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Effects of Low-protein, *DL*-methionine and Lysine-supplemented Diets on Growth Performance, N-balance and Fur Characteristics of Blue Foxes (*Alopex lagopus*) during the Growing-furring Period

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Abstract: An experiment was carried out to examine the effects of low-protein diets supplemented with different levels of *DL*-methionine (Met) and Lysine (Lys) on growth performance and fur characteristics of growing-furring blue foxes in order to find the optimal dietary supplementation levels of Met and Lys. For two protein levels, conventional 27% (P27) and low 19% (P19), the measured protein contents of the diets were 271.2 and 189.4 g/kg on dry matter basis, respectively, and the low-protein diets were supplemented with Met (0.3%, 0.5%, 0.7%) and Lys (0.4%, 0.6%, 0.8%). An entirely random experimental design was adopted with two factors (3×3) and totally 10 groups (P27, L1M1, L1M2, L1M3, L2M1, L2M2, L2M3, L3M1, L3M2 and L3M3). From mid-September to pelting, based on the average daily gain, daily N retention, N retention ratio and the performance of blue foxes in different groups, 0.6% Met supplementation in low-protein diet was optimum; based on the daily N retention, N biological value and the quality of the fur, 0.3% and 0.5% Lys supplementation were optimum; based on the N apparent digestibility and daily N output, 0.3% Lys supplementation was optimum. No significant differences were observed in fur characteristics of blue foxes in all groups ($P>0.05$). In this experiment, the performance of blue foxes in L1M2 (0.3% Lys×0.6% Met) group was better than that in the other groups, which indicates that low-protein diets supplemented with *DL*-methionine and lysine for blue foxes can be beneficial to reduce feed expenses and nitrogen emission to the environment.

Key words: low-protein; growth performance; fur characteristic; blue fox (*Alopex lagopus*)
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Blue fox (*Alopex lagopus*) is by nature a carnivorous species with a high protein requirement, and diets used in commercial blue fox production characteristically contain high levels of animal protein. Feed constitutes the greatest individual item of cost, amounting to 50% of the total production costs in fox farming[1]. As protein is the most expensive dietary nutrient, any reduction in protein level contributes to a saving in production costs, and moreover, to a reduction in nitrogen emission. In the light of findings in growing-furring silver foxes, Harris et al.[2] concluded that 28% of protein in dry matter (DM) was sufficient for growth as that in higher protein levels. A reduction in protein levels, from 45% to 22% of metabolic energy (ME), was reported not to affect body weight. However, a protein level below 28%-30% of ME significantly reduced body length at pelting[3]. The established blue fox nutrition requirements do not contain any data on amino acids. In mink, another carnivorous furbearing animal, researches on dietary protein and

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amino acids are intensive[4-7]. These studies displayed that methionine (Met) was the first limiting amino acid for hair growth and thus for fur quality, and lysine (Lys) was the second limiting amino acid. There were some experiments which confirmed that animals fed diets of low-protein with some essential amino acid supplementations had no significant differences in their growth performance compared with those fed conventional diets[8]. Earlier feeding experiments showed that it was possible to decrease the recommended protein level during the growing-furring season of blue foxes[9-10]. The crucial point of this study is to evaluate the growth performance, N balance and fur characteristics of blue foxes fed low-protein diets supplemented with Met and Lys during the growing-furring period, and to quantify the optimal supplementation levels of amino acids in the low-protein diets.

1 MATERIALS AND METHODS

The experiment was carried out at the Fur Animals Experiment Station of Institute of Special Economic Animals and Plants of the Chinese Academy of Agricultural Sciences (44° N, 126° E) in Northeast China during the period from September 27 to December 13 in 2007. The experimental blue foxes were housed in standard roofed sheds with open sides in individual standard rearing cages (100 cm×70 cm×70 cm). The animals were exposed to natural temperature and photoperiod.

1.1 Animals and experimental design

One hundred and twenty male blue foxes were selected with similar weight [(5.34±0.57) kg] from different litters in an attempt to minimize genetic variation. The animals were allotted to 10 treatments of 12 animals each and fed twice a day at 08:00 and 16:00. Water and experimental diets were provided for *ad lib* consumption throughout the trial. Before the formal experiment, the animals were accustomed to the experimental feed for two weeks. The design of the experimental diets was shown in Table 1. Two experimental dietary protein levels added different proportions of Lys and Met became 10 treatments. The blue foxes were raised in cages of standard size (100 cm × 80 cm×80 cm). All training and testing of the animals were performed by the same person. Body weights were recorded at monthly intervals. Daily feed consumption of blue foxes was measured by offering a known quantity of feed and weighing residues the next day. The animals were pelted in accordance with common farming practice. Skin length was calculated from tip of nose to base of tail.

Eight blue foxes' blood was collected from heart in vacuum tubes in each group at the end of the trial, and centrifuged at 5 000 r/min for 10 min. Serum separated from blood was packed in Eppendorf centrifuge tubes, and then kept at −20 ℃ until analysis.

Table 1　The design of the experiment (DM basis)　%

Items	Control	L1M1	L1M2	L1M3	L2M1	L2M2	L2M3	L3M1	L3M2	L3M3
Protein	27					19				
Lys	0.4	0.3	0.3	0.3	0.5	0.5	0.5	0.7	0.7	0.7
Met	0.6	0.4	0.6	0.8	0.4	0.6	0.8	0.4	0.6	0.8

1.2 Experimental diets

Ten experimental diets were prepared at the same time before the start of the experiment and stored frozen at −20 ℃ in cold storage for later analysis. All diet samples were collected for chemical analyses before freezing. Diet composition was given in Table 2. Analyzed values of the diets and respective digestibility values were obtained from 17 weeks old male kits in N-balance experiments.

1.3 N-balance experiments

The N-balance experiments were carried out

with eight 21-week old male blue foxes from each group. The feces and urine collection period lasted for 3 days (October 14 – 16, 2007). The animals were raised in metabolism cages constructed for separate collection of feces and urine. Feces and urine were collected quantitatively everyday and kept frozen for later analysis. To avoid ammonia evaporation from the urine and feces, 20 mL of 5% sulphuric acid solution was added to the urine collection bottles, and the urine collection trays were sprayed with 20% citric acid solution once everyday. In the N-balance calculations, retained N was determined as ingested N – (fecal N + urinary N).

1.4 Chemical analyses

The chemical compositions of feed and feces were analyzed by standard methods. Dry matter (DM), ash, crude protein (CP), calcium, and phosphorus contents were analyzed according to AOAC procedures[11]. The calculation of ME content and the proportional composition of ME were based on the digestibility coefficients achieved and the following ME values: protein 18.8 MJ/kg, fat 39.8 MJ/kg and carbohydrate 17.6 MJ/kg[12]. The concentrations of amino acids were determined on an Agilent 1100 high performance liquid chromatograph (Agilent Technologies, Santa Clara, CA). All chromatographic procedures were performed at room temperature, and the samples and standards were evaluated in duplicates as described by Sedgwick et al.[13]. Serum urea nitrogen (SUN) concentration and serum total protein were measured according to the method of Bardford[14] using the kit (Nanjing Jiancheng Biotechnology Co., Ltd.) as the standard.

1.5 Calculations and statistical analyses

The apparent total tract digestibility (AD) coefficient of nutrient was calculated as follows: $AD = (a - b)/a$, where a is the intake of nutrient from feed, and b is the nutrient in feces. Statistical analyses of experimental data were performed with the general linear model (GLM) procedure of the SAS statistical package[15]. The differences among groups were tested by means of the least significant difference method in the statistical model.

Table 2 Composition of the diets (DM basis) %

Ingredients	Control diet	Basal experiment diet
Extruded soybean	18.0	20.0
Extruded corn	34.0	42.0
Soybean meal	9.0	5.0
Bone and meat meal	10.0	6.0
Corn germ meal	7.5	10.0
Fish meal	16.0	12.0
Lysine	0.4	
DL-methionine	0.6	
Soybean oil	3.5	4.0
Premix	1.0	1.0
Total	100.0	100.0

The premix provided the following per kg of diet: Ca ($CaHPO_4 \cdot 2H_2O$) 6.4 mg, P [$Ca(H_2PO_4)_2 \cdot H_2O$] 4.4 mg, Mg (MgO) 1.6 mg, Na (NaCl) 24 mg, Fe ($FeSO_4 \cdot H_2O$) 16 mg, Cu ($CuSO_4 \cdot 5H_2O$) 4.0 mg, Zn ($ZnSO_4 \cdot H_2O$) 10 mg, Mn ($MnSO_4 \cdot H_2O$) 12 mg, VA 300 IU, VB_1 0.15 mg, VB_2 0.40 mg, VB_6 0.30 mg, folic acid 0.30 mg, nicotinic acid 1.60 mg, *D*-pantothenic acid 1.3 mg.

2 RESULTS

2.1 Feed intake and growth performance

2.1.1 Average daily feed intake (ADFI)

ADFI was affected by dietary protein levels with amino acid supplements, and the values were all significantly higher in the L2M2, L1M2 and L2M3 groups than in the control group ($P<0.01$,

Table 4). The ADFI values were affected by Lys levels ($P<0.01$), and were higher in L1 and L2 than in L3. There was no Met effect or Met-Lys interaction on the ADFI ($P>0.05$).

2.1.2 Body weight and average daily gain (ADG)

Initial body weight, final body weight and ADG were similar among the groups ($P>0.05$, Table 4). Different Met and Lys levels did not affect the ADG ($P>0.05$), but the ADG was reduced with dietary Lys increased, and L1M2 was the highest one. There was no Met-Lys interaction effect on the ADG ($P>0.05$).

2.1.3 Feed conversion rate (FCR) and digestibility of DM

FCR and DM digestibility were of no significant difference among groups ($P>0.05$, Table 4). Different levels of Lys, Met and the interaction between Lys and Met had no effects on FCR and DM digestibility ($P>0.05$).

Table 4 Effects of low-protein, Lys and Met-supplemented diets on performance of blue foxes

Treatment		Initial weight/kg	Final weight/kg	ADG/(g/d)	ADFI/(g/d)	F/G	DM digestibility/%
Control		5.35±0.65	8.69±0.67	55.67±6.71	334.88±5.37^{CD}	6.37±1.40	62.34±2.98
L1M1		5.34±0.61	8.57±0.44	53.61±5.33	350.48±29.04^{BC}	6.63±0.64	58.78±2.05
L1M2		5.34±0.83	8.79±0.84	57.51±5.27	364.93±14.17^{AB}	6.51±1.20	63.48±4.96
L1M3		5.35±0.84	8.71±0.81	56.90±8.21	355.73±17.33^{BC}	6.31±0.88	58.82±2.23
L2M1		5.34±0.55	8.77±0.49	57.17±7.62	350.92±14.01^{BC}	6.31±1.56	60.06±5.72
L2M2		5.34±0.71	8.65±0.83	55.17±4.93	382.02±19.68^{A}	6.87±1.59	59.57±2.07
L2M3		5.35±0.95	8.43±0.46	51.33±4.12	359.76±16.02^{B}	6.90±1.00	60.06±5.90
L3M1		5.34±0.66	8.52±0.84	53.00±4.74	335.80±20.31^{CD}	6.92±1.01	60.55±1.72
L3M2		5.35±0.66	8.64±0.48	54.83±5.17	327.11±13.80^{D}	6.05±0.96	59.65±6.75
L3M3		5.35±0.99	8.44±0.49	51.71±10.80	323.15±24.49^{D}	6.19±0.96	61.77±2.29
L1		5.34±0.76	8.69±0.69	55.78±6.27	356.99±20.18^{A}	6.48±0.90	60.36±3.08
L2		5.34±0.73	8.61±0.59	54.56±5.55	356.31±16.57^{A}	6.73±1.38	59.90±4.56
L3		5.34±0.77	8.53±0.60	53.11±6.90	328.83±19.46^{B}	6.42±0.97	60.59±3.58
M1		5.34±0.61	8.69±0.59	55.83±5.89	341.20±20.12	6.65±1.07	59.85±3.16
M2		5.34±0.73	8.62±0.71	54.67±5.12	351.00±15.83	6.51±1.25	60.96±4.59
M3		5.34±0.92	8.53±0.58	52.94±7.71	346.21±19.21	6.47±0.95	59.99±3.47
P-value	C & T	0.113 8	0.152 4	0.395 9	0.000 1	0.898 1	0.545 7
	Lys	0.881 3	0.834 1	0.588 6	0.000 4	0.713 3	0.871 0
	Met	0.965 2	0.616 5	0.851 4	0.212 5	0.903 1	0.660 1
	Lys×Met	0.169 8	0.261 3	0.843 3	0.230 3	0.559 7	0.238 4

The values are the mean±SD, and each of the estimated requirement levels is based on 8 blue foxes. C & T means the control group compared with the other groups. In the same column of the same stage, values with different small letter superscripts mean significant difference ($P<0.05$), with different capital letter superscripts mean significant difference ($P<0.01$), and with the same or no letter superscripts mean no significant difference ($P>0.05$). The same as below.

2.2 N-balance

2.2.1 N intake

Daily N intake of the control group was significantly higher than the other groups ($P<0.01$, Table 5). N intake was affected by different levels of Lys ($P<0.01$), and L2 group was the highest one. Different levels of Met and interaction between Lys and Met did not affect the N intake ($P>0.05$), and M2 was the highest one.

2.2.2 Fecal nitrogen (FN)

FN of the control group was higher than most low-protein groups, except for L2M2, L2M3 and L3M1 ($P<0.05$). FN was affected by different levels of Lys ($P<0.05$), and L2 and L1 groups were the highest and the lowest, respectively. Different levels of Met and the interaction between Lys and Met had no effects on FN of different groups ($P>0.05$, Table 5).

2.2.3 Urine nitrogen (UN)

UN of the control group was significantly higher than all low-protein groups ($P<0.01$), and L3M2 group was the lowest one. Different Lys levels had significant effects on UN of each group ($P<0.05$), and L1 and L2 groups were the highest and the lowest, respectively. There were no significant differences of UN among different levels of Met and the interaction between Lys and Met ($P>0.05$, Table 5).

2.2.4 N retention

N retention was affected by dietary protein levels with amino acid supplements ($P<0.01$, Table 5). The Lys levels of L1 and L2 were notably higher than that of L3 ($P<0.01$). Different Met levels and the interaction between Lys and Met had no significant effects on N retention ($P>0.05$), and N retention was the highest in the M2 level.

2.2.5 N retention ratio

There were no significant differences of N retention ratio among all the treatments ($P>0.05$, Table 5), but the groups of L1M1 and L2M1 were higher than the control group. Different Lys levels had remarkable effects on N retention ratio of each group ($P<0.01$), and L1 and L2 were significantly higher than L3 ($P<0.01$). Different Met levels and the interaction between Lys and Met had no significant effects on N retention ratio ($P>0.05$), and M2 was the highest one.

2.2.6 SUN

SUN was significantly higher in L2M2 group than that in the control and the other groups ($P<0.01$, Table 5). There were significant differences among different Lys levels ($P<0.01$), and L1 was the highest one. There were no significant differences among different Met levels ($P>0.05$). The interaction between Lys and Met had significant effects on SUN of each groups ($P<0.01$).

2.3 Body length and fur characters

No significant differences were observed in body length, dry fur length and guard fur length among the control and the other low-protein groups ($P>0.05$, Table 6). Different Lys levels, Met levels and the interaction between Lys and Met also had no effects on the three items of different groups ($P>0.05$). The under fur length of the control group was significantly lower than some of the low-protein groups ($P<0.05$).

Table 6 Effects of low-protein, Lys and Met-supplemented diets on fur characteristics of blue foxes cm

Treatments		Body length	Dry fur length	Guard fur length	Under fur length
Control		67.12±2.70	107.14±2.97	7.30±1.38	4.88±0.85[a]
L1M1		64.88±1.81	105.29±2.75	7.10±0.81	5.45±0.41[ab]
L1M2		68.44±2.72	106.67±3.20	7.31±0.30	5.59±0.19[b]
L1M3		67.38±2.50	105.83±2.04	7.45±0.97	5.31±0.35[ab]
L2M1		67.25±2.43	107.43±4.03	7.26±0.77	5.29±0.26[ab]
L2M2		67.81±2.70	105.86±4.10	7.34±0.54	5.32±0.19[ab]
L2M3		68.62±2.67	105.57±2.30	7.06±0.57	5.59±0.22[b]
L3M1		67.62±1.41	102.14±4.85	7.76±1.29	5.55±0.23[b]
L3M2		67.00±1.51	106.57±2.82	7.21±0.97	5.40±0.31[ab]
L3M3		66.38±2.00	104.43±2.37	7.26±0.51	5.80±0.54[b]
L1		67.90±2.34	105.90±2.66	7.29±0.69	5.45±0.31
L2		67.90±2.60	106.29±3.47	7.22±0.62	5.40±0.22
L3		67.00±1.64	104.38±3.34	7.41±0.92	5.58±0.36
M1		67.75±1.88	104.95±3.87	7.38±0.95	5.43±0.30
M2		67.58±2.31	106.35±3.37	7.29±0.60	5.44±0.23
M3		67.46±2.39	105.25±2.23	7.26±0.68	5.57±0.37
P-value	C & T	0.831 8	0.159 4	0.992 3	0.019 5
	Lys	0.288 0	0.156 1	0.838 4	0.366 2
	Met	0.903 5	0.366 4	0.933 4	0.510 1
	Lys×Met	0.493 1	0.211 6	0.796 6	0.284 8

3 DISCUSSION

3.1 Feed intake and growth performance

Feed intake can be affected by many factors, including animal growth period, feed energy, the balance of amino acids and other nutrients, palatability, ion concentrations in intestines, and feedstuff digestibility and so on. Feed intake of some animals would be reduced if protein in diets was absent[17], and this may induce the synthesis of digestive enzyme being decreased and the body protein being broken down. Chickens and rats can slightly improve their intake to compensate the shortage of amino acids when fed the diets with inadequate amino acids[18]. The ADFI of this study was affected by different diets and may be influenced more by different dietary Lys levels. Our results suggest that supplemental Met could improve the digestibility of low-protein diets for blue foxes, which shows good agreement with Dahlman's results[19]. Our results are also consistent with the results reported in swine and poultry that free amino acids are used as efficiently as protein-bound ones, and it is therefore possible to obtain the same performance with low-protein, amino acid-supplemented diets as with high-protein controls[20-21].

3.2 N-balance

N intake was decreased with dietary protein level being reduced. It may be related to the increased dietary content of extruded corn. The results were consistent with earlier findings[22-23]. According to the experiment, N excretion in urine declined significantly ($P<0.01$) when the protein level in the diet was lowered from 27% to 19% (Table 5). The results were consistent with the earlier findings that N excretion declined noticeably along with a reduction in dietary protein in pigs[24]. N retention and N retention ratio were not significantly different among most groups. Our results showed very good agreement with the view that the lower the protein level in the diet, the better the utilization of N and the smaller the proportion excreted. A previous study showed that in 21-week-old blue foxes, N retention did not differ in relation to dietary protein level and even 15% of protein was sufficient to satisfy the animals' requirement[19]. The protein requirement of blue foxes at this age is likely to be determined mainly by hair growth, suggested by studies of Blomstedt[25-26] and also by recent studies in mink[27]. SUN of L2M2 was the highest, 9.22 g/d, which was significantly higher than that in other groups ($P<0.01$). Malmolf[28] reported that SUN concentration was related to the status of protein metabolism and the balance of amino acids in the animal, and it decreased when amino acids were well balanced[29]. Our results showed that when the dietary protein level decreased from 27% to 19% with Met and Lys supplements, the performance of blue foxes was not affected, but the N excretion was decreased. This discovery was confirmed by the present research on blue foxes which showed that there was a 36.8% reduction in N excretion when the protein level declined from about 320 to 250 g/kg (DM basis)[19].

3.3 Fur parameters

The development of hair and the quality of fur were crucially affected by dietary factors in later growing-furring period of mink in recent studies[27]. In our study, there were no significant differences observed in body length, dry fur length and guard fur length among all groups except for under fur length. The body length of L1M2 and L2M2 (68.44 and 68.62 cm, respectively) was higher compared with other groups. In an earlier study of blue foxes, low-protein diets added with Met, Lys, or both, did not affect fur quality compared with high-protein control group[30]. These results were consistent with previous studies that Met supplementation improved the overall fur quality[31] and skin length[9] of blue foxes fed the lowest protein diet, and brought them up to the level of animals in the highest protein diet group. From the result we conclude that sulfur containing amino acids associated with other essential amino acids may be a more important factor for the development of winter fur during the last months of the growing-furring period, while lysine, correspondingly, the less.

4 CONCLUSIONS

① Compared with the high protein level group, there were no significant effects on growth performance and fur quality of growing-furring blue foxes when the protein level of their diets with Lys and Met supplements was reduced from 27% to 19% on DM basis.

② Reducing the percentage of CP in dietary DM by 1 unit, on average, led to a 4.1 percentage unit decline in the daily amount of N excreted in urine. In absolute amounts, an approximately 1.72 g decline in N excretion per blue fox per day can be achieved by reducing the dietary protein level from about 27% to 19% of DM.

③ Considering growth performance and fur quality, the group L1M2 was the best one. When the dietary protein was decreased to 19% (DM basis), the diet supplemented with 0.6% (DM basis) Met and 0.3% (DM basis) lysine was optimum. The optimal dietary lysine and Met levels to attain the best growth performance and fur characters were 1 035 and 2 070 mg/d, respectively, during growing-furring period.

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低蛋白质饲粮中添加 *DL*－蛋氨酸和赖氨酸对冬毛期蓝狐生产性能、氮平衡及毛皮质量的影响

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摘 要： 本文旨在探讨低蛋白质饲粮中添加 *DL*－蛋氨酸和赖氨酸对冬毛生长期蓝狐生长性能、氮平衡和毛皮质量的影响，并研究低蛋白质饲粮中蛋氨酸和赖氨酸的最适添加量。对照组饲粮蛋白质水平为 27%（P27），低蛋白质饲粮蛋白质水平为 19%（P19），选择健康的生长后期雄性蓝狐 120 只（17 周龄左右），随机分成 10 组，每组 12 只。本试验采用 3×3 双因子交叉试验设计，有 3 个赖氨酸水平（0.3%、0.5%、0.7%）和 3 个含硫氨基酸水平（0.4%、0.6%、0.8%），试验组编号分别为 P27、L1M1、L1M2、L1M3、L2M1、L2M2、L2M3、L3M1、L3M2 和 L3M3，饲养试验试验期为 61 d（2007－10－12～2007－12－12）。结果表明，如果考虑蓝狐平均日增重、日氮沉积、氮沉积率，0.6%的蛋氨酸水平最佳；如果考虑日氮沉积和毛皮质量，0.3%和 0.5%的赖氨酸水平最佳；如果考虑氮的表观消化率、日粪氮排出量，0.3%的赖氨酸水平最佳；各处理蓝狐的毛皮质量与对照组差异不显著（$P>0.05$）。综合各项指标，L1M2（0.3% Lys×0.6% Met）组蓝狐生产性能最佳；低蛋白质饲粮中添加蛋氨酸和赖氨酸不影响冬毛期蓝狐的生产性能；应用低蛋白质饲粮降低了排泄物中氮的含量，减轻了环境污染，节约了蛋白质资源。[**动物营养学报，2010，22(6)：1614-1624**][**中文全文见《动物营养学报》网站（www. ChinaJAN. com）中文版 2010 年 22 卷 6 期**]

关键词： 低蛋白质；生长性能；毛皮性能；蓝狐

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