Effects of Dietary Vitamin B2 Supplementation Level on Growth Performance, Nutrient Digestibility, and Nitrogen Metabolism in Growing Mink (Postprint)

Authors: Mu Linlin, Zhong Wei, Zhang Haihua, Weixiao Nan, Zhang Xinyu, Chen Shuangshuang, Li Guangyu

Date: 2018-12-25T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary vitamin B2 supplementation levels on growth performance, nutrient digestibility, and nitrogen metabolism in growing minks. Ninety-six healthy male short-haired black minks at (65±5) days of age with similar body weight [(1,213.38±72.72) g] were randomly divided into 6 groups with 16 replicates per group and 1 mink per replicate. A single-factor experimental design was adopted, and the dietary vitamin B2 supplementation levels for each group were 0 (Group I), 2.5 (Group II), 5.0 (Group III), 10.0 (Group IV), 20.0 (Group V), and 40.0 mg/kg (Group VI), respectively. The preliminary period lasted 7 days, and the formal experimental period lasted 58 days. The results showed: 1) Dietary vitamin B2 supplementation level significantly affected final body weight and feed-to-gain ratio ($P<0.05$), and extremely significantly affected average daily gain ($P<0.01$). Among all groups, Group V had the highest average daily gain and final body weight, and the lowest feed-to-gain ratio. The average daily gain of Group V was significantly or extremely significantly higher than that of Groups I, II, III, and IV ($P<0.05$ or $P<0.01$). The feed-to-gain ratio of Groups V and VI was significantly or extremely significantly lower than that of Group I ($P<0.05$ or $P<0.01$). The final body weight of Groups V and VI was significantly higher than that of Group I ($P<0.05$). 2) Dietary vitamin B2 supplementation level extremely significantly affected crude protein digestibility and crude fat digestibility ($P<0.01$), but had no significant effect on dry matter intake, dry matter excretion, and dry matter digestibility ($P>0.05$). Group VI had the highest crude fat digestibility, while Group I had the lowest; Group I was extremely significantly lower than Groups II, V, and VI ($P<0.01$). Group V had the highest crude protein digestibility, which was extremely significantly higher than that of Group I (P<0.01). 3) Dietary vitamin B2 supplementation level significantly affected nitrogen retention, net protein utilization, and protein biological value in growing minks (P<0.05), but had no significant effect on nitrogen intake, fecal nitrogen, and urinary nitrogen (P>0.05). Group V had the highest net protein utilization, which was significantly higher than that of Groups I and II (P<0.05), with no significant differences from Groups III, IV, and VI (P>0.05). Group V had the highest protein biological value; Groups V and VI were significantly higher than Group I (P<0.05), with no significant differences from Groups II, III, and IV (P>0.05). Group VI had the highest nitrogen retention, while Group I had the lowest; Group I was significantly lower than Groups V and VI (P<0.05), with no significant differences from Groups II, III, and IV (P>0.05). Based on comprehensive analysis of all indicators, under the conditions of this experiment, a dietary vitamin B2 supplementation level of 20.0 mg/kg (corresponding to a dietary vitamin B2 level of 23.35 mg/kg) could result in better growth performance and higher nutrient digestibility and nitrogen utilization in growing minks.

Full Text

Effects of Dietary Vitamin B2 Level on Growth Performance, Nutrient Digestibilities and Nitrogen Metabolism of Growing Minks

MU Linlin[1], ZHONG Wei[1], ZHANG Haihua[2], NAN Weixiao[1], ZHANG Xinyu[1], CHEN Shuangshuang[1], LI Guangyu[1]*

[1] State Key Laboratory of Special Economic Animal Molecular Biology, Institute of Special Economic Animal and Plant Sciences, Chinese Academy of Agricultural Sciences, Changchun 130112, China
[2] College of Animal Science and Technology, Hebei Normal University of Science and Technology, Qinhuangdao 066004, China

Abstract

This experiment was conducted to evaluate the effects of dietary vitamin B2 level on growth performance, nutrient digestibilities, and nitrogen metabolism in growing minks. Ninety-six healthy short-haired black male minks aged (65±5) days with similar initial body weights [(1,213.38±72.72) g] were randomly allocated to six groups with 16 replicates per group and one mink per replicate. A single-factor experimental design was employed with dietary vitamin B2 supplementation levels of 0 (Group I), 2.5 (Group II), 5.0 (Group III), 10.0 (Group IV), 20.0 (Group V), and 40.0 mg/kg (Group VI). The adaptation period lasted 7 days, followed by a 58-day formal experimental period.

The results showed: (1) Dietary vitamin B2 level significantly affected final body weight and feed-to-gain ratio (P<0.05) and extremely significantly affected average daily gain (P<0.01). Group V exhibited the highest average daily gain and final weight, and the lowest feed-to-gain ratio. The average daily gain of Group V was significantly or extremely significantly higher than that of Groups I, II, III, and IV ($P<0.05$ or $P<0.01$). The feed-to-gain ratio of Groups V and VI was significantly or extremely significantly lower than that of Group I ($P<0.05$ or $P<0.01$). The final weight of Groups V and VI was significantly higher than that of Group I ($P<0.05$). (2) Dietary vitamin B2 level extremely significantly affected crude protein digestibility and ether extract digestibility ($P<0.01$), but had no significant effects on dry matter intake, dry matter output, or dry matter digestibility ($P>0.05$). Group VI showed the highest ether extract digestibility, while Group I showed the lowest, with Group I being extremely significantly lower than Groups II, V, and VI ($P<0.01$). Group V demonstrated the highest crude protein digestibility, which was extremely significantly higher than that of Group I ($P<0.01$). (3) Dietary vitamin B2 level significantly affected nitrogen deposition, net protein utilization, and protein biological value ($P<0.05$), but had no significant effects on nitrogen intake, fecal nitrogen, or urinary nitrogen ($P>0.05$). Group V exhibited the highest net protein utilization, which was significantly higher than that of Groups I and II ($P<0.05$), with no significant differences compared to Groups III, IV, and VI ($P>0.05$). Group V showed the highest protein biological value, with Groups V and VI being significantly higher than Group I ($P<0.05$), and no significant differences compared to Groups II, III, and IV ($P>0.05$). Group VI had the highest nitrogen deposition, while Group I had the lowest, with Group I being significantly lower than Groups V and VI ($P<0.05$). Based on comprehensive evaluation of all indicators, a dietary vitamin B2 supplementation level of 20.0 mg/kg (resulting in a total dietary vitamin B2 level of 23.35 mg/kg) supported optimal growth performance and achieved high nutrient digestibility and nitrogen utilization in growing minks under the conditions of this experiment.

Keywords: minks; vitamin B2; growth performance; nutrient digestibility; nitrogen metabolism

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Introduction

Vitamin B2 (riboflavin) is an essential water-soluble vitamin for organism metabolism that primarily exists in two forms in vivo: flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The active components FMN and FAD serve as prosthetic groups of flavoenzymes and widely participate in the absorption and utilization of carbohydrates, lipids, and proteins, thereby promoting nutrient digestion and absorption, influencing animal growth and development, and enhancing immune organ development as well as immune cell proliferation, differentiation, and maturation, while improving antioxidant capacity. Vitamin B2 deficiency in pregnant minks can cause stillbirth, though its impact on male fertility is relatively minor. Insufficient vitamin B2 levels lead to slow growth, reduced hemoglobin content, and gray or white underfur. Excess vitamin B2 is excreted in urine and generally does not cause toxicity, whereas deficiency produces various deficiency symptoms.

Current research on vitamin B2 is extensive in pigs and poultry. Zhang et al. reported that dietary supplementation with 14.4 mg/kg vitamin B2 significantly improved average daily gain and reduced feed-to-gain ratio in broilers, promoting growth. The Danish Fur Breeders Association recommends a dietary vitamin B2 level of 2.0–3.5 mg/kg, while NRC (1982) recommends 1.5 mg/kg as the minimum requirement to prevent deficiency symptoms. However, due to differences in mink breeds, feed composition, and structure between domestic and international contexts, these recommended levels may not be suitable for Chinese mink breeds. Therefore, this study investigated the effects of dietary vitamin B2 level on growth performance, nutrient digestibility, and nitrogen metabolism in minks to determine the optimal supplementation level and provide a theoretical basis for vitamin nutrition in China' s mink industry.

1.1 Experimental Animals and Design

Ninety-six healthy short-haired black male minks aged (65±5) days with similar body weights [(1,213.38±72.72) g] were randomly divided into six groups, ensuring that minks from the same litter were not placed in the same group to eliminate genetic errors. Each group comprised 16 replicates with one mink per replicate. The experiment employed a single-factor randomized design with six vitamin B2 supplementation gradients: 0 (Group I, control), 2.5 (Group II), 5.0 (Group III), 10.0 (Group IV), 20.0 (Group V), and 40.0 mg/kg (Group VI). The adaptation period lasted 7 days, and the formal experimental period lasted 58 days. The animal feeding trial was conducted at the Key Field Scientific Observation Station of Wild Biological Resources in Changbai Mountain, Ministry of Agriculture.

1.2 Experimental Diets and Materials

The basal diet was formulated using fish meal, chicken meal, meat meal, extruded corn, corn gluten meal, soybean meal, and other ingredients, supplemented with minerals and vitamins (excluding vitamin B2) required for mink growth and development. The diet was prepared according to NRC (1982) and relevant literature regarding mink nutritional requirements. The composition and nutrient levels of the basal diet are presented in Table 1 . The vitamin B2 used in the experiment was purchased from Zhejiang NHU Co., Ltd., with an active ingredient content of 82.8%.

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

Item	Content
Ingredients	
Extruded corn meal	

Item	Content
Soybean meal	
Meat meal	
Fish meal	
Corn gluten meal	
Chicken meal	
Premix[1]	
Lysine	
Methionine	
Calcium hydrogen phosphate	
Soybean oil	
Salt	
Total	
Nutrient levels[2]	
Metabolizable energy (MJ/kg)	
Crude protein	
Ether extract	
Vitamin B2 (mg/kg)	

[1]The premix provided the following per kg of diet: VA 10,000 IU, VE 60 mg, VK 1.6 mg, VB 20 mg, VB 10 mg, VB 0.1 mg, nicotinic acid 40 mg, pantothenic acid 20 mg, folic acid 1 mg, biotin 0.5 mg, choline 400 mg, Fe 80 mg, Zn 60 mg, Mn 15 mg, Cu 10 mg, I 0.5 mg, Se 0.2 mg, Co 0.3 mg.

[2]Metabolizable energy was a calculated value, while the others were measured values.

1.3 Feeding Management

Prior to the experiment, mink cages, water boxes, and feed trays were disinfected, and minks were vaccinated. Each mink was housed individually in a single cage. Experienced farm staff fed the minks twice daily at 07:00 and 14:00, ensuring ad libitum access to feed and water.

1.4.1 Growth Performance Indicators

During the experimental period, feed provision and leftovers were recorded daily. Minks were weighed at the beginning and end of the experiment to calculate average daily feed intake, average daily gain, and feed-to-gain ratio.

1.4.2 Digestion and Metabolism Trial

A digestion and metabolism trial was conducted from August 15 to August 17, 2017, lasting 3 days using the total fecal collection method. Before urine collection, 20 mL of 10% sulfuric acid was added to collection buckets to fix nitrogen for subsequent nitrogen content determination. Daily fecal collections were weighed, and 10% sulfuric acid solution equivalent to 5% of fresh weight was added along with a small amount of toluene as a preservative, then stored at -20°C for later analysis. Urine and feces collected over the 3 days were thoroughly mixed separately before sampling. Fecal samples were first sterilized at 80°C for 2 hours, then dried at 65°C to constant weight, ground to pass through a 40-mesh sieve, and prepared as air-dry samples for laboratory analysis.

1.4.3 Measurement Methods and Calculation Formulas

Dry matter content in feed and feces was determined using the 105°C drying method according to GB/T 6435-2006. Ether extract content was determined using the Soxhlet extraction method according to GB/T 6435-2006. Crude protein content in feed, feces, and urine was determined using the Kjeldahl method according to GB/T 6435-1994. The vitamin B2 content in the basal diet was measured by high-performance liquid chromatography at the Ministry of Agriculture's Special Economic Animal and Plant Products Quality Supervision, Inspection and Testing Center.

Nutrient digestibility and nitrogen metabolism indicators were calculated using the following formulas:

- Nutrient digestibility (%) = [(Nutrient intake - Total nutrient in feces) / Nutrient intake] × 100
- Nitrogen deposition (g/d) = Nitrogen intake - Fecal nitrogen - Urinary nitrogen
- Net protein utilization (%) = (Nitrogen deposition / Nitrogen intake) × 100
- Protein biological value (%) = [Nitrogen deposition / (Nitrogen intake - Fecal nitrogen)] × 100

Statistical Analysis

Results are expressed as "mean ± standard deviation." Data were organized using Excel 2013 and subjected to one-way analysis of variance using the GLM procedure in SAS 9.4 software. Duncan' s multiple comparison test was used for post-hoc analysis. Differences were considered significant at $P<0.05$ and extremely significant at $P<0.01$.

2.1 Effects of Dietary Vitamin B2 Level on Growth Performance of Growing Minks

As shown in Table 2 , dietary vitamin B2 level extremely significantly affected average daily gain ($P<0.01$) and significantly affected final weight and feed-to-gain ratio ($P<0.05$) in minks. Group V exhibited the highest final weight and average daily gain, and the lowest feed-to-gain ratio. The average daily gain of Group

V was extremely significantly higher than that of Group I (P<0.01) and significantly higher than that of Groups II, III, and IV (P<0.05), while no significant differences were observed among Groups I, II, III, and IV (P>0.05). The feed-to-gain ratio of Group V was extremely significantly lower than that of Group I (P<0.01), and Group VI was significantly lower than Group I (P<0.05), with no significant difference between Groups V and VI (P>0.05). The final weight of Groups V and VI was significantly higher than that of Group I (P<0.05).

Table 2 Effects of dietary vitamin B2 level on growth performance of growing minks

Group	Initial weight (g)	Final weight (g)	ADG (g/d)	ADFI (g/d)	Feed/gain ratio
I	1,201.00±16.50	2,080.00±58.50	15.16±0.81	118.08±5.86	8.05±0.60
II	1,205.83±16.40	2,139.17±44.93	16.09±0.60	122.32±3.35	7.15±0.36
III	1,237.14±18.88	2,188.57±40.16	16.17±0.42	113.42±4.70	7.22±0.42
IV	1,205.45±18.80	2,139.09±62.12	16.10±0.81	114.74±4.84	7.21±0.16
V	1,216.88±24.87	2,301.25±62.97	18.70±0.86	125.30±3.49	6.14±0.17
VI	1,226.00±13.18	2,277.00±46.17	18.12±0.70	125.95±4.54	6.83±0.37

In the same column, values with different small letter superscripts indicate significant difference (P<0.05), while different capital letter superscripts indicate extremely significant difference (P<0.01). Values with the same or no superscripts indicate no significant difference (P>0.05). The same notation applies to subsequent tables.

2.2 Effects of Dietary Vitamin B2 Level on Nutrient Digestibilities of Growing Minks

As shown in Table 3 , dietary vitamin B2 level extremely significantly affected crude protein digestibility and ether extract digestibility (P<0.01), but had no significant effects on dry matter intake, dry matter output, or dry matter digestibility (P>0.05). When dietary vitamin B2 supplementation ranged from 10.0 to 40.0 mg/kg, ether extract digestibility showed an increasing trend with higher vitamin B2 levels. Group VI exhibited the highest ether extract digestibility, while Group I showed the lowest, with Group I being extremely significantly lower than Groups II, V, and VI (P<0.01) but not significantly different from Group IV (P>0.05). Group V demonstrated the highest crude protein digestibility, which was extremely significantly higher than that of Group I (P<0.01). Crude protein digestibility showed an increasing trend with vitamin B2 supplementation levels between 5.0 and 20.0 mg/kg.

Table 3 Effects of dietary vitamin B2 level on nutrient digestibilities of growing minks

Group	DM intake (g/d)	DM output (g/d)	CP digestibility (%)	EE digestibility (%)	DM digestibility (%)
I	120.36±5.62	24.52±1.50	79.67±0.50	74.09±0.80	84.80±1.23
II	121.35±3.11	23.00±0.70	80.95±0.85	74.00±0.66	89.47±0.66
III	110.50±5.07	21.30±1.90	80.75±1.68	74.40±0.92	88.42±0.77
IV	113.21±3.88	23.19±1.55	79.60±0.98	75.48±0.27	86.32±0.98
V	125.30±3.49	22.32±1.20	82.12±0.95	78.54±1.22	89.65±1.77
VI	119.63±6.11	21.57±0.90	81.80±0.56	76.44±0.47	90.26±0.59

2.3 Effects of Dietary Vitamin B2 Level on Nitrogen Metabolism of Growing Minks

As shown in Table 4 , dietary vitamin B2 level significantly affected nitrogen deposition, net protein utilization, and protein biological value (P<0.05), but had no significant effects on nitrogen intake, fecal nitrogen, or urinary nitrogen (P>0.05). Nitrogen deposition increased with higher vitamin B2 supplementation, with Group VI showing the highest value and Group I the lowest. Group I was significantly lower than Groups V and VI (P<0.05) but not significantly different from Groups II, III, and IV (P>0.05). Group V exhibited the highest net protein utilization, which was significantly higher than that of Groups I and II (P<0.05) but not significantly different from Groups III, IV, and VI (P>0.05). Protein biological value showed an increasing trend with vitamin B2 supplementation from 0 to 20.0 mg/kg, with Group V achieving the highest value. Groups V and VI were significantly higher than Group I (P<0.05) but not significantly different from Groups II, III, and IV (P>0.05).

Table 4 Effects of dietary vitamin B2 level on nitrogen metabolism of growing minks

Group	Nitrogen intake (g/d)	Urinary nitrogen (g/d)	Fecal nitrogen (g/d)	Nitrogen deposition (g/d)	NPU (%)	Protein biological value (%)
I	7.06±0.25	3.69±0.22	1.28±0.15	2.08±0.20	29.50±[illegible]	[illegible]±3.31
II	7.17±0.20	3.46±0.24	1.47±0.05	2.24±0.20	31.15±[illegible]	[illegible]±3.46
III	7.00±0.17	3.20±0.19	1.44±0.11	2.36±0.21	33.71±[illegible]	[illegible]±3.57
IV	6.75±0.30	2.90±0.27	1.45±0.15	2.50±0.18	37.13±[illegible]	[illegible]±3.68
V	7.33±0.28	3.05±0.26	1.27±0.07	3.00±0.29	40.79±[illegible]	[illegible]±3.74
VI	7.38±0.29	2.94±0.32	1.41±0.09	3.04±0.24	39.20±[illegible]	[illegible]±3.90

3.1 Effects of Dietary Vitamin B2 Level on Growth Performance of Growing Minks

Vitamin B2, as an important micronutrient with regulatory functions, can promote animal growth and development. Wang et al. demonstrated that increasing dietary vitamin B2 supplementation significantly improved average daily gain and growth performance while reducing feed-to-gain ratio in caged ducklings. Zhang et al. and Tang found that dietary vitamin B2 supplementation significantly increased average daily gain and decreased feed-to-gain ratio in broilers. Li reported that feeding broilers a diet containing 10.5 mg/kg vitamin B2 significantly improved body weight and feed utilization under high-temperature conditions. The present study showed that increasing vitamin B2 supplementation led to increased final weight and average daily gain, and decreased feed-to-gain ratio in growing minks, with the 20.0 mg/kg supplementation group (Group V) achieving the highest final weight and average daily gain and the lowest feed-to-gain ratio. These findings align with previous literature, indicating that appropriate vitamin B2 supplementation promotes mink growth and development.

3.2 Effects of Dietary Vitamin B2 Level on Nutrient Digestibilities of Growing Minks

Previous studies have shown that dietary vitamin B2 level had no significant effects on average daily feed intake in 12-month-old Yili horses or growing rex rabbits, but significantly affected feed intake in 1-14-day-old Beijing ducks. Barile et al. reported that vitamin B2 is mainly absorbed in the small intestine, with partial absorption in the large intestine. When dietary proteins are hydrolyzed and denatured, vitamin B2 is released from the brush border membrane of intestinal epithelial cells to participate in corresponding physiological processes. The present results indicate that dietary vitamin B2 level had extremely significant effects on crude protein and ether extract digestibility in growing minks, with overall increasing trends as supplementation levels rose. This may be related to the involvement of the active component FAD in fatty acid -oxidation, promoting lipid oxidation and decomposition in mitochondria, thereby improving ether extract digestibility. The increase in crude protein digestibility may be attributed to enhanced activity of glutamate dehydrogenase in the amino acid oxidative deamination process, which is influenced by cellular energy charge when ATP is hydrolyzed to ADP during physiological activities, thereby accelerating amino acid oxidative degradation. The specific mechanisms of nutrient digestion require further investigation. Under the conditions of this experiment, a dietary vitamin B2 supplementation level of 20.0 mg/kg yielded optimal nutrient digestibility in minks.

3.3 Effects of Dietary Vitamin B2 Level on Nitrogen Metabolism of Growing Minks

Nitrogen metabolism indicators reflect the utilization of dietary protein by animals. After ingesting nitrogen-containing diets, animals decompose, utilize, and absorb nitrogen, with a portion being used for protein synthesis and storage while the unabsorbed portion is excreted as fecal nitrogen to maintain nitrogen balance. Limited reports exist on changes in nitrogen metabolism indicators with dietary vitamin B2 supplementation in monogastric animals. Zhou reported that protein deposition rate increased with higher dietary vitamin B2 levels. In this study, nitrogen deposition increased with dietary vitamin B2 supplementation, while net protein utilization and protein biological value showed initial increases followed by decreases, consistent with the trend in crude protein digestibility. This suggests that vitamin B2 promotes protein absorption and utilization, but the subsequent decline may indicate that vitamin B2 enhances nitrogen metabolism only within a certain range, beyond which it may inhibit protein absorption and utilization.

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4 Conclusion

Based on comprehensive evaluation of all indicators, a dietary vitamin B2 supplementation level of 20.0 mg/kg (resulting in a total dietary vitamin B2 level of 23.35 mg/kg) supports optimal growth performance and achieves high nutrient digestibility and nitrogen utilization in growing minks under the conditions of this experiment.

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