# Effects of Dietary Inosine Monophosphate Supplementation in Breeder Hens on Hatching Egg Quality, Growth Performance, and Serum Biochemical Parameters of Broiler Offspring from 1 to 21 Days of Age (Postprint)

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## Abstract

This experiment aimed to investigate the effects of supplementing different levels of inosine monophosphate (IMP) in hen diets on egg quality, growth performance of 1-21 day-old offspring broilers, and serum biochemical indices. A total of 480 healthy 20-week-old Arbor Acres broiler breeder hens were randomly assigned to a control group (basal diet), Group I (basal diet + 0.2% IMP), Group II (basal diet + 0.5% IMP), and Group III (basal diet + 1.0% IMP). A total of 480 newly hatched offspring broilers were randomly selected and correspondingly divided into control, Group I, Group II, and Group III based on the IMP supplementation level in their maternal diets, with 6 replicates per group and 20 birds per replicate. The experimental period lasted 21 days. The results showed that compared with the control group, there were no significant differences in egg quality indices of hens at the end of 32 weeks, growth performance indices of 1-21 day-old offspring broilers, or serum protein metabolism and enzyme activity-related indices in any of the treatment groups ($P>0.05$). Compared with the control group, serum high-density lipoprotein cholesterol (HLD-C) content in 21-day-old offspring broilers in Group III was significantly increased ($P<0.05$), while serum total cholesterol (T-CHO), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) contents showed no significant differences among all treatment groups ($P>0.05$). Compared with the control group, serum triiodothyronine (T3) content in 21-day-old offspring broilers in Groups II and III was significantly increased ($P<0.05$), serum thyroxine (T4) and insulin-like growth factor-1 (IGF-1) contents in Group II were significantly increased ($P<0.05$), while serum growth hormone (GH) content

showed no significant differences among all treatment groups (P>0.05). Compared with the control group, serum immunoglobulin M (IgM), interleukin-2 (IL-2), and interleukin-6 (IL-6) contents in 21-day-old offspring broilers in Group III showed an upward trend (P>0.05). These results indicate that dietary IMP supplementation in hens enhanced lipid metabolism in offspring broilers and increased serum levels of growth- and immune-related hormones and immune factors; comprehensively considering egg quality, growth performance, and serum biochemical indices, supplementation of 0.5%-1.0% IMP in hen diets demonstrated optimal effects.

## Full Text

# Effects of Dietary Inosine Monophosphate Acid of Hens on Hatching Egg Quality, Growth Performance and Serum Biochemical Indexes of Offspring Broilers Aged from 1 to 21 Days

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### Abstract

This experiment was conducted to investigate the effects of dietary supplementation with different levels of inosine monophosphate acid (IMP) in hen diets on hatching egg quality, growth performance, and serum biochemical indexes of offspring broilers aged from 1 to 21 days. Four hundred and eighty 20-week-old healthy Arbor Acres (AA) broiler breeders were randomly allocated into four groups: a control group (basal diet), Group I (basal diet + 0.2% IMP), Group II (basal diet + 0.5% IMP), and Group III (basal diet + 1.0% IMP). After hatching, 480 offspring broilers were randomly selected and correspondingly divided into control, Group I, Group II, and Group III based on the IMP supplementation levels in their maternal diets, with 6 replicates per group and 20 broilers per replicate. The experimental period lasted for 21 days. The results showed that compared with the control group, there were no significant differences in hatching egg quality indexes of hens at 32 weeks of age, growth performance indexes, or serum protein metabolism and enzyme activity-related indexes of offspring broilers aged 1-21 days across all experimental groups (P>0.05). Compared with the control group, the serum high-density lipoprotein cholesterol (HDL-C) content in 21-day-old offspring broilers from Group III was significantly increased (P<0.05), while serum total cholesterol (T-CHO), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) contents showed no sig-

nificant differences among all experimental groups (P>0.05). Compared with the control group, serum triiodothyronine (T3) content in 21-day-old offspring broilers from Groups II and III was significantly elevated (P<0.05), and serum thyroxine (T4) and insulin-like growth factor-1 (IGF-1) contents in Group II were significantly increased (P<0.05), whereas serum growth hormone (GH) content showed no significant differences among all experimental groups (P>0.05). Compared with the control group, serum immunoglobulin M (IgM), interleukin-2 (IL-2), and interleukin-6 (IL-6) contents in 21-day-old offspring broilers from Group III exhibited an increasing trend (P>0.05). These results suggest that dietary IMP supplementation in hens enhances lipid metabolism in offspring broilers and elevates serum levels of growth- and immune-related hormones and immune factors. Based on comprehensive evaluation of hatching egg quality, growth performance, and serum biochemical indexes, dietary supplementation with 0.5% to 1.0% IMP in hen diets yielded the optimal effects.

**Keywords:** inosine monophosphate acid; maternal effect; growth performance; hatching egg quality; serum biochemical indexes

## Introduction

Maternal effects primarily refer to the regulation of offspring nutrient metabolism by maternal nutrition levels in livestock and poultry. For poultry, growth and development during early life stages are entirely dependent on nutrients deposited in the egg. Maternal nutrition levels can regulate the nutrient content in eggs, thereby influencing the levels of growth regulatory factors in the blood and tissues of offspring, ultimately affecting nutrient utilization efficiency and exerting long-term impacts on offspring growth and development. Consequently, modulating maternal exogenous additive levels to improve offspring growth performance is considered a safe and effective approach.

Inosine monophosphate acid (IMP), a type of nucleotide, is widely involved in life processes including cell structure, energy supply, and metabolism. Its umami-enhancing capacity is 40 times stronger than that of monosodium glutamate, and IMP content is internationally recognized as an important indicator for measuring meat flavor. While IMP has been widely applied as a flavor enhancer, research on its use as a feed additive remains limited. Zhang et al. reported that dietary supplementation with exogenous IMP could improve broiler growth performance, increase endogenous IMP deposition, and enhance meat quality. Wang et al. demonstrated that dietary mononucleotide supplementation could increase IMP content in broiler muscle and improve meat quality. Our research team previously found that dietary IMP supplementation significantly affected broiler growth performance, protein and lipid metabolism levels, and related enzyme activities. However, no studies have been reported on the effects of exogenous IMP supplementation levels in hen diets on offspring broilers.

Therefore, this experiment aimed to investigate the effects of dietary supplementation with different IMP levels on hatching egg quality, offspring broiler growth performance, and serum biochemical indexes, while providing a theoretical basis for further exploring maternal effects between maternal nutrition and offspring.

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## Materials and Methods

### 1.1 Experimental Materials

The 5' -inosine monophosphate disodium used in this experiment, with a purity of 97.0% or higher, was purchased from CJ CheilJedang Bio.

### 1.2 Experimental Animals and Design

Four hundred and eighty 20-week-old healthy Arbor Acres (AA) broiler breeders were randomly divided into four groups: a control group (basal diet), Group I (basal diet + 0.2% IMP), Group II (basal diet + 0.5% IMP), and Group III (basal diet + 1.0% IMP). Artificial insemination was performed when the hens reached 50% egg production rate. When the egg production rate reached 80% (at 32 weeks of age), 2,000 hatching eggs were collected and incubated. After hatching, 480 offspring broilers were randomly selected and correspondingly divided into control, Group I, Group II, and Group III based on the IMP supplementation levels in their maternal diets, with 6 replicates per group and 20 broilers per replicate. The experimental period lasted for 21 days. The basal diet was a corn-soybean meal type diet, and its composition and nutrient levels are shown in Table 1 .

### 1.3 Measurement Indicators

**1.3.1 Hatching Egg Quality** At the end of 32 weeks of age, 10 hatching eggs were randomly selected from each replicate to determine egg quality indexes. Eggshell strength, eggshell thickness, albumen height, Haugh unit, and yolk color were measured using a series of egg quality analyzers produced by ORKA, Israel. Egg shape index was measured using an egg shape index analyzer produced by Fujitsubo, Japan.

**1.3.2 Growth Performance** The body weight of offspring broilers was measured at 1 day of age, and fasting body weight was measured at 21 days of age by replicate. Feed intake and residual feed were accurately recorded to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G) for each group.

**1.3.3 Serum Biochemical Indexes** At 21 days of age, 2 healthy broilers were randomly selected from each replicate, and blood was collected from the

jugular vein to separate serum. Serum total protein (TP), albumin (ALB), total cholesterol (T-CHO), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) contents, as well as alkaline phosphatase (ALP) and aspartate aminotransferase (AST) activities, were determined using a FULLY automatic biochemical analyzer (Italy). The assay kits were purchased from Zhongsheng Beikong Biotechnology Co., Ltd. Serum immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), interleukin-2 (IL-2), interleukin-6 (IL-6), interleukin-12 (IL-12), triiodothyronine (T3), thyroxine (T4), growth hormone (GH), and insulin-like growth factor-1 (IGF-1) contents were determined by enzyme-linked immunosorbent assay (ELISA). The assay kits were purchased from Bethyl Company, USA.

### 1.4 Statistical Analysis

Data were analyzed using one-way ANOVA with SPSS 20.0 software. Results were expressed as "mean ± standard error." Duncan' s multiple comparison test was used to compare means among groups, with $P<0.05$ as the criterion for significant difference.

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## Results

### 2.1 Effects of Dietary IMP of Hens on Growth Performance of Offspring Broilers

As shown in Table 2 , compared with the control group, there were no significant differences in average daily gain, average daily feed intake, or feed-to-gain ratio of offspring broilers aged 1-21 days across all experimental groups ($P>0.05$).

### 2.2 Effects of Dietary IMP of Hens on Hatching Egg Quality

As shown in Table 3 , compared with the control group, there were no significant differences in average egg weight, egg shape index, eggshell strength, eggshell percentage, albumen height, yolk color, Haugh unit, yolk percentage, or eggshell thickness of hatching eggs from 32-week-old hens across all experimental groups ($P>0.05$).

### 2.3 Effects of Dietary IMP of Hens on Serum Protein Metabolism and Enzyme Activity of Offspring Broilers

As shown in Table 4 , compared with the control group, there were no significant differences in serum TP and ALB contents or AST and ALP activities in 21-day-old offspring broilers across all experimental groups ($P>0.05$).

### 2.4 Effects of Dietary IMP of Hens on Serum Lipid Metabolism of Offspring Broilers

As shown in Table 5 , compared with the control group, serum HDL-C content in 21-day-old offspring broilers from Group III was significantly increased ($P<0.05$), while no significant differences were observed among other groups ($P>0.05$). Serum T-CHO, TG, and LDL-C contents showed no significant differences compared with the control group across all experimental groups ($P>0.05$).

### 2.5 Effects of Dietary IMP of Hens on Serum Hormone Contents of Offspring Broilers

As shown in Table 6 , compared with the control group, serum T3 content in 21-day-old offspring broilers from Groups II and III was significantly increased ($P<0.05$), and serum T4 and IGF-1 contents in Group II were significantly increased ($P<0.05$). No significant differences were observed among other groups ($P>0.05$). Serum GH content showed no significant differences compared with the control group across all experimental groups ($P>0.05$).

### 2.6 Effects of Dietary IMP of Hens on Serum Immune Indexes of Offspring Broilers

As shown in Table 7 , compared with the control group, serum IgM, IL-2, and IL-6 contents in 21-day-old offspring broilers from Group III showed an increasing trend, though the differences were not significant ($P>0.05$).

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## Discussion

### 3.1 Effects of Dietary IMP of Hens on Growth Performance of Offspring Broilers

Inosine monophosphate, as a dietary nucleotide, is closely related to animal growth, immunity, and intestinal health. It is the main product in the de novo synthesis of nucleotides, and supplementation with exogenous nucleotides may be beneficial during rapid growth and stress conditions, as it is immediately used as a precursor to synthesize other functional nucleotides upon generation. Exogenous IMP can alleviate the pressure of de novo nucleotide synthesis and increase feed intake by stimulating taste receptors. Research on IMP application in broiler diets is limited. Our previous study found that dietary supplementation with exogenous IMP could reduce the feed-to-gain ratio in broilers during the early growth stage. However, this experiment found that dietary supplementation with 0.2%, 0.5%, and 1.0% IMP in hen diets had no significant effect on the growth performance of offspring broilers aged 1-21 days. This may be because the IMP supplementation level in hen diets failed to trigger maternal effects, thus exerting no regulatory effect on offspring broiler growth. The underlying mechanism requires further investigation.

### 3.2 Effects of Dietary IMP of Hens on Hatching Egg Quality

During early life stages, avian growth and development are entirely dependent on nutrients in the egg. Maternal nutrition levels can affect the nutrient content of hatching eggs, thereby influencing the physiological status of chicks during hatching. During eggshell formation, calcium carbonate crystals interact with eggshell matrix proteins to form eggshells with specific thickness and strength. Eggshell strength and percentage are primarily influenced by genetic factors and calcium-phosphorus metabolism, with calcium metabolism regulation in laying hens being crucial for high-quality eggshell formation. Albumen height and Haugh unit are indicators of egg freshness, with higher Haugh units indicating better albumen viscosity. The results of this experiment showed that dietary IMP supplementation in hens had no significant effect on hatching egg quality, possibly because the IMP supplementation level did not affect calcium, phosphorus, and protein absorption and utilization. However, comprehensive analysis of offspring broiler growth performance and blood biochemical indexes suggests that dietary IMP supplementation in hens promoted offspring broiler growth and development, possibly due to IMP deposition in hatching eggs. Further research is needed to measure this deposition.

### 3.3 Effects of Dietary IMP of Hens on Serum Protein Metabolism and Enzyme Activity of Offspring Broilers

Total protein performs important functions including nutrient transport, osmotic pressure and acid-base balance maintenance, and humoral immunity participation. Albumin content is an important indicator reflecting protein synthesis capacity and liver function. Our previous study found that exogenous IMP could increase serum TP and globulin contents in broilers. However, this experiment found that dietary IMP supplementation in hens had no significant effect on serum TP and ALB contents in offspring broilers. Alkaline phosphatase is closely related to poultry bone metabolism, while serum AST activity increases significantly only when liver cells are severely damaged, causing mitochondrial membrane injury. The results of this experiment showed that although serum ALP and AST activities in offspring broilers showed some variation, all values remained within normal ranges with no significant differences among groups, indicating that dietary IMP supplementation in hens had no negative effects on offspring broiler liver function and protein metabolism was normal.

### 3.4 Effects of Dietary IMP of Hens on Serum Lipid Metabolism and Hormone Contents of Offspring Broilers

High-density lipoprotein cholesterol is a serum protein rich in phospholipids that transports cholesterol from peripheral tissues to be converted into bile acids or directly excreted through bile from the intestine. In contrast, low-density lipoprotein cholesterol transports cholesterol to cells throughout the body and to the liver for bile acid synthesis. Triglyceride is a component of blood lipids, and its content reflects lipid absorption, metabolism, and utilization, with lower

values indicating higher fat utilization efficiency. Our previous study found that dietary IMP supplementation in broilers could increase serum HDL-C content and reduce TG content. This experiment similarly found that compared with the control group, dietary supplementation with 1.0% IMP in hens increased serum HDL-C content in 21-day-old offspring broilers, though it had no significant effect on TG and T-CHO contents, indicating that 1.0% IMP supplementation enhanced lipid absorption and utilization. Thyroxine (T4) and triiodothyronine (T3) primarily regulate material and energy metabolism, with T3 promoting energy metabolism and T4 stimulating thyroid synthesis and secretion of T3. The results showed that dietary supplementation with 0.5% and 1.0% IMP in hens increased serum T3 and T4 contents in offspring broilers. We speculate that maternal IMP may promote energy metabolism in offspring broilers. Growth hormone promotes metabolism and protein synthesis, affecting animal growth performance, while IGF-1 secretion is closely related to body growth. The results showed that dietary supplementation with 0.5% IMP in hens significantly increased serum IGF-1 content in offspring broilers but had no significant effect on serum GH content, suggesting that IMP supplementation has certain growth-promoting effects. However, this was inconsistent with the growth performance results in this experiment, possibly because the elevated hormone levels had not yet produced effects on 1-21-day-old offspring broiler growth. Whether it affects growth performance during later stages requires further investigation.

### 3.5 Effects of Dietary IMP of Hens on Serum Immune Indexes of Offspring Broilers

Exogenous nucleotides can promote lymphocyte maturation, activation, and proliferation, and alleviate immunosuppression caused by cortisol-induced reduction in lymphocyte numbers and phagocytic capacity under stress conditions. Immunoglobulin M is the first antibody secreted during humoral immunity. It not only interacts with foreign antigens but also reacts with self-antigens such as nucleic acids, heat shock proteins, carbohydrates, and phospholipids, and is therefore considered to play an important role in autoimmune responses. The results showed that dietary supplementation with 1.0% IMP in hens increased serum IgM content in offspring broilers but had no significant effect on IgA and IgG contents. Interleukins are important information molecules in the immune system and play crucial roles in immune regulation. Interleukin-2 is a polypeptide immune regulatory factor produced by activated T lymphocytes that promotes T and B lymphocyte proliferation and differentiation and specific antibody production, ultimately promoting effective immune responses mediated by IgA and increasing secretory immunoglobulin A. Interleukin-6, as an inflammatory factor, primarily enhances anti-infection effects by promoting inflammatory response. The results showed that dietary supplementation with 1.0% IMP in hens increased serum IL-2 and IL-6 contents in offspring broilers. We speculate that maternal IMP may promote lymphocytes in offspring broilers, thereby increasing related immune indexes in serum.

## Conclusion

Based on the experimental results, the following conclusions can be drawn: (1) Dietary IMP supplementation in hens had no significant effects on hatching egg quality or growth performance of offspring broilers aged 1-21 days. (2) Dietary supplementation with 1.0% IMP in hens significantly increased serum HDL-C content in 21-day-old offspring broilers. (3) Dietary supplementation with 0.5% IMP in hens significantly increased serum T3, T4, and IGF-1 contents in 21-day-old offspring broilers, while 1.0% IMP supplementation increased serum IgM, IL-2, and IL-6 contents. (4) Based on comprehensive evaluation of all indexes, dietary supplementation with 0.5% to 1.0% IMP in hen diets produced optimal effects.

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