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Effect of Dietary Nucleotide Supplementation on the Immune Response of White Leghorn Layers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Immune competence, as indicated by the levels of antibody titres, represents a vital component in the production of layer poultry. It contributes significantly to enhancing disease resistance, thereby reducing production losses associated with infections and promoting overall performance in poultry

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farming. An experiment was conducted to evaluate the effect of dietary nucleotide supplementation on the immune response of White Leghorn layers, with a specific focus on antibody titre against sheep red blood cells (sRBC) over a 40-week period. A total of 160 birds, aged 15 weeks, were randomly assigned to four dietary treatments (T1: basal diet for layer chicken phase I (BD), T2: BD + 0.5 g nucleotide/kg, T3: BD + 0.75 g nucleotide/kg, T4: BD + 1.0 g nucleotide/kg) in a completely randomized design, with five replicates per treatment and eight birds per replicate. Following a two-week adaptation period (15–16 weeks) for nucleotide supplementation, the birds were fed experimental layer diets from 17 to 40 weeks of age. The humoral immune response to sRBCs was assessed using the haemagglutination (HA) test. Blood was collected at 15 weeks of age from six birds in each treatment group to quantify the pre-inoculation titre value. A primary and booster dose of 25% sheep RBC suspension was administered at 17 weeks of age, followed by additional blood collections at three-week intervals from 18 to 40 weeks of age to estimate the humoral immune response. The results showed no significant differences in antibody titres during the early weeks (15–27 weeks of age). However, significant improvements in antibody titres were observed in T2, T3, and T4 groups at 36 and 40 weeks, with these groups exhibiting significantly higher titres ($p<0.05$) compared to the control group (T1). Dietary supplementation with nucleotides at 0.5, 0.75, and 1.0 g/kg of diet enhanced humoral immunity, as indicated by elevated antibody responses in later weeks. These findings underscore the importance of dietary nucleotide supplementation, to strengthen the immune system in layer birds. Enhanced antibody titres, particularly in later production phases, contribute to improved disease resistance and sustained productivity, emphasizing the value of nutritional interventions in optimizing health and performance in commercial layer production.

Keywords: Nucleotide; white leghorn layers; sheep RBC; immune response.

1. INTRODUCTION

The poultry industry is a cornerstone of India's agribusiness sector, representing one of the fastest-growing livestock industries globally [1]. With India ranking as the second-largest producer of eggs and a significant player in global meat production, the sector contributes substantially to the nation's economy [2]. The per capita availability of eggs in 2023-24 is 103 per annum [1], remaining below the Indian Council of Medical Research (ICMR) recommendation of 180 eggs annually, highlighting a significant gap in meeting domestic nutritional needs. This gap in consumption underscores the potential for further growth in production to meet both domestic demand and export opportunities.

White Leghorn layers, renowned for their prolific egg-laying capacity and efficient feed utilization, is key to India's egg production status. Optimizing their productivity requires a multifaceted approach, integrating genetics, nutrition, biosecurity and environmental management. Nutritional interventions, in particular, play a critical role in improving both the quantity and quality of egg production. Given their high metabolic demands and susceptibility to stress during peak production periods, White Leghorn layers are especially poised to benefit from targeted dietary strategies like nucleotide supplementation.

Dietary nucleotide supplementation has emerged as a promising nutritional tool in poultry production. Nucleotides, the building blocks of DNA and RNA, are vital for cellular energy metabolism, protein synthesis and immune function [3]. Although the body can synthesize nucleotides, their demand increases during stress, rapid growth or illness [4]. Supplementation, often derived from yeast extracts, has been shown to support gut health, enhance immune responses and improve overall performance, addressing challenges such as disease outbreaks and declining productivity [5].

Current challenges in poultry immune health, including the prevalence of infectious diseases and the overuse of antibiotics, highlight the importance of immune-supportive strategies. Studies have demonstrated that nucleotide supplementation benefits poultry by promoting intestinal health, optimizing nutrient absorption, and bolstering immune defenses. These effects lead to improved survivability, egg production and economic returns.

This study examines the impact of dietary nucleotide supplementation on the immune response of White Leghorn layers, with a focus

on its role in enhancing antibody production and disease resistance. By evaluating key immune parameters and their relationship to egg production, the findings aim to underscore the potential of nucleotide supplementation as a sustainable strategy for improving the health, productive performance and economic viability of layer birds.

2. MATERIALS AND METHODS

An experiment was conducted to study the impact of dietary nucleotide supplementation on humoral immune response of White Leghorn layers at Avian Research Station, Thiruvazhamkunnu, Palakkad District, Kerala Veterinary and Animal Sciences University, Kerala.

2.1 Experimental Design

The experiment was conducted over 24 weeks, from May to October 2023. A total of 160 White Leghorn layer birds were randomly selected at 15 weeks of age and assigned to four treatment groups using a completely randomized design. Each treatment group included five replicates with eight birds per replicate. Following a two-week adaptation period (from 15 to 16 weeks) for nucleotide supplementation, the birds were fed with experimental layer diets from 17 to 40 weeks of age. The four dietary treatments are detailed in Table 1.

2.2 Housing and Management

All birds were kept in individual layer cages under standard management practices, including a 16-hour photoperiod maintained throughout the experimental period, with *ad libitum* access to feed and water. Birds were vaccinated with inactivated ND vaccine at16 weeks of age through the intramuscular route and all biosecurity measures were implemented to maintain uniform health conditions throughout the experimental period. The data was collected over 24 weeks from 17 to 40 weeks of age.

2.3 Experimental Diet

Feed ingredients and additives available in the feed mill of Avian Research Station, Thiruvazhamkunnu were utilized for the formulation of experimental diets. Commercial nucleotide supplement (NucleoproC- 20 per cent nucleotide) extracted from yeast *Saccharomyces cerevisiae* bought from Exotic Bio-solutions Pvt. Limited, Maharashtra was utilized for the study. It was stored in an air-tight container as per the precautions advised by the manufacturer.

The basal diet was formulated to meet nutrient requirements for layer chicken phase I specified by [6]. The ingredient composition of the basal diet is provided in Table 2. The feed ingredients were tested for proximate analysis as per [7] guidelines and the results are presented in Table 3. A weighed quantity of experimental diets was provided throughout the research period to ensure *ad libitum* feeding at all times.

2.4 Humoral Response to Sheep RBC

2.4.1 Preparation of sheep RBC (sRBC) antigen

Sheep blood was collected from a sheep farm located in Saravanmpatti, Coimbatore district, Tamil Nadu. The blood, collected in Alsever's solution (1:1 *v/v*, prepared and stored at 4°C), was centrifuged at 3,000 rpm for 10 minutes to sediment the RBCs. The RBCs were washed three times with PBS (phosphate-buffered saline, pH 7.2) by adding an equal volume of PBS and centrifuging at 3,000 rpmfor 10 minutes to remove other serum components. The supernatant was carefully collected without mixing with other serum components. After the final wash, the packed cells were prepared into a 25 per cent *v/v* solution by adding 25 mL of packed sheep RBCs to 75 mL of PBS.

Table 1. Treatment groups and their respective diets

Treatment groups	Experimental diet	No. of replicates/ treatment	No. of birds in each replicate
T_1	Basal diet (Control)	5	8
T_2	Basal diet supplemented with 0.5 g nucleotide per kg of diet	5	8
T_3	Basal diet supplemented with 0.75 g nucleotide per kg of diet	5	8
T_4	Basal diet supplemented with 1.0 g nucleotide per kg of diet	5	8

Table 2. Ingredient composition of basal diet, (%)

S.No.	Ingredients	Percentage (%)
1	Yellow maize	52.15
2	De-oiled rice bran	15.10
3	Soybean meal	21.70
4	Calcite	3.90
5	Shell grit	5.00
6	Di calcium phosphate	1.30
7	DL-Methionine	0.20
8	L-Lysine	0.20
9	Sodium bicarbonate	0.15
10	Salt	0.30
Total		**100.00**
Feed supplements (g/100 kg)		
9	Vitamin premix	100
10	Toxin binder	100
11	Liver tonic	30
12	Choline chloride	200
13	Trace mineral mix	150
14	Enzyme	50

Table 3. Chemical composition of the basal diet (Dry matter basis)

Sl. No.	Nutrient	Percentage (%)
1	Dry matter	89.98
2	Crude protein	19.06
3	Ether extract	2.67
4	Crude fiber	2.54
5	Total ash	14.63
6	Acid insoluble ash	0.82
7	NFE	60.28
8	Calcium	3.27
9	Total phosphorus	0.68
10	ME (kcal/kg)	2603.41
11	Lysine	0.75
12	Methionine	0.40

2.4.2 Administration of sRBC antigen

At 17 weeks of age, six birds from each treatment group were selected randomly and 1 mL of 25 per cent sheep RBC suspension was injected intramuscularly into the thigh muscle [8]. A booster dose of 1 mL of 25 per cent sheep RBC antigen was administered one week after the primary dose [9].

2.4.3 Harvesting of immune sera from sRBCsensitised birds

For the HA test, 2 mL of blood was collected in a sterilised serum vial from six birds in each treatment group at 15 weeks of age to quantify the pre-inoculation titre value. Subsequently, the blood collection was performed at three-week intervals from 18 to 40 weeks of age to estimate the humoral immune response. Sera were collected by centrifuging the tube at 3,000 rpm for 5 minutes and stored at -20^{o}C.

2.4.4 Estimation of antibody titre against sRBCs

The antibody titre in the serum of individual birds was determined by the HA test using a one per cent sRBC suspension prepared by mixing 1 mL of packed sRBCs and 99 mL of PBS. The test was performed in round bottom (U-shaped) microtitre plates. In each well of the titre plate, 25 µL of PBS was added, followed by 25 µL of serum in the first well of each row. Two-fold serial dilutions were performed up to the penultimate well and the final 25 µL of serum was discarded. An equal volume (25 µL) of one per cent sRBC suspension was added to all wells

and thoroughly mixed with the serum samples. The plates were then incubated at 37°C for 20-30 minutes. The highest dilution that exhibited complete agglutination (button-shaped clumping of RBCs, indicating a haemagglutination reaction) was recorded as the titre and expressed as $\log_2$ (n).

2.5 Statistical Analysis

Data were analyzed statistically using SPSS version 24.0. An Analysis of Variance (ANOVA) was conducted to determine the significance of differences among the treatment groups. The level of significance was set at $p<0.05$ and the results were presented as mean ± standard error.

3. RESULTS

The mean antibody titre against sRBC of White Leghorn layers in different dietary treatment groups from 15th to 40th weeks of age is presented in Table 4 and Fig. 1.

The mean antibody titre against sRBC in T1, T2, T3 and T4 groups at the 15th week of age was 1.67, 1.50, 1.50 and 1.17 $\log_2$, respectively without showing any significant variation among the treatment groups. The mean antibody titre against sRBC in T1, T2, T3 and T4 groups at 18 and 21 weeks of age was 6.67, 6.17, 6.67 and 6.00 $\log_2$, and 6.83, 6.33, 5.00 and 7.33 $\log_2$, respectively and the statistical analysis revealed no significant difference among the treatment groups. The mean antibody titre against sRBC in T1, T2, T3 and T4 groups at 24 and 27 weeks of age was 5.17, 6.50, 5.17 and 6.00 $\log_2$, and 5.33, 5.33, 4.83 and 5.33 $\log_2$, respectively, without any significant variation among the treatment groups.

The mean antibody titre against sRBC in T1, T2, T3 and T4 groups at 30 and 33 weeks of age was 3.83, 4.83, 2.83 and 4.00 $\log_2$, and 3.33, 4.50, 3.83 and 4.83 $\log_2$, respectively. Statistical analysis of the data revealed no significant difference in the mean antibody titre against sRBC among the treatment groups. The mean antibody titre against sRBC in T1, T2, T3 and T4 groups at the 36 weeks of age was 2.00, 4.33, 4.33 and 4.83 $\log_2$, respectively. The statistical analysis revealed that the antibody titre in T2, T3 and T4 was significantly higher ($p<0.05$) than in T1, with no significant differences among T2, T3 and T4 groups.

The mean antibody titre against sRBC in T1, T2, T3 and T4 groups at the 40 weeks of age was 1.83, 4.83, 4.67 and 4.83 $\log_2$, respectively. The statistical analysis revealed that the antibody titre in T2, T3 and T4 was significantly higher ($p<0.05$) than in T1, with no significant differences among T2, T3 and T4 groups.

Table 4. Mean (±SE) antibody titre against sheep RBC of White Leghorn layers in different dietary treatment groups, $\log_2$

Age (weeks)	T_1 (Control)	T_2 (0.5 g/kg nucleotide)	T_3 (0.75 g/kg nucleotide)	T_4 (1 g/kg nucleotide)	F - value	p-value
15	1.67 ± 0.42	1.50 ± 0.22	1.50 ± 0.34	1.17 ± 0.16	0.47	0.70 ns
18	6.67 ± 0.49	6.17 ± 0.98	6.67 ± 0.33	6.00 ± 1.03	0.20	0.90 ns
21	6.83 ± 0.87	6.33 ± 1.08	5.00 ± 0.51	7.33 ± 0.55	1.60	0.22 ns
24	5.17 ± 0.90	6.50 ± 0.84	5.17 ± 0.40	6.00 ± 0.25	0.98	0.42 ns
27	5.33 ± 0.88	5.33 ± 0.76	4.83 ± 0.30	5.33 ± 0.42	0.15	0.93 ns
30	3.83 ± 1.01	4.83 ± 0.87	2.83 ± 0.30	4.00 ± 0.68	1.15	0.35 ns
33	3.33 ± 0.42	4.50 ± 0.84	3.83 ± 0.47	4.83 ± 0.40	1.41	0.27 ns
36	2.00 b ± 0.36	4.33 a ± 0.80	4.33 a ± 0.49	4.83 a ± 0.47	5.18	0.01**
40	1.83 b ± 0.40	4.83 a ± 0.70	4.67 a ± 0.33	4.83 a ± 0.40	9.37	0.001**

Mean values bearing different superscripts within a row differ significantly ($p<0.05$)
*ns-non significant; **highly significant*

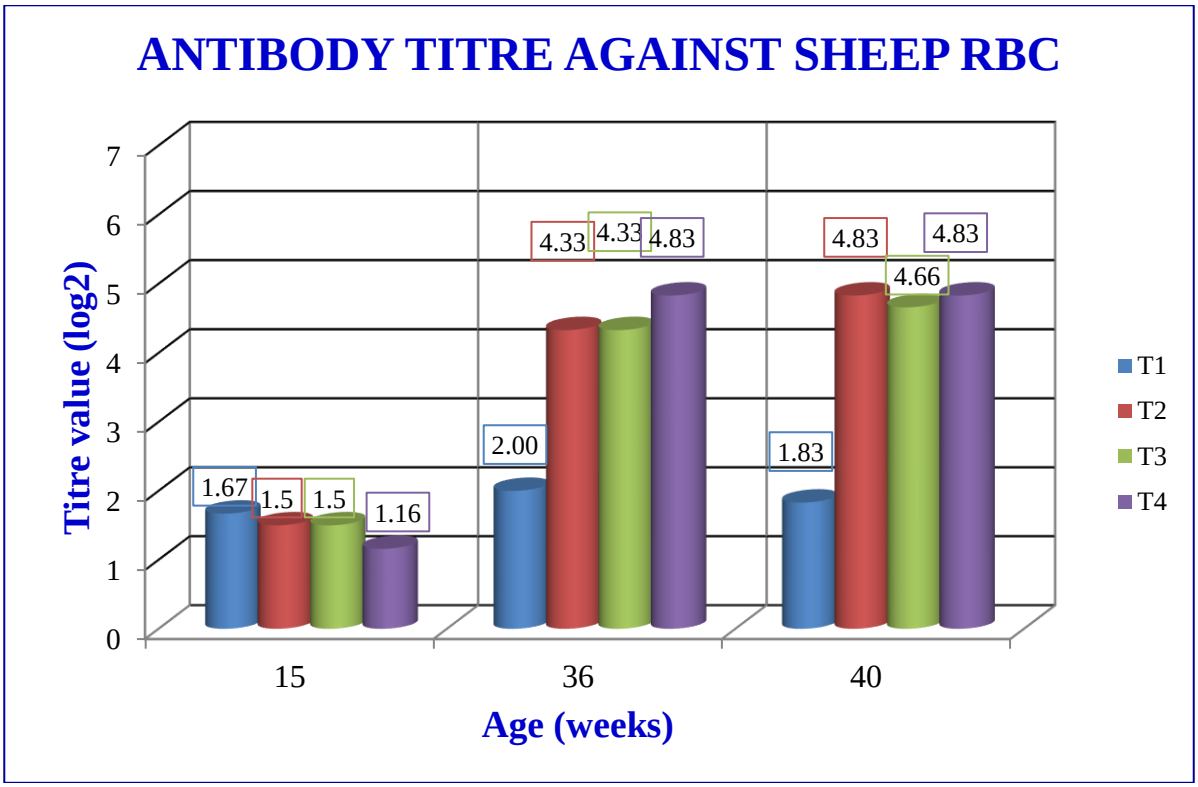

Fig. 1. Antibody titre against sheep RBC in different dietary treatment groups

4. DISCUSSION

The observed immune benefits align with existing research underscoring the role of nucleotide supplementation in enhancing immune responses in poultry. For instance, increased IgM titers were noted in birds fed yeast cell extract post-sRBC inoculation [10]. Likewise, improved antibody production was found in hens fed yeast [11,12] and [13] Yalcin et al. [12], Further studies highlighted that nucleotide-supplemented diets elevated IgA levels, which support humoral immunity by creating a protective barrier on the intestinal mucosal layer [14] and [15]. This protective role is crucial, as IgA activity in the gut contributes to intestinal mucosal immunity, a key defense against pathogens. Similarly, [16,17] and [18], Rady et al. [17].

A possible reason for the improved disease resistance in poultry could be the enhanced production of immunoglobulin A (IgA) associated with dietary nucleotide supplementation. Nucleotides play a crucial role in the proliferation and differentiation of B cells into plasma cells, which produce IgA. This immunoglobulin forms a protective layer on the intestinal mucosal membranes, safeguarding against pathogens [19], Sauer et al. [18], and [20], Jyonouchi [19]. Research found that nucleosides increase cytokine production, aiding B cell differentiation and enhancing IgA secretion [21] Zhou et al. [20]. The resulting increase in IgA production likely strengthens mucosal immunity, thereby contributing to greater disease resistance in poultry and promoting overall health and growth in production settings.

The delayed immune response improvements observed after 36 weeks may be due to the time required for dietary nucleotides to elicit measurable effects on immune function, including B cell proliferation and immunoglobulin production. Sustained supplementation likely enhances gut health and nutrient absorption over time, improving intestinal integrity and villi development, which supports humoral immunity. Additionally, physiological maturity during peak production periods may heighten metabolic and immune responsiveness to dietary interventions, aligning with the increased nucleotide demand under stress or heightened physiological activity.

However, some studies have shown no significant effects of nucleotide supplementation on immune response. For example, [22] and [23] reported no significant differences in HA-HI titer responses or antibody titre against Newcastle

virus, respectively. Similarly, [24] found that total sRBC-specific antibody levels were unaffected by yeast RNA supplementation.

Variations in results may be attributed to differences in experimental conditions, including the type, purity, and dosage of nucleotide sources used. Yeast cell extracts, autolyzed yeast or purified nucleotides may differ in their bioavailability and efficacy. Differences in bird health status and baseline immune competence could also influence outcomes. Birds with higher baseline immunity or less exposure to environmental stressors may exhibit less pronounced responses to supplementation. Furthermore, variations in study design, such as the timing and frequency of blood sampling or differences in antigen inoculation protocols, could impact the sensitivity of immune response measurements. Another consideration is the duration of supplementation. Studies reporting no significant effects might not have allowed sufficient time for the cumulative benefits of nucleotide supplementation to manifest. Additionally, environmental factors, such as housing conditions, biosecurity measures and the presence of subclinical infections, could interact with dietary interventions, masking or amplifying observed effects.

5. CONCLUSION

In conclusion, this study highlights the significant role of dietary nucleotide supplementation at 0.5, 0.75 and 1 g/kg levels in enhancing the immune response of birds, particularly in promoting long-term antibody production and boosting humoral immunity. The elevated antibody titres observed in nucleotide-supplemented groups, especially in later weeks, align with previous research demonstrating the immune-enhancing potential of nucleotides in avian species. Although some studies report limited effects, the overall evidence supports the positive influence of nucleotides on immune function, notably through increased IgA secretion. These findings emphasize the dual benefits of nucleotide supplementation in improving immune function and overall performance in poultry production, with potential economic implications such as reduced disease management costs, lower reliance on antibiotics and improved egg production efficiency. Future research could explore the impact of nucleotide supplementation on other performance metrics, including egg quality and feed conversion efficiency or investigate its efficacy under different environmental conditions and stress levels to further optimize its application in commercial poultry farming.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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