Effects of Dietary Tryptophan Supplementation Levels on Growth Performance, Nitrogen Metabolism, Nutrient Digestibility, Fur Quality, and Serum Parameters of White Mink during the Winter Fur Period (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary tryptophan supplementation levels on growth performance, nitrogen metabolism, nutrient digestibility, fur quality, and serum indices of white mink during the winter fur period. Sixty healthy male white mink aged (120±5) days 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 0.26%), 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, and the positive control group (Group VI) was fed a basal diet with 36% crude protein (tryptophan level of 0.27%). The preliminary period was 5 days, and the experimental period was 70 days. The results showed that: 1) There were no significant differences among groups in average daily gain, average daily feed intake, dry matter digestibility, crude protein digestibility, crude fat digestibility, and nitrogen deposition of white mink ($P>0.05$), no significant differences in fur quality scores among groups ($P>0.05$), and no significant differences in serum aspartate aminotransferase activity and insulin-like growth factor-I content among groups ($P>0.05$). 2) Serum total cholesterol content in Group V was significantly higher than that in Groups II, III, IV, and VI ($P<0.05$); serum immunoglobulin M content in Groups IV and V was significantly higher than that in Group II ($P<0.05$); serum immunoglobulin A content in Groups IV and V was significantly higher than that in Groups II, III, and VI ($P<0.05$), and Group VI was significantly lower than Group I ($P<0.05$); serum immunoglobulin G content in Group VI was significantly lower than that in Groups I, II, III,

and IV (P<0.05). It can be concluded that when dietary crude protein level is 34% and tryptophan level is 0.26%, the basic nutritional requirement for tryptophan of white mink during the winter fur period can be met, with satisfactory growth performance.

Full Text

Effects of Dietary Tryptophan Supplemental Level on Growth Performance, Nitrogen Metabolism, Nutrient Digestibility, Fur Quality and Serum Parameters of White Minks during Winter Fur-Growing Period

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Abstract

This study was conducted to investigate the effects of dietary tryptophan (Trp) supplemental level on growth performance, nitrogen metabolism, nutrient digestibility, fur quality, and serum parameters of white minks during the winter fur-growing period. Sixty healthy male white minks aged (120±5) days were randomly allocated into 6 groups with 10 replicates per group and one mink per replicate. Minks in the negative control group (Group I) were fed a basal diet containing 34% crude protein (CP) with a Trp level of 0.26%. Experimental groups were fed the basal diet supplemented with 0.1% (Group II), 0.3% (Group III), 0.5% (Group IV), and 0.7% Trp (Group V), respectively. The positive control group (Group VI) received a basal diet containing 36% CP with a Trp level of 0.27%. The pre-trial period lasted 5 days, followed by a 70-day formal trial period. The results showed: 1) No significant differences were observed among all groups in average daily gain, average daily feed intake, dry matter digestibility, crude protein digestibility, ether extract digestibility, or nitrogen deposition (P>0.05). Similarly, no significant differences were found in fur quality scores or in serum glutamic-oxaloacetic transaminase activity and insulin-like growth factor-I (IGF-I) content (P>0.05). 2) Serum total cholesterol content in Group V was significantly higher than in Groups II, III, IV, and VI (P<0.05). Serum immunoglobulin M (IgM) content in Groups IV and V was significantly higher than in Group II (P<0.05). Serum immunoglobulin A (IgA) content in Groups IV and V was significantly higher than in Groups II, III, and VI (P<0.05), while Group VI was significantly lower than Group I (P<0.05). Serum immunoglobulin G (IgG) content in Group VI was significantly lower than in Groups I, II, III, and IV (P<0.05). In conclusion, a dietary Trp level of 0.26% with 34% crude

protein can satisfy the basic Trp nutritional requirements of white minks during the winter fur-growing period, yielding satisfactory growth performance.

Keywords: winter fur-growing period; white minks; tryptophan; growth performance; serum biochemical parameters

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Introduction

Tryptophan is an essential amino acid in animals that serves not only as a substrate for protein synthesis but also as a regulator of protein synthesis. Its metabolite, 5-hydroxytryptamine (5-HT), regulates feed intake by acting on the hypothalamic feeding center, thereby modulating animal growth performance. Additionally, as a limiting amino acid for immune-related proteins, tryptophan can increase -globulin content and participate in neuro-endocrine-immune network regulation [1]. The immunomodulatory effects of tryptophan can also be mediated through insulin-like growth factors (IGFs) and 5-HT, as well as melatonin, with IGFs promoting the differentiation of early T lymphocytes into mature T cells [2]. Recent studies have shown that pigs fed low-protein diets exhibited optimal growth performance at a digestible L-tryptophan level of 0.146%, while performance decreased when L-tryptophan levels exceeded 0.152% [3]. Wei et al. [4] reported that dietary L-tryptophan supplementation significantly increased spleen index and enhanced humoral immunity by stimulating immunoglobulin G (IgG) and immunoglobulin M (IgM) secretion in Yangzhou geese. However, no studies have been reported on the effects of dietary tryptophan levels in white minks during the winter fur-growing period. Therefore, this experiment was designed to investigate the effects of dietary tryptophan supplemental levels on growth performance, nitrogen metabolism, nutrient digestibility, fur quality, serum biochemical parameters, and serum immune parameters in white minks during the winter fur-growing period, aiming to determine the appropriate tryptophan supplemental level for male white minks and provide a scientific basis for establishing feeding standards for minks in China.

1. Materials and Methods

1.1 Experimental Animals Sixty male white minks aged (120±5) days with similar body weights were selected at the Animal Experimental Base of the Institute of Economic Animal and Plant Science, Chinese Academy of Agricultural Sciences.

1.2 Experimental Diets A basal diet for minks during the winter fur-growing period was formulated based on previous research on mink nutritional requirements [5]. The composition and nutrient levels are presented in Table 1 .

1.3 Experimental Design The 60 experimental minks were randomly divided into 6 groups with 10 replicates per group and one mink per replicate. Initial body weights were adjusted among groups to ensure no significant differences through analysis of variance (P>0.05). The negative control group (Group I) received a basal diet containing 34% crude protein with a Trp level of 0.26%. Experimental groups were fed the basal diet supplemented with 0.1% (Group II), 0.3% (Group III), 0.5% (Group IV), and 0.7% Trp (Group V), respectively. The positive control group (Group VI) received a basal diet containing 36% crude protein with a Trp level of 0.27%. The experimental design is summarized in Table 2 . The pre-trial period lasted 5 days, followed by a 70-day formal trial period.

1.4 Animal Management All experimental minks were housed individually in cages and fed twice daily at 07:30 and 14:30 with ad libitum access to feed and water. Actual feed intake was recorded daily. The experiment was conducted from September 19, 2016, to December 3, 2016.

1.5 Digestion and Metabolism Trial The digestion and metabolism trial was conducted from October 10 to October 12, 2016, lasting 3 days. Six minks with similar body weights were selected from each group for the trial. The total feces collection method was employed, with management practices identical to daily feeding routines. Collected feces were weighed daily, and 5% of the fresh weight was mixed with 10% sulfuric acid solution. A small amount of toluene was added as a preservative, and samples were stored at -20°C. The 3-day fecal collections were then mixed, dried at 65°C to constant weight, ground to pass through a 40-mesh sieve, and prepared as air-dried samples for laboratory analysis.

1.6 Sample Collection and Analysis 1.6.1 Serum Preparation

At the end of the feeding trial, five minks from each group were selected for cardiac blood collection. Ten milliliters of blood were collected from each mink into procoagulant tubes. After serum separation, samples were centrifuged at 3,500 r/min for 10 minutes at 4°C. The separated serum was aliquoted into 1.5 mL Eppendorf tubes and stored at -80°C for later analysis.

1.6.2 Fur Quality Assessment

Prior to slaughter, fur quality was evaluated based on a scoring system: overall quality (1–12 points), color (1–5 points), luster (1–5 points), evenness (1–5 points), and softness (1–5 points), with the sum constituting the total fur quality score [6-7], recorded to 0.01 precision. Body length was measured from the nose tip to the tail root using a tape measure (precision: 0.1 cm). Guard hair and underhair lengths were measured with a ruler, and the guard hair to underhair ratio was calculated.

1.6.3 Analytical Methods

Tryptophan content was determined by spectrophotometry according to GB/T 15400-94. Amino acid content in diets was measured by hydrochloric acid hydrolysis following GB/T 5009.124-2003. Dry matter content in basal diets was determined by oven drying at 105°C according to GB/T 6435-2006. Crude protein content was measured by the Kjeldahl method (GB/T 6432-1994) [8]. Calcium content was determined by potassium permanganate titration (GB/T 6436-2002) [9], and total phosphorus by ammonium vanadomolybdate colorimetry (GB/T 6437-2002) [10]. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities, and IgG, immunoglobulin A (IgA), and IgM contents were measured using kits from Nanjing Jiancheng Bioengineering Institute following the manufacturer' s instructions. Serum triglyceride (TG) and total cholesterol (TCHO) contents were determined using kits from Wako Pure Chemical Industries, Japan. Serum insulin-like growth factor-I (IGF-I) content was measured by enzyme-linked immunosorbent assay (ELISA).

1.7 Statistical Analysis Data were analyzed using the GLM procedure of SAS 9.1.3 software. Duncan' s multiple range test was used for post-hoc comparisons. Differences were considered significant at $P<0.05$ and non-significant at $P>0.05$.

2. Results

2.1 Effects of Dietary Tryptophan Supplemental Level on Growth Performance As shown in Table 3 , dietary tryptophan supplemental level had no significant effects on average daily gain (ADG), average daily feed intake (ADFI), or feed-to-gain ratio (F/G) of white minks during the winter fur-growing period ($P>0.05$).

2.2 Effects of Dietary Tryptophan Supplemental Level on Nitrogen Metabolism and Nutrient Digestibility Table 4 shows that dry matter digestibility in Groups I–V exhibited a decreasing trend with increasing dietary tryptophan levels, though no significant differences were observed among groups ($P>0.05$). No significant differences were found in crude protein digestibility, ether extract digestibility, nitrogen intake, fecal nitrogen, urinary nitrogen, or nitrogen deposition among groups ($P>0.05$). Group IV exhibited significantly higher net protein utilization and protein biological value compared to Groups II and VI ($P<0.05$), with no significant difference from Group I ($P>0.05$).

2.3 Effects of Dietary Tryptophan Supplemental Level on Fur Quality Table 5 indicates that no significant differences were observed in fur quality scores among all groups ($P>0.05$). Similarly, no significant differences were found in body length or guard hair length ($P>0.05$). However, underhair length in Group V was significantly higher than in Group III ($P<0.05$), and the guard hair to underhair ratio in Group V was significantly lower than in Group II ($P<0.05$).

2.4 Effects of Dietary Tryptophan Supplemental Level on Serum Biochemical Parameters As presented in Table 6 , serum TCHO content in Group V was significantly higher than in Groups II, III, IV, and VI ($P<0.05$). No significant differences were observed in serum TG content among groups ($P>0.05$). Serum ALT activity in Group VI was significantly higher than in Group II ($P<0.05$). No significant differences were found in serum AST activity or IGF-I content among groups ($P>0.05$).

2.5 Effects of Dietary Tryptophan Supplemental Level on Serum Immune Parameters Table 7 shows that serum IgM content in Groups IV and V was significantly higher than in Group II ($P<0.05$). Serum IgA content in Groups IV and V was significantly higher than in Groups II, III, and VI ($P<0.05$), with Group VI showing the lowest level and being significantly lower than Group I ($P<0.05$). Serum IgG content gradually increased from Groups I to IV, began to decrease in Group V, and was significantly lower in Group VI compared to Groups I, II, III, and IV ($P<0.05$). No significant differences were observed in immune organ indices (liver index and spleen index) among groups ($P>0.05$).

3. Discussion

3.1 Effects on Growth Performance and Nutrient Digestibility Tryptophan regulates feed intake through two pathways: 5-HT and the gut regulatory peptide ghrelin. It acts on hypothalamic feeding centers while simultaneously increasing ghrelin secretion and intestinal emptying rate, thereby enhancing feed intake. Tryptophan also increases glucose absorption rate, promoting energy accumulation and weight gain [11-12]. In this study, no significant differences were observed in average daily feed intake, average daily gain, or feed-to-gain ratio among groups, possibly because the carnivorous diet of minks contained relatively high tryptophan levels (0.26%), which could maintain amino acid balance and meet basic nutritional requirements. Previous studies have reported that insufficient dietary tryptophan significantly reduces average daily gain, feed conversion ratio, and nitrogen deposition in pigs, while also decreasing serum insulin and IGF-I content [13-15]. IGF-I is an important endocrine hormone that participates in various metabolic activities and regulates growth, reproduction, cell differentiation, and metabolism, reflecting nutritional and growth status [16]. Studies in pigs, chickens, and cattle have demonstrated positive correlations between serum IGF-I content and body weight [17-19]. In this experiment, no significant differences in serum IGF-I content were observed among groups, consistent with the relatively stable growth performance of minks during the winter fur-growing period. Dietary tryptophan supplemental level had no significant effects on dry matter digestibility, crude protein digestibility, ether extract digestibility, nitrogen intake, fecal nitrogen, urinary nitrogen, or nitrogen deposition. Group IV showed the highest net protein utilization and protein biological value, indicating optimal protein utilization, though not significantly different from Group I.

3.2 Effects on Fur Quality Minks are carnivorous animals primarily raised for fur production, possessing high economic value. Tryptophan regulates mammalian fur growth through melatonin synthesis. Melatonin is synthesized by pineal gland cells from blood tryptophan via hydroxylase enzymes, and increased melatonin secretion promotes fur maturation and rapid development of dense winter coats [20]. Body length, body weight, and fur quality are primary determinants of economic profitability in fur animal production, with fur quality evaluated based on color, guard hair and underhair length, guard hair diameter, hair density, and evenness [21]. Brsting et al. [22] reported that when dietary essential amino acid content is low, a dietary protein level of 40% is required to ensure fur quality; conversely, when essential amino acid content is high, 30% dietary protein suffices. This study evaluated overall fur quality, color, luster, and evenness, finding no significant differences among groups, indicating that tryptophan as an essential amino acid was present at adequate levels. In terms of overall evenness, Group V showed the best guard hair and underhair evenness, while Group III showed the poorest.

3.3 Effects on Serum Biochemical Parameters Serum TCHO content reflects fat deposition status in animals, with appropriate amino acid levels reducing serum TCHO content. In this study, Group V exhibited the highest serum TCHO content, possibly due to excessive tryptophan supplementation disrupting amino acid balance and consequently affecting fat deposition. Groups II, III, and IV, with dietary tryptophan levels of 0.36%–0.76%, showed lower serum TCHO content, indicating better fat deposition status than the negative control group with 0.26% tryptophan. The high-protein Group VI showed serum TCHO content similar to experimental groups, suggesting that protein degradation to amino acids may be involved in this process. Akiba et al. [23] reported that tryptophan supplementation in broilers reduced liver total fat content while affecting serum TCHO and TG levels. TG reflects fat synthesis intensity, and appropriate tryptophan supplementation can reduce fat synthesis. In this study, Group V showed lower serum TG content, reduced fat synthesis intensity, and decreased fat deposition, though no significant differences in ether extract digestibility were observed among groups, warranting further investigation into the relationship between fat synthesis and deposition.

ALT and AST are primarily distributed in hepatocytes, with ALT mainly in the cytoplasm and AST in both cytoplasm and mitochondria. Serum activities of these enzymes reflect liver protein synthesis capacity and hepatic function [24]. Under normal conditions, serum activities of these enzymes are low, with highest activities in heart and liver tissues. When tissues are damaged, large amounts of transaminases are released into serum, increasing enzyme activities, thus allowing assessment of organ function based on serum enzyme activity changes [25]. In this study, Group II showed the lowest serum ALT and AST activities, indicating the strongest hepatic tissue activity, while Group VI showed the highest ALT activity, possibly due to the burden imposed by high dietary protein levels on liver function.

3.4 Effects on Serum Immune Parameters As a limiting amino acid for immune-related proteins, tryptophan plays a crucial role in humoral immune regulation. Elevated dietary tryptophan levels promote immunoglobulin synthesis and enhance immunity [26]. The primary catabolic pathway of tryptophan is the kynurenine pathway. Studies have shown that in immune regulation, certain kynurenine metabolites can inhibit pro-inflammatory cytokine interleukin-17 (IL-17) production, suppress T helper 17 (Th17) cell maturation and differentiation, promote CD4+ T cell conversion to regulatory T cells, and reduce inflammatory responses [27], thereby enhancing immunity. Key factors in tryptophan metabolism involved in immune regulation include indoleamine 2,3-dioxygenase (IDO), quinolinic acid, 5-HT, and melatonin. In this study, Group IV showed the highest immunoglobulin content, indicating that a dietary tryptophan level of 0.76% optimized immune function in minks. When dietary tryptophan exceeded 0.76%, immune function declined, possibly due to excessive tryptophan burdening hepatic metabolism, affecting liver function, and triggering hepatic immune responses. Comparison between Groups I and VI demonstrated that high dietary protein levels did not enhance mink immunity.

Immune organs are structural tissues that execute immune functions, serving as sites for lymphocyte and other immune cell development, differentiation, proliferation, and immune response generation. Generally, increased immune organ indices indicate faster immune system maturation. Qiu [28] found in mice that tryptophan supplementation within a certain range promoted spleen development and increased spleen index. In this study, no significant differences were observed in immune organ indices among groups, though Group IV showed slightly higher liver index than other groups. Experimental group liver indices were similar to the high-protein positive control group, suggesting that both high protein levels and increased tryptophan supplementation could enhance immune organ indices. Group IV showed the highest spleen index, exceeding that of the high-protein positive control group, suggesting that protein may enhance immunity through hepatic mechanisms.

4. Conclusion

A dietary tryptophan level of 0.26% with 34% crude protein can satisfy the basic tryptophan nutritional requirements of white minks during the winter fur-growing period, yielding satisfactory growth performance. When dietary tryptophan level reaches 0.76%, amino acid metabolism becomes imbalanced, liver function is compromised, and immune function is activated.

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