# Effects of Dietary Protein and Fat Levels on Growth Performance, Nutrient Digestibility, and Nitrogen Metabolism in Growing Female Mink: Postprint

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## Abstract

This experiment aimed to investigate the effects of dietary protein and fat levels on growth performance, nutrient digestibility, and nitrogen metabolism of female mink during the growing period, and to determine the appropriate levels of protein and fat in diets for growing female mink. A 2$×$3 factorial design was adopted, with two protein levels of 32% (L) and 36% (H) and three fat levels of 10% (L), 20% (M), and 30% (H), resulting in six experimental diets. According to protein and fat levels, the six experimental diets were designated as LL, LM, LH, HL, HM, and HH. Ninety healthy female mink at 60 days of age were selected and randomly divided into six groups with 15 replicates per group and one mink per replicate. The preliminary period lasted 7 d, and the formal experimental period lasted 60 d. The results showed that the final body weight and average daily gain of mink in the LH group were the highest, significantly higher than those in the LL and HL groups ($P<0.05$), and showed an increasing trend with increasing dietary fat levels. Both average daily feed intake and feed conversion ratio were lowest in the LH group; the average daily feed intake of mink in the 30% fat level group was extremely significantly lower than that in the 20% fat level group ($P<0.01$); feed conversion ratio increased with increasing dietary fat levels, and the 30% fat level group was extremely significantly lower than the 10% and 20% fat level groups ($P<0.01$). The dry matter digestibility and fat digestibility of mink in the LL and HL groups were extremely significantly lower than those in other groups ($P<0.01$); the protein digestibility of mink in the HH group was significantly higher than that in the LL, LH, and HL groups ($P<0.05$). With increasing dietary protein levels, protein digestibility and fat digestibility increased significantly ($P<0.05$) and extremely significantly ($P<0.01$), respectively; the dry matter digestibility and protein digestibility of the 10% fat level group were extremely significantly lower than those of the

20% and 30% fat level groups (P<0.01); with increasing dietary fat levels, fat digestibility showed an increasing trend; carbohydrate digestibility was lowest in the 30% fat level group, extremely significantly lower than that in the 10% and 20% fat level groups (P<0.01). Nitrogen intake of mink was highest in the HM group, extremely significantly higher than that in other groups (P<0.01); fecal nitrogen excretion was highest in the HL group, significantly higher than that in the LH and HH groups (P<0.05); urinary nitrogen excretion was lowest in the LH group, while nitrogen retention and nitrogen biological value were highest in the LH group. As dietary protein level increased from 32% to 36%, nitrogen intake and urinary nitrogen excretion increased extremely significantly (P<0.01), while nitrogen biological value decreased significantly (P<0.05). The 20% fat level group had the highest nitrogen intake, extremely significantly higher than that in the 30% fat level group (P<0.01); both fecal nitrogen excretion and urinary nitrogen excretion were lowest in the 30% fat level group, showing significant (P<0.05) or extremely significant (P<0.01) differences from the 10% and 20% fat level groups; with increasing dietary fat levels, nitrogen retention showed a trend of first increasing then decreasing, while nitrogen biological value showed an increasing trend. Based on comprehensive analysis of all indicators, under the conditions of this experiment, when dietary protein level was 32% and fat level was 20%-30%, growing female mink exhibited optimal growth performance, and could reduce urinary nitrogen excretion while improving the utilization efficiency of dietary protein by mink.

## Full Text

# Effects of Dietary Protein and Fat Levels on Growth Performance, Nutrient Digestibility and Nitrogen Metabolism of Female Minks during the Growing Period

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### Abstract

This experiment was conducted to investigate the effects of dietary protein and fat levels on growth performance, nutrient digestibility, and nitrogen metabolism of female minks during the growing period, and to determine the appropriate dietary protein and fat levels for this production stage. A 2$×$3 factorial design was employed with two protein levels [32% (L) and 36% (H)] and three fat levels [10% (L), 20% (M), and 30% (H)], resulting in six experimental diets designated as LL, LM, LH, HL, HM, and HH according to their protein and fat content. Ninety healthy 60-day-old female minks

were randomly allocated into six groups with 15 replicates per group and one mink per replicate. The study consisted of a 7-day adaptation period followed by a 60-day formal experimental period. The results showed that the LH group achieved the highest final body weight and average daily gain, which were significantly higher than those of the LL and HL groups ($P<0.05$) and showed an increasing trend with rising dietary fat levels. The LH group also exhibited the lowest average daily feed intake and feed-to-gain ratio. The 30% fat level group showed extremely significantly lower average daily feed intake compared with the 20% fat level group ($P<0.01$), while feed-to-gain ratio decreased as dietary fat level increased, with the 30% fat level group being extremely significantly lower than both the 10% and 20% fat level groups ($P<0.01$). Dry matter digestibility and fat digestibility in the LL and HL groups were extremely significantly lower than in other groups ($P<0.01$), while protein digestibility in the HH group was significantly higher than in the LL, LH, and HL groups ($P<0.05$). As dietary protein level increased, protein digestibility and fat digestibility increased significantly ($P<0.05$) and extremely significantly ($P<0.01$), respectively. The 10% fat level group showed extremely significantly lower dry matter digestibility and protein digestibility compared with the 20% and 30% fat level groups ($P<0.01$). Fat digestibility increased with rising dietary fat levels, while carbohydrate digestibility was lowest in the 30% fat level group, being extremely significantly lower than in the 10% and 20% fat level groups ($P<0.01$). Nitrogen intake was highest in the HM group, being extremely significantly higher than in all other groups ($P<0.01$), while fecal nitrogen excretion was highest in the HL group, significantly higher than in the LH and HH groups ($P<0.05$). Urinary nitrogen excretion was lowest in the LH group, whereas nitrogen deposition and biological value of nitrogen were highest in this group. As dietary protein level increased from 32% to 36%, nitrogen intake and urinary nitrogen excretion increased extremely significantly ($P<0.01$), while nitrogen biological value decreased significantly ($P<0.05$). The 20% fat level group showed the highest nitrogen intake, extremely significantly higher than the 30% fat level group ($P<0.01$). Both fecal and urinary nitrogen excretion were lowest in the 30% fat level group, with significant ($P<0.05$) or extremely significant ($P<0.01$) differences compared with the 10% and 20% fat level groups. Nitrogen deposition increased initially then decreased with rising dietary fat levels, while nitrogen biological value showed an increasing trend. Based on comprehensive evaluation of all indices, female minks during the growing period achieved optimal growth performance with reduced urinary nitrogen excretion and improved dietary protein utilization when fed diets containing 32% protein and 20%–30% fat under the conditions of this experiment.

**Keywords:** growing period; female mink; protein; fat; growth performance

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Mink are valuable fur-bearing animals, and protein and fat are important nutritional components in mink diets that directly affect feeding costs, growth development, and fur quality. Research on the appropriate protein-to-fat ratio

in mink diets is significant for guiding rational diet formulation and improving economic efficiency in mink production. Currently, numerous studies have investigated protein requirements in mink [1-3], and reports on fat and energy requirements are also available [4-6]. However, research on the optimal dietary protein-to-fat ratio remains scarce. Studies have demonstrated that metabolizable energy from fat has higher productive value than that from other nutrients [7]. Adding non-protein energy sources such as fat to animal diets can replace partial protein for energy supply, thereby improving dietary protein utilization [8-9], enhancing animal performance, and reducing feed costs. Therefore, this experiment was conducted to determine appropriate dietary protein and fat levels for growing minks by feeding different protein and fat levels and evaluating growth performance, nutrient digestibility, and nitrogen metabolism indices, thereby establishing a foundation for improving nutritional standards and providing scientific basis for rational diet formulation in mink production.

## 1 Materials and Methods

### 1.1 Experimental Animals

Ninety healthy female standard black minks of similar body weight at (60$±$3) days of age during the growing period were selected at the Fur Animal Base of the Changbai Mountain Wildlife Resources Key Field Scientific Observation and Experiment Station of the Ministry of Agriculture for this study.

### 1.2 Experimental Design

The 90 experimental minks were randomly divided into six groups with 15 replicates per group and one mink per replicate. A 2$×$3 factorial design was employed with two protein levels [32% (L) and 36% (H)] and three fat levels [10% (L), 20% (M), and 30% (H)], resulting in six experimental diets. According to protein and fat levels, the six diets were designated as LL, LM, LH, HL, HM, and HH and fed to the six experimental groups, respectively.

### 1.3 Experimental Diets

Extruded corn, corvina, chicken offal, pork, and soybean oil were used as main ingredients to formulate experimental diets with corresponding protein and fat levels based on the experimental design. Diet composition and nutrient levels are presented in Table 1 .

**Table 1 Composition and nutrient levels of experimental diets (air-dry basis), %**

*Note: 1) One kilogram of premix contained the following: VA 200,000 IU, VD3 40,000 IU, VE 5,000 IU, VB1 125 mg, VB2 200 mg, VB6 200 mg, VB12 2.5 mg, VK3 40 mg, VC 7,500 mg, niacin acid 500 mg, pantothenic acid 800 mg, folic acid 100 mg, choline 10,000 mg, biotin 7.5 mg, Fe 2,000 mg, Cu 500 mg, Mn 400 mg, Zn 1,500 mg, I 15 mg, Se 5 mg, Co 7.5 mg. 2) Crude protein,*

*crude fat, Ca, and TP were measured values, while carbohydrate and ME were calculated values [10].*

### 1.4 Feeding Management

Prior to the experiment, minks were vaccinated against canine distemper and parvovirus. Experimental minks were housed individually in cages and fed twice daily at 08:00 and 16:00 with free access to feed and water. Actual feed intake was recorded daily. The adaptation period lasted 7 days, followed by a 60-day formal experimental period.

### 1.5 Digestion and Metabolism Trial

Four days after the start of the formal experimental period, eight healthy minks of similar body weight were selected from each group for a 4-day digestion and metabolism trial. The total feces collection method was used with a specialized mink feces-urine collection device. For urine collection, 2 mL of 10% sulfuric acid was added per 100 mL for nitrogen fixation, plus 4 drops of toluene as preservative, and samples were stored at -20°C. For feces collection, 10% sulfuric acid was added at 5% of fresh weight for nitrogen fixation, with a small amount of toluene as preservative, and samples were stored at -20°C. Urine and feces collected over the 4 days were thoroughly mixed separately. Feces 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-dry samples for laboratory analysis.

### 1.6 Measured Indices and Methods

Body weight was measured on day 1 as initial weight, then every 15 days in the morning before feeding, and at the end of the experiment as final weight. Average daily gain (ADG) was calculated for each mink and each group. Daily feed allowance and refusals were recorded to calculate individual and group average daily feed intake (ADFI). Feed-to-gain ratio (F/G) was calculated based on group average daily gain and average daily feed intake.

Sample analysis: Dry matter content was determined by oven-drying at 105°C according to GB/T 6435-2006; crude fat content by Soxhlet extraction according to GB/T 6433-2006; crude protein content by Kjeldahl method according to GB/T 6432-1994; crude ash content according to GB/T 6438-2007; calcium content according to GB/T 6436-2002; and total phosphorus content according to GB/T 6437-2002.

### 1.7 Calculation Formulas

Carbohydrate content (%) = 100 - crude ash content - crude fat content - crude protein content
Average daily feed intake (g/d) = total feed intake during experimental period

/ number of days
Average daily gain (g/d) = (final weight - initial weight) / number of days
Feed-to-gain ratio = average daily feed intake / average daily gain
Nutrient digestibility (%) = [(nutrient intake - fecal nutrient excretion) / nutrient intake] × 100
Nitrogen deposition (g/d) = nitrogen intake - fecal nitrogen output - urinary nitrogen output
Biological value of nitrogen (%) = [nitrogen deposition / (nitrogen intake - fecal nitrogen output)] × 100

### 1.8 Data Analysis

Data were statistically analyzed using SAS 9.0 software with two-way ANOVA for significance testing. Results are expressed as "mean ± standard deviation," where $P<0.05$ indicates significant difference, $P<0.01$ indicates extremely significant difference, and $P>0.05$ indicates no significant difference.

## 2 Results

### 2.1 Effects of Dietary Protein and Fat Levels on Growth Performance of Female Minks during the Growing Period

As shown in Table 2 , no significant differences were observed in initial weight among groups ($P>0.05$). The LH group achieved the highest final weight and average daily gain, which were significantly higher than those of the LL and HL groups ($P<0.05$) but not significantly different from the LM, HM, and HH groups ($P>0.05$). Average daily feed intake was lowest in the LH group, significantly lower than in the LM and HM groups ($P<0.05$) but not significantly different from other groups ($P>0.05$). Feed-to-gain ratio was lowest in the LH group, being extremely significantly lower than all other groups ($P<0.01$), while the HH group was also extremely significantly lower than the LL, HL, and HM groups ($P<0.01$). Dietary protein level had no significant effect on final weight, average daily gain, average daily feed intake, or feed-to-gain ratio ($P>0.05$). However, dietary fat level extremely significantly affected final weight, average daily gain, average daily feed intake, and feed-to-gain ratio ($P<0.01$). Final weight and average daily gain showed an increasing trend with rising dietary fat levels, with the 30% fat level group being extremely significantly higher than the 10% fat level group ($P<0.01$). The 30% fat level group showed the lowest average daily feed intake, being extremely significantly lower than the 20% fat level group ($P<0.01$). Feed-to-gain ratio decreased as dietary fat level increased, with the 30% fat level group being extremely significantly lower than both the 10% and 20% fat level groups ($P<0.01$). The interaction between dietary protein and fat levels had no significant effect on final weight, average daily gain, average daily feed intake, or feed-to-gain ratio ($P>0.05$).

**Table 2 Effects of dietary protein and fat levels on growth performance of female minks during the growing period**

*Note: In the same column, 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). The same applies below.*

### 2.2 Effects of Dietary Protein and Fat Levels on Nutrient Digestibility of Female Minks during the Growing Period

As shown in Table 3 , dry matter digestibility in the LL and HL groups was extremely significantly lower than in other groups (P<0.01). Protein digestibility was highest in the HH group and lowest in the LL group, with the HH group being significantly higher than the LL, LH, and HL groups (P<0.05). Fat digestibility in the LL and HL groups was extremely significantly lower than in all other groups (P<0.01), with the LL group showing the lowest value. Dietary protein level had no significant effect on dry matter digestibility or carbohydrate digestibility (P>0.05) but significantly affected protein digestibility (P<0.05) and extremely significantly affected fat digestibility (P<0.01). The 36% protein level group showed significantly higher protein digestibility than the 32% protein level group (P<0.05) and extremely significantly higher fat digestibility (P<0.01). Dietary fat level extremely significantly affected dry matter digestibility, protein digestibility, fat digestibility, and carbohydrate digestibility (P<0.01). The 10% fat level group showed the lowest dry matter digestibility and protein digestibility, being extremely significantly lower than the 20% and 30% fat level groups (P<0.01). Fat digestibility increased with rising dietary fat levels, with the 30% fat level group being significantly higher than the 10% and 20% fat level groups (P<0.01). Carbohydrate digestibility was lowest in the 30% fat level group, being extremely significantly lower than the 10% and 20% fat level groups (P<0.01). The interaction between dietary protein and fat levels had no significant effect on dry matter digestibility, protein digestibility, or carbohydrate digestibility (P>0.05) but significantly affected fat digestibility (P<0.05).

**Table 3 Effects of dietary protein and fat levels on nutrient digestibility of female minks during the growing period, %**

### 2.3 Effects of Dietary Protein and Fat Levels on Nitrogen Metabolism of Female Minks during the Growing Period

As shown in Table 4 , nitrogen intake was highest in the HM group, being extremely significantly higher than all other groups (P<0.01), while the LH group showed the lowest value, being extremely significantly lower than other groups (P<0.01). Fecal nitrogen output was highest in the HL group, significantly higher than in the LH and HH groups (P<0.05). Urinary nitrogen output in the LH group was significantly lower than all other groups except the LM group (P<0.05). Nitrogen deposition was highest in the LH group, significantly higher than in the LL and HL groups (P<0.05). Nitrogen biological value was highest in the LH group, not significantly different from the LM group (P>0.05) but

significantly higher than all other groups (P<0.05). Dietary protein level had no significant effect on fecal nitrogen output (P>0.05) but significantly affected nitrogen biological value (P<0.05) and extremely significantly affected nitrogen intake and urinary nitrogen output (P<0.01). As dietary protein level increased from 32% to 36%, nitrogen intake and urinary nitrogen output increased extremely significantly (P<0.01), while nitrogen biological value decreased significantly (P<0.05). Dietary fat level had extremely significant effects on nitrogen intake, fecal nitrogen output, nitrogen deposition, and nitrogen biological value (P<0.01) and a significant effect on urinary nitrogen output (P<0.05). The 20% fat level group showed the highest nitrogen intake, extremely significantly higher than the 30% fat level group (P<0.01). Both fecal and urinary nitrogen output were lowest in the 30% fat level group, with fecal nitrogen output being extremely significantly lower than the 10% and 20% fat level groups (P<0.01) and urinary nitrogen output being significantly lower (P<0.05). Nitrogen deposition increased initially then decreased with rising dietary fat levels, with the 20% fat level group being extremely significantly higher than the 10% fat level group (P<0.01). Nitrogen biological value increased with rising dietary fat levels, with both the 20% and 30% fat level groups being extremely significantly higher than the 10% fat level group (P<0.01). The interaction between dietary protein and fat levels had no significant effect on nitrogen intake, fecal nitrogen output, urinary nitrogen output, nitrogen deposition, or nitrogen biological value (P>0.05).

**Table 4 Effects of dietary protein and fat levels on nitrogen metabolism of female minks during the growing period**

## 3 Discussion

### 3.1 Effects of Dietary Protein and Fat Levels on Growth Performance of Female Minks during the Growing Period

Fat constitutes a high proportion of mink diets, and its level determines dietary energy concentration. Research has shown that animal feed intake is directly affected by dietary energy level [11]. The present results demonstrated that average daily feed intake decreased with increasing dietary fat levels, consistent with findings in other animals [12-13]. The LH group achieved the highest final weight and average daily gain while showing the lowest average daily feed intake and feed-to-gain ratio. Previous studies have found that at a certain dietary energy level, lower dietary protein levels provide higher non-protein digestible energy, reducing nitrogen loss and increasing nitrogen accumulation in the body [14], which aligns with our results. Lower final weight and average daily gain in minks fed high-protein diets may be attributed to the fact that when dietary fat reaches a certain level, protein is not required as an energy source. Excess protein beyond its essential functions requires energy for excretion, thereby affecting body weight. The high final weight in the LH group led to higher average daily gain, and combined with the lowest average daily feed intake, resulted in the lowest feed-to-gain ratio. However, since final weight and

average daily gain in the LH group were not significantly different from the LM group, optimal growth performance in growing female minks was achieved when dietary protein level was 32% and fat level ranged between 20%–30%, particularly at 30% fat. These findings are consistent with Hoie [15], who reported that dietary fat levels of 7%–33% could improve mink growth performance.

### 3.2 Effects of Dietary Protein and Fat Levels on Nutrient Digestibility of Female Minks during the Growing Period

The present results showed that dry matter digestibility in the LL and HL groups was significantly lower than in other groups. Dietary protein level had no significant effect on dry matter digestibility, whereas dietary fat level extremely significantly affected dry matter digestibility, with the 10% fat level group showing the lowest value. Previous research indicates that dietary composition alters nutrient digestibility by changing gastrointestinal transit time [16]. The lowest dry matter digestibility in the 10% fat level group may be due to relatively higher carbohydrate levels in low-fat diets promoting gastrointestinal motility and shortening retention time. In this experiment, protein digestibility increased with rising dietary protein levels, consistent with findings in pre-mating female minks by Jiang et al. [3]. Li et al. [6] reported that dietary fat level had no significant effect on protein digestibility in minks, which differs from our results showing an increasing trend in protein digestibility with dietary fat level. This discrepancy may be because the highest fat level in Li et al. [6] was 20%, whereas our study reached 30%. Fat level affects dietary energy concentration, and high fat levels reduce feed intake, thereby decreasing nitrogen intake. To meet growth requirements, minks may increase protein digestibility [17]. Studies on mink have shown that most fats have high digestibility, with fat digestibility in mixed diets ranging from 80%–90% and averaging 85% or higher [18-20], which aligns with our findings. Moreover, fat digestibility increased with rising dietary fat levels. Carbohydrate digestibility decreased with increasing dietary fat levels, consistent with the trend in dry matter digestibility. This may be because dietary protein and fat digestibility are both high, and since minks have low carbohydrate requirements, the carbohydrate in high-protein, high-fat diets can meet their needs, with the body regulating carbohydrate digestibility through feed intake. The specific mechanisms require further investigation. Comprehensive analysis indicates that nutrient digestibility in minks is primarily affected by dietary fat and energy levels, with optimal digestibility of all nutrients achieved at a dietary fat level of 20%.

### 3.3 Effects of Dietary Protein and Fat Levels on Nitrogen Metabolism of Female Minks during the Growing Period

In this experiment, nitrogen intake increased extremely significantly with rising dietary protein levels but showed an initial increase then decrease with increasing dietary fat levels, clearly indicating that changes in nitrogen intake were mainly caused by differences in dietary protein levels and feed intake [3]. Under

our experimental conditions, the LH group showed the lowest fecal nitrogen output. Studies have demonstrated a strong correlation between protein intake and urinary nitrogen excretion, with excess protein supply or amino acid imbalance being important causes of high urinary nitrogen output and reduced nitrogen utilization efficiency [21]. Approximately 80% of nitrogen is excreted via urine in growing minks [22]. In this experiment, urinary nitrogen output increased with dietary protein level across all groups, indicating that minks can regulate protein and energy metabolism, using excess protein for energy and excreting it through urine. Under our conditions, the LM and LH groups showed relatively low urinary nitrogen output. Nitrogen biological value measures the degree of dietary protein utilization and animal protein requirements [23]. In this experiment, nitrogen biological value decreased with increasing dietary protein levels but increased with rising dietary fat levels, indicating that lower dietary protein levels and higher fat levels improved nitrogen biological value under our experimental conditions. Based on comprehensive evaluation of these indices, optimal protein utilization with reduced urinary nitrogen excretion was achieved when growing female minks were fed diets containing 32% protein and 20%–30% fat.

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