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Original research article

Effects of dietary methionine supplementation on growth performance of cubs, nutrient digestibility, nitrogen metabolism and serum biochemical indicators of female blue foxes (*Alopex lagopus*)

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ABSTRACT

The objective of the present study was to investigate the effects of methionine (Met) supplementation on growth performance of cubs, nutrient digestibility, nitrogen metabolism and serum biochemical parameters of female blue foxes. One hundred primiparous female blue foxes that were similar in breeding date, pedigree, age, and weight were selected for the trial. The foxes were randomly assigned to four groups ($n = 25$ each group) and fed diets supplemented with Met at 2 (Met2), 4 (Met4), 6 (Met6) and 8 g/kg (Met8), respectively, for 40 days. Our data showed that body weights at 20 and 40 d were significantly higher in the Met4 group than in the Met2 group ($P < 0.05$). The Met4 group also had the highest apparent digestibility of dry matter and crude protein compared with either the Met2, Met6, or Met8 group ($P < 0.05$). The serum Met and isoleucine (Ile) concentrations were significantly higher in the Met4 group than in the Met6 or Met8 group ($P < 0.05$). In summary, these data indicate that supplementary Met improves growth performance of cubs likely due to increased crude protein and dry matter and increased nitrogen retention of female blue foxes. The optimal amount of Met supplementation is 10 g/kg basal diet.

1. Introduction

Methionine (Met) plays a key role in metabolism as a donor of active methyl groups. It does so after conversion into S-adenosylmethionine. Following release of the active methyl group, S-adenosylmethionine is formed which may then undergo hydrolysis to produce homocysteine (D'Mello, 2003). Homocysteine is needed for the synthesis of protein in animal hair. It has been shown that Met is the most important essential amino acid for mink and fox. Dietary Met supplementation is thought to enhance protein synthesis in the body and stimulate weight gain. The results of this study indicate that diets for blue foxes should be supplemented with Met. The experimental diets contribute to improving the performance traits of foxes, and they have no adverse influence on the health status of animals. The good condition of blue foxes results in higher body weight gains and better parameters of conformation and pelt quality (Gugolek et al., 2004). According to Gugolek et al. (2012), blue foxes show an increased demand for Met. However, research concerning the effects of Met supplementation on reproduction in fur animals is lacking. One study showed that the mammary gland is a site of conversion with 20% of milk protein Met derived from the analogue (Lobley and Lapierre, 2001). Research on blue foxes by Dahlman et al. (2003) and Zhang et al. (2013) showed that the apparent digestibility of crude fat (CF) increased when Met levels was optimal. Higher digestibility might be related to the composition or activation of the digestive enzymes involved (Dahm and Jones, 1994). As-, Med The above suggest that

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the demand for sulfur amino acids in blue foxes may be higher than expected, probably due to higher productivity resulting from lactating and personal digestion and metabolism. The main objective of this study was to determine the effect of increased dietary Met supplementation on reproduction performance of blue foxes.

2. Materials and methods

The experiment was conducted at the Fur Animal Breeding Base of the Institute of Special Economic Animal and Plant Sciences, the Chinese Academy of Agricultural Sciences (44.02_N, 126.15_E) in the northeast of China. Animals were housed outdoors individually in conventional cages (100 cm long × 80 cm wide × 80 cm high), with an addition of nest boxes (60 cm long × 50 cm wide × 42 cm high). The animals used in this experiment were managed according to the requirements of the National Experimental Animals Protection Law, and the animal protocol was approved by Animal Use and Care committee. Met used in this study was supplied by Sumitomo Chemical Co., Ltd.

2.1. Animals and experimental design

One hundred primiparous female blue foxes that were similar in breeding date, pedigree, age, and weight were selected for the trial. The blue foxes were randomly assigned to four groups ($n = 25$ each group). They were fed diets which were supplemented with Met at 2 (Met2), 4 (Met4), 6 (Met6) and 8 g/kg (Met8), respectively, for 40 days. The animals had free access to feed and water. The main protein sources of the diets were of good protein quality origin: Peru fish meal, bone meat meal, corn protein meal and soybean meal (Table 1). All diets met the nutrient requirements of suckling foxes (NRC 1982). Daily feed consumption was recorded for each animal.

2.2. Growth performance of cubs

The body weight of cubs was recorded right after they were born, prior to feeding, accurate to 1 g, using digital scale. Then the cubs were weighed every ten days. The duration of the lactation period was calculated as from birth to forty days of age.

Table 1
Ingredient and chemical composition of basal diet.

Ingredient, %	Amount	Chemical composition[2]	Amount
Extruded corn	18.4	Metabolizable energy, MJ/kg DM	20.35
Soybean meal	23.0	Dry matter, %	94.88
Meat and bone meal	24.0	Crude protein, % of DM	40.56
Fish meal	18.0	Crude fat, % of DM	13.86
Corn gluten meal	4.0	Crude carbohydrate, % of DM	30.18
Cheese meal	1.0	Ash, % of DM	10.28
Soybean oil	10.0	Lysine, % of DM	1.96
Methionine	0.2	Methionine, % of DM	0.57
Lysine	0.2	Cysteine, % of DM	0.7
NaCl	0.2	Calcium, % of DM	2.99
Premix[1]	1.0	Phosphorus, % of DM	1.85
Total	100.0		

[1] Contained the following per kilogram of premix: vitamin A, 1,000,000 IU, vitamin D, 3,200,000 IU, vitamin E 18,000 IU, vitamin B_1, 600 mg, vitamin B_2, 1,000 mg, vitamin B_6, 1,000 mg, vitamin B_{12}, 10 mg, vitamin K_3, 200 mg, vitamin C, 50,000 mg, nicotinic acid 4,000 mg, pantothenic acid 4,000 mg, biotin, 30 mg, folic acid, 300 mg, choline, 60,000 mg, Fe, 10,000 mg, Cu, 800 mg, Mn, 2,000 mg, Zn 8,000 mg, I 50 mg, Se, 20 mg, Co, 50 mg.
[2] Metabolizable energy was calculated according to NRC (1982), other values were actual analysis.

2.3. Digestion and nitrogen metabolism experiment

On d 20 of the feeding period, about 20 d before weaning, eight animals from each treatment were selected randomly and transferred to and housed individually in metabolic crates that allowed separation of urine and feces to determine nutrient digestibility and N balance. The digestive experiment lasted for 3 d and the excretions were collected each day. Feed samples were sampled for further analysis. Before feeding, daily fecal output was collected, and weighed, and then 10% of the output was kept for subsequent analysis. Urine samples were collected in plastic containers, weighed, and recorded, and then 20% of the urine samples were kept to evaluate N retention. To avoid ammonia evaporation from the urine, 10 mL sulfuric acid for every 100 mL urine was added to the urine collection bottles and five drops of methylbenzene were added to prevent decaying. All samples were dried at 55°C in a forced-air oven to reach a constant weight, air equilibrated, and then ground to pass 1 mm screen and kept for further analysis. All samples were stored at −20°C before chemical analysis.

2.4. Chemical analysis

The chemical composition of the diets and feces were analyzed by standard methods as reported elsewhere. Dry matter (DM) was determined by drying feed or fecal samples at 105°C to constant weight (method 2001.12, AOAC). Nitrogen was measured by FOSS Kjeltec 8400, crude protein in the feed and feces was calculated as N × 6.25, and fat (AOAC Procedure 7.052) was determined by employing the instruments Kjeltec Auto 1030 Analyser and Soxtec 1043 from Tecator Comp. Calcium was estimated by the titrimetric method, number 6.011 of AOAC, and phosphorus by a colorimetric method (method 968.08, AOAC). The metabolisable energy (ME) content was calculated on the basis of the concentrations of crude protein, crude fat and carbohydrates; digestibility coefficients were calculated from Danish standard values for the individual feedstuffs; and the following values for the content of ME per unit of digestible nutrients (kJ/g) were used: crude protein, 18.8; ether extract, 39.8; and crude carbohydrates, 17.6. Nutrient digestibility and nitrogen metabolism were calculated by established methods as described elsewhere. The analyzed chemical compositions of the diets are shown in Table 1.

2.5. Blood samples

Eight female blue foxes were selected randomly from each group and their blood were taken immediately after anesthesia on d 30 of the experiment. Blood samples were collected in non-heparinized tube. The activities of serum alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and the contents of serum total protein (TP), albumin (ALB), globulin (GLOB), serum urea nitrogen (SUN), (the kits above were supplied by ZhongshengBeikong Biotechnology LLC, Beijing, P. R. China) were analyzed by an automatic biochemistry analyzer (Hitachi 7020, Hitachi High Technologies, Inc., Ibaraki, Japan).

2.6. Statistical analysis

Data were analyzed using the one-way ANOVAG Procedure and groups means were used Duncan's multiple comparison of SAS (SAS Institute, Cary, NC, USA, 2002). The following model was used:

$$Yij = \mu + di + \varepsilon ij,$$

Where Yij is the observation; μ is the general mean; di is the effect of Met level ($i = 1, 2, 3, 4$); εij is the random error.

Data was mean ± SD.

3. Results

3.1. Growth performance of cubs

The effects of dietary Met supplementation on growth performance of cubs are shown in Table 2. Body weights at birth, 10, 30 d after birth were not significant different among groups supplemented with Met ($P > 0.05$). However, body weights at 20 and 40 d were significantly higher in the Met4 group than in the Met2 group ($P < 0.05$). The Met2 group had the lowest individual body weight at 20 d compared with the Met4, Met6, and Met8 groups ($P < 0.05$), while Met4 group had the highest value.

Table 2
Effects of dietary Met supplementation on growth performance of cubs.

Item	Met2	Met4	Met6	Met8	*P*-value
Average litter number	10.00 ± 2.41	11.00 ± 2.09	11.33 ± 3.50	10.50 ± 2.39	
BW at birth, g	81.55 ± 12.03	80.90 ± 12.35	80.53 ± 9.09	78.27 ± 11.70	0.538
BW at 10 d, g	183.83 ± 26.28	217.21 ± 32.66	191.62 ± 42.91	174.86 ± 29.45	0.183
BW at 20 d, g	348.65 ± 46.25^{b}	437.69 ± 45.47^{a}	389.49 ± 84.66^{ab}	377.22 ± 76.25^{ab}	0.158
BW at 30 d, g	569.47 ± 114.74	718.65 ± 120.15	642.21 ± 137.33	635.08 ± 103.63	0.204
BW at 40 d, g	875.46 ± 114.74^{b}	1087.33 ± 188.78^{a}	992.21 ± 142.95^{a}	933.32 ± 111.23^{a}	0.100

Met2 = 2 g Met/kg diet; Met4 = 4 g Met/kg diet; Met6 = 6 g Met/kg diet; Met8 = 8 g Met/kg diet.
^{a,b} Within a row, means with different superscripts differ ($P < 0.05$); $n = 25$ treatments.

3.2. Nutrition digestibility

The effects of dietary Met supplementation on nutrient digestibility of female blue foxes are shown in Table 3. The digestibility of DM and CP was significantly higher in the Met4 group than in the Met2 group ($P < 0.05$). No significant difference was observed in the apparent digestibility of CF among groups ($P > 0.05$).

3.3. Nitrogen metabolism

The effects of dietary Met supplementation on nitrogen metabolism of female blue foxes are shown in Table 4. There were no significantly differences in N intake, urinary N, or nitrogen retained among the four treatments ($P > 0.05$). Fecal N in the Met8 group was significantly lower than in the Met2 group ($P < 0.01$).

3.4. Serum free amino acid

The effects of dietary Met supplementation on serum free amino acid concentrations of female blue foxes are shown in Table 5. The Met and Ile concentrations were significantly higher in the Met4 group than in the Met6 and Met8 groups ($P < 0.05$). The differences were not significant in other amino acid concentrations among groups, while the highest values were seen in Met4 group ($P > 0.05$).

Table 3
Effects of dietary Met supplementation on nutrient digestibility (%) of female blue foxes.

Item	Met2	Met4	Met6	Met8	*P*-value
DM	74.97 ± 3.47^{a}	78.91 ± 4.07^{b}	77.27 ± 6.18^{b}	77.07 ± 2.71^{b}	0.010
CP	76.36 ± 3.70^{a}	81.11 ± 3.49^{b}	79.01 ± 6.00^{b}	80.21 ± 2.67^{b}	0.007
EE	93.11 ± 0.99	95.23 ± 1.90	94.16 ± 2.18	93.06 ± 1.54	0.014

DM = dry matter; CP = crude protein; EE = crude fat.
Met2 = 2 g Met/kg diet; Met4 = 4 g Met/kg diet; Met6 = 6 g Met/kg diet; Met8 = 8 g Met/kg diet.
^{a,b} Within a row, means with different superscripts differ ($P < 0.05$); $n = 8$ treatment.

3.5. Serum biochemical parameters

The effects of dietary Met supplementation on blood serum biochemical parameters of female blue foxes are shown in Table 6. No significant differences were observed in serum ALT and AST activity among Met supplemented groups ($P > 0.05$). The serum concentrations of ALB and TP increased with the Met supplementation initially and then decreased with higher amount of Met supplementation ($P < 0.05$), and the highest values for these parameters were seen in the Met4 group.

4. Discussions

Dietary Met supplementation is thought to enhance protein synthesis in the body and stimulate weight gain. In the nutrition of fur-bearing animals, a particularly important role is played by sulfur amino acid considered limiting (Dahlman et al., 2002a, 2000b, 2003). The dietary sulfur amino acids content affected milk production, growth rate and feed efficiency in rabbits (Taboada et al., 1996). One study indicated that the mammary gland is a site of conversion with 20% of milk protein methionine derived from the analogue (Lobely and Lapierre, 2001). In our study, individual body weight at 40 d is significantly higher in the Met4 group than in other groups. As in actual production, before group up, the cubs gain nutrients by breast milk up to 25 d of age, after that they obtain nutrients partly by themselves and partly from their mothers. The Met is the important factor affecting milk yield, milk protein content during this period.

The dry matter intake of fox is usually affected by the palatability and energy levels of the diet (Wu et al., 2014). Met supplementation in diets improved the apparent digestibility of CP and DM. Similar results were obtained by Blaza et al. (1982) in growing dogs where digestibility of CP and DM improved significantly when Met supplementation was increased in the diet. In this study, the apparent digestibility of DM and CP were highly dependent on the Met supplementation of the diet. The influence of Met on the apparent digestibility of CP has been studied in great detail. Research on blue foxes by Dahlman et al. (2003) and Zhang et al. (2013) showed that the apparent digestibility of CP increased when Met supplementation was optimal. Higher digestibility might be related to the composition or activation of the digestive enzymes involved (Dahm and Jones, 1994). As a precursor of other amino acids-cystine and taurine, Met could have been a limiting factor in the synthesis or function of an enzyme essentially associated with the degradation processes in question.

On N retention, no previous data in the breeding season of blue foxes is available. The variation between the animals was high and, accordingly, no significant differences between the diets could be found. The high requirement for SAA, Met in particular, is in line with their effects on developing hair and fur quality, and also on

Table 4
Effects of dietary Met supplementation on nitrogen metabolism of female blue foxes.

Item	Met2	Met4	Met6	Met8	*P*-value
N intake, g/d	29.46 ± 3.92	29.92 ± 9.94	26.10 ± 3.49	22.95 ± 5.18	0.178
Fecal N, g/d	8.11 ± 1.23^{Aa}	5.84 ± 2.78^{ABab}	5.32 ± 1.87^{ABb}	4.62 ± 1.47^{Bb}	0.029
Urinary N, g/d	7.79 ± 1.38	8.16 ± 1.58	7.53 ± 1.69	7.23 ± 2.36	0.439
Nitrogen retained, g/d	13.56 ± 2.53	15.93 ± 5.97	13.25 ± 4.11	11.10 ± 3.92	0.314

^{a,b} Within a row, means with different superscripts differ ($P < 0.05$); ^{A,B}With in a row, means with different capital letter superscripts differ ($P < 0.01$); $n = 8$ treatment.
Met2 = 2 g Met/kg diet; Met4 = 4 g Met/kg diet; Met6 = 6 g Met/kg diet; Met8 = 8 g Met/kg diet.

Table 5
Effects of dietary Met supplementation on serum free amino acid concentrations (nmol/ml) of female blue foxes.

Item	Met2	Met4	Met6	Met8	*P*-value
Supplement AA					
Met	55.29 ± 14.68^{ab}	60.06 ± 14.16^{a}	48.39 ± 10.42^{b}	47.50 ± 2.92^{b}	0.035
AA associated with Met metabolism					
Thr	195.27 ± 28.74	202.05 ± 24.21	190.58 ± 29.29	197.62 ± 11.58	0.524
Gly	396.31 ± 60.87	422.20 ± 65.86	412.70 ± 82.19	396.22 ± 64.47	0.749
Ser	345.24 ± 29.05	345.81 ± 49.67	332.74 ± 36.21	339.79 ± 32.31	0.079
Urea Cycle AA					
Arg	235.28 ± 54.12	242.64 ± 14.42	214.37 ± 75.19	224.68 ± 21.55	0.417
Branched-chain AA					
Ile	92.46 ± 21.65^{ab}	119.97 ± 25.14^{a}	85.41 ± 22.87^{b}	80.66 ± 10.96^{b}	0.052
Leu	124.20 ± 19.76	132.67 ± 22.68	127.57 ± 13.92	120.31 ± 24.27	0.333
Val	196.86 ± 27.65	248.94 ± 37.57	203.64 ± 29.87	193.18 ± 22.16	0.205
Remaining indispensable AA					
Lys	166.56 ± 36.43	189.46 ± 50.69	176.73 ± 55.96	168.09 ± 10.99	0.362
Phe	84.93 ± 17.37	99.24 ± 17.63	86.50 ± 19.42	84.79 ± 7.79	0.270
His	96.12 ± 9.27	100.44 ± 9.72	85.47 ± 13.91	84.36 ± 8.60	0.326
Tyr	56.10 ± 5.40	60.68 ± 11.67	54.20 ± 7.93	55.04 ± 4.43	0.064
Remaining dispensable AA					
Glu	113.79 ± 41.04	117.18 ± 32.35	109.48 ± 16.18	112.72 ± 22.18	0.258
Ala	500.01 ± 34.28	532.26 ± 56.94	490.26 ± 104.04	490.14 ± 22.66	0.049
Asp	14.78 ± 5.62	16.86 ± 2.18	14.69 ± 3.74	14.63 ± 0.84	0.056
Pro	183.36 ± 29.95	187.43 ± 37.22	188.96 ± 39.31	189.10 ± 18.86	0.159

^{a,b} Within a row, means with different superscripts differ ($P < 0.05$); $n = 8$ treatment.
Met2 = 2 g Met/kg diet; Met4 = 4 g Met/kg diet; Met6 = 6 g Met/kg diet; Met8 = 8 g Met/kg diet.

growth (Dahlman et al., 2002). In our study, the protein level is relatively high, excessive Met supplementation can inhibit the growth of animals (Abe et al., 2000).

The Met and Ile concentrations were significantly higher in the Met4 group than in the Met6 or Met8 group. As pointed out by Susenbeth and Lucanus (2005), the piglets fed diets supplemented with Tyr were able to increase serum tryptophan concentration. When the amino acid in diets cannot meet the need of the animal, serum free amino acids concentration is low, satisfying the need of animals can decrease the serum free amino acid concentration. This is due to the amino acids in different tissues and organs in the body of the diversion ratio tend to be more reasonable and improve the utilization efficiency of the original.

In the present study, serum urea nitrogen was not affected by dietary Met levels, a finding that is consistent with the report by Lorek et al. (2005) in that synthetic Met had no effect on serum urea nitrogen levels, which has also been found in other reports. However, a study has been found that adding Met can reduce serum urea nitrogen in sheep (Wright and Loerch, 1988). What was certain is that a good amino acid balance has a beneficial influence on blood serum biochemical parameters of female blue foxes. The observed changes in their activity showed that Met supplementation contributed to regulating metabolism in the liver and supported liver function in foxes. Comparable results were obtained by Fau et al. (1987) in other carnivorous animals, namely cats, and by Rana et al. (2000) in rats.

Table 6
Effects of dietary Met supplementation on blood serum biochemical parameters of female blue foxes.

Item	Met2	Met4	Met6	Met8	*P*-value
ALT, U/L	144.15 ± 25.41	162.66 ± 33.47	141.28 ± 13.14	144.34 ± 5.48	0.202
AST, U/L	67.25 ± 15.86	77.31 ± 18.48	64.29 ± 10.53	64.62 ± 13.54	0.096
SUN, mmol/L	8.64 ± 1.09	8.57 ± 1.42	8.66 ± 0.91	8.67 ± 0.89	0.138
ALB, g/L	32.04 ± 3.99^{ab}	36.08 ± 5.04^{a}	31.22 ± 0.63^{ab}	30.32 ± 0.70^{b}	0.060
TP, g/L	70.40 ± 1.04^{a}	76.89 ± 6.94^{ab}	68.66 ± 14.73^{ab}	64.41 ± 2.41^{b}	0.061

^{a,b} Within a row, means with different superscripts differ ($P < 0.05$); $n = 8$ treatment.
ALT = alanine transaminase; AST = aspartate aminotransferase; SUN = serum urea nitrogen; ALB = albumin; TP = total protein.
Met2 = 2 g Met/kg diet; Met4 = 4 g Met/kg diet; Met6 = 6 g Met/kg diet; Met8 = 8 g Met/kg diet.

5. Conclusions

The results of this feeding trial indicate that Met plays an important role in increasing growth performance of cubs. Our data also demonstrate that Met supplementation improves apparent digestibility of dry matter and crude protein in female blue foxes. The optimal dietary Met supplementation is 10 g/kg basal diet.

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