Effects of Feed Flavoring Agents on Feed Intake, Nutrient Digestibility, Nitrogen Metabolism and Growth Performance in Growing Blue Foxes Postprint

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Abstract

This experiment aimed to investigate the effects of four feed flavorings (liver flavor, milk flavor, sweetener, and intestine flavor) on feed intake, nutrient digestibility, nitrogen metabolism, and growth performance of growing male blue foxes. A single-factor experimental design was employed, in which 50 healthy growing male blue foxes were selected and randomly allocated into 5 groups with 10 replicates per group, with no significant difference in body weight among individual animals across groups. The control group (Group I) was fed the basal diet, while the experimental groups (Groups II, III, IV, and V) were fed experimental diets supplemented with liver flavor, milk flavor, sweetener, and intestine flavor feed flavorings in the basal diet at supplementation levels of 500, 500, 120, and 500 mg/kg, respectively. The experimental results showed: 1) Compared with Group I, Groups II and IV extremely significantly increased dry matter intake ($P<0.01$), while Group III significantly increased dry matter intake ($P<0.05$). 2) Compared with Group I, Group IV significantly decreased dry matter excretion ($P<0.05$); the dry matter digestibility of Groups II, III, IV, and V increased by 4.34%, 9.07%, 9.40%, and 5.23%, respectively, but the differences were not significant ($P>0.05$); the protein digestibility of Groups III, IV, and V was extremely significantly increased ($P<0.01$); the fat digestibility of Group IV was significantly increased ($P<0.05$). 3) Compared with Group I, there were no significant differences in nitrogen intake, urinary nitrogen, and protein biological value among the other groups ($P>0.05$); Group IV significantly decreased fecal nitrogen ($P<0.05$) and significantly increased nitrogen retention and net protein utilization ($P<0.05$). 4) There were no significant differences in body weight and total weight gain of blue foxes among all groups ($P>0.05$); there were no significant differences in average daily gain among groups ($P>0.05$), but Group

I was the lowest, and Groups II, III, IV, and V were 7.99%, 3.27%, 6.19%, and 7.37% higher than Group I, respectively; the feed-to-gain ratio from low to high was in the order of Groups IV, III, II, V, and I, but there were no significant differences among groups (P>0.05). Under the conditions of this experiment, dietary supplementation with liver flavor, milk flavor, and sweetener all increased the dry matter intake and nutrient digestibility of blue foxes, and improved feed utilization efficiency, with the sweetener showing the best effect.

Full Text

Effects of Feed Flavoring Agents on Feed Intake, Nutrient Digestibility, Nitrogen Metabolism, and Growth Performance of Growing Male Blue Foxes

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Abstract: This experiment investigated the effects of four feed flavoring agents—liver flavor, milk flavor, sweetener, and bowel flavor—on feed intake, nutrient digestibility, nitrogen metabolism, and growth performance in growing male blue foxes. Using a single-factor experimental design, fifty healthy growing male blue foxes were randomly allocated into five groups with ten replicates per group. The control group (Group I) received a basal diet, while the experimental groups (Groups II, III, IV, and V) received the basal diet supplemented with liver flavor, milk flavor, sweetener, and bowel flavor at concentrations of 500, 500, 120, and 500 mg/kg, respectively. The results showed that: (1) Compared with Group I, dry matter intake was extremely significantly increased in Groups II and IV (P<0.01) and significantly increased in Group III (P<0.05). (2) Dry matter output was significantly reduced in Group IV compared with Group I (P<0.05). Dry matter digestibility in Groups II, III, IV, and V increased by 4.34%, 9.07%, 9.40%, and 5.23%, respectively, though these differences were not significant (P>0.05). Protein digestibility was extremely significantly higher in Groups III, IV, and V (P<0.01), while fat digestibility was significantly higher in Group IV (P<0.05). (3) Regarding nitrogen metabolism, no significant differences were observed among groups in nitrogen intake, urinary nitrogen, or protein biological value (P>0.05). However, Group IV exhibited significantly lower fecal nitrogen (P<0.05) and significantly higher nitrogen retention and net protein utilization (P<0.05). (4) No significant differences were found in body weight or total weight gain among all groups (P>0.05). Average daily gain did not differ significantly between groups (P>0.05), though Group I showed the lowest value, with Groups II, III, IV, and V exhibiting increases of 7.99%, 3.27%, 6.19%, and

7.37%, respectively, compared with Group I. The feed-to-gain ratio from lowest to highest was Groups IV, III, II, V, and I, though differences were not significant (P>0.05). Under the conditions of this experiment, dietary supplementation with liver flavor, milk flavor, and sweetener improved dry matter intake, nutrient digestibility, and feed utilization in blue foxes, with sweetener showing the best effect.

Keywords: blue fox; feed flavoring agent; feed intake; digestibility; nitrogen metabolism; growth performance

Introduction

The blue fox (*Vulpes lagopus*) belongs to the Canidae family and possesses highly sensitive olfactory and gustatory systems. Wild blue foxes exhibit diverse dietary preferences, primarily consuming animal-based foods such as rodents, frogs, fish, and birds, while occasionally ingesting berries, plant seeds, and roots. Since the introduction of blue foxes to China in the 1950s, artificial feeding has relied predominantly on animal-based ingredients (fish meal, chicken viscera) and plant-based ingredients (corn, soybean meal), resulting in relatively monotonous diets. The growing period represents a critical developmental stage in blue foxes, making it essential for researchers to consider both nutritional requirements and sensory preferences to enhance feed intake and utilization.

Feed flavoring agents, also known as flavor enhancers or palatability enhancers, are non-nutritive feed additives derived from either chemical synthesis or natural plant extraction. These compounds improve feed taste, mask undesirable odors in raw materials, enhance palatability, stimulate appetite, increase feed conversion efficiency, and promote animal welfare. The application of feed flavoring agents has generated significant economic benefits and attracted considerable attention in the livestock industry. In recent years, various flavoring agents have been widely used in piglet and poultry diets, demonstrating recognized improvements in feed intake, conversion rates, and meat quality. However, research on flavoring agents for fur-bearing animals remains scarce, and suitable flavor types for blue foxes lack empirical support, with current practices based primarily on anecdotal experience from farmers.

Based on the feeding characteristics of blue foxes, this study evaluated the effects of supplementing basal diets with liver flavor, milk flavor, sweetener, and bowel flavor. The objective was to identify appropriate flavoring agents for growing blue foxes and investigate their impacts on feed intake, nutrient digestibility, nitrogen metabolism, and growth performance, thereby providing a scientific basis for the application of feed flavoring agents in blue fox production.

1.1 Experimental Animals and Management

Fifty healthy 12-week-old male blue foxes with an average initial body weight of (3.15$±$0.21) kg were selected and randomly divided into five groups (one control and four experimental groups) using a single-factor design. Each group

comprised ten replicates with one fox per replicate, and no significant differences in initial body weight were observed among groups (P>0.05). The experiment consisted of a 7-day preliminary period followed by a 52-day formal experimental period. All foxes received routine immunizations and were housed individually in cages with ad libitum access to water. Fixed personnel fed the animals twice daily at 07:00 and 15:00. Body weight was measured every 13 days in the morning after overnight fasting. Feed intake was recorded precisely to calculate feed-to-gain ratio.

1.2 Experimental Diets

The basal diet was formulated using fish meal, extruded corn, soybean meal, and corn gluten meal as primary ingredients. Based on previous research by Cui et al. on optimal protein requirements for growing blue foxes, the basal diet contained 31% crude protein (Group I). Experimental groups received the basal diet supplemented with liver flavor (500 mg/kg, Group II), milk flavor (500 mg/kg, Group III), sweetener (120 mg/kg, Group IV), or bowel flavor (500 mg/kg, Group V). These supplementation levels were recommended by DadHank Biotechnology Co., Ltd. based on extensive research data. The composition and nutrient levels of the basal diet are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis)

Item	Content
Ingredients	
Extruded corn	
Soybean meal	
Meat-bone meal	
Corn gluten meal	
Fish meal	
$CaHPO_4$	
NaCl	
Soybean oil	
Premix[1])	
Total	
Nutrient levels	
Crude protein (CP)	
Crude fat (EE)	
Crude ash	
Calcium (Ca)	
Carbohydrate	
Metabolizable energy (ME)/(MJ/kg)[2])	

[1]) The premix provided the following per kg of diet: VA 940,000 IU, VD_3 250,000 IU, VE 4,000 mg, VK_3 200 mg, VB_1 200 mg, VB_2 500 mg, VB_6 300 mg, VB_{12}

1.5 mg, VC 12,000 mg, folacin 100 mg, nicotinamide 2,000 mg, pantothenic acid 1,000 mg, biotin 10 mg, ethoxyquin 10 mg, choline chloride 30,000 mg, Fe (as ferrous sulfate) 4,000 mg, Zn (as zinc sulfate) 3,200 mg, Se (as sodium selenite) 12 mg, Cu (as copper sulfate) 500 mg.

[2]) ME was a calculated value, while other values were measured.

1.3 Digestion and Metabolism Trial

On day 7 of the formal experimental period, six healthy foxes with good body condition from each group were selected for a 3-day digestion and metabolism trial using the total fecal collection method. Management during this period remained consistent with routine practices. Collected feces were weighed, mixed with 5% sulfuric acid solution (10% concentration), dried, ground, and passed through a 40-mesh sieve before storage at -20°C. Urine was collected with 2 mL of 10% sulfuric acid added per 100 mL to fix nitrogen, then filtered and stored at -20°C.

1.4 Test Indicators and Methods

Daily feed intake was recorded accurately to obtain total feed consumption during the experimental period. Initial body weight was measured using a Shanghai Yingzhan electronic scale (15 kg capacity, 0.01 kg precision). Fasting body weight was measured every 13 days in the morning to determine weight gain and calculate average daily gain (ADG). Dry matter, crude protein, crude ash, crude fat in diets and feces, and crude protein in urine were determined according to Zhang (2003). Calculations were performed as follows:

- Dry matter digestibility (%) = 100 × [(dry matter intake - dry matter output) / dry matter intake]
- Protein digestibility (%) = 100 × [(protein intake - fecal protein content) / protein intake]
- Fat digestibility (%) = 100 × [(fat intake - fecal fat content) / fat intake]
- Nitrogen retention (g/d) = nitrogen intake - fecal nitrogen - urinary nitrogen
- Net protein utilization (%) = 100 × (nitrogen retention / nitrogen intake)
- Protein biological value (%) = 100 × [nitrogen retention / (nitrogen intake - fecal nitrogen)]

1.5 Data Processing

Data were analyzed using SAS 9.2 software with one-way ANOVA. Duncan' s multiple range test was used for post-hoc comparisons. Results are expressed as means ± standard error. Significance was declared at $P<0.05$ and extreme significance at $P<0.01$.

Results

2.1 Effects of Different Flavoring Agents on Feed Intake and Nutrient Digestibility

As shown in Table 2 , compared with Group I, dry matter intake in Groups II, III, and IV was significantly or extremely significantly increased ($P<0.05$ or $P<0.01$). Specifically, Groups II and IV were extremely significantly higher than Groups I and V ($P<0.01$), while Group III was significantly higher than Groups I and V ($P<0.05$). Dry matter output in Group IV was significantly lower than in Groups I, II, and III ($P<0.05$). Although dry matter digestibility improved with all flavoring agents, the differences were not significant ($P>0.05$), with increases of 4.34%, 9.07%, 9.40%, and 5.23% in Groups II, III, IV, and V, respectively. Protein digestibility was highest in Group IV (72.62%) and was extremely significantly higher than in Groups I and II ($P<0.01$), while Groups III and V were significantly higher than Groups I and II ($P<0.05$). Fat digestibility was also highest in Group IV (90.69%), being significantly higher than Group I ($P<0.05$) and extremely significantly higher than Group II ($P<0.01$).

Table 2 Effects of flavoring agents on nutrient digestibility of growing blue foxes

Item	Group I	Group II	Group III	Group IV	Group V	P-value
Item	Group I	Group II	Group III	Group IV	Group V	P-value
Dry matter intake (g/d)	292.00$±5.28 ^{bB} |303.02±2.71 ^{aA} |301.91±7.67 ^{aAB} |302.79±5.75 ^{aA} |288.60±6.03 ^{bC} |||*Drymatteroutput(g/d)*|88.83±6.10 ^a |91.49±5.15 ^a |87.81±7.72 ^a |73.22±11.53 ^b |84.91±9.49 ^{ab} |||*Drymatterdigestibility*(±2.40|71.82±6.43|75.07±6.77|75.30±3.06|72.43±5.28|||*Proteindigestibility* ^{bC} |59.52±7.32 ^{bBC} |70.49±9.14 ^{aAB} |					

In the same row, values with different small letter superscripts indicate significant difference (P<0.05), while different capital letter superscripts indicate extremely significant difference (P<0.01). The same notation applies below.

2.2 Effects of Flavoring Agents on Nitrogen Metabolism

As presented in Table 3 , nitrogen intake did not differ significantly among groups compared with Group I (P>0.05), though Group II was significantly higher than Group V (P<0.05). Fecal nitrogen was lowest in Group IV, being significantly lower than Groups I, II, and III (P<0.05), while Group V was significantly lower than Group II (P<0.05). Urinary nitrogen showed no significant differences among groups (P>0.05). Nitrogen retention was greater in Groups III and IV, being significantly higher than Group I (P<0.05). Net protein utilization was highest in Group IV, significantly exceeding Group I (P<0.05). Protein biological value did not differ significantly among groups (P>0.05).

Table 3 Effects of flavoring agents on nitrogen metabolism of growing blue foxes

Item	Group I	Group II	Group III	Group IV	Group V	P-value
Item	Group I	Group II	Group III	Group IV	Group V	P-value
Nitrogen in-take (g/d)	14.49$±0.26 ^{ab}	15.05±0.12 ^a	14.79±0.59 ^{ab}	14.68±0.89 ^{ab}	14.08±0.66 ^b	
$Fecal nitrogen (g/d)$	5.19±0.46 ^{ab}	5.40±0.50 ^a	5.24±0.70 ^{ab}	4.27±0.70 ^c	4.47±0.36 ^{bc}	
$Urinary nitrogen (g/d)$	3.42±0.63	3.45±0.80	3.33±0.63	3.42±0.75	3.87±0.60	
$Nitrogen retention (g/$						
	6.05±0.58 ^b	7.05±0.98 ^a	6.98±0.84 ^a			

2.3 Effects of Flavoring Agents on Growth Performance of Growing Blue Foxes

As shown in Table 4 , no significant differences were observed in body weight or total weight gain among all groups (P>0.05). Average daily gain did not differ significantly between groups (P>0.05), though Group I showed the lowest value. Groups II, III, IV, and V exhibited increases of 7.99%, 3.27%, 6.19%, and 7.37%, respectively, compared with Group I. The feed-to-gain ratio from lowest to highest was Groups IV, III, II, V, and I, though differences were not significant (P>0.05).

Table 4 Effects of flavoring agents on growth performance of growing blue foxes

Item	Group I	Group II	Group III	Group IV	Group V	P-value
Body weight (kg)						
90 days of age	3.12$±0.25	3.11±0.14	3.17±0.21	3.18±0.22	3.16±0.23		
	103*daysofage*	4.08±0.32	4.11±0.20	4.02±0.29		
	to − *gainratio*	6.67±0.59	6.12±0.82	6.01±1.13	5.67±1.39	6.14±$0.49

Discussion

3.1 Effects of Flavoring Agent Types on Feed Intake

The odor, taste, and texture of food serve as sensory cues that influence feed intake based on animal preferences. The present results demonstrate that diets supplemented with liver flavor, milk flavor, and sweetener exhibited palatability-enhancing effects for growing blue foxes compared with the basal diet and bowel-flavored diet. Previous studies by our research group found that blue foxes during the pre-mating period showed high preference indices for liver flavor, which increased both feed intake and feeding speed. The growing period represents a rapid growth phase following weaning, and numerous studies indicate that animals continue to prefer flavors similar to maternal milk post-weaning. Our previous research demonstrated that milk flavor supplementation in growing blue fox diets achieved a preference index of 1.50 and significantly increased feeding speed, whereas milk flavor showed no effect on feed intake during the pre-mating period, possibly due to reduced dependence on milk flavor with maturity. Sweeteners are the most widely used flavoring agents in livestock production, as most animals share a common preference for sweet substances, a finding confirmed in blue foxes. Bowel flavor showed no apparent palatability-enhancing effect in this study, indicating that not all flavoring agents are effective. The efficacy of flavoring agents depends on type, basal diet composition, supplementation method, and dosage. Inappropriate aroma or excessively strong odor may produce adverse effects. Animals exhibit different flavor preferences across physiological stages, with varying sensory sensitivity and threshold levels. Therefore,

flavoring agent selection and efficacy differ according to growth stage and characteristics.

3.2 Effects of Different Flavoring Agents on Nutrient Digestibility and Nitrogen Metabolism

Pavlov stated that "appetite is digestive juice." When feed aroma is appealing and palatable, animals exhibit excitation of feeding centers, triggering conditioned reflexes that increase secretion of saliva, gastric juice, intestinal fluid, pancreatic juice, and bile, along with elevated levels of protease, amylase, and lipase. Enhanced gastrointestinal motility and mechanical digestion improve nutrient digestion and absorption, increase digestibility, promote gastric emptying, and stimulate subsequent feed intake. Platel et al. reported that certain spices like turmeric and capsaicin increased digestive juice secretion and enzyme activity in rats, while others like fennel and mustard produced opposite effects. Lü et al. demonstrated that milk and sweet flavoring agents increased activities of pepsin, total pancreatic protease, total lipase, and total amylase in pigs, while upregulating mRNA expression and serum secretion of feeding-regulating factors including neuropeptide Y (NPY) in the hypothalamus and ghrelin in gastric mucosa. The current study found that milk flavor and sweetener supplementation improved dry matter, protein, and fat digestibility, with protein digestibility reaching significant levels. Although liver flavor significantly increased feed intake without significantly improving nutrient digestibility, the additional secreted enzymes likely digested the increased feed consumption, resulting in improved nutrient utilization compared with the control group.

Nitrogen metabolism is fundamental to animal physiology. Dietary nitrogen is either deposited in tissues for utilization or excreted as fecal and urinary nitrogen. Deposited nitrogen serves as the primary building material for tissue cells and functional substances including enzymes, hormones, and antibodies. In fur-bearing animals like blue foxes, nitrogen deposition and utilization are critically important for hair growth and development. The present results show that liver flavor, milk flavor, and sweetener supplementation increased nitrogen retention, net protein utilization, and protein biological value, with nitrogen retention reaching significant levels for milk flavor and sweetener, and net protein utilization achieving significance for sweetener. However, the molecular mechanisms underlying the complex regulation of feeding centers, stimulation of feed intake, and modulation of feeding behavior and weight gain by flavoring agents in blue foxes require further investigation.

3.3 Effects of Different Flavoring Agents on Growth Performance of Growing Blue Foxes

The growing period is crucial for body development in blue foxes. The most direct method to increase body weight is enhancing feed intake to obtain more energy, with surplus energy stored as fat. Body growth during this period directly influences pelt size at pelting. Feed palatability, comprising taste, aroma,

and texture characteristics, affects feed intake and can be improved through flavoring agents. Mou reported that sweetener supplementation significantly improved growth performance and feed conversion in weaned piglets. Liu demonstrated that milk flavor, sweetener, and flavoring agents increased total weight gain in piglets. Li et al. found that supplementation with grass-flavored agents in basal diets improved feed intake, average daily gain, and feed conversion in lambs. In the current study, supplementation with liver flavor, milk flavor, and sweetener reduced feed-to-gain ratio, with sweetener producing the lowest ratio and thus maximizing economic benefits.

Under the conditions of this experiment, dietary supplementation with liver flavor, milk flavor, and sweetener improved dry matter intake, nutrient digestibility, and feed utilization in growing blue foxes, with sweetener demonstrating the best overall effect.

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