Effects of Dietary Arginine Supplementation Level on Production Performance, Nutrient Digestibility, and Nitrogen Metabolism in Female Blue Foxes during the Winter Fur Period: Postprint

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Abstract

This experiment was conducted to investigate the effects of dietary arginine supplementation levels on production performance, nutrient digestibility, and nitrogen metabolism in female blue foxes during the winter fur period. Sixty healthy female blue foxes at 120 days of age with similar body weight were randomly allocated to 6 groups, with 10 replicates per group and 1 fox per replicate. The dietary arginine supplementation levels for each group were 0 (control group), 0.2%, 0.4%, 0.6%, 0.8%, and 1.0%, respectively. The preliminary period lasted 7 days, and the formal experimental period lasted 80 days. The results showed that: 1) The average daily gain of blue foxes in the 0.6% supplementation group was extremely significantly higher than that of all other groups ($P < 0.01$). Body length and pelt length increased by 1.42% and 1.26% compared with the control group, respectively ($P > 0.05$), and the feed-to-gain ratio was extremely significantly lower than that of the control group ($P < 0.01$). The average daily feed intake of blue foxes in the 0.4% supplementation group was significantly lower than that of the control group ($P < 0.05$). 2) Dietary arginine supplementation level extremely significantly affected the fat digestibility of blue foxes ($P < 0.01$), with the fat digestibility of all groups increasing as dietary arginine supplementation level increased. The dry matter digestibility of the 0.6% supplementation group was extremely significantly higher than that of the control group and the 0.2% and 0.4% supplementation groups ($P < 0.01$). Dietary arginine supplementation level had no significant effect on protein digestibility and carbohydrate digestibility of blue foxes ($P > 0.05$). 3) The nitrogen intake of blue foxes in the 0.4% supplementation group

was significantly lower than that of all other groups except the 0.6% supplementation group ($P < 0.05$). The 0.6% supplementation group exhibited the lowest fecal nitrogen and urinary nitrogen contents, while showing the highest nitrogen retention, net protein utilization, protein biological value, and protein efficiency ratio. Based on comprehensive evaluation of all indicators, supplementation of 0.6% arginine in the diet of female blue foxes during the winter fur period (total dietary arginine level of 2.04%) can increase average daily gain and reduce feed-to-gain ratio.

Full Text

Effects of Dietary Arginine Supplemental Level on Performance, Nutrient Digestibility and Nitrogen Metabolism of Female Blue Foxes during Fur Development Period

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Abstract

This experiment was conducted to investigate the effects of dietary arginine supplemental level on performance, nutrient digestibility and nitrogen metabolism of female blue foxes during the fur development period. Sixty healthy female blue foxes aged 120 days with similar body weight were randomly divided into 6 groups, with 10 replicates per group and 1 fox per replicate. The dietary arginine supplemental levels for each group were 0 (control group), 0.2%, 0.4%, 0.6%, 0.8% and 1.0%, respectively. The pre-trial period lasted 7 days, and the formal trial period lasted 80 days. The results showed: 1) The average daily gain of blue foxes in the 0.6% supplemental group was extremely significantly higher than that in other groups ($P < 0.01$). Body length and fur length increased by 1.42% and 1.26% compared with the control group ($P > 0.05$), and the feed-to-gain ratio was extremely significantly lower than that in the control group ($P < 0.01$). The average daily feed intake of blue foxes in the 0.4% supplemental group was significantly lower than that in the control group ($P < 0.05$). 2) Dietary arginine supplemental level had an extremely significant effect on fat digestibility of blue foxes ($P < 0.01$), with fat digestibility increasing as dietary arginine supplemental level increased. The dry matter digestibility in the 0.6% supplemental group was extremely significantly higher than that in the control group and 0.2% and 0.4% supplemental groups ($P < 0.01$). Dietary arginine supplemental level had no significant effect on protein digestibility and carbohydrate digestibility ($P > 0.05$). 3) The nitrogen intake of blue foxes in

the 0.4% supplemental group was significantly lower than that in other groups except the 0.6% supplemental group ($P < 0.05$). The 0.6% supplemental group had the lowest fecal nitrogen and urinary nitrogen content, while nitrogen retention, net protein utilization, biological value of protein and protein efficiency ratio were all highest in this group. Based on comprehensive evaluation of all indicators, supplementing 0.6% arginine to the diet of female blue foxes during the fur development period (total dietary arginine level of 2.04%) can improve average daily gain and reduce feed-to-gain ratio.

Keywords: arginine; blue fox; fur development period; performance; nutrient digestibility; nitrogen metabolism

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Introduction

In most mammalian studies, arginine is considered a semi-essential or conditionally essential amino acid that requires dietary supplementation when animals are young or diseased [1]. Arginine is widely present in meat foods. For carnivores, arginine is an essential amino acid, and adequate dietary arginine is necessary to ensure normal growth and development [2]. The blue fox is a carnivorous fur-bearing animal whose diet is characterized by high protein and high fat content, with a relatively high proportion of animal-derived feed ingredients [3]. Previous research has thoroughly investigated the protein nutritional requirements of blue foxes during various periods and applied these findings to farming practices. However, the issue of dietary amino acid balance is often neglected in production. Insufficient or excessive supplementation of certain essential amino acids can prevent effective absorption and utilization of dietary protein, and may even cause metabolic disorders that hinder full expression of animal performance.

Studies on arginine nutrition in other mammals and humans have found that insufficient arginine intake in fetuses or young animals can cause growth retardation [4] and hyperammonemia [5]. Arginine deficiency in adult mammals can affect reproductive function [6-7]. Additionally, arginine can improve animal immunity [8]. Furthermore, Czarnecki et al. [9] found in canids that adult dogs fed an arginine-free diet developed orotic acidemia and elevated blood ammonia levels. The NRC (1982) [10] standards for mink and fox nutritional requirements state that arginine plays a crucial role in fur development of fur-bearing animals and may affect subsequent fur quality.

Previous studies have shown that supplementing arginine to the basal diet of growing female blue foxes can increase average daily gain and reduce feed-to-gain ratio [11]. Thus, research on arginine nutrition in blue foxes is significant for normal development, healthy growth, and fur quality improvement. However, recent studies using blue foxes as experimental animals to investigate arginine nutritional requirements during various growth periods remain scarce, and neither domestic nor international sources have clearly established optimal arginine lev-

els in dry powder diets for blue foxes. Therefore, this experiment supplemented different levels of arginine to blue fox diets during the fur development period to study the effects of dietary arginine supplemental level on performance, nutrient digestibility and nitrogen metabolism of female blue foxes, preliminarily explore the nutritional role of arginine, and provide a scientific basis for rational application of arginine in dry powder diets and improvement of blue fox feeding standards in China.

1.1 Experimental Design and Management

Sixty healthy female local improved breed blue foxes aged 120 days were selected and randomly divided into 6 groups, with 10 replicates per group and 1 fox per replicate. Differences in initial body weight among replicates were not significant ($P > 0.05$). A single-factor randomized experimental design was adopted, with arginine supplemental levels in the basal diet of 0 (Group I), 0.2% (Group II), 0.4% (Group III), 0.6% (Group IV), 0.8% (Group V) and 1.0% (Group VI), respectively. The pre-trial period lasted 7 days, and the formal trial period lasted 80 days. The entire trial was conducted under natural outdoor lighting conditions and managed by dedicated personnel. Foxes were housed individually in cages and fed twice daily (morning and evening) with free access to water. The composition and nutrient levels of the basal diet, formulated according to NRC (1982) [10] standards for mink and fox nutritional requirements, are shown in Table 1 . The L-arginine used in this experiment was produced by Wuxi Jinghai Amino Acid Co., Ltd., with a purity of $ $99.0%.

1.2 Digestion-Metabolism Trial

Seven healthy blue foxes with normal feed intake and defecation were selected from each group for a digestion-metabolism trial conducted from day 29 to day 32 of the experiment. The total feces and urine collection method was used to continuously collect feces and urine for 4 days. Before urine collection, 20 mL of 10% sulfuric acid solution was added to the collection bucket to fix nitrogen. Urine samples collected over 4 days were uniformly mixed, filtered, and stored at -20 ℃ for later use. Fecal samples collected over 4 days were uniformly mixed, sprayed with a small amount of 10% sulfuric acid solution for nitrogen fixation, dried at 65 ℃ to constant weight, ground to pass through a 40-mesh sieve, and stored for later use.

1.3 Slaughter Trial

After the feeding trial was completed, 7 healthy blue foxes were randomly selected from each group for euthanasia. Each blue fox was intramuscularly injected with 10 mL of 10 mg/mL succinylcholine chloride. After confirming death, the fur was collected.

1.4 Measurement Indicators and Methods

After the formal trial began, the health status of experimental blue foxes was observed daily, feed intake and leftover feed were recorded, and feed consumption was calculated. Body weight was measured every 21 days in the morning after fasting, and daily weight gain was calculated. After euthanasia, body length was measured from nose tip to tail base. After fur scraping and oil removal, fur length was measured on the stretching board from nose tip to tail base.

Dry matter content in feed and fecal samples was determined using the 105 ℃ drying method according to GB/T 6435-2006. Crude protein content was determined using the Kjeldahl method according to GB/T 6432-1994 with a Foss Kjeltec 8400 automatic Kjeldahl analyzer. Crude fat content was determined using the Soxhlet extraction method according to GB/T 6433-2006 with a Buchi B-811 Soxhlet extractor. Crude ash content was determined using the 550 ℃ incineration method according to GB/T 6438-1992. Amino acid content was determined using the hydrochloric acid hydrolysis method according to GB/T 18246-2000 with a Hitachi L-8900 automatic amino acid analyzer. Carbohydrate content in this experiment was obtained by calculation [12].

1.5 Calculation Formulas

Feed-to-gain ratio = Dry matter intake (g) / Average daily gain (g)
Total carbohydrate = Total dry matter - Total protein - Total fat - Total ash
Dry matter digestibility (%) = [(Dry matter intake - Total fecal dry matter) / Dry matter intake] × 100
Protein digestibility (%) = [(Protein intake - Total fecal protein) / Protein intake] × 100
Fat digestibility (%) = [(Fat intake - Total fecal fat) / Fat intake] × 100
Carbohydrate digestibility (%) = [(Carbohydrate intake - Total fecal carbohydrate) / Carbohydrate 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
Protein efficiency ratio = Daily gain (g) / Nitrogen intake (g)

1.6 Data Statistical Analysis

Experimental data were expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA with SPSS 21.0 software. Duncan' s multiple comparison test was used for significance testing. $P < 0.01$ indicated extremely significant difference, $P < 0.05$ indicated significant difference, and $P > 0.05$ indicated no significant difference.

2.1 Effects of Dietary Arginine Supplemental Level on Performance of Female Blue Foxes during Fur Development Period

As shown in Table 2 , dietary supplementation with 0.2%, 0.4%, 0.6% and 0.8% arginine increased final body weight of blue foxes, while 1.0% arginine supplementation decreased final body weight. The 0.6% supplemental group had the highest average daily gain, which was extremely significantly higher than that in other groups ($P < 0.01$). The 0.4% supplemental group had the lowest average daily feed intake, which was significantly lower than that in other groups except the 0.6% supplemental group ($P < 0.05$). The 0.6% supplemental group had the lowest feed-to-gain ratio, which was extremely significantly lower than that in other groups except the 0.4% supplemental group ($P < 0.01$). Regarding fur quality, the 0.6% supplemental group had optimal body length and fur length, which increased by 1.42% and 1.26% compared with the control group ($P > 0.05$).

2.2 Effects of Dietary Arginine Supplemental Level on Nutrient Digestibility of Female Blue Foxes during Fur Development Period

As shown in Table 3 , the 0.6% supplemental group had the highest dry matter digestibility, which was extremely significantly higher than that in the control group and 0.2% and 0.4% supplemental groups ($P < 0.01$). Dietary arginine supplemental level had an extremely significant effect on fat digestibility of blue foxes ($P < 0.01$), with fat digestibility increasing as dietary arginine supplemental level increased. The 1.0% supplemental group showed a 3.97% improvement in fat digestibility compared with the control group ($P < 0.01$). Dietary arginine supplemental level had no significant effect on protein digestibility and carbohydrate digestibility ($P > 0.05$).

2.3 Effects of Dietary Arginine Supplemental Level on Nitrogen Metabolism of Female Blue Foxes during Fur Development Period

As shown in Table 4 , regarding nitrogen intake, the 0.4% supplemental group had the lowest value, which was significantly lower than that in other groups except the 0.6% supplemental group ($P < 0.05$). The 0.6% supplemental group had the lowest fecal nitrogen and urinary nitrogen content, with fecal nitrogen content significantly lower than that in the control group ($P < 0.05$). The 0.6% supplemental group had the highest nitrogen retention, which was 13.57% higher than that in the control group ($P < 0.05$). Net protein utilization, biological value of protein and protein efficiency ratio were all highest in the 0.6% supplemental group and lowest in the 1.0% supplemental group.

3.1 Effects of Dietary Arginine Supplemental Level on Performance of Female Blue Foxes during Fur Development Period

Carnivores have weak endogenous arginine synthesis capacity and require exogenous arginine supplementation [13]. Studies on carnivores such as dogs [14],

domestic cats [15] and mink [16] have found that arginine can promote animal growth, increase average daily gain, and maintain stable nitrogen metabolism. The results of this experiment showed that supplementing the basal diet with 0.2%, 0.4%, 0.6% and 0.8% arginine improved average daily gain, body length and fur length of female blue foxes during the fur development period to varying degrees, and reduced feed-to-gain ratio, indicating that appropriate arginine supplementation has growth-promoting effects on blue foxes. Wan et al. [17] reported that arginine supplementation in dry powder diets can reduce feed-to-gain ratio of mink during the fur development period. Studies on mice [18] and piglets [19] found that dietary arginine supplementation can increase daily gain. The results of this experiment are basically consistent with these related reports. The improvement effects of different arginine supplemental levels on performance of blue foxes during the fur development period varied, with the 0.6% supplemental level being significantly superior to other groups. Larger fur size and better fur integrity result in higher fur quality and economic value [20]. Since there are body size differences between local improved breed blue foxes and Finnish original breed blue foxes [21], performance is better when daily arginine intake per kilogram body weight for each female blue fox during the fur development period is between 0.95-1.04 g.

However, excessive arginine intake may affect the absorption and metabolism of lysine and other amino acids [22], disrupting amino acid balance. Southern et al. [23] demonstrated that excessive arginine supplementation in pig diets reduced plasma lysine and histidine contents, and this adverse effect of excess arginine could not be alleviated by lysine supplementation, hindering animal performance. Supplementing 1.0% arginine to the basal diet reduced average daily gain, body length and fur length of female blue foxes during the fur development period, indicating that excessive arginine supplementation is not conducive to performance expression in blue foxes.

3.2 Effects of Dietary Arginine Supplemental Level on Nutrient Digestibility and Nitrogen Metabolism of Female Blue Foxes during Fur Development Period

This study showed that arginine supplementation to the basal diet could improve dry matter digestibility of blue foxes. This may be because arginine has intestinal health-improving effects, specifically by promoting intestinal morphological development, maintaining small intestinal villus structure [24], increasing digestive enzyme activity [25], enhancing intestinal immune barrier function [26], and reducing intestinal microbial translocation [27]. Therefore, maintaining intestinal health is more conducive to nutrient absorption and utilization, thereby improving dry matter digestibility.

This experiment found that as dietary arginine supplemental level increased, fat digestibility of blue foxes during the fur development period also continuously improved. This may be related to the involvement of arginine metabolite nitric oxide in lipid metabolism. Arginine is the sole precursor for nitric oxide

synthesis in the body, and nitric oxide produced under the action of nitric oxide synthase promotes white fat decomposition [28]. Tan et al. [29] found that feeding 110-day-old finishing pigs a diet supplemented with 1.0% arginine for 60 days reduced body fat levels. Blue foxes begin seasonal fat storage during the fur development period to resist cold, while arginine intake accelerates body fat metabolism, promotes fat absorption, and consequently improves fat digestibility. The effect of arginine on lipase activity has not been reported, so whether arginine supplementation in the diet increases intestinal lipase activity in blue foxes requires further investigation.

Arginine is hydrolyzed into urea and ornithine under the action of arginase, which is an important process in the urea cycle [30]. The urea cycle in the liver can convert excess ammonia into urea for excretion, avoiding ammonia toxicity and maintaining nitrogen balance. In this experiment, as dietary arginine supplemental level increased, urinary nitrogen excretion of blue foxes first decreased then increased, while nitrogen retention showed a trend of first increasing then decreasing, similar to the trend in average daily gain. This may be because as arginine supplemental level approached the appropriate level, dietary amino acid composition gradually reached balance to meet animal requirements. However, excessive arginine supplementation in the diet may affect nutrient absorption and utilization, disrupt nitrogen balance, and interfere with nitrogen metabolism, resulting in higher urinary and fecal nitrogen contents in the 1.0% supplemental group than in other groups and reducing nitrogen retention.

Under the conditions of this experiment, comprehensive evaluation of performance, nutrient digestibility and nitrogen metabolism indicators of blue foxes during the fur development period indicated that supplementing 0.6% arginine to the diet of female blue foxes (total dietary arginine level of 2.04%) can increase average daily gain and reduce feed-to-gain ratio.

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