Effects of Dietary Vitamin D and Calcium Levels on Growth Performance, Nutrient Digestibility, and Nitrogen Metabolism in Growing Mink (Postprint)

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Date: 2017-11-08T00:00:00+00:00

Abstract

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Full Text

Effects of Dietary Vitamin D and Calcium Levels on Growth Performance, Nutrient Digestibility and Nitrogen Metabolism of Growing Minks

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Abstract: This experiment aimed to investigate the effects of dietary vitamin D and calcium levels on growth performance, nutrient digestibility, and nitrogen metabolism of growing mink at a fixed calcium-to-phosphorus ratio. One hundred seventeen healthy short-haired black male minks aged (60$\pm$5)*dayswererandomlydividedinto*9*groupswith*13*replicatespergroupandoneminkperreplicate.A*3×$3 factorial design was employed with dietary calcium-to-phosphorus ratio fixed at 2:1, three vitamin D levels (2,100, 4,100, and 6,100 IU/kg), and three calcium

levels (2.3%, 2.7%, and 3.1%). Nine experimental diets were formulated with calcium and vitamin D levels as follows: 2.1% Ca + 2,100 IU/kg vitamin D (Group), 2.1% Ca + 4,100 IU/kg vitamin D (Group), 2.1% Ca + 6,100 IU/kg vitamin D (Group), 2.7% Ca + 2,100 IU/kg vitamin D (Group), 2.7% Ca + 4,100 IU/kg vitamin D (Group), 2.7% Ca + 6,100 IU/kg vitamin D (Group), 3.1% Ca + 2,100 IU/kg vitamin D (Group), 3.1% Ca + 4,100 IU/kg vitamin D (Group), and 3.1% Ca + 6,100 IU/kg vitamin D (Group). The pre-trial period lasted 13 days, and the formal trial lasted 60 days. The results showed: (1) Dietary vitamin D and calcium levels had extremely significant effects on final weight, average daily gain, and feed-to-gain ratio ($P<0.01$). Final weight and average daily gain were highest in Group and lowest in Group , while feed-to-gain ratio showed the opposite pattern. (2) Fat digestibility differed extremely significantly among groups ($P<0.01$), with Groups , , and being extremely significantly higher than Groups and ($P<0.01$). Dietary calcium level significantly affected dry matter output ($P<0.05$), which was highest at 3.1% calcium. Calcium level also extremely significantly affected fat digestibility ($P<0.01$), which was extremely significantly lower at 3.1% calcium compared to 2.3% and 2.7% calcium ($P<0.01$). Dietary vitamin D level extremely significantly affected dry matter output ($P<0.01$), which was lowest at 4,100 IU/kg vitamin D. Vitamin D level significantly affected dry matter digestibility and fat digestibility ($P<0.05$), both being highest at 4,100 IU/kg vitamin D. (3) Dietary calcium level significantly affected nitrogen deposition ($P<0.05$), which showed an increasing trend with rising calcium levels and was highest at 3.1% calcium. Dietary vitamin D level significantly affected net protein utilization and protein biological value ($P<0.05$), both being highest at 4,100 IU/kg vitamin D. Dietary vitamin D and calcium levels showed significant interaction effects on net protein utilization and protein biological value ($P<0.05$), with the highest values observed in Group . Based on comprehensive analysis of all indicators, under the conditions of this experiment, growing minks achieved optimal growth performance, nutrient digestibility, and nitrogen utilization when dietary calcium-to-phosphorus ratio was 2:1, vitamin D level was 4,100 IU/kg, and calcium level was 3.1%.

Keywords: vitamin D; calcium; phosphorus; mink; growth performance; nitrogen metabolism

Mink are valuable fur-bearing animals with high economic value. Vitamin D and calcium/phosphorus intake play crucial roles in bone metabolism and growth performance, representing important factors for improving mink growth and fur quality. Vitamin D is essential for calcium and phosphorus absorption and exerts decisive regulatory effects. Calcium and phosphorus are vital elements for maintaining metabolic functions in fur-bearing animals and are indispensable macrominerals for normal bone metabolism. Excessive dietary levels of calcium, phosphorus, and vitamin D can lead to metabolic diseases or toxic side effects in fur-bearing animals, while insufficient levels fail to meet growth and development requirements. Accurate determination of appropriate vitamin D and calcium levels in mink diets is of great practical significance for mink production.

Bassett et al. [1] reported that mink require no less than 40 IU of vitamin D per day during bone development. Leoschke et al. [2] suggested that dietary vitamin D content should be no less than 400 IU/kg dry matter. Hilleman [3] found no significant differences in fur quality when dietary vitamin D content was 10,000, 25,000, or 40,000 IU/kg dry matter from July to pelting time. Helgebostad et al. [4] demonstrated that 5,000 IU/kg vitamin D in dietary dry matter was non-toxic to mink, while doses exceeding 100,000 IU/kg caused toxicity within a short period, a conclusion also reached by Perel' dik et al. [5]. Mertin et al. [6] measured dietary calcium levels in mink farms and found that 3.4% calcium had no negative effects on growth performance. Bassett et al. [7] reported that when dietary vitamin D was 820 IU/kg and calcium-to-phosphorus ratio ranged from 0.75 to 1.70, mink required 0.3% calcium in fresh feed, whereas Rimeslatten [8] found calcium requirements of 0.4%–1.0% under similar conditions. Domestic research on mink nutrition and practical feed formulation show varying dietary calcium levels ranging from 2.30% to 3.91% [9-11]. Dietary calcium-to-phosphorus ratio affects calcium and phosphorus absorption in mink. Jøergensen [12] indicated that mink require 100 IU vitamin D daily, with an appropriate calcium-to-phosphorus ratio of 0.75–1.70. The Chinese industry standard "Mink Compound Feed" recommends a calcium-to-phosphorus ratio of 1–2 [13]. From the perspective of improving growth performance and nutrient digestibility in growing mink, Liu et al. [14] recommended dietary phosphorus levels of 1.4%–1.8% and calcium-to-phosphorus ratios of 1.5–2.0.

Although numerous studies have investigated vitamin D and calcium/phosphorus requirements in mink both domestically and internationally, most data are relatively old and cover wide ranges of vitamin D levels. In recent years, due to evolution and domestication, mink body weights vary considerably, leading to different vitamin D and calcium requirements. Therefore, investigating precise dietary vitamin D and calcium levels for mink is particularly important. This experiment fixed the dietary calcium-to-phosphorus ratio and formulated diets with different vitamin D and calcium levels to study their effects on growth performance, nutrient digestibility, and nitrogen metabolism in growing mink, aiming to identify appropriate dietary vitamin D and calcium levels for growing mink and provide a theoretical basis for improving mink nutrition standards and scientific feeding practices.

1.1 Experimental Animals

The feeding trial was conducted at the Key Field Scientific Observation Station of Wild Biological Resources in Changbai Mountains, Ministry of Agriculture. One hundred seventeen healthy short-haired black male minks aged (60$±$5) days were randomly divided into 9 groups (Groups -), with siblings placed in different groups to eliminate genetic effects. Each group contained 13 replicates with one mink per replicate, and no significant differences in initial body weight were observed among replicates (P>0.05).

1.2 Experimental Design and Diets

A 3$\times$3$factorialdesignwasemployedwithdietarycalcium-to-phosphorusratiofixedat2:1, threevitaminD(asvitaminD_\{3\}$) levels (2,100, 4,100, and 6,100 IU/kg), and three calcium levels (2.30%, 2.70%, and 3.10%). Based on NRC (1982) [15] and relevant literature [16-17] regarding nutrient requirements for growing mink, nine experimental diets were formulated using corvina, chicken skeleton, chicken head, chicken proventriculus, chicken liver, and extruded corn as ingredients. Diet composition and nutrient levels are presented in Table 1 .

Group received diet containing 2.30% calcium and 2,100 IU/kg vitamin D; Group : 2.30% calcium and 4,100 IU/kg vitamin D; Group : 2.30% calcium and 6,100 IU/kg vitamin D; Group : 2.70% calcium and 2,100 IU/kg vitamin D; Group : 2.70% calcium and 4,100 IU/kg vitamin D; Group : 2.70% calcium and 6,100 IU/kg vitamin D; Group : 3.10% calcium and 2,100 IU/kg vitamin D; Group : 3.10% calcium and 4,100 IU/kg vitamin D; and Group : 3.10% calcium and 6,100 IU/kg vitamin D. Dietary calcium levels were adjusted using zeolite powder and calcium hydrogen phosphate, while vitamin D was added as vitamin D_3 purchased from Zhejiang Xinweipu Additive Co., Ltd. The pre-trial lasted 13 days, and the formal trial lasted 60 days.

Table 1 Composition and Nutrient Levels of Experimental Diets (Air-Dry Basis), %

Note: The table content appears to be incomplete in the original text. The translation would include the full table if complete data were provided.

1.3 Management

Prior to the experiment, mink were vaccinated against canine distemper and parvovirus. All experimental mink were housed individually in cages and fed twice daily at 07:30 and 15:30 with free access to water. Daily feed intake was recorded, and health status was observed and recorded throughout the formal trial period.

1.4 Digestion-Metabolism Trial

The digestion-metabolism trial was conducted from August 16 to August 18, 2016, for three days using the total fecal collection method. Management during this period was identical to daily management. Urine was collected daily, with 20 mL of 10% sulfuric acid added to collection buckets before collection to fix nitrogen for subsequent nitrogen content determination. Feces were collected daily, weighed, and mixed with 10% sulfuric acid solution at 5% of fresh weight plus a small amount of toluene as preservative, then stored at -20°C. Three-day urine and fecal samples were thoroughly mixed separately. Fecal samples were 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-dried samples for laboratory analysis.

1.5 Measurements and Methods

At the start of the formal trial, initial body weight was measured on day 1, followed by fasting weight measurement every 15 days before morning feeding. Final weight was measured at trial conclusion to calculate individual and group average daily gain. Daily feed allowance and residual feed were recorded to calculate individual and group average daily feed intake. Basal diet and fecal samples were analyzed for dry matter, crude protein, crude fat, crude ash, calcium, and phosphorus content [18]. Vitamin D levels were determined by high-performance liquid chromatography according to GB/T 17818-2010.

Nutrient digestibility was calculated as:
Nutrient digestibility (%) = [(Nutrient intake - Total fecal nutrient)/Nutrient intake] × 100

Carbohydrate content = Dry matter content - Crude protein content - Crude fat content - Crude ash content

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

Data were expressed as "mean ± standard deviation," processed using Excel 2010, and analyzed using the General Linear Model (GLM) procedure in SAS 9.4 software for two-way ANOVA with interaction, followed by Duncan' s multiple comparison test. $P<0.05$ was considered significant, and $P<0.01$ was considered extremely significant.

2.1 Effects of Dietary Vitamin D and Calcium Levels on Growth Performance of Growing Minks

As shown in Table 2 , extremely significant differences were observed among groups in final weight, average daily gain, and feed-to-gain ratio ($P<0.01$). Group showed the highest final weight and average daily gain and the lowest feed-to-gain ratio. Dietary calcium level had extremely significant effects on final weight, average daily gain, and feed-to-gain ratio ($P<0.01$). Final weight and average daily gain showed an increasing trend with rising calcium levels, being extremely significantly lower at 2.3% calcium compared to 3.1% calcium ($P<0.01$). Feed-to-gain ratio showed a decreasing trend, being extremely significantly higher at 2.3% calcium compared to 2.7% and 3.1% calcium ($P<0.01$). Dietary vitamin D level also had extremely significant effects on final weight, average daily gain, and feed-to-gain ratio ($P<0.01$). At 4,100 IU/kg vitamin D, final weight and average daily gain were significantly higher than at 2,100 and 6,100 IU/kg ($P<0.05$), while feed-to-gain ratio was extremely significantly lower ($P<0.01$). A significant interaction between dietary vitamin D and calcium levels was observed for feed-to-gain ratio ($P<0.01$).

Table 2 Effects of Dietary Vitamin D and Calcium Levels on Growth Performance of Growing Minks

Note: The table contains detailed data for each group showing initial weight, final weight, average daily gain, average daily feed intake, and feed-to-gain ratio. In the same column and item, values with no letter or the same letter superscripts indicate no significant difference (P>0.05), different lowercase letters indicate significant difference (P<0.05), and different uppercase letters indicate extremely significant difference (P<0.01). This applies to all tables.

2.2 Effects of Dietary Vitamin D and Calcium Levels on Nutrient Digestibility of Growing Minks

As shown in Table 3 , extremely significant differences were observed among groups in dry matter output and fat digestibility (P<0.01), and significant differences in dry matter digestibility (P<0.05). Group had the lowest dry matter output and highest dry matter digestibility, while Group had the highest fat digestibility. Dietary calcium level extremely significantly affected fat digestibility (P<0.01) and significantly affected dry matter output (P<0.05). Fat digestibility was extremely significantly lower at 3.1% calcium compared to 2.3% and 2.7% calcium (P<0.01), while dry matter output was significantly higher at 3.1% calcium compared to 2.7% calcium (P<0.05). Dietary vitamin D level extremely significantly affected dry matter output (P<0.01) and significantly affected dry matter digestibility and fat digestibility (P<0.05). Dry matter output was extremely significantly lower at 4,100 IU/kg vitamin D compared to 2,100 and 6,100 IU/kg (P<0.01), while dry matter digestibility was significantly lower (P<0.05). Additionally, fat digestibility was significantly higher at 4,100 IU/kg vitamin D compared to 2,100 IU/kg (P<0.05). A significant interaction between dietary vitamin D and calcium levels was observed for fat digestibility (P<0.01). No significant effects were observed on dry matter intake, protein digestibility, or carbohydrate digestibility (P>0.05), though protein digestibility, carbohydrate digestibility, and fat digestibility showed quadratic trends of increasing then decreasing with vitamin D level, all peaking at 4,100 IU/kg vitamin D.

Table 3 Effects of Dietary Vitamin D and Calcium Levels on Nutrient Digestibility of Growing Minks

Note: The table presents data for dry matter intake, dry matter output, dry matter digestibility, protein digestibility, fat digestibility, and carbohydrate digestibility across all groups.

2.3 Effects of Dietary Vitamin D and Calcium Levels on Nitrogen Metabolism of Growing Minks

As shown in Table 4 , no significant differences were observed among groups in nitrogen intake, fecal nitrogen, or urinary nitrogen (P>0.05). However, extremely significant differences were found in nitrogen deposition (P<0.01), with

Group being highest and Group being lowest, and Group was significantly higher than all other groups (P<0.05). Additionally, net protein utilization differed extremely significantly among groups (P<0.01), with Group being extremely significantly higher than Groups and (P<0.01). Protein biological value showed significant differences (P<0.05), with Group being significantly higher than Groups , , , , and (P<0.05). Dietary calcium level extremely significantly affected nitrogen deposition (P<0.01), which showed an increasing trend with rising calcium levels and was extremely significantly higher at 3.1% calcium compared to 2.3% calcium (P<0.01). Calcium level had no significant effects on nitrogen intake, fecal nitrogen, urinary nitrogen, net protein utilization, or protein biological value (P>0.05). Vitamin D level had no significant effects on nitrogen intake, fecal nitrogen, or urinary nitrogen (P>0.05), but extremely significantly affected nitrogen deposition (P<0.01) and significantly affected net protein utilization and protein biological value (P<0.05). Nitrogen deposition, net protein utilization, and protein biological value showed quadratic trends of increasing then decreasing with vitamin D level, with nitrogen deposition being extremely significantly higher at 4,100 IU/kg compared to 2,100 and 6,100 IU/kg (P<0.01), and net protein utilization and protein biological value being significantly higher at 4,100 IU/kg (P<0.05). Significant interactions between dietary vitamin D and calcium levels were observed for nitrogen deposition, net protein utilization, and protein biological value (P<0.05).

Table 4 Effects of Dietary Vitamin D and Calcium Levels on Nitrogen Metabolism of Growing Minks

Note: The table presents data for nitrogen intake, fecal nitrogen, urinary nitrogen, nitrogen deposition, net protein utilization, and protein biological value across all groups.

3.1 Effects of Dietary Vitamin D and Calcium Levels on Growth Performance of Growing Minks

Table 2 shows inconsistent average daily gains among groups after the trial, with extremely significant differences, indicating that different dietary vitamin D and calcium levels affect mink growth performance. Both calcium and vitamin D levels must be maintained within certain ranges to ensure rapid growth. Wang et al. [19] reported significant interaction effects of dietary calcium, phosphorus, and vitamin D levels on daily feed intake and daily weight gain in early-stage growing Beijing ducks, but not on feed-to-gain ratio. In this study, dietary calcium and vitamin D levels showed significant interaction effects on feed-to-gain ratio in growing mink, but not on average daily gain or average daily feed intake. Ji [20] found that average daily gain in Duroc-Landrace-Yorkshire crossbred piglets increased then decreased with rising dietary calcium levels, consistent with results in pigs by Wang et al. [21] and Lin et al. [22], indicating that appropriate high calcium can improve growth performance within a certain range. Vitamin D, an anti-rachitic vitamin, promotes calcium and phosphorus absorption and is essential for animal growth. Appropriate dietary vitamin D

levels promote growth, while chronic excessive supplementation causes toxic side effects. Atencio et al. [23] demonstrated that vitamin D improved broiler feed intake, weight gain, and reduced chick mortality, thereby enhancing growth performance, consistent with Yang [24]. However, Jia [25] found that broiler weight increased then decreased with rising dietary vitamin D levels, consistent with our results. Under our experimental conditions, average daily gain in mink showed an increasing trend with rising dietary calcium levels, suggesting that high calcium benefits growth performance in growing mink. The optimal calcium level of 3.1% is higher than previous findings [7-8], possibly due to differences in mink breed, size, dietary calcium-to-phosphorus ratio, and vitamin D levels, requiring further investigation. Compared to 2,100 IU/kg vitamin D, 4,100 IU/kg improved average daily gain, while further increase to 6,100 IU/kg reduced gain, suggesting that appropriate vitamin D levels promote growth but excessive levels are detrimental. Therefore, dietary calcium at 3.1% and vitamin D at 4,100 IU/kg provided optimal growth performance for growing mink.

3.2 Effects of Dietary Vitamin D and Calcium Levels on Nutrient Digestibility of Growing Minks

Research indicates that dietary vitamin D level has no significant effect on average daily feed intake in 3-5 month-old Rex rabbits [26] or 1-28 day-old ducklings [27], consistent with our results. In this trial, dry matter output in growing mink decreased then increased with rising calcium levels, while dry matter digestibility showed an increasing trend, consistent with body weight changes. With fixed calcium-to-phosphorus ratio, increased calcium level means increased phosphorus level. Takeuchi et al. [28] reported that phosphorus participates in ATP energy supply, enhancing fatty acid activation, and increased phosphorus helps enhance β-oxidation and glycogen production, thereby reducing fat deposition and increasing protein deposition. However, excessive calcium can form insoluble soaps with fatty acids in the intestine, reducing saturated fat digestibility [14,29-30]. The primary physiological function of vitamin D is regulating calcium and phosphorus absorption to maintain normal blood calcium and phosphorus levels. Appropriate vitamin D promotes calcium and phosphorus absorption, but excessive intake causes toxicity, manifested as polyuria, hypercalciuria, decreased appetite or anorexia, and growth stagnation [25]. In this study, fat digestibility in growing mink increased then decreased with rising calcium levels, being 2% lower at 3.1% calcium than at 2.7% calcium, consistent with previous research. Fat digestibility also increased then decreased with vitamin D level, possibly because appropriate vitamin D promotes calcium absorption and improves fat digestibility, while excessive vitamin D increases blood calcium, causing deposition in joints, blood vessels, heart, intestinal walls, and other tissues, leading to liver overload, tissue/organ degeneration and calcification, and reduced fat digestibility. Dietary calcium and vitamin D levels had no significant effect on carbohydrate digestibility, with average carbohydrate digestibility of 81.16% across groups, higher than results in mink by Zhang et al. [10], possibly because our dietary energy was lower than the 17.08 MJ/kg recommended

by NRC (1982) [15], prompting animals to increase carbohydrate digestibility to meet energy demands, requiring further investigation. Under our experimental conditions, dietary calcium at 2.7% and vitamin D at 4,100 IU/kg favored optimal nutrient digestibility in growing mink.

3.3 Effects of Dietary Vitamin D and Calcium Levels on Nitrogen Metabolism of Growing Minks

Research indicates no significant interaction between dietary crude protein and phosphorus levels [31]. Liu et al. [14] reported that different calcium and phosphorus levels had no significant effect on fecal or urinary nitrogen in growing mink, but nitrogen deposition increased then decreased with rising phosphorus levels, and increased phosphorus levels improved nitrogen utilization, net protein utilization, and protein biological value. Mudd et al. [32] suggested that nitrogen deposition is more affected by dietary phosphorus than calcium levels. Vipperman et al. [33] found that high calcium reduced nitrogen deposition, primarily because calcium, phosphorus, and fat form saponins that inhibit some amino acid functions. Under our experimental conditions, nitrogen deposition continuously increased with rising calcium levels, as our highest calcium level was within the appropriate range, consistent with previous findings. Dietary calcium level had no significant effect on net protein utilization or protein biological value, possibly because phosphorus gradient changes were small with fixed calcium-to-phosphorus ratio. Nitrogen deposition, net protein utilization, and protein biological value increased then decreased with vitamin D level, possibly because appropriate vitamin D promotes phosphorus absorption and improves nitrogen utilization, while excessive vitamin D increases blood calcium and phosphorus, reducing nitrogen utilization. Under our experimental conditions, calcium at 3.1% and vitamin D at 4,100 IU/kg provided the highest nitrogen utilization in growing mink.

Based on comprehensive analysis of all indicators, under the conditions of this experiment, growing mink achieved optimal growth performance, nutrient digestibility, and nitrogen utilization when dietary calcium-to-phosphorus ratio was 2:1, vitamin D level was 4,100 IU/kg, and calcium level was 3.1%.

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