Effects of Dietary Methionine Level on Growth Performance, Serum Immune Indices, and Antioxidant Parameters in 9-10 Month-Old Porcupines (Postprint)

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Date: 2017-10-23T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary methionine levels on growth performance, serum immune indices, and antioxidant indices of 9-10-month-old porcupines, and to estimate their methionine requirement using a quadratic regression model. Ninety-six 9-month-old porcupines with similar body weight were selected and randomly divided into 6 groups with 4 replicates per group and 4 porcupines per replicate, and fed experimental diets with methionine levels of 0.25%, 0.45%, 0.65%, 0.85%, 1.05%, and 1.25%, respectively. The pre-trial period was 7 days, and the formal trial period was 50 days. The results showed that: 1) Dietary methionine level had no significant effect on average daily gain (ADG) and average daily feed intake (ADFI) of porcupines ($P > 0.05$), but had a significant effect on feed-to-gain ratio (F/G) ($P < 0.05$). With the increase of dietary methionine level, F/G showed a quadratic curve trend of first decreasing and then increasing, reaching the minimum value of 3.63 at a methionine level of 0.85%. 2) Dietary methionine level had no significant effects on serum immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM) contents, and superoxide dismutase (SOD) activity of porcupines ($P > 0.05$), but significantly affected serum glutathione peroxidase (GSH-Px) activity ($P < 0.05$) and extremely significantly affected serum malondialdehyde (MDA) content ($P < 0.01$). With the increase of dietary methionine level, serum GSH-Px activity showed a quadratic curve trend of first increasing and then decreasing, reaching the maximum value at a methionine level of 0.85%. With the increase of dietary methionine level, serum MDA content showed a quadratic curve trend of first decreasing and then increasing; specifically, the 0.85% and 1.05% groups were extremely significantly lower than the 0.25%, 0.45%, and 0.65% groups ($P < 0.01$), and the 1.25% group was extremely significantly lower than the 0.25% and 0.45% groups (P < 0.01). In conclusion, adding appropriate amounts of methionine to the diet can improve the growth performance and enhance the antioxidant capacity of 9–10-month-old porcupines. According to the quadratic regression model estimation, the methionine requirements for achieving the lowest F/G, highest serum GSH-Px activity, and lowest serum MDA content in 9–10-month-old porcupines were 0.76%, 0.87%, and 0.96%, respectively.

Full Text

Effects of Dietary Methionine Level on Growth Performance, Serum Immune Indices and Antioxidant Indices of 9 to 10-Month-Old Porcupine

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Abstract

This study was conducted to investigate the effects of dietary methionine (Met) level on growth performance, serum immune indices and antioxidant indices of 9 to 10-month-old porcupine, and to determine the Met requirement using quadratic regression models. Ninety-six 9-month-old porcupines with similar body weight were randomly assigned to 6 groups with 4 replicates per group and 4 individuals per replicate. Porcupines in the 6 groups were fed experimental diets containing 0.25%, 0.45%, 0.65%, 0.85%, 1.05% and 1.25% Met, respectively. The adaptation period lasted 7 days, followed by a 50-day formal trial period. The results showed: 1) Dietary Met level did not significantly affect average daily gain (ADG) and average daily feed intake (ADFI) (P > 0.05), but significantly affected feed/gain ratio (F/G) (P < 0.05). As dietary Met level increased, F/G exhibited a quadratic trend of first decreasing then increasing, reaching its minimum value of 3.63 at 0.85% Met level. 2) Dietary Met level had no significant effects on serum immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM) contents or superoxide dismutase (SOD) activity (P > 0.05), but significantly affected serum glutathione peroxidase (GSH-Px) activity (P < 0.05) and extremely significantly affected serum malondialdehyde (MDA) content (P < 0.01). Serum GSH-Px activity showed a quadratic trend of first increasing then decreasing as dietary Met level increased, reaching its maximum at 0.85% Met level. Serum MDA content exhibited a quadratic trend of first decreasing then increasing; the 0.85% and 1.05% groups were extremely significantly lower than the 0.25%, 0.45% and 0.65% groups ($P < 0.01$), while the 1.25% group was extremely significantly lower than the 0.25% and 0.45% groups ($P < 0.01$). In conclusion, dietary supplementation with appropriate Met can improve growth performance and enhance antioxidant capacity in 9 to 10-month-old porcupines. Based on quadratic regression models, the estimated Met requirements for achieving minimum F/G, maximum serum GSH-Px activity and minimum serum MDA content are 0.76%, 0.87% and 0.96%, respectively.

Keywords: methionine; porcupine; growth performance; serum immune indices; antioxidant function

Introduction

Porcupine (*Hystrix hodgsoni*), also known as arrow pig or spiny pig, belongs to the order Rodentia and is a rare animal with high economic value, including edible, medicinal and ornamental uses. With improving living standards, porcupine meat—known as "animal ginseng"—has become popular in high-end hotels and restaurants in major Chinese cities, creating an expanding domestic market that has stimulated rapid development of porcupine farming. Methionine is an essential sulfur-containing amino acid for animal growth that serves as the first limiting amino acid in corn-soybean meal diets for poultry and the second limiting amino acid for swine. In addition to participating in protein and other amino acid synthesis, methionine can promote animal growth and improve immunity and plays an important role in molecular oxidative defense mechanisms. While methionine has been extensively studied in poultry and livestock production, research on porcupine nutritional requirements has started relatively late due to their short history of domestication, and studies on porcupine methionine requirements are rarely reported. This experiment used 9-month-old porcupines to investigate the effects of dietary methionine level on growth performance, serum immune indices and antioxidant indices from 9 to 10 months of age, thereby determining the optimal dietary methionine level for growth and providing a theoretical basis for porcupine production practices.

Materials and Methods

1.1 Experimental Animals and Grouping A single-factor completely randomized block design was used. Ninety-six healthy 9-month-old porcupines with similar body weight were randomly divided into 6 groups with 4 replicates per group and 4 individuals per replicate. The average body weight per replicate was (29.90 ± 4.10) kg.

1.2 Experimental Diets Corn, soybean meal and wheat bran were used as main ingredients to formulate a basal diet according to relevant literature . Methionine was added in the form of DL-methionine while other nutrients remained consistent. The premix was self-prepared by the porcupine farm. The basal diet composition and nutrient levels are shown in Table 1 . The six groups were fed experimental diets with methionine levels of 0.25%, 0.45%, 0.65%, 0.85%, 1.05% and 1.25%, respectively.

1.3 Feeding Management The feeding trial was conducted at the Duijin Porcupine Breeding Base in Taoyuan County. After a 7-day adaptation period, the formal 50-day trial began. Pelleted feed was provided throughout the trial period with ad libitum access to feed and water. Porcupine houses had cement floors with one feed trough and one automatic waterer per pen. Deworming, disinfection and disease prevention were carried out according to routine farm management practices. Pens were cleaned daily to maintain cleanliness and dryness.

1.4 Sample Collection and Preparation At the end of the trial, fasting body weight was measured by replicate. One porcupine close to the average weight of each replicate was selected and anesthetized by intramuscular injection of Sumianxin II, then 5 mL blood was collected from the anterior vena cava. The same dose of Luxingning II was injected intramuscularly to revive the animal. Blood samples were left to stand for 30 minutes, then centrifuged at 3,000 r/min for 10 minutes. Serum was collected and aliquoted into Eppendorf tubes, then stored at -20°C.

1.5 Measurements 1.5.1 Growth Performance

At the beginning and end of the trial, porcupines were fasted (with free access to water) for 12 hours before weighing. Initial and final body weights were measured individually by pen, and feed consumption was recorded by replicate to calculate average daily feed intake (ADFI), average daily gain (ADG) and feed/gain ratio (F/G).

1.5.2 Serum Immune Indices

Serum immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM) contents were determined by immunoturbidimetry at the Hunan Provincial Veterinary Drug Engineering Center Laboratory using assay kits purchased from Nanjing Jiancheng Bioengineering Institute.

1.5.3 Serum Antioxidant Indices

Serum glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities and malondialdehyde (MDA) content were determined using assay kits from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions at the Hunan Provincial Veterinary Drug Engineering Center Laboratory.

1.6 Data Processing Experimental data were analyzed by one-way ANOVA using SPSS 19.0 statistical software, with Duncan' s multiple comparison test. Results were expressed as means and standard errors, with $P < 0.05$ as the significance threshold. Quadratic curves were fitted through regression analysis using the model: $Y = aX^2 + bX + c$, where Y is the dependent variable and X is dietary methionine level. When significant quadratic effects were obtained, the methionine level for maximum quadratic effect (Xmax = -b/2a) was calculated according to Neter et al. [17], and this value was multiplied by 95% to obtain the appropriate methionine requirement.

Results

2.1 Effects of Dietary Methionine Level on Growth Performance of 9 to 10-Month-Old Porcupine As shown in Table 2 , dietary methionine level had no significant effects on ADG and ADFI ($P > 0.05$), but significantly affected F/G ($P < 0.05$). With increasing dietary methionine level, F/G showed a quadratic trend of first decreasing then increasing, with the 0.85% group being significantly lower than the 0.25%, 0.45% and 1.25% groups ($P < 0.05$), but not significantly different from the 0.65% and 1.05% groups ($P > 0.05$).

2.2 Effects of Dietary Methionine Level on Serum Immune Indices of 9 to 10-Month-Old Porcupine As shown in Table 3 , dietary methionine level had no significant effects on serum IgA, IgG and IgM contents ($P > 0.05$).

2.3 Effects of Dietary Methionine Level on Serum Antioxidant Indices of 9 to 10-Month-Old Porcupine As shown in Table 4 , dietary methionine level had no significant effect on serum SOD activity ($P > 0.05$). Dietary methionine level significantly affected serum GSH-Px activity ($P < 0.05$), which showed a quadratic trend of first increasing then decreasing with increasing dietary methionine level. The 0.85% group had the highest activity, significantly higher than the 0.25% and 0.45% groups ($P < 0.05$), but not significantly different from the 0.65%, 1.05% and 1.25% groups ($P > 0.05$). Dietary methionine level extremely significantly affected serum MDA content ($P < 0.01$), showing a quadratic trend of first decreasing then increasing. The 0.85% and 1.05% groups were extremely significantly lower than the 0.25%, 0.45% and 0.65% groups ($P < 0.01$), not significantly different from the 1.25% group ($P > 0.05$), while the 1.25% group was extremely significantly lower than the 0.25% and 0.45% groups ($P < 0.01$).

2.4 Estimation of Optimal Methionine Requirement Using Quadratic Regression Model As shown in Table 5 , the estimated methionine requirements at 95% of maximum response for achieving minimum F/G, maximum serum GSH-Px activity and minimum serum MDA content in 9 to 10-month-old porcupines were 0.76%, 0.87% and 0.96%, respectively.

Discussion

Currently, there are few reports on methionine requirements for porcupines domestically or internationally, with requirements often based on farming experience or reference to other animals. The quadratic regression model estimated that dietary methionine levels at 95% of maximum response for minimum F/G, maximum serum GSH-Px activity and minimum serum MDA content in 9 to 10-month-old porcupines were 0.76%, 0.87% and 0.96%, respectively—higher than recommended levels for pigs and poultry, such as 0.30% for piglets [18], 0.38% for 42-62-day-old Campbell ducks [19], 0.42% for 5-8-week-old Jinghong laying hens [20], and 0.48% for laying hens [21]. However, fur-bearing animals have higher methionine requirements, such as 0.91% for silver foxes during winter fur growth [22], and 0.99%, 1.03% and 0.98% for blue foxes during winter fur period, pre-breeding and breeding period, respectively [23-25]. Porcupines are covered with hard spines throughout their body, particularly dense and thick on the back and hips (reaching over 20 cm in length and about 6 mm in diameter), with shorter and softer spines on limbs and abdomen. Part of the porcupine's methionine requirement is used for spine growth, making reference to fur-bearing animals' methionine requirements practically meaningful for porcupine farming.

Methionine is one of the essential amino acids for animals, and meeting methionine requirements helps achieve optimal production performance. Numerous studies have shown that appropriate methionine supplementation in basal diets can significantly increase poultry weight gain, improve feed utilization and carcass quality, and promote feather growth [26-29]. Cheng [18] reported that dietary methionine levels (0.18%, 0.24%, 0.30%, 0.36%, 0.42% and 0.48%) significantly affected piglet growth performance. Zhang and Li [30] reported that dietary methionine supplementation significantly increased ADG and feed conversion ratio (FCR) in young rabbits without significantly affecting ADFI. Our results showed that dietary methionine level had no significant effect on ADFI but significantly affected F/G in 9 to 10-month-old porcupines, consistent with the above studies, though ADG was not significantly affected. Some studies have also found that dietary methionine level does not affect animal production performance; Ye et al. [19] reported that within the tested methionine range (0.28%-0.48%), production performance of 42-62-day-old Campbell ducks was not significantly affected. Additionally, methionine deficiency or excess can inhibit animal growth performance; Song et al. [20] reported that dietary methionine levels of 0.37%-0.44% yielded the best growth performance in 5-8-week-old Jinghong laying hens, with decreased performance below or above this range. In this study, the lowest F/G was obtained at 0.85% dietary methionine, subjectively identified as the appropriate level for 9 to 10-month-old porcupines. Regression analysis revealed a significant quadratic relationship between F/G and dietary methionine level, with F/G showing a quadratic trend of first decreasing then increasing as methionine level increased, indicating that appropriate methionine supplementation improved feed utilization efficiency in porcupines. The regression model estimated that 0.76% dietary methionine would optimize growth performance in 9 to 10-month-old porcupines, which is lower than the subjectively observed value from trial results and more meaningful for reducing feeding costs and environmental pollution.

Protein and amino acids play important roles in maintaining normal immune function [31-32]. Hou et al. [33] reported that methionine mainly affects humoral immune responses, with dietary methionine level significantly affecting serum IgG content and anti-sheep red blood cell (SRBC) antibody titers in early-weaned piglets. Wu [34] found that methionine deficiency significantly or extremely significantly decreased serum IgG, IgA and IgM contents in chicks compared with the control group. Tsiagbe et al. [10] showed that adding 0.25% methionine to a basal diet (0.35% methionine, 0.37% cystine) significantly increased serum anti-SRBC antibody titers and IgG content in broilers after SRBC intraperitoneal injection, while excessive supplementation (0.45%) had no significant effect on post-vaccination antibody titers. Liu et al. [35] reported similar findings that different methionine forms increased serum immunoglobulin and complement contents in broiler breeders. In this study, dietary methionine level had no significant effects on serum IgA, IgG and IgM contents in porcupines, inconsistent with the above reports. This may be related to whether animals are under disease or immune stress conditions, where more amino acids may be mobilized for immunoglobulin synthesis to achieve higher antibody levels in the short term, while only maintaining lower levels under normal physiological conditions [30]. It may also be related to experimental animals, vaccination programs and feeding environments, requiring further investigation.

Another important function of methionine is serving as a cysteine precursor for reduced glutathione (GSH) and taurine synthesis [11-13,36], acting as an antioxidant in molecular oxidative defense mechanisms [37]. SOD and GSH-Px are important enzymes in the antioxidant system, playing key roles in scavenging free radicals, preventing oxidative damage and maintaining cell structure, and can serve as markers of antioxidant status. MDA is a product of cell membrane lipid peroxidation, and its content can indirectly reflect the degree of cell damage [38]. Ma et al. [21] reported that appropriate methionine could increase serum SOD activity and enhance antioxidant function in laying hens. Liu et al. [35] showed that dietary methionine supplementation in different forms improved serum antioxidant function in broiler breeders, significantly increasing liver and kidney GSH-Px and SOD activities while decreasing MDA content. Ye et al. [39] reported that dietary methionine significantly increased serum GSH and SOD activities and decreased MDA content in 21-day-old Lion-head geese, with serum GSH-Px activity increasing (though not significantly) as methionine level increased. Our results showed that dietary methionine level significantly affected serum GSH-Px activity and extremely significantly affected serum MDA content in porcupines, consistent with the above studies. However, dietary methionine level had no significant effect on serum SOD activity, inconsistent with previous reports, possibly because free radical scavenging capacity in porcupines is mainly reflected by serum GSH-Px activity rather than SOD activity. Comprehensive analysis indicates that appropriate dietary methionine supplementation can enhance antioxidant capacity and reduce lipid peroxidation in 9 to 10-month-old porcupines, though the mechanism requires further investigation.

Conclusion

Dietary supplementation with appropriate methionine can improve growth performance and enhance antioxidant capacity in 9 to 10-month-old porcupines. Based on quadratic regression models, the estimated methionine requirements for achieving minimum F/G, maximum serum GSH-Px activity and minimum serum MDA content are 0.76%, 0.87% and 0.96%, respectively.

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