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Asian Journal of Animal and Veterinary Advances



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## **Supplemental Dietary Methionine Affects the Pelt Quality and Nutrient Metabolism of Raccoon Dogs (*Nyctereutes procyonoides*)**

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### **ABSTRACT**

Essential amino acids, particularly those containing the element sulfur, are required by small canids to produce a high quality pelt. The experiments were designed to estimate the effect of a diet supplemented with the sulfur-containing amino acid methionine on the nutrient metabolism and pelt quality of raccoon dogs (*Nyctereutes procyonoides*). Seventy-five male raccoon dogs with similar body weights were randomly assigned to five dietary groups of 15 each during the winter fur growth period. The diet for the control group contained 24% protein while the diets for groups 1 to 4 contained 20% protein plus 0.15, 0.35, 0.55 and 0.75 g methionine per 100 g dry matter, respectively, for a 60-day period. As a result, the body weights in group 4 were clearly reduced compared to the other groups ( $p < 0.05$ ). Dry matter intake in the control group was significantly higher than for groups 1 and 2 ( $p < 0.05$ ), but was similar to groups 3 and 4. Although, serum methionine level was similar among all groups, group 2 showed significantly higher total protein in serum ( $p < 0.05$ ) than the control group; while serum urea nitrogen in group 2 was lower than that of groups 3 or 4 ( $p < 0.05$ ). The pelt length in the control group and in group 2 was significantly longer than the other groups. The density of guard hair and fiber in the controls and groups 1 and 2 was remarkably higher compared with that in groups 3 and 4 ( $p < 0.05$ ). These results suggest that a certain amount of supplemental methionine may reduce the total protein requirement in the diet without affecting the pelt quality of raccoon dogs.

**Key words:** Raccoon dogs, pelt performance, methionine, nutrient metabolism, dry matter intake, crude protein

### **INTRODUCTION**

The Ussuriensis raccoon dog (*Nyctereutes procyonoides ussuriensis*), also known as the one of raccoon dog, has unique pelt characteristics. The hair color at the nape is black and the body is mostly yellowish-brown or a little saffron, similar to raccoons (*Procyon lotor*). Recently, raccoon dogs have been raised in captivity in China for the economic value of its pelt and their numbers have increased rapidly. To date, the population of farmed raccoon dogs has reached about 13 million in China, mainly in the northeast and eastern coast area. It has been shown that methionine is the most important essential amino acid for mink and fox (Dahlman *et al.*, 2002) and like the raccoon dog, these are canine animals. Sulfur-containing amino acids are involved in the methylation process, participate in the regulation of oxidative status and may also affect metabolism and cell

functions (Tesseraud *et al.*, 2009). Supplemented methionine had significant effect on feather quality of broiler chicken (Halder and Roy, 2007). Al-Maya (2006) also reported that added methionine rather than total protein content led to better immune response in broiler chicks. Studies have reported that feed intake, weight gain and feed efficiency are significantly maximized with addition of methionine (Pillai *et al.*, 2006; Ahmed and Abbas, 2011) while increasing feed intake only showed no significant difference (Saki *et al.*, 2007). Either insufficient or too much sulfur-containing amino acids in the diet of these animals could lead to certain functional disorders (Takahashi and Horiguchi, 1991). Since excessive protein will be deaminated, transformed into fatty acid and deposited as fat, diet with high protein may result in slow weight-gain and decreased feed-to-meat conversion (Parvin *et al.*, 2010). Thus, the addition of a certain amount of methionine in the conventional diet of the raccoon dog may affect nutrient metabolism and pelt quality. This study was aimed to evaluate the effect of protein-reduced diet with variable amounts of supplemental methionine on the nutrient metabolism and pelt quality of raccoon dog.

## MATERIALS AND METHODS

All animal procedures were approved by The Wild Animal and Plant Subcommittee of the China Association of Agriculture Science Societies (WAPS CAASS) and all experiments were performed according to the animal health and well-being regulations.

**Animal care and management:** Seventy-five, 4-month old male raccoon dogs of similar body weight ( $5.13 \pm 0.25$  kg) were randomly assigned to five dietary treatments as described above. All animals were held and fed in individual cages (100 L $\times$ 70 W $\times$ 70 H cm<sup>3</sup>). The feeding time and amount were recorded every day. Animals received water ad libitum and were immunized regularly. The experimental period encompassed the winter-fur-growing season, from the end of September to the end of November, 2008, a total of 60 days.

**Experimental design and rations:** In accordance with the National Research Council (1982), the basic protein level in the experimental diets was 20% crude protein with supplemented methionine 0.15, 0.35, 0.55 and 0.75% and lysine level of 0.65 g/100 g Dry Matter (DM).

The control diet consisted of conventional rations containing 24% crude protein, 0.45% lysine and 0.15% methionine. The actual methionine component (supplemented plus existing methionine) in the control diet and four experimental diets (groups 1 to 4) was 0.6, 0.5, 0.7, 0.9, 1.1 g/100 g DM, respectively and lysine component practically was 1.59 g/100 g DM in all diets. The diets were powder rations consisting of extruded corn, soybean meal, meat, bone meal and fish meal. The complete diet components are shown in Table 1. One part diet powder was mixed with three parts water.

**Sample collection and handling:** The raccoon dogs were weighed on days 30 and 58 after the start of experimental feeding and the average weight was calculated. The total feces for 3 days were collected as a measure of the apparent macronutrient digestibility on day 45 of the experiment. During the fecal collection period, total feces were removed from the plate under the cages, weighed, homogenized and sampled for laboratory analysis. Feed and fecal samples were dried at 65°C for 48 h, ground in a mill to pass a 2 mm screen and analyzed. Blood samples from raccoon dogs fasted for 12 h were collected via hind leg puncture after 40 days and refrigerated overnight. After centrifugation for 20 min at 5000 $\times$ g, serum was collected and frozen at -20°C for

Table 1: Composition (%) of experimental basal diets (dry matter) and analysis of nutrient content of diets

Ingredient (%)	Control	Experimental groups receiving supplemental methionine (g/100 g DM)			
		Group 1	Group 2	Group 3	Group 4
Extruded coru	37.750	47.440	47.440	47.440	47.440
Soybean meal	05.500	4.750	04.750	04.750	04.750
Meat and bone meal	12.000	7.000	07.000	07.000	07.000
Distillers dried grains with soluble	09.500	9.500	09.500	09.500	09.500
Coru gluten feed	15.000	16.500	16.500	16.500	16.500
Fish meal	13.500	6.500	06.500	06.500	06.500
Chicken oil	04.650	6.010	06.010	06.010	06.010
Lysine	00.450	0.650	00.650	00.650	00.650
Methionine (added)	00.150	0.150	00.350	00.550	00.750
Additives <sup>1</sup>	01.000	1.000	01.000	01.000	01.000
Salt	00.500	0.500	00.500	00.500	00.500
Total	100.000	100.000	100.000	100.000	100.000
<b>Nutrient requirements of raccoon dogs in winter during the fur-production period<sup>2</sup></b>					
Metabolizable energy ME (MJ kg <sup>-1</sup> )	13.96				
Crude protein CP (g/100 g DM)	24.58				
Crude fat C fat (g/100 g DM)	9.29				
Calcium Ca (g/100 g DM)	1.00				
Phosphor P (g/100 g DM)	0.72				
Lysine Lys (g/100 g DM 2)	1.60				
Methionine Met (g/100 g DM 2)	0.91				

<sup>1</sup>Additives provided per kilogram of diet: vitamin A, 198 mg; vitamin D<sub>3</sub>, 32.5 mg; vitamin E, 3000 mg; vitamin K<sub>3</sub>, 40 mg; vitamin B<sub>1</sub>, 300 mg; vitamin B<sub>2</sub>, 460 mg; vitamin B<sub>6</sub>, 180 mg; vitamin B<sub>12</sub>, 4 mg; vitamin C, 9000 mg; folic acid 50 mg; niacin acid 2 000 mg; pantothenic acid 1 500 mg; biotin 42 mg; choline chloride 60 mg; Fe 3 800 mg; Zn 3200 mg; Mn 1600 mg; I50 mg; Se 10 mg; Cu 500 mg.

<sup>2</sup> Lysine and methionine are feed-grade, content>99.00%. <sup>3</sup> According to Nutrient Requirements of National Research Council (1982)

later analysis. At the end of the experiment, all raccoon dogs were sacrificed and the pelts were collected 30 min later for evaluation of pelt properties.

**Chemical analysis:** Feed and fecal samples were analyzed for DM, crude protein and crude fat using methods of the Association of Official Analytical Chemists (AOAC, 1990). Crude protein was determined using a Gerhardt Kjeldahl determination device, the Protein Distillation Unit B-324 (Büchi, Swiss). Crude fat was determined using an ether extracting method. Total protein and serum albumin level were measured in serum samples. Urea nitrogen was detected using an assay kit from Nanjing Jiancheng Science and Technology and amino acid was analyzed by an Automatic Amino Acid Analyzer L-8800 (Hitachi, Japan).

**Pelt properties:** Pelt weight and the length from the nose tip to the tail base were measured. The length of guard hair and under hair was also recorded. Hair density was counted under a microscope on a vertical section of skin with a net micrometer. The degree of under hair regularity was defined as the average length of under hair at the neck, dorsocentral and lumbosacral regions.

**Statistical analysis:** For multiple comparisons, the one-way Analysis of Variance (ANOVA) was performed and the differences between the means were compared using Duncan's multiple-range test and SAS 8.2 (SAS, Cary, NC) software. Treatment effects were considered significant at p<0.05 and trends were observed at p<0.10, unless otherwise noted.

## RESULTS

**Body weight, Dry Matter Intake (DMI) and apparent nutrient digestibility:** At the end of the experiment, the body weight of the controls and groups 1, 2, 3 remained at similar levels, while the body weight of group 4 decreased significantly compared to the control ( $p < 0.05$ ; Table 2).

Dry Matter Intake (DMI) was significantly higher in the control than in the experimental groups ( $p < 0.05$ ), this led to the higher weight in the control group compared with other groups. Dry matter digestibility (DMD), Crude Protein Digestibility (CPD) and Crude Fat Digestibility (CFD) were not significantly different among the control and experimental groups ( $p > 0.05$ ). According to the results, apparent nutrient digestibility was not affected by either a high protein diet mixed with low methionine, or a low protein diet mixed with high methionine.

**Blood parameters:** Table 3 shows the effect of different levels of methionine on blood parameters. The serum total protein in group 2 was the highest among all groups and significantly higher than the control ( $p < 0.05$ ). Serum albumin was similar in all treatment groups. Blood Urea Nitrogen (BUN) decreased markedly in group 2 as compared with groups 3 or 4 ( $p < 0.05$ ) but was not significantly different between the control and group 1. The methionine concentration in serum remained at a similar level among all groups but the concentration of lysine increased considerably in group 4 when compared with the control ( $p < 0.05$ ) and other groups ( $p < 0.05$ ).

**Pelt properties:** The body length and pelt weight was similar in all groups (Table 4). However, the pelt length was longer in the control and group 2 than that in groups 1, 3 and 4 ( $p < 0.05$ ). Density of guard hair and fiber in the control and groups 1 and 2 was obviously superior compared to groups 3 and 4 ( $p < 0.05$ ). The degree of under fur regularity in group 2 was clearly superior when compared with group 4 ( $p < 0.001$ ). These results indicated that in the fur-growing period for raccoon dogs the diet of group 2 which contained 0.35 g methionine/100 g DM (total 0.7 g methionine/100 g DM), improved pelt quality.

Table 2: Effect of methionine level on body weight, DMI, DMD, CPD, CFD of raccoon dogs (Mean±SD)

Items	Control	Group 1	Group 2	Group 3	Group 4
Body weight (kg)	7.57±0.93 <sup>a</sup>	7.02±0.87 <sup>ab</sup>	7.26±0.82 <sup>ab</sup>	6.79±0.96 <sup>ab</sup>	6.90±0.88 <sup>b</sup>
DMI (kg/day)	0.34±0.03 <sup>a</sup>	0.28±0.07 <sup>bc</sup>	0.26±0.12 <sup>bc</sup>	0.32±0.06 <sup>ab</sup>	0.28±0.05 <sup>abc</sup>
DMD (% DM)	0.68±0.04 <sup>a</sup>	0.73±0.04 <sup>a</sup>	0.77±0.14 <sup>a</sup>	0.75±0.06 <sup>a</sup>	0.75±0.05 <sup>a</sup>
CPD (% DM)	0.67±0.05 <sup>a</sup>	0.69±0.06 <sup>a</sup>	0.73±0.16 <sup>a</sup>	0.67±0.08 <sup>a</sup>	0.68±0.07 <sup>a</sup>
CFD (% DM)	0.77±0.05 <sup>a</sup>	0.80±0.11 <sup>a</sup>	0.69±0.10 <sup>a</sup>	0.74±0.06 <sup>a</sup>	0.78±0.05 <sup>a</sup>

DMI: Dry matter DMD; Dry matter digestibility, CPD; Crude protein digestibility, CFD; Crude fat digestibility. <sup>a, b, c</sup>In the same row, values with same superscripts mean no significant difference ( $p > 0.05$ ), with different letter superscripts mean significant difference ( $p < 0.05$ )

Table 3: Effect of methionine level on the serum index

Item	Control	Group 1	Group 2	Group 3	Group 4
Total protein (g dL <sup>-1</sup> )	7.65±1.28 <sup>a</sup>	9.94±1.42 <sup>ab</sup>	10.48±0.82 <sup>b</sup>	8.88±0.84 <sup>ab</sup>	9.00±1.17 <sup>ab</sup>
Serum albumin (g L <sup>-1</sup> )	39.38±7.25 <sup>a</sup>	35.65±5.64 <sup>a</sup>	37.86±9.54 <sup>a</sup>	42.64±5.77 <sup>a</sup>	45.29±9.36 <sup>a</sup>
Urea nitrogen (mg L <sup>-1</sup> )	5.50±0.48 <sup>ab</sup>	5.39±0.96 <sup>ab</sup>	4.64±1.16 <sup>a</sup>	5.75±1.14 <sup>b</sup>	5.80±1.04 <sup>b</sup>
Methionine (mg L <sup>-1</sup> )	0.27±0.05 <sup>a</sup>	0.28±0.04 <sup>a</sup>	0.26±0.03 <sup>a</sup>	0.27±0.05 <sup>a</sup>	0.32±0.07 <sup>a</sup>
Lysine (mg L <sup>-1</sup> )	0.80±0.14 <sup>a</sup>	0.79±0.15 <sup>a</sup>	0.77±0.09 <sup>ab</sup>	0.86±0.02 <sup>a</sup>	0.64±0.09 <sup>a</sup>

<sup>a, b, c</sup>In the same row, values with same superscripts mean no significant difference ( $p > 0.05$ ), with different letter superscripts mean significant difference ( $p < 0.05$ )

Table 4: Effect of methionine level on pelt properties

Item	Control	Group 1	Group 2	Group 3	Group 4
Body length (cm)	65.57±2.59 <sup>ab</sup>	64.57±3.32 <sup>ab</sup>	60.77±2.59 <sup>ab</sup>	64.78±3.12 <sup>ab</sup>	67.14±2.77 <sup>a</sup>
Pelt length (cm)	94.43±3.72 <sup>a</sup>	93.58±7.13 <sup>ab</sup>	94.65±5.12 <sup>a</sup>	90.00±6.49 <sup>b</sup>	93.07±2.34 <sup>ab</sup>
Pelt weight (g)	665.67±83.92 <sup>a</sup>	675.71±89.31 <sup>a</sup>	678.08±68.54 <sup>a</sup>	594.17±92.11 <sup>a</sup>	632.86±66.15 <sup>ab</sup>
Density of guard hair and fiber (root/cm <sup>-2</sup> )	1839.57±597.20 <sup>ab</sup>	1957.76±572.36 <sup>a</sup>	1883.16±675.70 <sup>ab</sup>	1303.64±187.97 <sup>c</sup>	1387.51±277.49 <sup>bc</sup>
Average length of under hair (cm)	5.70±0.41 <sup>bc</sup>	6.07±0.33 <sup>a</sup>	5.86±0.36 <sup>ab</sup>	5.62±0.53 <sup>bc</sup>	5.34±0.78 <sup>c</sup>

<sup>a, b, c</sup>In the same row, values with same superscripts mean no significant difference (p>0.05), with different letter superscripts mean significant difference (p<0.05)

## DISCUSSION

Pelt quality is the most important factor affecting pelt price. Pelt quality is determined by its length and color, the density and length of guard hair and fiber and cleanliness. Both pelt and animal weight closely correlate with pelt quality, indicating that the estimated pelt quality, in fact, can be derived directly from its weight (Korhonen and Harri, 1985). Hair growth and hair properties in pelts are very dependent on dietary protein during the period from 22 weeks of age until pelting, irrespective of the supply in the preceding periods (Rasmussen and Borsting, 2000). Since sulfur-containing amino acids enhance keratin biosynthesis, methionine is the most important essential amino acid required in the diet of an animal farmed for its fur. However, too much methionine intake may cause an amino acid imbalance. In this study, the pelt properties of raccoon dogs fed with 0.15 to 0.35 g supplemental methionine/100 g DM diets were superior to those of the other dietary groups, suggesting that a suitable supplemental methionine could improve pelt quality.

Dry matter intake which affects the animals' growth and performance greatly, can be influenced by many factors including the number of calories, the balance of amino acids and other nutrients, palatability, the concentration of ions in the intestines and food digestibility. In this study, dry matter intake was higher in the control and the possible reason might be that these rations consisted of more fish meal and high protein and therefore had better palatability, leading to the higher weight than others. The present results confirmed the previous study that feed intake increase will lead to weight gain accordingly (Abdelrahman, 2009). Apparent digestibility is the percent of absorbed feed relative to total intake. Theoretically, when the apparent nutrient digestibility increases, the animal's performance will improve (Khampa *et al.*, 2009). In this study, although DMI in the control group was higher than in the other groups, the apparent nutrient digestibility, body weight and body length were similar. These findings are in line with the findings in layer chicken by Halder and Roy (2007). Therefore, rations containing 20 g protein plus 0.355% methionine per 100 g DM did not make any significant difference with the conventional diet with 24 g protein per 100 g DM.

Levels of BUN are a measure of an organism's protein metabolism. There was a significant negative correlation between the amino acid balance in diets and BUN (Coma *et al.*, 1995). The decrease of BUN indicates enhanced nitrogen recovery (Owusu-Asiedu *et al.*, 2003). In this study, the BUN in groups supplied with 0.55 and 0.75 g methionine/100 g DM was higher than in the others. This may be due to the degradation of excessive methionine to ammonia, which was subsequently absorbed into the blood through the gastric and intestinal wall. Serum total protein is the result of absorption by the intestines and synthesis by the liver and includes mostly albumin and globulin, serum total protein in groups with supplemental methionine was greater than that of the control, serum total protein levels of group 3 were higher than other groups, it can

be concluded that 0.35 g methionine/100 g diet level of diet positively affects feed efficiency. These findings are in line with the previous report by Tete *et al.* (2010) on feeding different diet on layer type chick.

The methionine cycle is of major importance for cellular metabolism. Among its numerous roles is the regulation of the balance between methionine and cysteine for protein synthesis. The level of free amino acid in serum can reflect the metabolism of protein *in vivo* and usually remains stable when the amount of amino acid in the diet meets the animals' needs (Girard *et al.*, 2005). In this study, high methionine inhibited the level of serum lysine, in line with a previous study by Ozkan *et al.* (2011) in swine. These results suggest that supplemental 0.35 g methionine/100 g DM improves protein synthesis.

## CONCLUSION

The pelt quality of the raccoon dog can be improved by feeding with a balanced composition of amino acids in low-protein rations. In this study, we found that the diet containing 20 g protein +0.7 g methionine /100 g DM gave raccoon dogs a better performance and higher pelt quality.

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