups were identical. In group I, fish oil and soybean oil were added to the diet, with an n-6/n-3 ratio of 3.00; in group II, fish oil and corn oil were added, with an n-6/n-3 ratio of 6.03; in group III, fish oil and corn oil were added, with an n-6/n-3 ratio of 9.01; and in group IV, corn oil and soybean oil were added, with an n-6/n-3 ratio of 18.04. The pre-experimental period was 7 d, and the experimental period was 46 d. The results showed that: 1) At 125 days of age, the body weights of groups III and IV were significantly higher than that of group II (P<0.05). During 102-125 days of age, the average daily gains of groups I, III, and IV were all extremely significantly higher than that of group II (P<0.01), and the feed-to-gain ratios of groups I, III, and IV were all significantly lower than that of group II (P<0.05). The

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dietary n-6/n-3 PUFA ratio had no significant effect on growth performance during 126–147 days of age (P>0.05). 2) The dry matter intake of groups I and III was significantly higher than that of group II (P<0.05). The dry matter digestibility of groups I, III, and IV was significantly higher than that of group II (P<0.05). The crude protein digestibility of groups III and IV was extremely significantly higher than that of group II (P<0.01). The gross energy digestibility of groups III and IV was significantly higher than that of group II (P<0.05). The crude fat digestibility of group III was significantly higher than that of group II (P<0.05). The dietary n-6/n-3 PUFA ratio had no significant effect on carbohydrate digestibility (P>0.05). 3) The nitrogen intake of groups I and III was extremely significantly higher than that of groups II and IV (P<0.01), and the fecal nitrogen content of group IV was significantly lower than that of groups I and II (P<0.05). The dietary n-6/n-3 PUFA ratio had no significant effect on urinary nitrogen content, nitrogen retention, net protein utilization, or protein biological value (P>0.05). In summary, when the dietary n-6/n-3 PUFA ratio was 9.01 or 18.04, blue foxes obtained better growth performance; however, considering feed cost and storage stability, the mixture of corn oil and soybean oil was superior.

Keywords: n-6/n-3; polyunsaturated fatty acids; blue fox; growth performance; digestibility; nitrogen metabolism

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Fat has high productive energy and is the main source of energy supplied to animals in feed. Adding fat to diets can not only improve feed palatability, provide essential fatty acids, promote the absorption of fat-soluble vitamins, and improve the appearance and glossiness of the fur, but also reduce dust during feed processing and decrease environmental pollution[⁀1-2]. Different fat sources differ in their fatty acid compositions. If different oils and fats are mixed in certain proportions, the complementary effects of fatty acids can be exerted, thereby meeting animals' requirements for multiple fatty acids, especially essential fatty acids.

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Author biography: Zhong Wei (1980–), female, from Yongji, Jilin, associate researcher, engaged in research on nutrient metabolism of special economic animals. E-mail: zhongwei8015@163.com
* Corresponding author: Li Guangyu, researcher, doctoral supervisor, E-mail: tcslgy@126.com

important [3]. Polyunsaturated fatty acids (PUFA), as constituent components

of fat, have important effects on fat utilization and overall nutritional value, thereby directly affecting animal growth performance [4]. A large number of studies have reported the effects of adding different fats and oils on livestock and poultry production performance and on the fatty acid content of products [6–12]. However, most reports on fur-bearing animals have focused on the effects on production of adding different levels of the same fat source, or adding different fat sources at the same level [13–15]. There have been relatively few reports on the effects of adding mixed oil sources on the growth performance of blue foxes. The growing period is the stage during which the skeleton and muscle of blue foxes grow most rapidly; adding fat at an appropriate ratio can significantly improve their growth performance [5], and the nutritional role of fat is mainly reflected in the regulation by its PUFA. Therefore, this study aimed to investigate the effects of different dietary n-6/n-3 PUFA ratios on growth performance, nutrient digestibility, and nitrogen metabolism in growing male blue foxes, in order to clarify the appropriate ratio of n-6 to n-3 PUFA in diets for growing blue foxes and provide a theoretical reference for the precise formulation of feeds for fur-bearing animals.

1 Materials and Methods

1.1 Experimental Animals, Experimental Design, and Feeding Management

The experiment was completed at the Fur Animal Experimental Base of the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences. Forty-eight healthy male blue foxes, 102 days of age, with an average body weight of (2 370$±$24) g, were selected and randomly divided into 4 groups, with 12 replicates in each group and 1 blue fox per replicate. Except for differences in the fat composition of the diets, all other ingredients were the same among groups. Diets for group I were supplemented with fish oil and soybean oil, with an n-6/n-3 ratio of 3.00; diets for group II were supplemented with fish oil and corn oil, with an n-6/n-3 ratio of 6.03; diets for group III were supplemented with fish oil and corn oil, with an n-6/n-3 ratio of 9.01; and diets for group IV were supplemented with corn oil and soybean oil, with an n-6/n-3 ratio of 18.04.

The experimental animals were housed individually in cages. The experiment began on August 12, 2014 and ended on September 26, 2014. The preliminary trial lasted 7 d, and the formal trial lasted 46 d. The entire experimental period was divided into an early growing period (102–125 days of age) and a late growing period (126–147 days of age). The animals were fed once each at 08:00 and 15:00 every day, with free access to drinking water.

1.2 Experimental Diets

The diets were mainly composed of extruded corn, soybean meal, fish meal, meat meal, and other ingredients. The proportions of fatty acids were adjusted through the ratios of fats and oils in the diets. The composition, nutrient levels, and fatty acid contents of the experimental diets are shown in Table 1.

Table 1 Composition, nutrient levels and fatty acid contents of experimental diets (air-dry basis) %

Items	Groups	I	II	III	IV
Ingredients					
Extrusion corn		35.75	35.75	35.75	35.75
Soybean meal		12.00	12.00	12.00	12.00
Corn protein meal		9.00	9.00	9.00	9.00
Distillers dried grains with solubles		3.55	3.55	3.55	3.55

Item				
Fish meal	16.00	16.00	16.00	16.00
Meat meal	10.00	10.00	10.00	10.00
Lys	0.80	0.80	0.80	0.80
Met	0.40	0.40	0.40	0.40
Premix1)	1.00	1.00	1.00	1.00
Fish oil	6.85	6.77	6.11	
Corn oil		1.23	1.89	5.36
Soybean oil	1.15			2.64
CaHPO4	3.00	3.00	3.00	3.00
NaCl	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Nutrient levels2)				
ME/(MJ/kg)	14.12	14.00	14.16	14.04
CP	30.13	29.13	30.47	29.67
EE	10.11	10.07	10.42	10.36
Ash	8.63	9.28	8.88	9.49
CC	43.13	43.52	42.23	42.48
Lys	1.09	1.09	1.09	1.09
Met	0.76	0.76	0.76	0.76
Ca	1.578	1.673	1.807	1.821
P	1.043	1.048	1.168	1.182
Fatty acid contents3)				
SFA	0.681	0.626	0.584	0.311
MUFA	2.009	1.953	1.880	1.312
PUFA	1.028	1.110	1.441	4.417
n-3	0.257	0.158	0.144	0.232

Item				
n-6	0.772	0.952	1.298	4.185
n-6/n-3	3.00	6.03	9.01	18.04

1) One kilogram of premix contained: VA 300 000 IU, VD 3 200 000 IU, VE 4 000 mg, VK 350 mg, VB1 400 mg, VB2 500 mg, VB6 200 mg, VB12 4.2 mg, folic acid 50 mg, pantothenic acid 2 200 mg, biotin 1 600 mg, choline chloride 120 mg, VC 12 000 mg, Fe 4 000 mg, Zn 3 200 mg, Mn 1 600 mg, I 80 mg, Se 12 mg, Cu 500 mg.

2) CP, EE, ash, Lys, Met, Ca and P were measured values, while the others were calculated values.

3) n-3 mainly included α-linolenic acid, eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid, etc.; n-6 mainly included linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid and arachidonic acid, etc. SFA, MUFA, PUFA, n-3 and n-6 were measured values, while n-6/n-3 was a calculated value.

1.3 Digestion and metabolism trial

During the trial period, feed offered and refusals were accurately weighed and recorded daily to calculate feed intake; animal health and feed intake were observed.

condition. On day 23 of the formal trial, 8 blue foxes from each group with normal feed intake and defecation and similar physical condition were selected for the digestion and metabolism trial. The total feces collection method was used; self-made fecal trays and 5 L urine buckets were used to continuously collect feces and urine for 4 d. Before urine samples were collected, 10 mL of 10% H_2SO_4 was added to the urine buckets to fix nitrogen. For each fox, urine samples collected continuously for 4 d were mixed thoroughly, weighed, filtered through filter paper, and stored for later use. For each fox, fecal samples collected continuously for 4 d were mixed thoroughly and weighed; a portion of the fecal sample was collected, a small amount of 10% H_2SO_4 was added to fix nitrogen, and the sample was dried at 65 ℃ to constant weight to determine initial moisture. After grinding, the samples were passed through a 40-mesh sieve for determination of various nutrient components.

1.4 Determination indices and methods

The dry matter, crude protein, crude fat, crude ash, calcium, phosphorus contents and gross energy in the basal diet were determined. The dry matter, crude protein, crude fat, crude ash contents and gross energy in fecal samples were determined. The carbohydrate content in feed and fecal samples was obtained by calculation. Urinary nitrogen content was determined in urine. Dry matter content was determined by drying at 105 ℃, according to GB/T 6435–2006; crude

protein content was determined by the Kjeldahl method, according to GB/T 6432–1994; crude fat content was determined by the Soxhlet extraction method, according to GB/T 6433–1994; crude ash content was determined by combustion at 550 ℃, according to GB/T 6438–1992; gross energy was determined by combustion using an IKA C2000 calorimeter (Germany); calcium content was determined by the ethylenediaminetetraacetic acid (EDTA) complexometric titration method, according to GB/T 6436–1992; phosphorus content was determined by the ammonium vanadomolybdate colorimetric method, according to GB/T6437–1992. Pretreatment of fatty acids in the diet was performed by the methyl esterification method, according to GB/T 21514–2008, and analysis was performed by the external standard method. Fatty acid contents were determined using a gas chromatography-mass spectrometry instrument (Agilent 7890A-7000B). Chromatographic conditions were as follows: chromatographic column, DB-5MS (30 m × 250 μm × 0.25 μm); column temperature: initial 55 ℃, held for 2 min, increased to 200 ℃ at 5 ℃/min, held for 1 min, then increased to 230 ℃ at 2 ℃/min, held for 3 min, then increased to 270 ℃ at 5 ℃/min, held for 10 min; injector temperature: 250 ℃; carrier gas: helium (99.999%) at 1.0 mL/min; injection volume: 1 μL; split ratio: 10 1. Mass spectrometry conditions: electron impact ionization (EI) source; ion source temperature 230 ℃; electron energy 70 eV; interface temperature 250 ℃; scanning mass range 50–500 m/z. The calculation formulas were as follows:

Average daily feed intake (g/d) = feed intake during the trial period/number of trial days;

Average daily gain (g/d) = (final weight − initial weight)/number of trial days;

Feed/gain ratio = average daily feed intake/average daily gain;

Nutrient digestibility (%) = [(nutrient intake−nutrient excretion)/nutrient intake]×100;

Nitrogen retention (g/d) = nitrogen intake − fecal nitrogen − urinary nitrogen;

Net protein utilization (%) = (nitrogen retention/nitrogen intake) × 100;

Biological value of protein (%) = [nitrogen retention/(nitrogen intake−fecal nitrogen)]×100.

1.5 Data processing and statistical analysis

The experimental data were organized using Excel 2003, and statistical analysis was performed using the GLM procedure in SPSS 9.13 software. Multiple comparisons were conducted using Duncan' s method, where (P<0.01) indicated an extremely significant difference, (P<0.05) indicated a significant difference, and (P>0.05) indicated no significant difference. Results are expressed as mean ± standard deviation.

2 Results

2.1 Effects of dietary n-6/n-3 PUFA ratio on growth performance of blue foxes during the growing period

As shown in Table 2, the dietary n-6/n-3 PUFA ratio had a significant effect on the body weight of blue foxes at 125 days of age ((P<0.05)); groups III and IV were significantly higher than group II ((P<0.05)), while the differences between group I and group II, and among groups III and IV, were not significant ((P>0.05)). There were no significant differences in body weight among groups at 147 days of age ((P>0.05)). The dietary n-6/n-3 PUFA ratio had an extremely significant effect on average daily gain of blue foxes from 102 to 125 days of age ((P<0.01)); groups I, III, and IV were all extremely significantly higher than group II ((P<0.01)), but the differences among groups I, III, and IV were not significant ((P>0.05)). The dietary n-6/n-3 PUFA ratio had significant effects on average daily feed intake and feed-to-gain ratio of blue foxes from 102 to 125 days of age ((P<0.05)); average daily feed intake in groups I and III was significantly higher than that in group II ((P<0.05)), and did not differ significantly from group IV ((P>0.05)); average daily feed intake did not differ significantly between groups II and IV ((P>0.05)); the feed-to-gain ratios of groups I, III, and IV were all significantly lower than that of group II ((P<0.05)), while the differences in feed-to-gain ratio among groups I, III, and IV were not significant ((P>0.05)). From 125 to 147 days of age, there were no significant differences among groups in average daily feed intake, average daily gain, or feed-to-gain ratio of blue foxes ((P>0.05)).

Table 2 Effects of dietary ratio of n-6/n-3 PUFA on growth performance of blue fox during the growth period

Days of age	Items	I	II	III	IV	(P)-value
Days of age	Items	I	II	III	IV	(P)-value
102	Initial body weight/kg	2.37$±0.24	2.37±0.22	2.36±0.20	2.36±0.23	0.999
125	*Bodyweight/kg*	3.53±0.27 ^{ab}	3.37±0.30 ^b	3.67±0.24 ^a	3.60±0.19 ^a	0.042
102–125	*ADG/(g/d)*	48.68±6.51 ^{Aa}	41.42±9.68 ^{Bb}	54.44±7.19 ^{Aa}	51.53±3.11 ^{Aa}	0.001
102–125	*ADFI/(g/d)*	260.00±1.25 ^a	251.63±10.23 ^b	259.02±1.86 ^a	255.73±5.97 ^{ab}	0.039
102–125	*F/G*	5.22±0.45 ^b				

125–147 | Average daily gain ADG/(g/d) | 48.77$±5.91|48.04±6.99|47.54±4.88|45.73±$8.99 | 0.733 |

|–|–:|–:|–:|–:|–:|–:|–:| | | Average daily feed intake ADFI/(g/d) | 257.99$±1.28|252.37±3.23|255.76±1.45|257.00±2 | 0.714 |

In the same row, values with different lowercase superscript letters indicate significant differences ($P < 0.05$), and values with different uppercase superscript letters indicate extremely significant differences ($P < 0.01$); values with the same letter or no letter indicate no significant difference ($P > 0.05$). The same applies below.

In the same row, values with different small letter superscripts mean significant difference ($P < 0.05$), and with different capital letter superscripts mean significant difference ($P < 0.01$), while with the same or no letter superscripts mean no significant difference ($P > 0.05$). The same as below.

2.2 Effects of the dietary n-6/n-3 PUFA ratio on nutrient digestibility in growing blue foxes

As shown in Table 3, the dietary n-6/n-3 PUFA ratio had a significant effect on dry matter intake in blue foxes ($P < 0.05$). Groups I and III were significantly higher than group II ($P < 0.05$), with no significant difference from group IV ($P > 0.05$); the difference between groups II and IV was not significant ($P > 0.05$). The dietary n-6/n-3 PUFA ratio had significant or extremely significant effects on dry matter digestibility, crude protein digestibility, and gross energy digestibility in blue foxes ($P < 0.05$ or $P < 0.01$). The dry matter digestibility of groups I, III, and IV was significantly higher than that of group II ($P < 0.05$), while differences among groups I, III, and IV were not significant ($P > 0.05$); the crude protein digestibility of groups III and IV was extremely significantly higher than that of group II ($P < 0.01$), the difference between groups I and III was not significant ($P > 0.05$), the difference between groups I and IV was extremely significant ($P < 0.01$), and the difference between groups I and II was not significant ($P < 0.05$); the gross energy digestibility of groups III and IV was significantly higher than that of group II ($P < 0.05$), did not differ significantly from group I ($P > 0.05$), the difference between groups I and II was not significant ($P > 0.05$), and the difference between groups III and IV was not significant ($P > 0.05$).

The crude fat digestibility of group III was significantly higher than that of group II ($P < 0.05$), with no significant difference from groups I and IV ($P > 0.05$); differences among groups I, III, and IV were not significant ($P > 0.05$). There were no significant differences in carbohydrate digestibility among groups ($P > 0.05$).

Table 3 Effects of dietary ratio of n-6/n-3 PUFA on nutrient digestibility of blue fox during the growing period

Items	Groups				P-value
	I	II	III	IV	
Dry matter intake DMI/(g/d)	260.00$±1.26 < $sup > a < $/sup > |251.63±10.23 < $sup > b < $/sup > |259.02±1.86 < $sup > a < $/sup > |255.73±5.97 < $sup > ab < $/sup >	0.014			
	|0.039||$Drymatterdigestibility < $br > $DMdigestibility/$±2.38 < $sup > a < $/sup > |59.06±3.81 < $sup > b < $/sup > |63.00±2.74 < $sup > a < $/sup > |64.42±$3.32a				

Items	I	II	III	IV	*P*-value
CP digestibility/%	54.67$±4.11 < *sup* > *BCbc* < */sup* > |51.42±5.21 < *sup* > *Cc* < */sup* > |58.23±4.84 < *sup* > *ABab* < */sup* > |60.24±4.84 < *sup* > *Aa* < */sup* >	0.034			
	|0.005||*EEdigestibility*/±1.86 < *sup* > *ab* < */sup* > |93.68±1.12 < *sup* > *b* < */sup* > |95.50±0.58 < *sup* > *a* < */sup* > |95.03±1.08 < *sup* > *ab* < */sup* >				
	|0.035||*CCdigestibility*/±8.65|65.51±4.08|71.11±2.92|69.04±2.90|0.219||*GEdigestibility*/±8.78 < *sup* > *ab* < */sup* > |65.90±3.88 < *sup* > *b* < */sup* > |72.80±2.69 < *sup* > *a* < */sup* > |72.71±$2.81a				

2.3 Effects of dietary n-6/n-3 PUFA ratio on nitrogen metabolism in growing blue foxes

As shown in Table 4, the dietary n-6/n-3 PUFA ratio had an extremely significant effect on nitrogen intake in blue foxes (P<0.01). Groups I and III were

extremely significantly higher than groups II and IV (P<0.01); group IV was extremely significantly higher than group II (P<0.01); and the difference between groups I and III was not significant (P>0.05). The dietary n-6/n-3 PUFA ratio had a significant effect on fecal nitrogen content in blue foxes (P<0.05), among which groups I and II were significantly higher than group IV (P<0.05), with no significant difference from group III (P>0.05), and there was no significant difference between groups I and II (P>0.05). There were no significant differences among groups in urinary nitrogen content, nitrogen retention, net protein utilization, or protein biological value (P>0.05).

Table 4 Effects of dietary ratio of n-6/n-3 PUFA on nitrogen metabolism of blue fox during the growing period

Items	I	II	III	IV	P-value
IN/(g/d)	12.53$±0.001 <	0.410			
	$sup >				
	$Aa <				
	$/sup >				
	|11.73±0.48 <				
	$sup >				
	$Cc <				
	$/sup >				
	|12.63±0.09 <				
	$sup >				
	$Aa <				
	$/sup >				
	|12.14±0.28 <				
	$sup >				
	$Bb <				
	$/sup > | <				
	0.001||$FN/(g/d)$|5.68±0.52 <				
	$sup > a <				
	$/sup >				
	|5.69±0.64 <				
	$sup > a <				
	$/sup >				
	|5.27±0.59 <				
	$sup > ab <				
	$/sup >				
	|4.83±0.61 <				
	$sup > b <				
	$/sup >				
	|0.023||$UN/(g/d)$|3.67±0.78|3.51±0.46|3.74±0.84|3.56±0.74|0.558||$RN/(g/d)$|3.18±0.50|2.93±0.60				

BV of protein/%

3 Discussion

3.1 Effects of dietary n-6/n-3 PUFA ratio on growth performance of blue foxes during the growing period

For fur-bearing animals, the growing period is the time when bones and muscles grow most rapidly; therefore, a large supply of nutrients such as protein and fat from exogenous feed is needed to meet the requirements for growth and development. In this experiment, the entire trial period was divided into 2 stages, namely the early growing period (12 August 2014–4 September 2014) and the late growing period (5 September 2014–26 September 2014). The dietary n-6/n-3 PUFA ratio had a significant effect on body-weight gain of blue foxes in the early growing period. The average daily gain of group III was 23.92% higher than that of group II, and the feed-to-gain ratio of group III was 25.93% lower than that of group II. The diets of groups II and III were composed of the same oil sources, fish oil and corn oil, but their proportions differed, resulting in different n-6/n-3 ratios. Because the average dry-matter intake of group III was significantly higher than that of group II, and because different dietary fatty-acid ratios affected the absorption and digestion of nutrients in the body, this may have led to the differences in body weight and feed-to-gain ratio between the 2 groups. The average daily gains of groups I and IV were 14.90% and 19.60% higher than that of group II, respectively, and the feed-to-gain ratios of groups I and IV were 17.20% and 23.14% lower than that of group II, respectively, indicating that different dietary oil-source compositions and n-6/n-3 PUFA ratios had significant effects on the growth of blue foxes in the early growing period. The growth trends of groups II, III, and IV in this experiment were consistent with the reports of Zhou Jimu[^{16}] and Tu Ting[^{17}] in fish, namely that, as the n-6/n-3 ratio increased, all indicators of growth performance were higher than those in the low-ratio group. The dietary n-6/n-3 PUFA ratio had no significant effect on the growth performance of blue foxes in the late growing period, which is consistent with literature reports in broilers and piglets[^{{{18}}^{-}}^{19}]. This may be because, in the late growing period, young foxes grow relatively slowly, and the addition of fats at different growth stages of animals produces different growth effects.

3.2 Effects of dietary n-6/n-3 PUFA ratio on nutrient digestibility of blue foxes during the growing period

Studies have shown that the combined use of vegetable oils and animal oils can improve the digestibility of animal oils and can exert a synergistic effect in animals. Adding a certain proportion of fat to the diet can slow the passage rate of chyme, increase digestion time, and thereby improve the digestion and absorption of carbohydrates and protein in the duodenum[^{20}]. Livestock

and poultry must rely on digestive enzymes in the digestive tract to break down macromolecular substances such as protein and fat into small-molecular substances before they can be absorbed and utilized; therefore, the activity of digestive enzymes and the retention time of feed in the digestive tract determine the digestive absorption rate of nutrients in animals[^{21}]. The results of this experiment are consistent with the above literature reports: different oil ratios had significant effects on the digestibility of crude protein and crude fat. The dietary n-6/n-3 PUFA ratio affected the absorption and utilization of protein by blue foxes, mainly because the addition of fat can promote the digestion and absorption of amino acids. Diets based on meat meal and meat-and-bone meal can increase amino-acid digestibility[^{22}]. Amino acids are the form in which dietary protein is degraded in the animal body; an increase in amino-acid digestibility means an increase in protein digestibility. Fatty-acid composition analysis results

The results showed that the three oils selected for the experiment were all long-chain fatty acids. Groups I, II, and III were mixed oils of fish oil with soybean oil and corn oil, respectively; group IV was a mixed oil of corn oil and soybean oil. Different oil ratios affected the palatability of the diet, resulting in differences among groups in dry matter intake. The experimental results showed that the digestibility of most nutrients in group II was significantly lower than that in groups III and IV; the digestibility of most nutrients did not differ significantly between group I and groups III and IV, but was lower than that in groups III and IV. This may have been because an excessively high proportion of fish oil affects nutrient absorption. These findings indicate that a reasonable fatty acid composition in the diet can not only promote the digestion and absorption of fat, but also improve the utilization efficiency of protein and energy and promote the growth and development of livestock and poultry[^{{{23}}^{-}}^{{{24}}^{].}} The dietary n-6/n-3 PUFA ratio affected nutrient metabolism in blue foxes, leading to differences among groups in nutrient digestibility.

3.3 Effects of Dietary n-6/n-3 PUFA Ratio on Nitrogen Metabolism in Growing Blue Foxes

The dietary n-6/n-3 PUFA ratio significantly affected nitrogen intake and fecal nitrogen content in growing blue foxes, but had no significant effect on urinary nitrogen content. Ultimately, differences in nitrogen deposition among groups were not significant, and net protein utilization and biological value of protein also showed the same trend. This indicates that, when blue foxes were fed diets with the same fat level, the dietary n-6/n-3 PUFA ratio caused changes in lipid metabolism. The balance between n-6 and n-3 fatty acids plays an important role in maintaining the stability of the internal environment and normal growth of animals, and can reduce the occurrence of cardiovascular disease and hyperlipidemia[^{{{25}}^{],}} but it had no direct effect on protein deposition in blue foxes.

4 Conclusion

When the dietary n-6/n-3 PUFA ratio was 9.01 or 18.04, blue foxes achieved relatively good growth performance. However, considering feed cost and rancidity stability, the mixed corn oil and soybean oil group was preferable.

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Dietary Ratio of n-6/n-3 Polyunsaturated Fatty Acid Affect Growing Performance, Nutrient Digestibility and Nitrogen Metabolism of Male Blue Foxes during Growth Period

ZHONG Wei LUO Jing ZHANG Ting SUN Weili LIU Hanlu SUN Xuyang LI Guangyu*

(State Key Laboratory of Special Economic Animal Molecular Biology, Institute of Special Animal and Plant Science, Chinese Academy of Agriculture Sciences, Changchun 130112, China)

Abstract: The aim of this trial was to investigate the effects of dietary ratio of n-6/n-3 polyunsaturated fatty acid (PUFA) on growing performance, nutrient digestibility and nitrogen metabolism of male blue foxes during growth period. Forty-eight 102-day-old male blue foxes with average body weight of (2 370$±$24) g were selected and assigned into 4 groups with 12 replicates in each group and 1 blue fox in each replicate. Except the composition and proportion of oil, the other ingredients in diets were the same. Diet of group supplemented with fish oil and soybean oil, n-6/n-3=3.00; diet of group supplemented with fish oil and corn oil, n-6/n-3=6.03; diet of group supplemented with fish oil and corn oil, n-6/n-3=9.01; diet of group supplemented with soybean oil and corn oil, n-6/n-3=18.04. The adjustment period lasted for 7 days, and the experimental period lasted for 46 days. The results showed as follows: 1) the body weight of groups and at 125 days of age was significantly higher than that of group (P<0.05), the average daily gain of groups , and at 102 to 125 days of age was significantly higher than that of group (P<0.01), the ratio of feed to gain of groups , and at 102 to 125 days of age was significantly lower than that of group (P<0.05). Dietary ratio of n-6/n-3 PUFA had no effects on growth performance at 126 to 147 days of age (P>0.05). 2) The dry matter intake of groups and was significantly higher than that of group (P<0.05), the dry matter digestibility of groups , and was significantly higher than that of group (P<0.05), the crude protein digestibility of groups and was significantly higher than that of group (P<0.01), the gross energy digestibility of groups and was

significantly higher than that of group II ($P < 0.05$), and the ether extract digestibility of group III was significantly higher than that of group II ($P < 0.05$).

The dietary n-6/n-3 PUFA ratio had no effect on carbohydrate digestibility ($P > 0.05$). 3) The nitrogen intake of groups I and III was significantly higher than that of groups II and IV ($P < 0.01$); the fecal nitrogen content of group IV was significantly lower than that of groups I and II ($P < 0.05$). The dietary n-6/n-3 PUFA ratio had no effects on urinary nitrogen content, nitrogen retention, net protein utilization, or the biological value of protein ($P > 0.05$). In conclusion, when the dietary n-6/n-3 PUFA ratio is 9.01 or 18.04, blue foxes can achieve better growth performance; however, considering feed cost and storage stability, the mixture of corn oil and soybean oil is more beneficial.

Key words: n-6/n-3; PUFA; blue fox; growth performance; digestibility; nitrogen metabolism

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*Corresponding author, professor, E-mail: tcslgy@126.com

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