

## Effects of Dietary Threonine Level on Growth Performance, Carcass Quality, Immune Function, and Serum Hormones in Pekin Ducklings (Post-print)

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### Abstract

This experiment was conducted to investigate the effects of dietary threonine levels on growth performance, carcass quality, immune function, and serum hormones in Beijing ducklings. A total of 320 healthy 1-day-old male Beijing ducks were randomly allocated to 5 groups with 8 replicates per group and 8 birds per replicate. The measured threonine levels in the diets of each group were 0.65% (control), 0.69%, 0.81%, 0.88%, and 0.98%, respectively, and the experimental period was 21 days. The results showed: 1) Dietary threonine level had a highly significant effect on average daily gain and average daily feed intake of ducklings ( $P < 0.01$ ), and the control group had significantly lower average daily gain and average daily feed intake than the other groups ( $P < 0.05$ ). 2) Dietary threonine level had a significant effect on breast muscle percentage of ducklings ( $P < 0.05$ ), and the control group had significantly lower breast muscle percentage than the 0.69%, 0.81%, and 0.88% level groups ( $P < 0.05$ ). 3) Dietary threonine level had no significant effect on bursa of Fabricius index, spleen index, or thymus index of ducklings ( $P > 0.05$ ). Dietary threonine level had a highly significant effect on bursa of Fabricius weight and thymus weight of ducklings ( $P < 0.01$ ); the control group had significantly lower bursa weight than the other groups ( $P < 0.05$ ), and the control group had significantly lower thymus weight than the 0.81% and 0.88% level groups ( $P < 0.05$ ). 4) Dietary threonine level had a significant effect on serum triiodothyronine (T3) content of ducklings ( $P < 0.05$ ); the 0.81% level group had the highest serum T3 content, which was significantly higher than that of the control and 0.98% level groups ( $P < 0.05$ ). 5) The threonine requirement showed quadratic relationships with average daily feed intake, average daily gain, and breast muscle percentage, and the threonine requirements of Beijing ducklings for maximum values of these three parameters were 0.860%, 0.852%, and 0.837%, respectively. In conclusion, dietary supplementation with

appropriate levels of threonine can improve growth performance, carcass quality, and immune function in ducklings.

## Full Text

### Abstract

This experiment was conducted to investigate the effects of dietary threonine level on growth performance, carcass traits, immune function, and serum hormone levels in Peking ducklings. A total of 320 one-day-old healthy male Peking ducks were randomly allocated to 5 groups with 8 replicates per group and 8 ducklings per replicate. The measured dietary threonine levels were 0.65% (control), 0.69%, 0.81%, 0.88%, and 0.98%, with an experimental period of 21 days. The results showed: 1) Dietary threonine level had a highly significant effect on average daily gain (ADG) and average daily feed intake (ADFI) of ducklings ( $P < 0.01$ ), with the control group showing significantly lower ADG and ADFI compared to other groups ( $P < 0.05$ ). 2) Dietary threonine level significantly affected breast meat percentage ( $P < 0.05$ ), with the control group exhibiting significantly lower values than the 0.69%, 0.81%, and 0.88% groups ( $P < 0.05$ ). 3) Dietary threonine level had no significant effect on bursa of Fabricius index, spleen index, or thymus index ( $P > 0.05$ ), but showed highly significant effects on bursa of Fabricius weight and thymus weight ( $P < 0.01$ ). The bursa weight in the control group was significantly lower than in other groups ( $P < 0.05$ ), while thymus weight in the control group was significantly lower than in the 0.81% and 0.88% groups ( $P < 0.05$ ). 4) Dietary threonine level significantly affected serum triiodothyronine (T3) content ( $P < 0.05$ ), with the 0.81% group showing the highest T3 level, significantly exceeding the control and 0.98% groups ( $P < 0.05$ ). 5) Threonine requirement demonstrated quadratic relationships with ADFI, ADG, and breast meat percentage. The estimated threonine requirements for maximizing these parameters were 0.860%, 0.852%, and 0.837%, respectively. These results indicate that dietary supplementation with appropriate threonine levels can improve growth performance, carcass traits, and immune function in Peking ducklings.

**Keywords:** threonine; ducklings; growth performance; carcass traits; immune function; serum hormone

### Introduction

As early as 1935, Rose et al. [1] isolated an unknown amino acid from hydrolyzed fibrin protein, naming it threonine due to its structural similarity to threose [2]. Subsequent research revealed that threonine is the last essential amino acid discovered in poultry and ranks as the fourth most important essential amino acid after methionine, lysine, and tryptophan, playing a crucial role in maintaining normal growth, development, and immune function [3]. While current research has primarily focused on threonine's effects on broiler performance, studies on

meat ducks remain limited. This experiment selected Peking ducklings as subjects to investigate the effects of dietary threonine level on growth performance, carcass traits, immune function, and serum hormone levels, and to estimate threonine requirements for 1-21 day-old Peking ducks using mathematical models, thereby providing theoretical guidance for production practices.

## 1. Materials and Methods

### 1.1 Experimental Animals and Design

A completely randomized design was employed. Three hundred and twenty healthy, uniform-weight, one-day-old male Peking ducks from the same hatch were randomly divided into 5 groups with 8 replicates per group and 8 ducks per replicate. Each replicate consisted of one cage, with each group receiving different experimental diets. The dietary threonine levels were formulated at 0.60%, 0.68%, 0.76%, 0.84%, and 0.92% (with measured values of 0.65%, 0.69%, 0.81%, 0.88%, and 0.98%, respectively). The 0.65% measured level served as the basal diet (control group), while the other four diets were prepared by supplementing L-threonine at 0.08%, 0.16%, 0.24%, and 0.32% to the basal diet. The basal diet's nutrient levels were formulated according to NRC (1994) nutritional requirements for meat ducks and practical production experience. The composition and nutrient levels of the basal diet are presented in Table 1.

**Table 1** Composition and nutrient levels of the basal diet (air-dry basis), %

Items	Content
<b>Ingredients</b>	
Corn	58.00
Peanut meal	31.00
Soybean oil	3.00
CaHPO <sub>4</sub>	1.80
Limestone	1.20
NaCl	0.30
Premix <sup>1</sup>	0.50
DL-Met	0.20
Lys · HCl	0.50
Filler <sup>2</sup>	3.50
<b>Total</b>	<b>100.00</b>
<b>Nutrient levels<sup>3</sup></b>	
ME/(MJ/kg)	12.13
CP	18.00
Ca	0.90
AP	0.40
Lys	1.10
Met	0.45
Thr	0.65

<sup>1</sup> The premix provided the following per kg of diet: VA 10,350 IU, VB<sub>1</sub> 2.76 mg, VB<sub>2</sub> 8.625 mg, VB<sub>6</sub> 4.6 mg, VB<sub>12</sub> 0.03 mg, VD<sub>3</sub> 2,760 IU, VE 27.6 IU, VK<sub>3</sub> 12.65 mg, biotin 0.184 mg, folic acid 1.61 mg, D-pantothenic acid 11 mg, nicotinic acid 30 mg, Cu (as copper sulfate) 8.12 mg, Fe (as ferrous sulfate) 90 mg, Mn (as manganese sulfate) 101.76 mg, Zn (as zinc oxide) 89.7 mg, I (as potassium iodide) 0.80 mg, Se (as sodium selenite) 0.30 mg.

<sup>2</sup> Bentonite served as a filler.

<sup>3</sup> ME was a calculated value, while others were measured values.

## 1.2 Management

The experiment was conducted at Beijing Chengyuan Shenglong Breeding Co., Ltd. in Changping District. Ducks were raised on wire-floor pens with automatic temperature and humidity control using heating furnaces and fans to maintain consistent conditions and ensure adequate ventilation. A 24-hour lighting regimen was implemented, with house temperature gradually decreasing from 31°C to 24°C. Water was supplied through nipple drinkers and feed was provided manually, with ad libitum access to both feed and water throughout the 21-day experimental period.

## 1.3 Measurements

**1.3.1 Growth Performance** Feed intake was recorded at 7, 14, and 21 days of age for each replicate. After a 12-hour fast, ducks were weighed to determine body weight gain and feed consumption. Average daily gain, average daily feed intake, and feed-to-gain ratio at 21 days were calculated.

**1.3.2 Carcass Traits** At 22 days of age, 3 ducks were randomly selected from each replicate, weighed, and slaughtered according to the “Poultry Production Performance Terminology and Measurement Methods” [4]. Breast meat percentage, leg meat percentage, and abdominal fat percentage were calculated using the following formulas:

- Breast meat percentage (%) = (breast muscle weight/live body weight) × 100
- Leg meat percentage (%) = (leg muscle weight/live body weight) × 100
- Abdominal fat percentage (%) = (abdominal fat weight/live body weight) × 100

**1.3.3 Immune Function** During slaughter, spleen, thymus, and bursa of Fabricius were collected and weighed immediately to calculate organ indices:

- Spleen index (g/kg BW) = spleen weight/live body weight
- Thymus index (g/kg BW) = thymus weight/live body weight
- Bursa of Fabricius index (g/kg BW) = bursa weight/live body weight

**1.3.4 Serum Hormones** At 21 days of age, 3 ducks per replicate were randomly selected for blood collection from the right jugular vein. Serum growth hormone (GH), triiodothyronine (T3), and thyroxine (T4) were measured by radioimmunoassay, while insulin-like growth factor-I (IGF-I) was determined by enzyme-linked immunosorbent assay (ELISA).

#### 1.4 Statistical Analysis

Data were analyzed using SAS 9.2 software through one-way ANOVA and regression analysis. Duncan's multiple range test was used for mean comparisons, with  $P < 0.05$  considered significant and  $P < 0.01$  highly significant. Results are expressed as "mean  $\pm$  standard deviation".

## 2. Results

### 2.1 Effects of Dietary Threonine Level on Growth Performance

As shown in Table 2, dietary threonine level had a highly significant effect on ADG and ADFI ( $P < 0.01$ ). The control group exhibited significantly lower ADG and ADFI compared to other groups ( $P < 0.05$ ). Compared to the 0.69% group, the 0.81% group showed significantly higher ADG and ADFI ( $P < 0.05$ ), while no significant differences were observed between the 0.81% and 0.88% groups ( $P > 0.05$ ). Dietary threonine level did not significantly affect feed-to-gain ratio ( $P > 0.05$ ), though it tended to increase initially then decrease with rising threonine levels, peaking in the 0.88% group.

**Table 2** Effects of dietary Thr level on growth performance of Peking ducklings

Dietary Thr level/%	ADG/g	ADFI/g	Feed-to-gain ratio
0.65	43.49 $\pm$ 2.13 <sup>d</sup>	76.69 $\pm$ 5.51 <sup>c</sup>	1.762 $\pm$ 0.060
P-value	<0.0001	<0.0001	0.065

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

### 2.2 Effects of Dietary Threonine Level on Carcass Traits

Table 3 shows that dietary threonine level significantly affected breast meat percentage ( $P < 0.05$ ). The control group had significantly lower breast meat percentage than the 0.69%, 0.81%, and 0.88% groups ( $P < 0.05$ ), while no significant differences were found among the 0.69%, 0.81%, 0.88%, and 0.98% groups ( $P > 0.05$ ). Dietary threonine level did not significantly affect leg meat percentage or abdominal fat percentage ( $P > 0.05$ ), though both parameters tended to increase then decrease with rising threonine levels, peaking in the 0.81% group.

**Table 3** Effects of dietary Thr level on carcass traits of Peking ducklings, %

Dietary Thr level/%	Breast meat rate	Leg meat rate	Abdominal fat rate
0.65	7.754 $\pm$ 0.144 <sup>b</sup>	5.871 $\pm$ 0.476	0.7728 $\pm$ 0.1845  0.69 8.853 $\pm$ 0.214 <sup>a</sup>  5.921 $\pm$ 0.692 0.7669 $\pm$ 0.214
P-value	0.001	0.754	0.476

### 2.3 Effects of Dietary Threonine Level on Immune Function

Table 4 indicates that dietary threonine level had no significant effect on bursa of Fabricius index, spleen index, or thymus index ( $P > 0.05$ ). However, both bursa and thymus indices tended to increase then decrease with rising threonine levels. The control group showed the lowest bursa and thymus indices, while the 0.88% group had the highest bursa and thymus indices but the lowest spleen index; the 0.69% group exhibited the highest spleen index.

**Table 4** Effects of dietary Thr level on immune organ index of Peking ducklings, g/kg BW

Dietary Thr level/%	Bursa index	Spleen index	Thymus index
0.65	2.10 $\pm$ 0.36	1.48 $\pm$ 0.17	0.40 $\pm$ 0.12  0.69 2.32 $\pm$ 0.33 1.60 $\pm$ 0.33 0.41 $\pm$ 0.09  0.81 2.35 $\pm$ 0.33
P-value	0.891	0.981	0.654

Table 5 reveals that dietary threonine level had a highly significant effect on bursa weight ( $P < 0.01$ ), with the control group showing significantly lower values than other groups ( $P < 0.05$ ), while no significant differences existed among the 0.69%, 0.81%, 0.88%, and 0.98% groups ( $P > 0.05$ ). Spleen weight was not significantly affected ( $P > 0.05$ ), though the control group had the lowest value and the 0.69% group the highest. Thymus weight was highly significantly affected ( $P < 0.01$ ), with the 0.81% and 0.88% groups showing significantly higher values than the control group ( $P < 0.05$ ). The 0.88% group had the highest average thymus weight, which was significantly higher than the 0.98% group ( $P < 0.05$ ).

**Table 5** Effects of dietary Thr level on immune organ weight of Peking ducklings, g

Dietary Thr level/%	Bursa weight	Spleen weight	Thymus weight
0.65	1.148 $\pm$ 0.243 <sup>b</sup>	0.765 $\pm$ 0.184	0.396 $\pm$ 0.130 <sup>c</sup>   0.69 1.491 $\pm$ 0.342 <sup>a</sup>  1.093 $\pm$ 1.177 0.455 $\pm$ 0.117
P-value	0.008	0.654	0.009

## 2.4 Effects of Dietary Threonine Level on Serum Hormone Indices

As shown in Table 5, dietary threonine level significantly affected serum T3 content ( $P < 0.05$ ), with the 0.81% group showing the highest T3 level, significantly exceeding the control and 0.98% groups ( $P < 0.05$ ). While T4 content was not significantly affected ( $P > 0.05$ ), the T3/T4 ratio was significantly influenced ( $P < 0.05$ ). The 0.81% group exhibited the highest T3/T4 ratio, significantly higher than the control, 0.69%, and 0.98% groups ( $P < 0.05$ ). Dietary threonine level did not significantly affect serum GH or IGF-I content ( $P > 0.05$ ).

**Table 5** Effects of dietary Thr level on serum hormone indices of Peking ducklings

Dietary Thr level/%	T3/(ng/mL)	T4/(ng/mL)	T3/T4	GH/(ng/mL)	IGF-I/(ng/mL)
0.65	0.885 $\pm$ 0.200 <sup>b</sup>	48.39 $\pm$ 4.19	1.829 $\pm$ 0.370 <sup>c</sup>	0.926 $\pm$ 0.175	115.10 $\pm$ 22.47
P-value	0.045	0.476	0.048	0.912	0.978

## 2.5 Estimation of Threonine Requirement for Peking Ducklings

Based on the experimental results, quadratic curve models were fitted with average daily feed intake ( $y_1$ ), average daily gain ( $y_2$ ), and breast meat percentage ( $y_3$ ) as dependent variables and threonine requirement ( $x$ ) as the independent variable:

$$y_1 = -448.84x^2 + 772.04x - 233.75 \quad (R^2 = 0.9430)$$

$$y_2 = -227.97x^2 + 388.39x - 111.8 \quad (R^2 = 0.9177)$$

$$y_3 = -3.4883x^2 + 5.8385x - 1.5485 \quad (R^2 = 0.8108)$$

The regression models indicate that threonine requirements for maximizing ADFI, ADG, and breast meat percentage in Peking ducklings were 0.860%, 0.852%, and 0.837%, respectively.

## 3. Discussion

### 3.1 Effects of Dietary Threonine Level on Growth Performance

Threonine regulates poultry growth performance, with animals exhibiting optimal requirements because performance improves as dietary threonine increases until reaching a plateau, after which performance declines. Optimal threonine levels enable maximum expression of growth potential. Numerous studies in broilers have demonstrated that appropriate threonine supplementation significantly improves feed intake, weight gain, and feed efficiency [5-7]. In meat ducks, Zhang et al. [8] reported that optimal threonine levels significantly increased ADFI and ADG while decreasing feed-to-gain ratio. Xie et al. [9] supplemented synthetic threonine to a basal diet containing 18.98% crude protein, elevating threonine from 5.0 to 8.2 g/kg, and found that appropriate threonine addition significantly improved weight gain, feed intake, and feed efficiency

in Peking ducks, with similar findings reported by Guo et al. [10] and Zhang et al. [11]. The current study also found that threonine supplementation significantly improved ADG and ADFI in ducklings, with a non-significant trend toward improved feed efficiency, consistent with Jiang et al. [12]. The estimated threonine requirements for optimal ADG (0.852%) and ADFI (0.860%) differed substantially from Guo et al. [10] (0.733% and 0.794%), but aligned closely with Zhang et al. [13] (0.86%). These discrepancies may stem from differences in dietary crude protein levels and other factors affecting threonine requirements [14], including age, amino acid ratios, diet digestibility, and feeding management.

### 3.2 Effects of Dietary Threonine Level on Carcass Traits

Breast meat percentage, leg meat percentage, and abdominal fat percentage are key indicators of poultry carcass quality, with abdominal fat comprising approximately 22% of total body fat and serving as the primary fat deposition site. This study demonstrated that increasing dietary threonine significantly improved breast meat percentage, with non-significant trends for leg meat and abdominal fat percentages. The improvement in breast muscle may be attributed to elevated serum T3 levels promoting protein synthesis and growth. Zhang et al. [11] reported that increasing threonine from 0.60% to 0.95% in 1-14 day-old Peking ducks significantly increased breast muscle weight and relative yield. Zhang et al. [13] found that elevating threonine from 0.64% to 0.72% in 15-35 day-old ducks increased breast weight by 1.0%. Maqbool [15] observed that higher threonine levels significantly increased breast meat and abdominal fat percentages in 1-14 day-old Peking ducks without affecting leg meat percentage. In broilers, Abbasi et al. [16] and Taghinejad-Roudbaneh et al. [17] reported that threonine supplementation significantly increased breast and leg meat percentages without affecting abdominal fat. Conversely, Barkley et al. [18] found that increased threonine significantly reduced abdominal fat in broilers. The non-significant effect on abdominal fat in this study may be because visible abdominal fat is absent in newborn ducklings, only appearing in the second week, and the 21-day experimental duration was insufficient to affect fat deposition. Alternatively, the basal threonine level may have been too high to elicit detectable effects on abdominal fat.

### 3.3 Effects of Dietary Threonine Level on Immune Function

Threonine is a major component and limiting amino acid of avian immunoglobulin G, playing a critical role in immune system development and function [19]. Adequate threonine enables appropriate immune responses to antigens, whereas deficiency suppresses immunoglobulin and lymphocyte production, impairing antibody formation and immune function [20]. Threonine promotes differentiation of bone marrow lymphocyte precursors into mature lymphocytes, thereby enhancing immune function and reducing morbidity and mortality. In broilers, Li et al. [21] reported that threonine significantly increased bursa and spleen indices. Corzo et al. [22] found that 0.51% dietary threonine did not signif-

icantly affect immune organ weights but levels below 0.51% reduced thymus weight. Wang et al. [20] observed that threonine supplementation significantly increased bursa index in 1-3 week-old broilers and spleen index in 4-6 week-old birds. Limited research exists on threonine' s effects in ducks. This study found that threonine supplementation significantly increased bursa and thymus weights but not spleen weight, possibly due to the relatively high basal threonine level.

### 3.4 Effects of Dietary Threonine Level on Serum Hormone Indices

Growth hormone (GH) promotes growth of all tissues except neural tissue and stimulates anabolism and protein synthesis. Triiodothyronine (T3) and thyroxine (T4) reflect metabolic status, promoting growth, tissue differentiation, and nutrient metabolism while affecting multiple organ systems. The GH-IGF-I axis is central to growth regulation, with GH stimulating protein synthesis and inhibiting degradation [23] while inducing increased circulating IGF-I, which mediates GH' s growth-promoting effects more potently than GH itself. This study found that appropriate threonine supplementation significantly increased serum T3 content, similar to Wang et al. [20] in broilers. Although GH and T4 were not significantly affected, both showed trends of increasing then decreasing with rising threonine levels. These changes suggest threonine may influence nutrient metabolism through modulation of serum hormone levels, while indicating that these hormones maintain dynamic equilibrium with only minor fluctuations.

## 4. Conclusions

Based on the experimental results, three main conclusions can be drawn: First, dietary threonine level significantly affects average daily gain and feed intake in Peking ducklings, with appropriate supplementation improving breast meat percentage, bursa weight, and thymus weight. Second, increasing dietary threonine appropriately enhances serum triiodothyronine content. Third, the threonine requirements for optimizing average daily feed intake, average daily gain, and breast meat percentage are 0.860%, 0.852%, and 0.837%, respectively.

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