

## Effects of Riboflavin Deficiency on Growth Performance, Organ Indices, and Plasma Biochemical Parameters in 1-28-Day-Old Peking Ducks (Postprint)

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### Abstract

This experiment was conducted to investigate the effects of riboflavin deficiency on growth performance, slaughter performance, organ indices, tissue riboflavin content, and plasma biochemical indices in 1- to 28-day-old Peking ducks. A single-factor completely randomized design was adopted. Three hundred sixty 1-day-old healthy male Peking ducks were selected and randomly divided into 3 groups: a riboflavin deficiency group, a pair-fed group (where feed intake was artificially controlled to match that of the riboflavin deficiency group), and an ad libitum control group, with 12 replicates per group and 10 ducks per replicate. Ducks in the riboflavin deficiency group were fed a riboflavin-deficient diet (measured riboflavin content of 1.38 mg/kg), while ducks in the pair-fed group and ad libitum control group were fed a riboflavin-adequate diet (supplemented with 10 mg/kg riboflavin based on the riboflavin-deficient diet). The experimental period lasted 28 days. The results showed that, compared with the pair-fed group and ad libitum control group, the riboflavin deficiency group exhibited significantly decreased average daily gain, breast muscle percentage, and leg muscle percentage ( $P < 0.05$ ), significantly increased feed-to-gain ratio and mortality rate ( $P < 0.05$ ), significantly increased liver index, heart index, and pancreas index ( $P < 0.05$ ), significantly decreased plasma riboflavin, liver riboflavin, and flavin mononucleotide contents ( $P < 0.05$ ), and significantly increased plasma aspartate aminotransferase activity as well as total cholesterol and triglyceride contents ( $P < 0.05$ ). It was concluded that riboflavin is an essential nutrient for the growth and development of Peking ducks; dietary riboflavin deficiency can reduce growth performance and tissue riboflavin content in Peking ducks during the early growth stage (1-28 days of age), increase liver index, and elevate plasma total cholesterol and triglyceride contents as well as aspartate aminotransferase activity.

## Full Text

### Abstract

This experiment was conducted to investigate the effects of riboflavin deficiency on growth performance, carcass traits, organ indexes, tissue riboflavin content, and plasma biochemical indices of Pekin ducks from 1 to 28 days of age. Using a one-factor completely randomized design, 360 one-day-old healthy male Pekin ducks were randomly divided into three groups: a riboflavin-deficient group, a pair-fed group (with artificially controlled feed intake matching the riboflavin-deficient group), and an ad libitum control group. Each group comprised 12 replicates with 10 ducks per replicate. Ducks in the riboflavin-deficient group were fed a riboflavin-deficient diet (measured riboflavin content: 1.38 mg/kg), while those in the pair-fed and ad libitum control groups received a riboflavin-sufficient diet (the riboflavin-deficient diet supplemented with 10 mg/kg riboflavin). The experimental period lasted 28 days.

The results showed that compared with the pair-fed and ad libitum control groups, the riboflavin-deficient group exhibited significantly lower average daily gain, breast muscle percentage, and leg muscle percentage ( $P < 0.05$ ), along with significantly higher feed-to-gain ratio and mortality ( $P < 0.05$ ). The liver index, heart index, and pancreas index were significantly elevated in the riboflavin-deficient group ( $P < 0.05$ ), while plasma riboflavin, liver riboflavin, and flavin mononucleotide contents were significantly reduced ( $P < 0.05$ ). Additionally, plasma aspartate transaminase activity and total cholesterol and triglyceride concentrations were significantly increased ( $P < 0.05$ ). These findings demonstrate that riboflavin is an essential nutrient for the growth and development of Pekin ducks. Dietary riboflavin deficiency during the early growth period (1-28 days of age) reduces growth performance and tissue riboflavin content while increasing liver index and elevating plasma total cholesterol, triglyceride concentrations, and aspartate transaminase activity.

**Keywords:** Pekin ducks; riboflavin; growth performance; plasma biochemical indices; organ indexes

### Introduction

Riboflavin is a crucial precursor for the synthesis of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). FMN and FAD serve as coenzymes for numerous flavoproteins involved in various metabolic processes, including fatty acid oxidation, the tricarboxylic acid cycle, mitochondrial electron transport, and amino acid degradation [1-2]. Dietary riboflavin deficiency significantly impairs poultry production performance, reducing average daily gain, average daily feed intake, and feed utilization efficiency [3-8]. In meat ducks, riboflavin deficiency markedly decreases growth performance and carcass traits, reduces plasma and liver riboflavin content, and significantly increases plasma alanine transaminase (ALT) and aspartate transaminase (AST) activities, ac-

accompanied by reduced feed intake [6-8]. However, it remains unclear whether these changes are directly attributable to riboflavin deficiency or secondary to reduced feed intake, warranting further investigation. Therefore, this study employed a pair-fed group (with controlled feed intake matching the riboflavin-deficient group) to examine the specific effects of dietary riboflavin deficiency on growth performance, organ indexes, tissue riboflavin content, and plasma biochemical indices in Pekin ducks, providing a reference for riboflavin application in duck feed formulation.

## 1. Materials and Methods

### 1.1 Experimental Diets

The basal diet was formulated according to the nutrient requirements for meat ducks in the NRC (1994) standards. Its composition and nutrient levels are presented in Table 1. The riboflavin content in the basal diet was measured as 1.38 mg/kg using high-performance liquid chromatography [9]. Riboflavin-deficient and riboflavin-sufficient diets were prepared by supplementing the basal diet with 0 and 10 mg/kg riboflavin, respectively.

### 1.2 Experimental Design and Management

A one-factor completely randomized design was employed. Three hundred sixty one-day-old healthy male Pekin ducks were randomly allocated to three groups: riboflavin-deficient, pair-fed, and ad libitum control, with 12 replicates per group and 10 ducks per replicate. The experimental period lasted 28 days (from 1 to 28 days of age). Ducks in the riboflavin-deficient group were fed the riboflavin-deficient diet, while those in the pair-fed and ad libitum control groups received the riboflavin-sufficient diet. Both the riboflavin-deficient and ad libitum control groups had free access to feed, whereas the pair-fed group was fed once daily at 08:30. Before feeding, residual feed from the previous day was collected and weighed from the riboflavin-deficient group to record feed intake, and the pair-fed group was provided with an equivalent amount of feed.

Ducks were raised on wire-floor pens with free access to water. House temperature was gradually reduced from 33°C to 22°C in phases during the experimental period. A 24-hour lighting schedule with artificial supplementation was implemented, and other routine management practices were followed.

### 1.3 Measurements

**1.3.1 Growth Performance** On day 28, fasting body weight and feed consumption were recorded per replicate to calculate average daily gain, average daily feed intake, and feed-to-gain ratio for each group from 1 to 28 days of age. Mortality was recorded throughout the experiment to calculate the mortality rate.

**1.3.2 Carcass Traits and Organ Indexes** On day 28, two ducks approaching the average body weight were selected from each replicate of each group. Live weight, breast muscle weight, leg muscle weight, abdominal fat weight, spleen weight, pancreas weight, liver weight, and heart weight were measured to calculate breast muscle percentage, leg muscle percentage, abdominal fat percentage, spleen index, pancreas index, liver index, and heart index (expressed as the ratio of absolute organ weight to live weight, in %). Liver samples were collected and stored at  $-20^{\circ}\text{C}$  for subsequent analysis.

**1.3.3 Determination of Riboflavin, FMN, and FAD Content** Riboflavin content in the basal diet was determined according to the method described in reference [9]. Plasma and liver riboflavin, FMN, and FAD contents were measured using the method described in reference [10].

**1.3.4 Plasma Biochemical Indices** On day 28, two ducks approaching the average body weight were selected from each replicate of each group. Blood samples (10 mL) were collected via cardiac puncture into anticoagulant-treated tubes and centrifuged at  $4^{\circ}\text{C}$  and 3,000 rpm for 10 minutes to prepare plasma, which was stored at  $-20^{\circ}\text{C}$ . Plasma ALT and AST activities and uric acid, total protein, albumin, total cholesterol, and triglyceride concentrations were measured using kits from Beijing Zhongsheng Beikong Biotechnology Co., Ltd. on a Hitachi 721 automatic biochemical analyzer.

## 1.4 Statistical Analysis

Experimental data (except mortality) are expressed as “mean  $\pm$  standard deviation.” Data were analyzed using SAS 9.0 statistical software, and differences among groups were compared using Duncan’s multiple range test. The significance level was set at  $P < 0.05$ .

## 2. Results

### 2.1 Effects of Riboflavin Deficiency on Growth Performance

The effects of riboflavin deficiency on growth performance of Pekin ducks from 1 to 28 days of age are shown in Table 2. Compared with the ad libitum control group, both the riboflavin-deficient and pair-fed groups exhibited significantly lower average daily gain and average daily feed intake ( $P < 0.05$ ), while the riboflavin-deficient group showed a significantly higher feed-to-gain ratio ( $P < 0.05$ ). The riboflavin-deficient group had significantly lower average daily gain and higher feed-to-gain ratio than the pair-fed group ( $P < 0.05$ ), but no significant difference in average daily feed intake ( $P > 0.05$ ). Additionally, mortality in the riboflavin-deficient group was significantly higher than in both the pair-fed and ad libitum control groups ( $P < 0.05$ ).

## 2.2 Effects of Riboflavin Deficiency on Carcass Traits

The effects of riboflavin deficiency on carcass traits of 28-day-old Pekin ducks are presented in Table 3 . Breast muscle percentage and leg muscle percentage in the riboflavin-deficient group were significantly lower than those in the pair-fed and ad libitum control groups ( $P<0.05$ ). Abdominal fat percentage in both the riboflavin-deficient and pair-fed groups was significantly lower than in the ad libitum control group ( $P<0.05$ ).

## 2.3 Effects of Riboflavin Deficiency on Organ Indexes

The effects of riboflavin deficiency on organ indexes of 28-day-old Pekin ducks are shown in Table 4 . Dietary riboflavin deficiency had no significant effect on spleen index compared with the pair-fed and ad libitum control groups ( $P>0.05$ ). However, pancreas index, heart index, and liver index in the riboflavin-deficient group were significantly higher than those in the pair-fed and ad libitum control groups ( $P<0.05$ ), with the pair-fed group also showing significantly higher pancreas and heart indexes than the ad libitum control group ( $P<0.05$ ).

## 2.4 Effects of Riboflavin Deficiency on Riboflavin, FMN, and FAD Content

The effects of riboflavin deficiency on plasma and liver riboflavin content in 28-day-old Pekin ducks are shown in Table 5 . Plasma and liver riboflavin contents in the riboflavin-deficient group were significantly lower than those in the pair-fed and ad libitum control groups ( $P<0.05$ ), with no significant difference between the latter two groups ( $P>0.05$ ). Liver FMN content in the riboflavin-deficient group was lower than in the ad libitum control group, while the ad libitum control group had lower liver FMN content than the pair-fed group, but these differences were not statistically significant ( $P>0.05$ ). Similarly, liver FAD content in both the riboflavin-deficient and ad libitum control groups was lower than in the pair-fed group, but the difference was not significant ( $P>0.05$ ).

## 2.5 Effects of Riboflavin Deficiency on Plasma Biochemical Indices

The effects of riboflavin deficiency on plasma biochemical indices of 28-day-old Pekin ducks are presented in Table 6 . Plasma AST activity and total protein, albumin, total cholesterol, and triglyceride concentrations in the riboflavin-deficient group were significantly higher than those in the pair-fed and ad libitum control groups ( $P<0.05$ ), with no significant differences between the latter two groups ( $P>0.05$ ). Plasma ALT activity and uric acid concentration in the riboflavin-deficient group were significantly higher than in the pair-fed group ( $P<0.05$ ) but did not differ significantly from the ad libitum control group ( $P>0.05$ ).

### 3. Discussion

#### 3.1 Effects of Riboflavin Deficiency on Growth Performance

Riboflavin deficiency significantly reduces feed intake in animals [11-16], and studies by Tang et al. [6-8] in Pekin ducks also demonstrated that dietary riboflavin deficiency markedly decreases average daily feed intake. Therefore, this experimental design included both a pair-fed group and an ad libitum control group to eliminate the confounding effect of feed intake. Most domestic and international studies have shown that riboflavin deficiency in poultry impairs growth and reduces feed utilization efficiency, while riboflavin supplementation significantly improves production performance [5,17-19]. The present results are consistent with these reports, showing that dietary riboflavin deficiency significantly reduced average daily gain and average daily feed intake while increasing feed-to-gain ratio in Pekin ducks. Furthermore, riboflavin deficiency significantly decreased breast muscle percentage and leg muscle percentage, aligning with previous findings [8].

In this study, despite identical feed intake between the riboflavin-deficient and pair-fed groups, the riboflavin-deficient group exhibited significantly lower average daily gain, indicating poorer feed conversion efficiency. This is consistent with research in rats [12-13,15,20] and pigs [16]. Since riboflavin participates in multiple metabolic processes including fatty acid oxidation, the tricarboxylic acid cycle, mitochondrial electron transport, and amino acid degradation, the reduced feed conversion efficiency may result from incomplete nutrient oxidation and decreased energy utilization due to riboflavin deficiency [21-23].

#### 3.2 Effects of Riboflavin Deficiency on Riboflavin Content

Plasma riboflavin concentration is a sensitive indicator of riboflavin nutritional status [24-27]. Research by Xu et al. [24] demonstrated that plasma riboflavin content in rats decreased rapidly after consuming a riboflavin-deficient diet, reaching only 8.1% of the control group after 4 weeks. In the present study, plasma riboflavin content in Pekin ducks fed a riboflavin-deficient diet for 4 weeks decreased to 9% of the ad libitum control group, confirming that plasma riboflavin content sensitively reflects riboflavin nutritional status in ducks. Additionally, liver riboflavin and FMN contents showed similar trends to plasma riboflavin content. However, liver FAD content in the riboflavin-deficient group did not differ significantly from the ad libitum control group, consistent with findings by Hustad et al. [26] in humans. This may be attributed to the body's priority in maintaining FAD supply [26,28].

#### 3.3 Effects of Riboflavin Deficiency on Organ Indexes and Plasma Biochemical Indices

The antioxidant function of riboflavin has been validated in numerous studies [18-19,29-30]. Levin et al. [30] confirmed that riboflavin deficiency reduces

cellular resistance to oxidative damage and alters membrane function and fluidity. Plasma ALT and AST are sensitive indicators of liver function, with their activities positively correlated with the degree of liver damage [31]. The significant increase in plasma AST activity in riboflavin-deficient ducks indicates liver cell membrane damage and enhanced membrane permeability, likely due to weakened antioxidant capacity and lipid peroxidation of liver cell membranes resulting from insufficient dietary riboflavin.

Organ indexes reflect organ development, metabolic activity, functional integrity, and overall health status. The significantly higher liver index in the riboflavin-deficient group compared with the pair-fed and ad libitum control groups is consistent with findings in other species [11-15,20], and the enlarged liver further confirms hepatic damage.

Studies in rats have shown that riboflavin deficiency can cause fatty liver [32], and the present study also found that riboflavin deficiency significantly increased plasma total cholesterol and triglyceride concentrations, leading to fat accumulation in blood. This may be due to reduced activity of acyl-CoA dehydrogenase (an FAD-dependent enzyme) and impaired fatty acid  $\beta$ -oxidation caused by riboflavin deficiency [33].

## Conclusions

1. Under the conditions of this experiment, dietary riboflavin deficiency reduced average daily gain, breast muscle percentage, and leg muscle percentage while increasing liver index, heart index, and pancreas index in Pekin ducks from 1 to 28 days of age.
2. Under the conditions of this experiment, dietary riboflavin deficiency decreased plasma and liver riboflavin content and increased plasma total cholesterol and triglyceride concentrations and AST activity in Pekin ducks from 1 to 28 days of age.

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