Effects of Dietary Riboflavin Supplementation Level on Meat Quality, Hair Follicle Development, and Immune Function in Growing Rex Rabbits (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary riboflavin supplementation levels on meat quality, hair follicle development, and immune performance in growing rex rabbits. One hundred sixty 3-month-old rex rabbits with similar body weight were selected and randomly divided into 4 groups with 40 replicates per group and 1 rabbit per replicate. Each group was fed a basal diet supplemented with 0 (control), 3, 6, and 12 mg/kg riboflavin, respectively. The experiment consisted of a 7-day preliminary period and a 53-day formal experimental period. The results showed that, compared with the control group, dietary supplementation with 6 and 12 mg/kg riboflavin significantly reduced the drip loss of rex rabbit muscle ($P<0.05$); dietary supplementation with 3, 6, and 12 mg/kg riboflavin significantly increased the total hair follicle density in rex rabbit skin ($P<0.05$); dietary supplementation with 3 and 6 mg/kg riboflavin significantly increased the primary hair follicle density in rex rabbit skin ($P<0.05$); dietary supplementation with 6 and 12 mg/kg riboflavin significantly increased the secondary hair follicle density in rex rabbit skin ($P<0.05$); and dietary supplementation with 3 and 6 mg/kg riboflavin significantly reduced the secondary to primary hair follicle density ratio in rex rabbit skin ($P<0.05$). Dietary riboflavin supplementation level had significant effects on thymus weight, thymus index, and serum contents of immunoglobulin G, immunoglobulin A, and interleukin-2 in rex rabbits ($P<0.05$), and all showed a trend of first increasing and then decreasing with increasing dietary riboflavin supplementation level, reaching maximum values in the 6 mg/kg supplementation group, which were significantly higher than those in the control group ($P<0.05$). It can be concluded that the appropriate dietary riboflavin supplementation level for 3- to 5-month-old growing rex rabbits is 6 mg/kg (the actual measured riboflavin level in the diet was 8.35 mg/kg).

Full Text

Abstract

This experiment was conducted to investigate the effects of dietary riboflavin supplementation on meat quality, hair follicle development, and immune performance in growing Rex rabbits. One hundred sixty 3-month-old Rex rabbits with similar body weight (1,860 ± 144 g) were randomly allocated to 4 groups, each consisting of 40 replicates with 1 rabbit per replicate. The groups were fed a basal diet supplemented with 0 (control), 3, 6, or 12 mg/kg riboflavin. The pre-trial period lasted 7 days, followed by a 53-day experimental period. The results showed that compared with the control group, supplementation with 6 and 12 mg/kg riboflavin significantly reduced muscle drip loss ($P < 0.05$). Dietary supplementation with 3, 6, and 12 mg/kg riboflavin significantly increased total hair follicle density in the skin ($P < 0.05$), while 3 and 6 mg/kg supplementation significantly increased primary hair follicle density ($P < 0.05$). Supplementation with 6 and 12 mg/kg significantly increased secondary hair follicle density ($P < 0.05$), and 3 and 6 mg/kg supplementation significantly decreased the ratio of secondary to primary hair follicle density ($P < 0.05$). Dietary riboflavin level significantly affected thymus weight, thymus index, and serum concentrations of immunoglobulin G (IgG), immunoglobulin A (IgA), and interleukin-2 (IL-2) ($P < 0.05$). All these parameters showed a quadratic response to increasing riboflavin supplementation, reaching maximum values at 6 mg/kg and significantly exceeding the control group ($P < 0.05$). In conclusion, the optimal dietary riboflavin supplementation level for 3- to 5-month-old growing Rex rabbits is 6 mg/kg (corresponding to an analyzed dietary riboflavin level of 8.35 mg/kg).

Keywords: Rex rabbits; riboflavin; meat quality; hair follicle development; immune performance

Introduction

Riboflavin is an essential water-soluble vitamin required for growth and development. It forms various flavoenzymes when bound to specific proteins as a coenzyme, participating in the metabolism of the three major nutrients. Riboflavin is also involved in iron metabolism and the synthesis of tryptophan and vitamin C, enhances immune function, strengthens liver function, and is essential for growth and tissue repair. Current riboflavin supplementation levels in Rex rabbit diets are largely based on established standards such as NRC (1977), which may not align with China' s domestic industry standards. Establishing scientifically sound feeding standards is essential for intensive Rex rabbit production. Our previous research investigated the effects of different dietary riboflavin levels (0–12 mg/kg) on growth performance in Rex rabbits and found no significant effects on body weight gain or feed intake [1]. However, riboflavin supplementation has been shown to promote growth in poultry and improve production performance in dairy cows [2,3], indicating substantial

species-specific differences in riboflavin requirements.

Riboflavin is first phosphorylated by flavokinase to form its coenzyme flavin mononucleotide (FMN). Subsequently, under the combined catalysis of pyrophosphorylase and FAD synthetase, ATP and FMN combine to generate flavin adenine dinucleotide (FAD). Riboflavin exists in three forms: FAD, FMN, and free vitamin B2. In biological tissues, FAD is covalently bound to specific proteins, forming flavoprotein enzymes that are closely associated with lipid, protein, and carbohydrate metabolism and participate in crucial redox reactions. However, whether dietary niacin supplementation affects immune organ development and cytokine secretion in rabbits remains unclear. Riboflavin deficiency inhibits hepatic flavoenzyme activity or reduces enzyme variety, leading to accumulation of liver fat and glycogen. Flavoenzymes are essential catalysts for fatty acid oxidation and unsaturated fatty acid metabolism, and riboflavin deficiency significantly decreases plasma and liver concentrations of unsaturated fatty acids such as arachidonic and linoleic acid [4]. Furthermore, riboflavin affects lipid metabolism by influencing fatty acid -oxidation [5]. Whether dietary riboflavin supplementation improves meat quality in Rex rabbits is currently unknown.

Rex rabbits are a dual-purpose breed valued for both meat and fur, with pelts of considerable economic value. Pelt quality is affected by size, thickness, and hair density. Riboflavin deficiency causes skin diseases, and our previous study found that 12 mg/kg riboflavin supplementation increased pelt area in Rex rabbits [1]. Additionally, studies in rats have shown that riboflavin affects hair follicle development [6]. However, whether dietary riboflavin supplementation influences hair follicle development in rabbits remains unknown. Therefore, this experiment was designed to determine the optimal dietary riboflavin level by measuring meat quality, hair follicle development, and immune performance indicators, which is crucial for accurately establishing riboflavin requirements in growing Rex rabbits.

Materials and Methods

Experimental Animals and Design

One hundred sixty healthy 3-month-old Rex rabbits with an average body weight of (1,860 ± 144) g, half male and half female, were randomly divided into 4 groups with 40 replicates per group (1 rabbit per replicate). The groups were fed a basal diet supplemented with 0 (control), 3, 6, or 12 mg/kg riboflavin (analyzed dietary riboflavin levels: 2.62, 4.43, 8.35, and 14.94 mg/kg, respectively). The basal diet was formulated according to NRC (1977) standards for rabbits. Diet composition and nutrient levels are presented in Table 1 . The pre-trial period lasted 7 days, followed by a 53-day experimental period.

Animal Management and Sample Collection

Rabbits were housed individually in cages under conventional management and immunization programs with natural lighting and ventilation. They had ad libitum access to water, and the rabbit house was disinfected every 3–5 days. At the end of the experiment, 8 rabbits were randomly selected from each group. Blood samples were collected immediately via cardiac puncture, incubated at 37°C for 40 minutes, then centrifuged at 3,000 r/min for 15 minutes to separate serum, which was stored at -20°C. After slaughter by cervical dislocation, skin samples were collected from the shoulder, back, and rump regions and fixed in paraformaldehyde solution for determination of hair follicle density (averaged across the three sites).

Laboratory Analyses

Immune Factor Determination Serum concentrations of immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA), and interleukin-2 (IL-2) were measured using enzyme-linked immunosorbent assay (ELISA) kits on a microplate reader. Kits were purchased from Nanjing Jiancheng Bioengineering Institute.

Hair Follicle Density Measurement Hair follicle density was determined using paraffin sectioning and hematoxylin-eosin (HE) staining, followed by microscopic image analysis. Primary and secondary hair follicle densities were recorded, and the ratio of secondary to primary hair follicle density was calculated.

Meat Quality Measurement **pH:** The pH of the longissimus dorsi muscle at the fifth rib was measured immediately after slaughter using a Mettler MP120 pH meter. The probe was inserted 3 mm into the muscle for reading, with a second measurement taken at 24 h postmortem.

Drip loss: At 2–3 h postmortem, the longissimus dorsi muscle between the 2nd and 3rd lumbar vertebrae was excised and cut into 2 cm-thick slices along the muscle fiber direction, then trimmed into 5 cm × 3 cm strips and weighed. Each strip was suspended in a paper cup using cotton thread (without contacting the cup walls). The cup opening was sealed with plastic wrap and stored at 4°C for 24 h, after which the sample was reweighed. Drip loss (%) = [(initial weight - final weight) / initial weight] × 100.

Meat color: Using a CR-10 colorimeter (Japan) in CIE-Lab mode, the longissimus dorsi muscle was cut to expose a fresh surface, and lightness (L*), redness (a*), and yellowness (b*) values were recorded.

Statistical Analysis

Data were analyzed using one-way ANOVA with SAS 8.0 statistical software. If treatment effects were significant, Duncan's multiple range test was used for post-

hoc comparisons. Data are expressed as means ± standard error. Significance was declared at $P < 0.05$.

Results

Effects of Dietary Riboflavin on Meat Quality

As shown in Table 2 , dietary riboflavin level significantly affected muscle L* value, a* value, and drip loss ($P < 0.05$). Drip loss decreased linearly with increasing riboflavin supplementation. The 12 mg/kg group had significantly lower a* value than other groups ($P < 0.05$), while the 3 mg/kg group had significantly lower L* value than the control group ($P < 0.05$). Dietary riboflavin had no significant effect on muscle pH or b* value ($P > 0.05$).

Effects of Dietary Riboflavin on Hair Follicle Development

As shown in Table 3 , compared with the control group, the 3, 6, and 12 mg/kg groups significantly increased total hair follicle density ($P < 0.05$). The 3 and 6 mg/kg groups significantly increased primary hair follicle density ($P < 0.05$), while the 6 and 12 mg/kg groups significantly increased secondary hair follicle density ($P < 0.05$). The 3 and 6 mg/kg groups significantly decreased the ratio of secondary to primary hair follicle density ($P < 0.05$). Figure 1 [Figure 1: see original paper] shows that riboflavin supplementation at 3, 6, and 12 mg/kg visibly increased hair follicle density compared with the control group.

Effects of Dietary Riboflavin on Immune Organs

As shown in Table 4 , dietary riboflavin level significantly affected thymus weight and thymus index ($P < 0.05$), both showing a quadratic response to supplementation and reaching maximum values at 6 mg/kg, which were significantly higher than the control group ($P < 0.05$). Riboflavin supplementation had no significant effect on spleen weight or spleen index ($P > 0.05$).

Effects of Dietary Riboflavin on Serum Immune Factors

As shown in Table 5 , dietary riboflavin level significantly affected serum IgG, IgA, and IL-2 concentrations ($P < 0.05$), all showing a quadratic response and reaching maximum values at 6 mg/kg supplementation, significantly higher than the control group ($P < 0.05$). Riboflavin supplementation had no significant effect on serum IgM concentration ($P > 0.05$).

Discussion

Effects on Meat Quality

Meat quality is comprehensively evaluated based on appearance (color), palatability (tenderness, flavor), nutritional value, and various physicochemical properties. Meat color is one of the most visually important traits influencing consumer

purchasing decisions. In this study, dietary riboflavin significantly affected muscle L* and a* values. The 3 mg/kg group had significantly lower L* value than the control, while the 12 mg/kg group had significantly lower a* value, indicating that riboflavin supplementation influences muscle color. However, the 6 mg/kg group showed no significant differences in L* or a* values compared with the control. Myoglobin is the primary protein determining meat color. Riboflavin level affects cellular ATP activity; riboflavin deficiency reduces intestinal mucosal ferritin reductase activity, impairing iron absorption and metabolism and consequently affecting myoglobin synthesis [8]. Some researchers suggest riboflavin can reduce meat color by inhibiting mammalian target of rapamycin (mTOR) expression and related protein synthesis [9].

As a flavoenzyme, riboflavin participates in crucial redox reactions including oxidative decarboxylation, dehydrogenation, and hydroxylation, which are closely related to lipid, protein, and carbohydrate metabolism. Studies have shown that riboflavin level affects NADH dehydrogenase activity, thereby influencing lactate production and muscle pH [10]. However, riboflavin supplementation did not affect muscle pH in this study. Le Bihan-Duval et al. [7] found a significant negative correlation between pH and breast muscle L* value, indicating complex relationships between pH and meat color. pH influences heme reactions, water-binding capacity of muscle proteins (directly affecting physical structure and lightness), and mitochondrial enzyme activity (altering heme oxidation). Rapid pH decline results in pale color, reduced water-holding capacity, and decreased tenderness due to inhibition of proteolytic enzymes.

Approximately three-quarters of muscle is water. Post-slaughter, water is expelled from myofibrillar spaces into extracellular spaces, where some is lost as drip. This lost water contains diluted sarcoplasmic proteins, resulting in nutrient loss. High drip loss is associated with lighter color, poorer tenderness and flavor, and flavor compound loss, reducing meat yield and affecting packaging appearance. Riboflavin supplementation significantly reduced drip loss, suggesting that optimal riboflavin levels improve water-holding capacity and meat quality. The color reduction observed may not be caused by changes in drip loss; the improved water-holding capacity may relate to reduced lipid and protein oxidation. Rabbit skeletal muscle contains abundant unsaturated fatty acids highly susceptible to oxidative stress. Lipid peroxidation damages muscle cell structural integrity and causes protein oxidation, reducing water-holding capacity [11].

Effects on Hair Follicle Development

Multiple factors including nutrition, age, breed, and sex affect fur growth, with prolonged nutritional deficiency causing slow growth and poor pelt quality. Riboflavin deficiency reduces hair quantity [12]. Since hair follicle density determines fur density in Rex rabbits, studying hair follicles has important theoretical and practical significance. This study found that 3, 6, and 12 mg/kg riboflavin supplementation increased primary, secondary, and total hair follicle densities,

demonstrating significant promotion of hair follicle development, particularly in the 6 mg/kg group where all parameters reached significance. Primary follicles differentiate and develop earlier, producing guard hairs, while secondary follicles differentiate later, producing down hairs. The reduced ratio of secondary to primary follicle density suggests that riboflavin accelerates follicle differentiation, increasing the proportion of guard hairs. Riboflavin not only nourishes hair follicles but also affects hormone synthesis that determines follicle development. B vitamins promote melatonin production, which plays a decisive role in hair follicle development in livestock [13].

Effects on Immune Performance

The thymus connects the immune-endocrine system with the nervous system and is a vital immune organ. Hematopoietic stem cells transported via blood circulation proliferate and differentiate into lymphocytes in the thymic cortex; only a small portion enters the medulla to develop into nearly mature T lymphocytes. The spleen, containing abundant lymphocytes and macrophages, is the largest immune organ and the site of B and T lymphocyte proliferation, differentiation, and immune response. It produces specific antibodies and plays anti-tumor roles, closely linking cellular and humoral immunity. This study showed that dietary riboflavin significantly affected thymus weight and index, consistent with findings in broilers and laying ducks [14,15], indicating that riboflavin promotes thymus development and immune function in growing Rex rabbits, with 6 mg/kg being the optimal level.

Immunoglobulins are globulins with antibody activity and important immune effectors that can be converted to antibodies upon antigen stimulation. IgG binds pathogens to neutralize infectivity, reduces toxins, and promotes phagocytosis. Serum IgA mediates opsonization and antibody-dependent cell-mediated cytotoxicity. IgM is the first antibody secreted and plays a crucial role in early immune response. Although present in small amounts, IgM has high binding valency and exceptional efficiency. IL-2, produced by activated CD4+ and CD8+ T cells upon antigen stimulation, promotes B cell growth and differentiation, regulates immune responses, and participates in antibody reactions, hematopoiesis, and tumor surveillance. Riboflavin supplementation promoted IgA and IgM secretion, indicating enhanced lymphocyte cytokine production that aligns with thymus development promotion, further confirming riboflavin' s immune-enhancing effects. The 6 mg/kg group showed significantly increased serum IgA, IgM, and IL-2 concentrations, suggesting this level is optimal.

In conclusion, dietary riboflavin supplementation promotes hair follicle development, improves meat quality, and enhances immune performance in growing Rex rabbits. Based on comprehensive evaluation, the optimal dietary riboflavin supplementation level for 3- to 5-month-old Rex rabbits is 6 mg/kg (analyzed dietary riboflavin level: 8.35 mg/kg).

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