# Effects of Dietary Tryptophan Supplementation Level on Growth Performance, Nitrogen Metabolism, and Amino Acid Digestibility in Growing Mink (Postprint)

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## Abstract

This experiment aimed to investigate the effects of dietary tryptophan supplementation levels on growth performance, nitrogen metabolism, and amino acid digestibility in growing mink. Sixty healthy male white mink with similar body weight [(0.83$±0.09)$kg$]at$(60±$5) days of age were selected and randomly divided into 6 groups with 10 replicates per group and 1 mink per replicate. The negative control group (Group I) was fed a basal diet with 34% crude protein (tryptophan level of 0.22%). The experimental groups were fed test diets supplemented with 0.1% (Group II), 0.3% (Group III), 0.5% (Group IV), and 0.7% tryptophan (Group V) based on the negative control basal diet. The positive control group (Group VI) was fed a basal diet with 36% crude protein (tryptophan level of 0.22%). The pre-trial period was 7 days, and the formal trial period was 60 days. The results showed: 1) There were no significant differences in average daily gain and average daily feed intake among groups during 60-120 days of age (P>0.05). The average daily gain of Groups III, IV, and VI was slightly higher than that of other groups. 2) The dry matter digestibility of Group I was significantly higher than that of Groups II, III, IV, and VI (P<0.05). The crude fat digestibility of Groups V and VI was significantly higher than that of Group IV (P<0.05). The urinary nitrogen of Groups IV, V, and VI was significantly higher than that of Group III (P<0.05). The nitrogen retention of Groups I and III was significantly higher than that of Group IV (P<0.05). The net protein utilization and protein biological value of Group III were significantly higher than those of Groups IV, V, and VI (P<0.05). 3) The digestibility of glutamic acid, phenylalanine, lysine, and proline in Group I was significantly higher than that in Group III (P<0.05). The isoleucine digestibility of Groups I and VI was significantly higher than that of Group III (P<0.05). The leucine digestibility

of Groups I and VI was significantly higher than that of Group III (P<0.05). The alanine digestibility of Group V was significantly higher than that of Group III (P<0.05). The methionine digestibility of Group VI was significantly higher than that of all other groups (P<0.05). The methionine digestibility of Groups I, IV, and V was significantly higher than that of Groups II and III (P<0.05). The tyrosine digestibility of Groups I, V, and VI was significantly higher than that of Groups III and IV (P<0.05). The cysteine digestibility of Groups I, V, and VI was significantly higher than that of Group IV (P<0.05). The histidine digestibility of Group V was significantly higher than that of Groups II and III (P<0.05). The tryptophan digestibility of Groups I, III, and VI was significantly higher than that of Group II (P<0.05). The arginine digestibility of Groups I, V, and VI was significantly higher than that of Groups II and III (P<0.05). The total amino acid digestibility of Group I was significantly higher than that of Group III (P<0.05). It can be concluded that, based on comprehensive evaluation of growth performance, nitrogen metabolism, and amino acid digestibility indices, the appropriate tryptophan supplementation level in mink diets is 0.3% (dietary tryptophan level of 0.52%) when the dietary crude protein level is 34%.

## Full Text

### Effects of Dietary Tryptophan Supplemental Level on Growth Performance, Nitrogen Metabolism and Amino Acid Digestibility of Minks during Growing Period

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**Abstract:** This experiment was conducted to investigate the effects of dietary tryptophan (Trp) supplemental level on growth performance, nitrogen metabolism, and amino acid digestibility in growing minks. Sixty healthy male white minks at (60$±5)*daysofagewithsimilarbodyweight*[(0.83±$0.09) kg] were randomly divided into 6 groups, with 10 replicates per group and 1 mink per replicate. The negative control group (Group ) was fed a basal diet containing 34% crude protein (CP) with a Trp level of 0.22%. The experimental groups were fed the basal diet supplemented with 0.1% (Group ), 0.3% (Group ), 0.5% (Group ), and 0.7% Trp (Group ), respectively. The positive control group (Group ) was fed a basal diet containing 36% CP with a Trp level of 0.22%. The pre-trial period lasted 7 days, followed by a 60-day formal trial period.

The results showed: 1) No significant differences were observed in average daily gain (ADG) or average daily feed intake (ADFI) among all groups during 60–120 days of age (P>0.05), though Groups , , and had slightly higher ADG than other groups. 2) Dry matter digestibility in Group  was significantly higher than in Groups , , , and  (P<0.05). Ether extract digestibility in Groups  and  was significantly higher than in Group  (P<0.05). Urinary nitrogen in Groups , , and  was significantly higher than in Group  (P<0.05). Nitrogen deposition in Groups  and  was significantly higher than in Group  (P<0.05). Net protein utilization (NPU) and biological value of protein in Group  were significantly higher than in Groups , , and  (P<0.05). 3) The digestibilities of glutamate, phenylalanine, lysine, and proline in Group  were significantly higher than in Group  (P<0.05). Isoleucine digestibility in Groups  and  was significantly higher than in Group  (P<0.05). Leucine digestibility in Groups  and  was significantly higher than in Group  (P<0.05). Alanine digestibility in Group  was significantly higher than in Group  (P<0.05). Methionine digestibility in Group  was significantly higher than in all other groups (P<0.05), while Groups , , and  had significantly higher methionine digestibility than Groups  and  (P<0.05). Tyrosine digestibility in Groups , , and  was significantly higher than in Groups  and  (P<0.05). Cysteine digestibility in Groups , , and  was significantly higher than in Group  (P<0.05). Histidine digestibility in Group  was significantly higher than in Groups  and  (P<0.05). Trp digestibility in Groups , , and  was significantly higher than in Group  (P<0.05). Arginine digestibility in Groups , , and  was significantly higher than in Groups  and  (P<0.05). Total amino acid digestibility in Group  was significantly higher than in Group  (P<0.05). In conclusion, based on comprehensive evaluation of growth performance, nitrogen metabolism, and amino acid digestibility, when dietary crude protein level is 34%, the appropriate dietary Trp supplemental level for growing minks is 0.3% (resulting in a dietary Trp level of 0.52%).

**Keywords:** growing period; minks; tryptophan; growth performance; nitrogen metabolism; amino acid digestibility

Tryptophan is a special essential amino acid for animals. It was first isolated from casein by Hokinst in 1902, with the chemical name $\alpha$-amino-$\beta$-indole propionic acid, and exists as L-type, D-type isomers, and racemic DL-tryptophan [1]. In animals, Trp binds to serum proteins through non-covalent bonds and participates in nucleic acid and protein synthesis. Wiertz et al. [2] reported that when dietary Trp level reached 0.20%, ribosomal activity in pig muscle increased, thereby promoting protein synthesis. Additionally, Trp serves as a precursor for 5-hydroxytryptamine (5-HT), niacin, melatonin, tryptamine, angiotensin, coenzyme I (NAD), coenzyme II (NADP), and other bioactive substances [3]. 5-HT acts on the feeding center in the hypothalamus to regulate animal feed intake [1]. Melatonin promotes immune balance by inducing macrophages to produce interleukins and interferons, thereby stimulating immune cells to release immune factors [4]. Supplementing appropriate levels of Trp in diets can increase feed intake, regulate lactation, enhance immunity, and improve antioxidant function. Wei et al. [5] reported that dietary Trp supplementation improved growth performance in Yangzhou geese. Ohtani et al. [6] found that adding 0.025% and 0.050% Trp to layer diets containing 0.15% Trp significantly improved egg production rate and feed utilization during weeks 53–82. Insufficient dietary Trp significantly reduces average daily gain, feed conversion ratio, and nitrogen deposition in pigs, while also decreasing plasma insulin and insulin-like growth factor-I (IGF-I) concentrations [7-9]. Minks are valuable fur-bearing animals, and research on their nutritional requirements has become increasingly clear in recent years, but studies on Trp requirements in minks have not been reported. Therefore, this experiment was conducted to investigate the effects of dietary Trp supplemental level on growth performance, nitrogen metabolism, and amino acid digestibility in growing minks, and to determine the Trp requirement for minks, providing a scientific basis for improving feeding standards in China.

### 1.1 Experimental Design and Management

The experiment was conducted at the Animal Experimental Base of the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences. A single-factor randomized design was employed. Sixty healthy male white minks at (60$\pm$5)*daysofagewithsimilarbodyweight*[(0.83$\pm$0.09) kg] were randomly divided into 6 groups with 10 replicates per group and 1 mink per replicate. The basal diet was formulated based on Danish mink diet composition, nutrient composition tables, and recent domestic research on mink nutritional requirements [10-11]. The composition and nutrient levels are shown in Table 1 . The negative control group (Group ) was fed a basal diet containing 34% crude protein (Trp level 0.22%). The experimental groups were fed the basal diet supplemented with 0.1% (Group ), 0.3% (Group ), 0.5% (Group ), and 0.7% Trp (Group ), respectively. The positive control group (Group ) was fed a basal diet containing 36% crude protein (Trp level 0.22%). The experimental design is shown in Table 2 . The pre-trial period lasted 7 days, and the trial period lasted 60 days. Minks were fed at 07:30 and 14:30 daily, with free access to feed and water.

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

| Items | Ingredients | Negative Control Group | Positive Control Group |
|---|---|---|---|
| Chicken liver | | | |
| Yellow croaker | | | |
| Chicken head | | | |
| Chicken glandular stomach | | | |

| Items | Ingredients | Negative Control Group | Positive Control Group |
|---|---|---|---|
| Chicken skeleton | | | |
| Extruded corn meal | | | |
| Premix[1] | | | |
| Fish meal | | | |
| **Total Nutrient Levels**[2] | | | |
| ME (MJ/kg) | | | |
| CP | | | |
| EE | | | |
| Ca | | | |
| TP | | | |
| Trp | | | |
| Lys | | | |
| Met | | | |

[1]Per kilogram of premix contained: VA 1,000,000 IU, $VD_3$ 200,000 IU, VE 18,000 IU, $VB_1$ 500 mg, $VB_2$ 1,000 mg, niacin 4,000 mg, pantothenic acid 4,000 mg, $VB_6$ 1,000 mg, $VB_{12}$ 10 mg, biotin 30 mg, folic acid 300 mg, $VK_3$ 200 mg, VC 50,000 mg, choline 6,000 mg, Fe 10,000 mg, Cu 800 mg, Mn 2,000 mg, Zn 8,000 mg, I 50 mg, Se 20 mg, Co 50 mg.

[2]CP, EE, Ca, TP, Trp, Lys, and Met were measured values, while ME was a calculated value.

**Table 2 Experimental Design**

| Groups | Trp Supplemental Level | CP Level |
|---|---|---|
| (Negative control) | 0 | 34% |
| | 0.1% | 34% |
| | 0.3% | 34% |
| | 0.5% | 34% |
| | 0.7% | 34% |
| (Positive control) | 0 | 36% |

### 1.2 Digestion and Metabolism Trial

At 42 days after the start of the experiment, 6 minks with similar body weight were selected from each group for a digestion and metabolism trial conducted from August 31 to September 2, 2016, lasting 3 days. The total feces collection method was used, and feeding management was the same as during the regular trial period. Daily feces collections were weighed, and 10% sulfuric acid solution was added at 5% of fresh weight with a small amount of toluene as preservative, then stored at -20°C. The 3-day feces samples were mixed uniformly, dried at 65°C to constant weight, ground, and passed through a sieve.

### 1.3 Measured Indices and Methods

Initial body weight of each mink was measured at the start of the experiment, and body weight was recorded every 15 days to calculate average daily feed intake, average body weight, and average daily gain. Dry matter content in diets was determined by oven-drying at 105°C according to GB/T 6435–2006. Crude protein content was determined by the Dumas combustion method according to ISO 16634-1:2008. Crude fat content was determined by Soxhlet extraction according to GB/T 6433–1994. Calcium content was determined by EDTA complexometric titration according to GB/T 6436–1992. Phosphorus content was determined by ammonium vanadate-molybdate colorimetry according to GB/T 6437–1992. Amino acid content in diets and feces was determined by hydrochloric acid hydrolysis according to GB/T 5009.124–2003. Trp content was determined by spectrophotometry according to GB/T 15400–94. Calculation methods for measured indices are detailed in reference [12].

### 1.4 Data Processing

Data were statistically analyzed using SAS 9.1.3 software with one-way ANOVA. Differences were considered significant at $P<0.05$ and non-significant at $P>0.05$.

### 2.1 Effects of Dietary Tryptophan Supplemental Level on Growth Performance of Growing Minks

As shown in Table 3 , no significant differences were observed in average daily feed intake among groups ($P>0.05$), though Group was slightly higher than other groups and Group was the lowest. At 60, 75, and 90 days of age, no significant differences were found in average body weight among groups ($P>0.05$). At 105 days of age, average body weight in Groups , , and was significantly higher than in Groups and ($P<0.05$), with Group showing the highest and Group the lowest average body weight. At 120 days of age, average body weight in Groups and was significantly lower than in Group ($P<0.05$). During 60–75 days of age, average daily gain in Groups , , and was significantly higher than in Groups , , and ($P<0.05$). During 76–90 days of age, average daily gain in Group was significantly higher than in Group ($P<0.05$). During 91–105 days of age, average daily gain in Groups , , and was significantly higher than in Groups , , and (P<0.05). During 106–120 days of age, average daily gain in Groups , , and was significantly higher than in Groups , , and (P<0.05). For the entire growing period (60–120 days of age), no significant differences were observed in average daily gain among groups (P>0.05), though Groups , , and were slightly higher than other groups.

**Table 3 Effects of Dietary Tryptophan Supplemental Level on Growth Performance of Minks during Growing Period**

<table>
<tr><th>Items</th><th>Groups</th><th>Day of Age</th><th>P-value</th></tr>
<tr><td>Average daily feed intake (g/d)</td><td colspan="3">: 386.00$±3| :<br>371.44±3| :<br>356.89±47| :<br>354.94±52| :<br>370.72±2| :<br>370.28±3|||Averagebodyweight(kg)|||||60days|0.84±0.08|0.83±0.08|0.83±0.10|0.83±0.09|0.83± [illegible]<br>0.0010||91–105days|26.38±4.7|27.73±4.9|27.74±3.3|16.50±3.0|16.83±2.4|19.34±3.2|||106–120 [illegible]</td></tr>
</table>

In the same row, values with different small letter superscripts indicate significant difference (P<0.05), while values with the same or no letter superscripts indicate no significant difference (P>0.05). The same applies below.

### 2.2 Effects of Dietary Tryptophan Supplemental Level on Nitrogen Balance and Nutrient Digestibility of Growing Minks

As shown in Table 4 , no significant differences were observed in nitrogen intake and fecal nitrogen among groups (P>0.05). Urinary nitrogen in Groups , , and was significantly higher than in Group (P<0.05). Nitrogen deposition in Groups and was significantly higher than in Group (P<0.05). Net protein utilization and biological value of protein in Group were significantly higher than in Groups , , and (P<0.05). Dry matter digestibility in Group was significantly higher than in Groups , , , and (P<0.05). No significant differences were observed in crude protein digestibility among groups (P>0.05). Ether extract digestibility in Groups and was significantly higher than in Group (P<0.05), with Group showing the lowest ether extract digestibility.

**Table 4 Effects of Dietary Tryptophan Supplemental Level on Nitrogen Metabolism and Nutrient Digestibilities of Minks during Growing Period**

<table>
<tr><th>Items</th><th>Groups</th><th>P-value</th></tr>
<tr><td>Nitrogen intake (g/d)</td><td>: 8.07$±0.80| :<br>7.77±0.75| :<br>7.46±0.98| :<br>7.43±1.09| :<br>7.75±0.54| :<br>8.25±0.69|||$Fecalnitrogen(g/d)$|0.91±0.18|0.94±0.16|0.96±0.16|0.96±0.17|0.94±0.14|1</td><td></td></tr>
</table>

### 2.4 Effects of Dietary Tryptophan Supplemental Level on Amino Acid Digestibility of Growing Minks

As shown in Table 5 , the digestibilities of glutamate, phenylalanine, lysine, and proline in Group were significantly higher than in Group (P<0.05). Isoleucine digestibility in Groups and was significantly higher than in Group (P<0.05). Alanine digestibility in Group was significantly higher than in Group (P<0.05). Cysteine digestibility in Groups , , and was significantly higher than in Group (P<0.05). Methionine digestibility in Group was significantly higher than in all other groups (P<0.05), while Groups , , and had significantly higher methionine digestibility than Groups and (P<0.05). Arginine digestibility in Groups , , and was significantly higher than in Groups and (P<0.05). Leucine digestibility in Groups and was significantly higher than in Group (P<0.05). Tyrosine digestibility in Groups , , and was significantly higher than in Groups and (P<0.05). Histidine digestibility in Group was significantly higher than in Groups and (P<0.05). Trp digestibility in Groups , , and was significantly higher than in Group (P<0.05). No significant differences were observed in aspartate, threonine, serine, glycine, and valine digestibility among groups (P>0.05). Total amino acid digestibility in Group was significantly higher than in Group (P<0.05).

**Table 5 Effects of Dietary Tryptophan Supplemental Level on Amino Acid Digestibilities of Minks during Growing Period**

<table>
<tr><th>Items</th><th>Groups</th><th>P-value</th></tr>
<tr><td>Aspartate (%)</td><td>: 92.60$±0.87| :<br>91.70±1.08| :<br>91.83±1.21| :<br>91.52±0.62| :<br>92.37±0.64| :<br>91.60±1.23|||$Threonine$(±0.97|89.67±1.48|89.69±1.34|89.77±1.02|90.16±0.98|90.63±1<br>0.0001||$Isoleucine$(±0.57$^{a}$|93.79±0.76$^{ab}$|93.40±0.50$^{b}$|93.90±0.61$^{ab}$|94.28±0.57$^{a}$|93.87±</td><td></td></tr>
</table>

### 3.1 Effects of Dietary Tryptophan Supplemental Level on Growth Performance of Growing Minks

Tryptophan, as an essential amino acid, promotes growth performance when supplemented at appropriate levels. Studies have shown that Trp influences animal feed intake by affecting the neurotransmitter 5-HT in the brain and regulating the secretion of gastrointestinal regulatory peptide ghrelin [3]. Ma et al. [13] reported that supplementing different levels of Trp in basal diets significantly increased average daily feed intake in broiler breeder chickens. Trp increases feed intake in piglets by elevating 5-HT content in the brain, and piglets fed high-Trp diets showed better growth performance than those fed low-Trp diets [14]. The present results showed that Trp supplemental level had no significant effect on average daily feed intake, with Groups and being slightly higher, possibly because the basal diet already contained relatively high Trp levels, making its effect on feed intake less pronounced. As mink age increased, the influence of Trp supplemental level became more apparent. At 106–120 days of age, Groups , , , and had similar average body weights. The overall trend indicated that as Trp supplemental level increased, average body weight gradually increased, but decreased when the supplemental level reached 0.5%, suggesting that appropriate Trp levels improve animal growth performance while excessive supplementation inhibits growth. Comparison between experimental and positive control groups revealed that high protein levels promoted mink growth performance, while appropriate Trp supplementation could reduce protein requirements.

### 3.2 Effects of Dietary Tryptophan Supplemental Level on Nitrogen Balance and Nutrient Digestibility of Growing Minks

The present results showed no significant differences in average daily feed intake among groups, with Group being slightly higher. Consequently, nitrogen intake in Group was also slightly higher than other groups. Group had higher dietary protein level, resulting in relatively higher nitrogen intake, but no significant differences were observed in nitrogen intake and fecal nitrogen among groups. Increased urinary nitrogen in Groups , , and led to reduced nitrogen deposition, net protein utilization, and biological value of protein. Groups and may have experienced amino acid imbalance due to excessive Trp supplementation, while Group had excess protein supply, as a correlation exists between protein intake and urinary nitrogen excretion. Pfeiffer et al. [15] confirmed that excess protein supply and amino acid imbalance cause increased urinary nitrogen excretion and changes in nitrogen utilization efficiency. Dietary Trp supplementation increases nitrogen deposition and reduces nitrogen-containing metabolites in feces, thereby decreasing environmental pollution from aquaculture waste [3]. Tryptophan can regulate liver protein synthesis [16], and international reports indicate that increasing dietary Trp level significantly improves protein deposition in pigs [17-18]. Wu et al. [19], Corzo et al. [20], and Xi et al. [21] also confirmed that supplementing appropriate amino acids improves amino acid balance, making protein synthesis rate exceed degradation rate and improving protein deposition efficiency. Zhang et al. [22] reported that the appropriate protein level for minks during 80–110 days of age is 34%. In this experiment, no significant differences were observed in protein digestibility among groups. At the same Trp level, crude protein digestibility in Group   was higher than in Group  , possibly because the 36% protein level in Group   exceeded the appropriate level, reducing digestibility. Mink diets with protein levels above 34% can improve ether extract digestibility, and in this experiment, Group   had the highest ether extract digestibility due to dietary protein level effects. Tryptophan plays an important role in regulating animal fat metabolism [21], and in this experiment, Group   with the highest Trp supplementation level had significantly higher ether extract digestibility than other experimental groups, similar to Group  , indicating that appropriate Trp supplementation facilitates fat digestion and absorption.

### 3.3 Effects of Dietary Tryptophan Supplemental Level on Amino Acid Digestibility of Growing Minks

The present results showed that Group   had the highest total amino acid digestibility, significantly higher than Group  , possibly due to effects of crude protein digestibility and dry matter digestibility. Total amino acid digestibility in Group   was slightly higher than in Group  , indicating that excessive dietary protein level affects amino acid digestion and absorption. Wu et al. [23] found that Trp absorption antagonizes arginine. In this experiment, as Trp supplemental level increased from Group   to  , arginine digestibility gradually increased, suggesting a positive correlation between Trp and arginine, with Trp digestibility showing the same trend as arginine. Groups   and   had similar arginine digestibility, indicating that high dietary protein level does not promote arginine absorption. Studies have shown that the optimal Trp to lysine ratio in piglet diets is 22% for best growth performance [24]. In this experiment, the optimal Trp to lysine ratio for male mink growth performance was 36%. Among neutral amino acids, for aliphatic amino acids (glycine, alanine, serine, threonine, valine, leucine, isoleucine), dietary Trp supplemental level affected the digestibility of alanine, leucine, and isoleucine. The results showed that Group   had the lowest digestibility for these three aliphatic amino acids, which increased slightly when Trp supplementation was reduced and improved significantly when Trp supplementation was increased. Leucine and isoleucine are isomers, and Trp had consistent effects on their digestibility. For aromatic amino acids (phenylalanine, tyrosine), sulfur-containing amino acids (methionine), heterocyclic amino acids (proline), and acidic amino acids (glutamate), Trp effects were consistent with those on aliphatic amino acids. In this experiment, proline digestibility decreased initially then increased with increasing Trp supplementation, with Group   showing the lowest proline digestibility while having the highest Trp digestibility, suggesting possible antagonism between proline and Trp as both are heterocyclic amino acids. In conclusion, a Trp level of 0.22% was beneficial for amino acid and protein nutrient digestion and absorption, meeting the requirements of the amino acid barrel theory. When Trp level reached 0.52% (0.3% supplementation), amino acid balance was disrupted, reducing digestibility of multiple amino acids. When Trp level exceeded 0.52%, amino acid digestibility improved, indicating that Trp as an essential limiting amino acid played a certain role, though the regulatory mechanism requires further investigation.

Under the conditions of this experiment, based on comprehensive evaluation of growth performance, nitrogen metabolism, and amino acid digestibility, when dietary crude protein level is 34%, the appropriate dietary Trp supplemental level for minks is 0.3% (resulting in a dietary Trp level of 0.52%).

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