

## Effects of Different Dietary Phosphorus Levels and Calcium to Phosphorus Ratios on Growth Performance, Nutrient Digestibility, and Nitrogen, Calcium, and Phosphorus Metabolism in Mink during the Winter Fur Period: Postprint

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### Abstract

This experiment was conducted to investigate the effects of different dietary phosphorus levels and calcium to phosphorus ratios on growth performance, nutrient digestibility, and nitrogen, calcium, and phosphorus metabolism in mink during the winter fur period. Ninety healthy female mink of similar body weight at  $(130 \pm 10)$  days of age were selected and randomly allocated to 9 groups with 10 replicates per group and 1 mink per factorial experimental design was employed, with three phosphorus levels of 1.0%, 1.4%, and 1.8%, and three calcium to phosphorus ratios of 1.0, 1.5, and 2.0, to formulate 9 experimental diets. The calcium and phosphorus levels of the 9 experimental diets were as follows: 1.03% calcium, 0.97% phosphorus (Group I), 1.47% calcium, 0.98% phosphorus (Group II), 1.98% calcium, 0.99% phosphorus (Group III), 1.45% calcium, 1.37% phosphorus (Group IV), 2.08% calcium, 1.38% phosphorus (Group V), 2.79% calcium, 1.38% phosphorus (Group VI), 1.81% calcium, 1.75% phosphorus (Group VII), 2.70% calcium, 1.79% phosphorus (Group VIII), and 3.59% calcium, 1.80% phosphorus (Group IX). The preliminary period lasted 10 days, and the experimental period lasted 67 days. The results showed that: 1) Dietary phosphorus level, calcium to phosphorus ratio, and their interaction had extremely significant effects on final body weight and average daily gain of mink during the winter fur period ( $P < 0.01$ ). Group V exhibited extremely significantly higher final body weight and average daily gain than the other groups ( $P < 0.01$ ). 2) Dry matter, protein, and fat digestibility showed a trend of increasing first and then decreasing with increasing dietary calcium to phosphorus ratio and phosphorus level, reaching maximum values in Group III. 3) Dietary phosphorus level, calcium to phosphorus ratio, and their interaction had no significant effects on

nitrogen intake, urinary nitrogen, fecal nitrogen, nitrogen retention, net protein utilization, or protein biological value in mink during the winter fur period ( $P > 0.05$ ). 4) Fecal calcium content increased correspondingly with increasing dietary phosphorus level, with extremely significant differences among groups ( $P < 0.01$ ); Groups VIII and IX had significantly or extremely significantly higher fecal calcium content than the other groups except Group VII ( $P < 0.05$  or  $P < 0.01$ ). Fecal phosphorus content increased correspondingly with increasing dietary phosphorus level, with significant or extremely significant differences among groups ( $P < 0.05$  or  $P < 0.01$ ); fecal phosphorus content was lowest when the dietary calcium to phosphorus ratio was 2.0, being extremely significantly lower than when the ratio was 1.0 and 1.5 ( $P < 0.01$ ); Groups VIII and IX had significantly or extremely significantly higher fecal phosphorus content than the other groups except Group VII ( $P < 0.05$  or  $P < 0.01$ ). Dietary phosphorus level and calcium to phosphorus ratio extremely significantly affected calcium digestibility ( $P < 0.01$ ). Calcium digestibility increased extremely significantly with increasing dietary calcium to phosphorus ratio ( $P < 0.01$ ). Group III exhibited the highest calcium digestibility, being extremely significantly higher than the other groups except Groups VI and IX ( $P < 0.01$ ). Dietary phosphorus level and calcium to phosphorus ratio had no significant effect on phosphorus digestibility ( $P > 0.05$ ), but their interaction had a significant effect on phosphorus digestibility ( $P < 0.05$ ). Phosphorus digestibility was lowest in Group I and highest in Group III. Based on comprehensive indices, and considering dietary cost reduction, environmental protection, and maintenance of growth performance in mink during the winter fur period, a dietary phosphorus level of 1.4% and a calcium to phosphorus ratio of 1.5-2.0 are appropriate.

## Full Text

### Effects of Different Dietary Phosphorus Levels and Calcium/Phosphorus Ratios on Growth Performance, Nutrient Digestibility, and Nitrogen, Calcium, and Phosphorus Metabolism of Minks during the Winter Fur-Growing Period

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## Abstract

This experiment was conducted to investigate the effects of different dietary phosphorus levels and calcium/phosphorus (Ca/P) ratios on growth performance, nutrient digestibility, and nitrogen, calcium, and phosphorus

metabolism in minks during the winter fur-growing period. Ninety healthy female minks aged (130 $\pm$ 10)dayswithsimilarbodyweightwererandomlydividedinto9groups,with10replicatesper factorial design was employed, with three phosphorus levels (1.0%, 1.4%, and 1.8%) and three Ca/P ratios (1.0, 1.5, and 2.0), resulting in nine experimental diets with the following calcium and phosphorus concentrations: 1.03% Ca, 0.97% P (Group I); 1.47% Ca, 0.98% P (Group II); 1.98% Ca, 0.99% P (Group III); 1.45% Ca, 1.37% P (Group IV); 2.08% Ca, 1.38% P (Group V); 2.79% Ca, 1.38% P (Group VI); 1.81% Ca, 1.75% P (Group VII); 2.70% Ca, 1.79% P (Group VIII); and 3.59% Ca, 1.80% P (Group IX). The experiment consisted of a 10-day adaptation period followed by a 67-day formal experimental period.

The results showed that: (1) dietary phosphorus level, Ca/P ratio, and their interaction had highly significant effects on final body weight and average daily gain ( $P<0.01$ ), with Group V showing significantly higher final weight and average daily gain than all other groups ( $P<0.01$ ); (2) dry matter, protein, and fat digestibility exhibited a trend of initially increasing then decreasing with rising dietary Ca/P ratios and phosphorus levels, reaching maximum values in Group III; (3) dietary phosphorus level, Ca/P ratio, and their interaction had no significant effects on nitrogen intake, urinary nitrogen, fecal nitrogen, nitrogen retention, net protein utilization, or protein biological value ( $P>0.05$ ); (4) fecal calcium content increased significantly with dietary phosphorus level ( $P<0.01$ ), with Groups VIII and IX showing significantly higher fecal calcium than all groups except VII ( $P<0.05$  or  $P<0.01$ ). Fecal phosphorus content also increased with dietary phosphorus level ( $P<0.05$  or  $P<0.01$ ), with the lowest values observed at a Ca/P ratio of 2.0, which was significantly lower than at Ca/P ratios of 1.0 and 1.5 ( $P<0.01$ ). Groups VIII and IX exhibited significantly higher fecal phosphorus than all groups except VII ( $P<0.05$  or  $P<0.01$ ). Dietary phosphorus level and Ca/P ratio significantly affected calcium digestibility ( $P<0.01$ ), which increased significantly with higher Ca/P ratios ( $P<0.01$ ). Group III showed the highest calcium digestibility, significantly exceeding all groups except VI and IX ( $P<0.01$ ). While dietary phosphorus level and Ca/P ratio did not significantly affect phosphorus digestibility ( $P>0.05$ ), their interaction had a significant effect ( $P<0.05$ ), with the lowest phosphorus digestibility in Group I and the highest in Group III.

Based on comprehensive evaluation of all indices, a dietary phosphorus level of 1.4% and Ca/P ratio of 1.5-2.0 are recommended as optimal for minks during the winter fur-growing period, considering feed cost reduction, environmental protection, and maintenance of growth performance.

**Keywords:** calcium/phosphorus ratio; minks; growth performance; nitrogen metabolism; calcium metabolism; phosphorus metabolism

## 1. Materials and Methods

### 1.1 Experimental Animals and Design

This experiment utilized 90 healthy female minks aged  $(130 \pm 10)$  days with similar body weight, randomly allocated to a factorial design was employed, establishing three phosphorus levels (1.0%, 1.4%, and 1.8%) and three Ca/P ratios (1.0, 1.5, and 2.0) based on recent literature and practical production considerations. Nine experimental diets were formulated according to these parameters and previous research on mink nutritional requirements. The specific calcium and phosphorus levels were: 1.03% Ca, 0.97% P (Group I); 1.47% Ca, 0.98% P (Group II); 1.98% Ca, 0.99% P (Group III); 1.45% Ca, 1.37% P (Group IV); 2.08% Ca, 1.38% P (Group V); 2.79% Ca, 1.38% P (Group VI); 1.81% Ca, 1.75% P (Group VII); 2.70% Ca, 1.79% P (Group VIII); and 3.59% Ca, 1.80% P (Group IX). The study included a 10-day adaptation period followed by a 67-day formal experimental period, conducted at the Key Field Scientific Observation and Experiment Station of Wild Biological Resources in Changbai Mountain, Ministry of Agriculture.

### 1.2 Feeding Management

Prior to the experiment, all minks were vaccinated against canine distemper and parvovirus. Minks were housed individually in cages, fed twice daily at 07:30 and 15:30 with ad libitum access to feed and water. Daily feed intake was recorded, and body weight was measured every 15 days in the morning before feeding, starting from the first day of the formal period. Health status was monitored and recorded daily.

### 1.3 Digestion and Metabolism Trial

After 42 days of the experiment, six minks with similar body weight were selected from each group for a digestion and metabolism trial conducted from October 27 to October 30, 2015 (4 days). The total fecal collection method was employed, with feeding management identical to routine practices. Urine was collected daily, with 20 mL of 10% sulfuric acid added to collection buckets to preserve nitrogen. Feces were collected daily, weighed, and preserved with 10% sulfuric acid (5% of fresh weight) and a small amount of toluene, then stored at  $-20^{\circ}\text{C}$ . After 4 days, urine and fecal samples were thoroughly mixed. Fecal samples were sterilized at  $80^{\circ}\text{C}$  for 2 hours, then dried at  $65^{\circ}\text{C}$  to constant weight, ground to pass through a 40-mesh sieve, and prepared as air-dried samples for laboratory analysis.

### 1.4 Measurement Indicators and Methods

Initial body weight was recorded on the first day of the formal period, with subsequent measurements every 15 days before morning feeding. Final body weight was recorded at the end of the experiment to calculate individual and group average daily gain (ADG). Daily feed provision and refusal were recorded

to calculate individual and group average daily feed intake (ADFI). Feed conversion ratio (F/G) was calculated from ADFI and ADG. Laboratory analyses included: dry matter content by oven drying at 105°C (GB/T 6435 2006), crude fat by Soxhlet extraction (GB/T 6433 2006), crude protein by Kjeldahl method (GB/T 6432 1994), calcium by EDTA titration (GB/T 6436 2002), and phosphorus by ammonium vanadate-molybdate colorimetry (GB/T 6437 2002).

### 1.5 Calculation Formulas

Average daily gain (g/d) = (final weight - initial weight) / experimental days  
Average daily feed intake (g/d) = total feed intake during experimental period / experimental days  
Feed conversion ratio = average daily feed intake / average daily gain  
Dry matter digestibility (%) = [(dry matter intake - dry matter excretion) / dry matter intake] × 100  
Protein digestibility (%) = [(protein intake - protein excretion) / protein intake] × 100  
Fat digestibility (%) = [(fat intake - fat excretion) / fat intake] × 100  
Calcium digestibility (%) = [(calcium intake - calcium excretion) / calcium intake] × 100  
Phosphorus digestibility (%) = [(phosphorus intake - phosphorus excretion) / phosphorus intake] × 100  
Nitrogen retention (g/d) = nitrogen intake - fecal nitrogen - urinary nitrogen  
Net protein utilization (%) = (nitrogen retention / nitrogen intake) × 100  
Protein biological value (%) = [nitrogen retention / (nitrogen intake - fecal nitrogen)] × 100

### 1.6 Statistical Analysis

Data were organized using Excel 2010 and analyzed using the General Linear Model (GLM) procedure in SAS 8.0 software for two-way ANOVA with interaction effects. Duncan's multiple range test was used for post-hoc comparisons, with  $P < 0.05$  considered significant and  $P < 0.01$  considered highly significant.

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## 2. Results

### 2.1 Effects of Dietary Phosphorus Levels and Ca/P Ratios on Growth Performance

As shown in , no significant differences were observed in average daily feed intake among groups ( $P > 0.05$ ). Dietary phosphorus level, Ca/P ratio, and their interaction had highly significant effects on final body weight, average daily gain, and feed conversion ratio ( $P < 0.01$ ). Final body weight in Group V was significantly or highly significantly higher than in other groups ( $P < 0.05$  or  $P < 0.01$ ). Average daily gain was highest in Group V and lowest in Group

IV, showing a quadratic trend of initially increasing with Ca/P ratio, peaking at a Ca/P ratio of 1.5, then decreasing. Feed conversion ratio was significantly higher in Group IV than in all other groups ( $P < 0.01$ ), while Group V had the lowest ratio ( $P < 0.01$ ).

## 2.2 Effects of Dietary Phosphorus Levels and Ca/P Ratios on Nutrient Digestibility

shows that no significant differences existed among groups in dry matter intake, dry matter excretion, or digestibility of dry matter, protein, and fat ( $P > 0.05$ ). However, dry matter, protein, and fat digestibility exhibited similar patterns, initially increasing then decreasing with rising dietary Ca/P ratios and phosphorus levels.

## 2.3 Effects of Dietary Phosphorus Levels and Ca/P Ratios on Nitrogen Metabolism

indicates that dietary phosphorus level, Ca/P ratio, and their interaction had no significant effects on nitrogen intake, urinary nitrogen, fecal nitrogen, nitrogen retention, net protein utilization, or protein biological value ( $P > 0.05$ ). Group III showed higher nitrogen retention, net protein utilization, and protein biological value than other groups, but differences were not significant ( $P > 0.05$ ). Nitrogen retention, net protein utilization, and protein biological value tended to increase with higher Ca/P ratios.

## 2.4 Effects of Dietary Phosphorus Levels and Ca/P Ratios on Calcium and Phosphorus Metabolism

demonstrates that fecal calcium content increased significantly with dietary phosphorus level ( $P < 0.01$ ), initially rising then declining with increasing Ca/P ratios. Group VIII had the highest fecal calcium content, significantly or highly significantly exceeding all groups except IX ( $P < 0.05$  or  $P < 0.01$ ), while Group III had the lowest content, representing a 152% reduction compared to Group VIII. Fecal phosphorus content also increased with dietary phosphorus level ( $P < 0.05$  or  $P < 0.01$ ), with the lowest values observed at a Ca/P ratio of 2.0, which was highly significantly lower than at Ca/P ratios of 1.0 and 1.5 ( $P < 0.01$ ). Groups VIII and IX showed significantly higher fecal phosphorus than all groups except VII ( $P < 0.05$  or  $P < 0.01$ ), while Group III had the lowest content, representing a 128% reduction compared to Group VIII.

Dietary phosphorus level and Ca/P ratio significantly affected calcium digestibility ( $P < 0.01$ ), which increased significantly with higher Ca/P ratios ( $P < 0.01$ ). Group III achieved the highest calcium digestibility, significantly exceeding all groups except VI and IX ( $P < 0.01$ ), while Groups I, IV, and VII had significantly lower calcium digestibility than other groups ( $P < 0.05$  or  $P < 0.01$ ). Although dietary phosphorus level and Ca/P ratio did not significantly affect phosphorus

digestibility ( $P>0.05$ ), their interaction had a significant effect ( $P<0.05$ ). Phosphorus digestibility was lowest in Group I and highest in Group III, with Group III significantly exceeding Groups I and II ( $P<0.01$ ).

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### 3. Discussion

#### 3.1 Effects on Growth Performance

Wang (2010) reported that dietary calcium and phosphorus levels significantly affected average daily gain and feed conversion ratio in 0-6 week-old broiler chickens, with highly significant interactive effects on average daily gain, average daily feed intake, and feed conversion ratio. In the present study, average daily gain in minks showed a quadratic trend with increasing Ca/P ratio, peaking then declining. At 1.8% phosphorus level, increasing dietary calcium reduced average daily gain, likely because excessive calcium impaired palatability, reduced feed intake, diluted dietary nutrient concentration, and interfered with absorption of other minerals such as manganese and zinc. Low-phosphorus diets also reduce feed intake, and the 1.0% phosphorus group in this study showed the lowest average daily gain, only half that of the 1.4% and 1.8% phosphorus groups, indicating that low phosphorus impaired weight gain.

Lu et al. (2000) demonstrated that low dietary phosphorus (0.381%) reduced weight gain and feed efficiency in broiler chickens. Guo et al. (1995) confirmed that low-phosphorus diet effects on broiler weight gain were closely related to dietary calcium levels, with high Ca/P ratios significantly reducing weight gain while low Ca/P ratios had no significant effect. In this study, Group V (2.08% Ca, 1.38% P) achieved the highest average daily gain and lowest feed conversion ratio, indicating that diets with low calcium, low phosphorus, and low Ca/P ratios were detrimental to mink growth, while high calcium, high phosphorus, and high Ca/P ratios also failed to optimize growth performance. Previous research found that a Ca/P ratio of 2.0 during July to pelting significantly reduced body weight in minks. However, other studies reported no significant effects of different calcium and phosphorus levels or Ca/P ratios on growth performance in pigs, with no significant interactive effects. From a production standpoint, calcium and phosphorus requirements can vary by 30% without affecting animal performance.

#### 3.2 Effects on Nutrient Digestibility

While dietary phosphorus level and Ca/P ratio did not significantly affect nutrient digestibility in minks during the winter fur-growing period, digestibility showed a trend of initially increasing then decreasing with rising phosphorus levels and Ca/P ratios, consistent with growth performance patterns. Increasing dietary calcium reduced fat digestibility because divalent metal ions, particularly calcium and magnesium, form insoluble soaps with fatty acids, reducing

saturated fatty acid digestibility. Elevated calcium also affected protein digestibility; Skrede (1978) found a linear relationship between ash content and protein digestibility in cod, with each 1% increase in ash reducing true nitrogen digestibility by 0.6%.

Phosphorus participates in energy metabolism as a component of ATP and phosphocreatine and is essential for substrate phosphorylation. Phosphorus deficiency also affects nutrient digestibility. Studies in fish demonstrated that dietary phosphorus deficiency disrupts metabolism and increases fat deposition. Vielma et al. (2002) suggested that inorganic phosphorus deficiency inhibits mitochondrial esterification of free fatty acids and acetyl-CoA, preventing acyl-CoA formation and reducing fat utilization as energy, with calcium having similar effects.

### 3.3 Effects on Nitrogen Metabolism

Nitrogen retention patterns were consistent with growth performance, with Group III (1.98% Ca, 0.99% P) showing the highest nitrogen retention, net protein utilization, and protein biological value. Dietary phosphorus level had minimal impact on nitrogen metabolism. Nitrogen retention, protein biological value, and net protein utilization increased with higher Ca/P ratios, peaking at a Ca/P ratio of 2.0, likely because higher ratios reduced urinary nitrogen excretion and increased nitrogen deposition, promoting nitrogen utilization. However, when Ca/P ratio exceeded 2.0, nitrogen retention decreased with further calcium increases, consistent with Shafey and McDonald (1991) who reported that excessive calcium significantly reduced nitrogen digestibility and metabolizable energy in chickens.

### 3.4 Effects on Calcium and Phosphorus Metabolism

Fecal phosphorus content increased continuously with dietary phosphorus level, while phosphorus digestibility showed a quadratic trend of initially increasing then decreasing. This reflects two intestinal phosphorus absorption pathways: paracellular transport and sodium-dependent transport. Active phosphorus absorption requires transport proteins with saturation kinetics; at low dietary phosphorus levels, absorption increases with dietary level, but excess phosphorus exceeds carrier capacity, resulting in incomplete absorption and environmental pollution. Fang et al. (2011) demonstrated that increasing dietary phosphorus from 0.16% to 1.10% progressively reduced Na/Pi-IIb mRNA expression in the duodenum, anterior jejunum, and posterior jejunum, indicating decreased carrier capacity and phosphorus absorption.

Fecal calcium content also increased significantly with dietary phosphorus level, while calcium digestibility initially increased then decreased, indicating that dietary phosphorus level affects calcium absorption. Calcium absorption adapts to physiological needs, increasing during deficiency and decreasing when calcium is abundant. At 1.0% phosphorus level, phosphorus digestibility increased with

dietary calcium, but at 1.4% and 1.8% phosphorus levels, increasing calcium reduced phosphorus digestibility, suggesting that phosphorus requirements were met and excessive calcium interfered with phosphorus absorption, consistent with Jongbloed (1987) in pigs. Dietary calcium and phosphorus levels must be maintained within appropriate ranges, as improper ratios severely affect absorption and utilization. The solubility product of calcium phosphate is constant; excess of either mineral impairs absorption of the other, and inadequate absorption of one affects bone formation while the excess mineral is excreted, causing waste.

Based on comprehensive evaluation of all indices, a dietary phosphorus level of 1.4% and Ca/P ratio of 1.5-2.0 are recommended as optimal for minks during the winter fur-growing period, balancing feed cost reduction, environmental protection, and growth performance maintenance.

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