

## Postprint: Optimal Dietary Tryptophan Requirement for Laying Ducks During Peak Laying Period

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### Abstract

This experiment aimed to determine the optimal tryptophan requirement for laying ducks during the laying period by investigating the effects of different dietary tryptophan levels on laying performance, egg quality, antioxidant indices, and slaughter indices. Nine hundred 120-day-old Shanma ducks were randomly allocated to 6 groups with 6 replicates per group and 25 ducks per replicate. Ducks in each group were fed experimental diets containing 0.12%, 0.16%, 0.20%, 0.24%, 0.28%, and 0.32% tryptophan, respectively, for a 20-week trial period. The results showed: 1) Dietary tryptophan level had no significant effect on laying rate, average egg weight, daily egg mass, feed-to-egg ratio, etc. ( $P>0.05$ ), but the 0.20% group numerically exhibited the highest laying rate and daily egg mass. Dietary tryptophan level had no significant effect on egg quality parameters including eggshell strength, Haugh unit, yolk weight, yolk percentage, eggshell weight, eggshell percentage, and eggshell thickness ( $P>0.05$ ), but significantly affected yolk color ( $P<0.05$ ), with the 0.20% group showing significantly lower yolk color than other groups ( $P<0.05$ ). 2) Dietary tryptophan level had no significant effect on serum albumin, total protein, and uric acid content ( $P>0.05$ ). 3) Dietary tryptophan level had no significant effect on serum and liver antioxidant indices (except for liver total superoxide dismutase) ( $P>0.05$ ). 4) Dietary tryptophan level had no significant effect on oviduct weight, oviduct length, and ovary weight ( $P>0.05$ ). Based on broken-line model fitting, a dietary tryptophan level of 0.20% could meet the nutritional requirements of laying ducks during peak production.

## Full Text

### Tryptophan Optimal Requirement for Laying Ducks in Peak Laying Period

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**Abstract:** This experiment investigated the effects of dietary tryptophan level on laying performance, egg quality, antioxidant parameters, and slaughter parameters of laying ducks to determine the optimal tryptophan requirement during peak production. Nine hundred 120-day-old Shanma ducks were randomly allocated into 6 groups with 6 replicates per group and 25 ducks per replicate. The six groups were fed experimental diets containing 0.12%, 0.16%, 0.20%, 0.24%, 0.28%, and 0.32% tryptophan for 20 weeks. The results showed that: 1) Dietary tryptophan level had no significant effects on egg production rate, average egg weight, daily egg mass, or feed conversion ratio ( $P > 0.05$ ), though the 0.20% group numerically achieved the highest egg production and daily egg mass. Tryptophan level did not significantly affect egg quality parameters including eggshell strength, Haugh unit, yolk weight, yolk percentage, eggshell weight, eggshell percentage, or shell thickness ( $P > 0.05$ ), but significantly influenced yolk color ( $P < 0.05$ ), with the 0.20% group showing significantly lower yolk color than other groups ( $P < 0.05$ ). 2) Dietary tryptophan level had no significant effects on serum albumin, total protein, or uric acid content ( $P > 0.05$ ). 3) Tryptophan level did not significantly affect serum and liver antioxidant parameters (except liver total superoxide dismutase activity) ( $P > 0.05$ ). 4) Dietary tryptophan level had no significant effects on oviduct weight, oviduct length, or ovary weight ( $P > 0.05$ ). Based on broken-line model fitting, a dietary tryptophan level of 0.20% was found to meet the nutritional requirements of laying ducks during peak production.

**Keywords:** laying ducks; tryptophan; laying performance; egg quality; antioxidant

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## Introduction

Tryptophan represents the third limiting amino acid in corn-soybean meal diets for poultry and plays a crucial role in maintaining normal physiological functions and production performance [1]. Research indicates that tryptophan not only

participates in protein synthesis but is also involved in multiple metabolic pathways. In the body, tryptophan can be metabolized into 5-hydroxytryptamine (5-HT), melatonin, niacin, nicotinamide adenine dinucleotide (NAD), and reduced NAD (NADH), which significantly influence feed intake behavior, fat deposition, and immune function [2]. Furthermore, studies have demonstrated that tryptophan promotes antioxidant capacity in poultry [3-4] and maintains production performance in low-protein diets. Current domestic research on tryptophan requirements in ducks has primarily focused on meat ducks [5] and growing ducks [6-7], with no published studies on tryptophan requirements for laying ducks during peak production. Therefore, this study utilized Longyan laying ducks to investigate the effects of different dietary tryptophan levels on laying performance, egg quality, antioxidant parameters, and slaughter parameters, aiming to establish tryptophan requirement parameters for laying ducks and provide a basis for formulating feeding standards.

## Materials and Methods

### Experimental Diets and Feeding Management

The experimental diets were formulated as corn-soybean meal type diets with six tryptophan levels: 0.12%, 0.16%, 0.20%, 0.24%, 0.28%, and 0.32%. The methionine content was 0.40% and lysine content was 0.86% across all diets. Diet composition and nutrient levels are presented in Table 1. Dietary metabolizable energy and crude protein levels were based on previous research from our group [8]. The analyzed tryptophan concentrations in the six diets were 0.15%, 0.16%, 0.17%, 0.22%, 0.24%, and 0.28%, respectively. A one-week pre-feeding period preceded the 20-week experimental period.

The experiment was conducted at the Laying Duck Research Facility of the Institute of Animal Science, Guangdong Academy of Agricultural Sciences. Nine hundred healthy Longyan laying ducks aged 120 days, with normal feed intake and similar body weight, were randomly divided into 6 groups using a single-factor completely randomized design. Each group consisted of 6 replicates with 25 ducks per replicate. Ducks were raised on the floor (water area:land area = 1:3). Temperature, humidity, and weather conditions were recorded daily at 07:00, 14:00, and 20:00.

### Sample Collection

On the day before the experiment ended, two ducks approaching the average body weight were selected from each replicate. Blood samples (5 mL) were collected from the wing vein, allowed to clot for 10 minutes, then centrifuged at 3,000 rpm for 15 minutes to obtain serum. After blood collection, ducks were euthanized and the abdominal cavity was opened. Liver samples were rapidly collected, snap-frozen in liquid nitrogen, and stored at -80°C for later analysis. The oviduct and ovary were separated, weighed, and oviduct length was measured.

## Measurements

**Feed Intake and Laying Performance** Feed was provided twice daily at 07:00 and 16:00. Under normal conditions, all experimental ducks maintained relatively consistent feed intake, with accurate records of feed offered and residual feed. Daily records were maintained for egg number, egg weight, broken eggs, abnormal eggs, and their weights. Daily feed intake, egg production rate, average egg weight, daily egg mass, feed conversion ratio, broken egg rate, and abnormal egg rate were calculated using the following formulas:

- Egg production rate =  $100 \times \text{number of eggs} / \text{number of experimental ducks}$
- Average egg weight =  $\text{total egg weight} / \text{number of eggs}$
- Daily egg mass =  $\text{egg production rate} \times \text{average egg weight}$
- Feed conversion ratio =  $\text{feed intake} / \text{daily egg mass}$
- Broken egg rate =  $100 \times \text{number of broken eggs} / \text{total number of eggs}$
- Abnormal egg rate =  $100 \times \text{number of abnormal eggs} / \text{total number of eggs}$

**Egg Quality** During peak production, egg samples were collected monthly. Four eggs (approaching average weight) were taken from each replicate for determination of egg shape index, eggshell strength, shell thickness, and shell percentage. Egg shape index was measured using a digital caliper to obtain longitudinal and transverse diameters, with the ratio of longitudinal to transverse diameter representing the shape index. Eggshell strength was measured using an eggshell force gauge (SN:EF0451 2011, ORKA Food Technology Ltd., Israel). Shell thickness was measured at the blunt end, middle, and pointed end using a digital micrometer (excluding the shell membrane), with the average of the three measurements calculated. All measurements were completed within 48 hours.

**Serum Parameters** Serum uric acid, albumin, and globulin contents were determined using a Beckman automatic biochemical analyzer (CX5, Beckman Instruments Inc., USA). Serum glutathione peroxidase (GSH-Px), superoxide dismutase (T-SOD) activities, total antioxidant capacity (T-AOC), and malondialdehyde (MDA) content were measured using assay kits from Nanjing Jiancheng Bioengineering Institute.

## Statistical Analysis

Data were analyzed using one-way ANOVA with SAS 8.1 software, with Duncan's multiple range test used for post-hoc comparisons. Results are expressed as means, with significance set at  $P < 0.05$ . Broken-line model analysis was used to fit the relationship between performance and dietary tryptophan level to determine the minimum tryptophan requirement for laying ducks during peak production.

## Results

### Effects of Dietary Tryptophan Level on Laying Performance

As shown in Table 2 , dietary tryptophan level had no significant effects on egg production rate, average egg weight, daily egg mass, average daily feed intake, feed conversion ratio, broken egg rate, or abnormal egg rate during peak production ( $P>0.05$ ). However, the 0.20% tryptophan group achieved the maximum values for both egg production rate and daily egg mass. Using broken-line model fitting (Figure 1 [Figure 1: see original paper]), the minimum dietary tryptophan requirement for peak laying ducks was estimated to be 0.20% based on egg production rate ( $R^2=0.999$ ,  $P=0.06$ ) and similarly 0.20% based on feed conversion ratio ( $R^2=0.999$ ,  $P=0.19$ ).

### Effects of Dietary Tryptophan Level on Egg Quality

Table 3 shows that dietary tryptophan level did not significantly affect eggshell strength, Haugh unit, yolk weight, yolk percentage, eggshell weight, eggshell percentage, or shell thickness ( $P>0.05$ ). However, tryptophan level significantly influenced yolk color ( $P<0.05$ ), with the 0.20% group exhibiting significantly lower yolk color compared to other groups ( $P<0.05$ ).

### Effects of Dietary Tryptophan Level on Serum Biochemical Parameters

Dietary tryptophan level had no significant effects on serum albumin, total protein, or uric acid content ( $P>0.05$ ) (Table 4 ).

### Effects of Dietary Tryptophan Level on Antioxidant Parameters

As shown in Table 5 , dietary tryptophan level did not significantly affect any serum antioxidant parameters ( $P>0.05$ ). In the liver, tryptophan level had no significant effects on T-AOC, GSH-Px activity, or MDA content ( $P>0.05$ ), but the 0.28% group showed significantly higher T-SOD activity than the 0.12% group ( $P<0.05$ ).

### Effects of Dietary Tryptophan Level on Slaughter Parameters

Dietary tryptophan level had no significant effects on oviduct weight, oviduct length, or ovary weight ( $P>0.05$ ) (Table 6 ).

## Discussion

### Dietary Tryptophan Requirement

The NRC [9] recommends dietary tryptophan levels of 0.13%-0.20% for White Leghorn laying hens, with daily requirements of 160 or 175 mg based on feed

intakes of 100 or 120 g, respectively. For breeding turkeys, the tryptophan requirement is 0.13% during laying, while Japanese quail require 0.19%. Although the NRC [9] provides tryptophan recommendations for meat ducks (Pekin ducks) at different growth stages, no data exist for laying ducks. Our study found that 0.20% dietary tryptophan optimized laying performance in peak laying ducks, consistent with requirements for quail and laying hens, though the daily tryptophan requirement for ducks (322 mg) was higher than other poultry species. Research indicates that dietary protein level interacts with tryptophan requirements, with higher protein levels increasing tryptophan needs in laying hens [10]. Studies have shown that supplementing appropriate tryptophan levels in low-protein diets (14%) significantly improved egg production, whereas no such effect was observed in normal (16%) or high-protein (18%) diets [10], confirming that protein level influences tryptophan requirements. Additionally, increasing dietary large neutral amino acids (isoleucine, alanine, leucine, phenylalanine, tyrosine) by 48% reduced tryptophan requirements by 21% [11], suggesting that large neutral amino acid levels may also affect tryptophan needs. Environmental conditions can alter tryptophan requirements; for example, laying hens under heat stress require 0.2%-0.4% dietary tryptophan [12], higher than NRC [9] recommendations (0.13%-0.20%). Research has also found that age does not affect tryptophan requirements in laying hens [13].

### **Effects of Dietary Tryptophan Level on Laying Performance and Feed Intake**

Our study found that 0.20% dietary tryptophan yielded the highest egg production rate and average egg weight, with production decreasing slightly as tryptophan levels increased. This may occur because at 0.20% tryptophan, the dietary amino acid profile achieves optimal balance, resulting in higher feed conversion efficiency and improved laying performance. However, exceeding this level creates amino acid imbalance, failing to further improve performance and potentially causing negative effects [14]. Similarly, Zhou [3] reported that feeding 300-day-old Roman laying hens diets with 0.16%-0.32% tryptophan significantly improved egg production with increasing levels, with the 0.20% group achieving the highest rate, though average egg weight and feed conversion ratio did not differ significantly among groups. Other studies have found that increasing dietary tryptophan significantly improved egg production when levels were below the requirement [10,15].

Broiler studies have shown that increasing dietary tryptophan from 0.12% to 0.24% significantly improved feed intake [16], and similar results were observed in laying hens [15,17]. This may be explained by tryptophan's conversion to 5-HT, which acts on the hypothalamic feeding center to regulate feed intake behavior [18]. However, research in meat ducks found that although tryptophan increased serum 5-HT levels [19], it did not affect feed intake. Our study also found that increasing dietary tryptophan had no significant effect on feed intake in laying ducks, suggesting that tryptophan may regulate feed intake differently

in ducks compared to chickens.

### **Effects of Dietary Tryptophan Level on Serum Biochemical and Antioxidant Parameters**

Body protein deposition depends on the balance between protein synthesis and catabolism. Serum total protein and albumin are important indicators of hepatic protein synthesis capacity, while uric acid, the end product of amino acid and purine metabolism in poultry, reflects protein catabolism—higher amino acid synthesis metabolism corresponds to lower serum uric acid content. In this study, dietary tryptophan level did not significantly affect serum albumin, globulin, or uric acid content, likely because the variation in dietary tryptophan was insufficient to influence protein metabolism. Studies in 42- to 56-day-old broilers also found that plasma total protein, albumin, and uric acid were unaffected by dietary tryptophan level [16], consistent with our findings.

We also observed that liver T-SOD activity increased significantly at 0.28% tryptophan, suggesting potential antioxidant capacity. Similarly, Zhou [3] reported that increasing dietary tryptophan significantly reduced serum MDA content and increased GSH-Px activity in laying hens, indicating improved antioxidant capacity. Wei et al. [4] found that 21-day-old Yangzhou geese fed 0.22% and 0.30% tryptophan showed significantly higher serum SOD activity and T-AOC compared to the 0.14% group, though tryptophan did not significantly affect serum MDA content or GSH-Px activity. Additionally, dietary tryptophan significantly increased serum T-AOC and GSH-Px and CAT activities while enhancing liver GSH-Px activity in meat ducks [19].

### **Effects of Dietary Tryptophan Level on Egg Quality**

Few studies have reported on tryptophan's effects on egg quality. Zhou [3] found that dietary tryptophan level did not significantly affect eggshell thickness, shell strength, or yolk color in chickens. Our study similarly found no significant effects on most egg quality parameters, except for yolk color, which was reduced in the 0.20% group. It remains unclear whether this result represents a chance finding or indicates that tryptophan potentially affects pigment deposition in egg yolk, warranting further investigation.

### **Conclusion**

Based on the results of this study, the following conclusions can be drawn: 1) Dietary tryptophan level did not significantly affect laying performance but significantly influenced yolk color, with the 0.20% group showing significantly lower yolk color than other groups, while other egg quality parameters were unaffected. 2) Dietary tryptophan level did not significantly affect serum biochemical or slaughter parameters, but the 0.28% level significantly increased liver T-SOD activity. 3) Based on egg production rate, a dietary tryptophan

level of 0.20% is recommended to meet the minimum nutritional requirement of laying ducks during peak production.

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