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Impact of dietary nucleotide supplementation on growth performance and survivability of white leghorn layers

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Abstract
An experiment was conducted at the Avian Research Station, Thiruvazhamkunnu, Kerala, to evaluate the impact of dietary nucleotide supplementation on growth performance and survivability of White Leghorn layers. A total of 160 birds, aged 15 weeks, were randomly assigned to four dietary treatments (T_1: basal diet, T_2: basal diet + 0.5 g nucleotide/kg, T_3: basal diet + 0.75 g nucleotide/kg, T_4: basal diet + 1.0 g nucleotide/kg) in a completely randomised design, with five replicates per treatment and 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 data for the experiment was collected over 24 weeks from 17 to 40 weeks of age, divided into six 28-day periods. Body weight at the 17thand 40thweeks of age and survivability across six experimental periods were recorded. In the 17th week, the treatment groups had no significant differences in body weight indicating the uniformity in the selection of birds for the experiment. However, in the 40th week, body weights were significantly higher ($p<0.01$) in T_3 and T_4 compared to T_1 and T_2. Similarly, weight gain between the 17thand 40th weeks was significantly higher ($p<0.01$) in T_3 (356.63 g) and T_4 (363.94 g) than in T_1 (242.30 g) and T_2 (258.96 g). Survivability varied across the six periods. The overall survivability was highest in T_2 (90.20%) and T_4 (90.20%), followed by T_1 (85.20%) and T_3 (85.20%). The results suggest that dietary nucleotide supplementation at 0.75 g/kg (T_3) and 1.0 g/kg (T_4) significantly improved growth performance, with marginal impacts on overall survivability. This study highlights the potential benefits of nucleotide supplementation in enhancing the growth performance of White Leghorn layers under standard management conditions.

Keywords: Nucleotide, white leghorn layers, body weight, survivability

1. Introduction

The poultry industry in India, a rapidly expanding sector of agribusiness, holds significant economic and nutritional importance. Among the various segments of poultry farming, egg production plays a central role, with White Leghorn layers being the cornerstone of this thriving industry. Renowned for their exceptional egg-laying capabilities and efficient feed conversion, White Leghorns contribute substantially to India's egg output, meeting the growing demand for protein-rich diets domestically and globally. However, achieving optimal productivity and sustainability requires a comprehensive understanding of factors that influence the health, body weight and survival of these prolific layers.

Dietary strategies, including targeted nutritional supplementation, have emerged as pivotal tools for enhancing the performance of layer birds. Among these strategies, nucleotide supplementation has garnered considerable interest due to its multifaceted benefits. Nucleotides are the building blocks of DNA and RNA, crucial for various biological processes. Composed of a sugar (ribose or deoxyribose), a nitrogenous base (purine or pyrimidine) and phosphate groups, they are vital for cellular energy metabolism through molecules like ATP. Nucleotides also act as coenzymes and regulators in metabolic pathways, aiding in the synthesis of carbohydrates, proteins, and lipids. They are essential for protein synthesis, lipid metabolism, and immune function. While nucleotides can be synthesized endogenously, their demand increases during rapid growth, stress or disease, making dietary supplementation beneficial for gut health and overall performance.

Supplementing White Leghorn diets with nucleotides has been shown to positively influence body weight and livability. By optimizing nutrient absorption, boosting immune resilience, and fostering a balanced intestinal microbiome, nucleotides not only enhance growth but also strengthen the birds' ability to withstand stressors such as disease, environmental challenges and management-related pressures. Improved body weight directly correlates with better productivity, while enhanced livability ensures the economic viability of poultry operations by reducing mortality losses.

This study aims to investigate the effects of dietary nucleotide supplementation on the body weight and livability of White Leghorn layers, providing insights into its potential as a sustainable and cost-effective strategy. By exploring these relationships, the study contributes to advancing nutritional interventions that support improved productivity and economic outcomes for the poultry industry.

2. Materials and Methods

An experiment was conducted to study the impact of dietary nucleotide supplementation on body weight and survivability of White Leghorn layers at Avian Research Station, Thiruvazhamkunnu, 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 15weeks of age and assigned to four treatment groups using a completely randomised 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 aT_{16} weeks of age through the intramuscular route and all biosecurity measures were implemented to maintain uniform health conditions throughout the experimental period. The data for statistical analysis were collected over 24 weeks from 17 to 40 weeks of age, divided into six 28-day periods.

2.3 Experimental diet

Feed ingredients and additives available in the feed mill of Avian Research Station, Thiruvazhamkunnu were utilised for the formulation of experimental diets. Commercial nucleotide supplement (NucleoproC- 20 percent nucleotide) extracted from yeast *Saccharomyces cerevisiae* bought from Exotic Biosolutions Pvt. Limited, Maharashtra was utilised 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 BIS (2007). The ingredient composition of the basal diet is provided in Table 2. The feed ingredients were tested for proximate analysis as per AOAC (2016) 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.

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	[1]Calcite	3.90
5	[2]Shell grit	5.00
6	Di calcium phosphate	1.30
7	[3]DL-Methionine	0.20
8	[4]L-Lysine	0.20
9	Sodium bicarbonate	0.15
10	Salt	0.30
Total		**100.00**
Feed supplements(g/100 kg)		
9	[5]Vitamin premix	100
10	[6]Toxin binder	100
11	[7]Liver tonic	30
12	[8]Choline chloride	200
13	[9]Trace mineral mix	150
14	[10]Enzyme	50

Note

1. Calcite - 38 percent calcium
2. Shell grit - 38 percent calcium
3. DL-Methionine containing 99 percent methionine (Met amino-R)
4. L-Lysine (HCL): containing 98.5 percent L Lysine Hydrochloride (Meihua)
5. Vitamin premix, each kg contains - Vitamin A - 22.50 MIU, Vitamin D3 - 4.50 MIU, Vitamin E - 70.00 g, Vitamin K3- 4.50 g, Vitamin B1- 6.00 g, Vitamin B2- 20.50 g, Vitamin B6- 8.00 g, Vitamin B12- 30.00 mg, Niacin - 75.00 g, Calcium D Pantothenate - 32.50 g, Folic acid - 4.50 g, Biotin - 210.00 mg (Nicomix, Primal Pharma Ltd).
6. Toxin binder- a mixture of activated hydrated sodium calcium almunio silicates (HSCAS), activated charcoal, mannan oligosaccharides, copper oxinate, organic acids (propionic acid, benzoic acid, acetic acid, sorbic acid), lipotropic agent, spirulina (AlusilMos Plus, Stallen South Asia Pvt Ltd).
7. Liver tonic- each kg contains Sorbital 35,000 mg, Choline chloride 60,000 mg, Betaine HCL 30,000 mg, Methionine 18,250 mg, Sodium chloride 20,000 mg, Magnesium chloride 50,000 mg, Vitamin PP 2000 mg, Inositol 10,000 mg, Glucosamine 40,000 mg, Silymarine 20,000 mg, Carnitine 90,000 mg, L-Asparatic acid 10,000 mg, L-Glutamaric acid 5,000 mg. (Dutchliv Gold, Aminorich Nutrients B.V.)

8. Choline chloride 60 percent: each kg contains a minimum of 600 g of choline chloride (Anichol 60, Jubilant Ingrevia Ltd)
9. Trace mineral mix each kg contains - Manganese 100 g, Zinc 85, Iron 90 g, Copper 15 g, Iodine 1.8 g, Organic Chromium 0.15 g, Selenium 0.45 g (Zenmak Nutrigencies and Health Pvt Ltd).
10. Enzyme: Composition - a blend of *Lactobacillus acidophilus*, *Saccharomyces cerevisiae*, xylanase, amylase, glucanase, cellulase, pectinase, lipase, protease, phytase (Alvizyme Plus, Alembic).

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

*Calculated value

2.4. Observations made
The following observations were made during the experiment.

2.4.1 Body weight
Individual body weight of all birds was recorded at 17and 40 weeks of age and the gain in body weight was calculated.

2.4.2. Survivability
The health status and mortality, if any, were monitored daily throughout the experimental period. The dead birds were subjected to detailed post-mortem examination to ascertain the cause of death. Data on the mortality of birds receiving different dietary treatments throughout the experimental period (17 to 40 weeks of age) and the percentage of survivability were calculated by using the following formula.

$$\text{Survivability (\%)} = \frac{\text{Number of birds survived} \times 100}{\text{Total number of birds at the beginning}}$$

3. Results
3.1. Body weight
3.1.1. Body weight at 17th and 40th week of age
Data on the mean body weight of White Leghorn layers at the 17th and 40th week of age in the different dietary treatment groups are presented in Table 4 and Fig 1.

The mean body weight of White Leghorn layers at the 17th week of age in the T_1, T_2, T_3 and T_4 groups was 1217.75, 1179.98, 1190.15 and 1175.90 g, respectively, without any significant variation among the treatment groups.

The mean body weight of White Leghorn layers at the 40th week of age in the T_1, T_2, T_3 and T_4 groups was 1460.05, 1438.94, 1546.78 and 1539.84 g, respectively. The statistical analysis revealed that the 40th week body weight of the T_3 and T_4 groups was significantly ($p<0.01$) higher compared to the T_1 and T_2 groups.

3.1.2. Body weight gain
The data on mean body weight gain betweenthe 17thand 40th week of age in the differentdietary treatment groups are presented in Table 4 and Fig. 1. The mean body weight gain of White Leghorn layers at 40th week of age in T_1, T_2, T_3 and T_4groups was 242.30, 258.96, 356.63 and 363.94 g, respectively. The statistical analysis revealed that weight gain at the 40th week of age is significantly higher ($p<0.01$) in the T_3 and T_4 groups than in the $T_1$and T_2 treatment groups.

Table 4: Mean (±SE) body weight of White Leghorn layers at 17th and 40th week of age and final weight gain in different dietary treatment groups, g

Parameters	T_1 (Control)	T_2 (0.5g/kg nucleotide)	T_3 (0.75g/kg nucleotide)	T_4 (1g/kg nucleotide)	p-value
17th-week body weight	1217.75± 13.74	1179.98± 8.98	1190.15± 32.70	1175.90± 15.57	0.463 ^{ns}
40th-week body weight	1460.05^b ± 15.26	1438.94^b± 28.42	1546.78^a± 22.98	1539.84^a± 15.35	0.004**
Final weight gain	242.30^b± 13.59	258.96^b± 24.58	356.63^a± 35.32	363.94^a± 12.20	0.002**

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

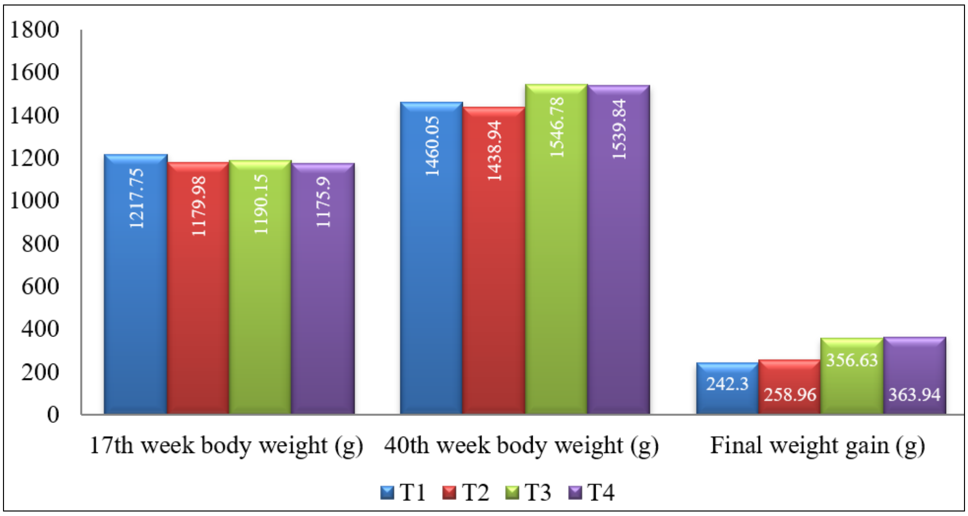

Fig 1: Body weight at the 17th and 40thweek of age and final weight gain in different dietary treatment groups

3.2. Survivability

The data on percent survivability of White Leghorn layers in different dietary treatment groups for six experimental periods (P1-P6) are presented in Table5.

During the first period (P1), 100 percent survivability was recorded in the T_1, T_3 and T_4 groups whereas T_2 recorded 97.60 percent survivability. In the second period (P2), 100 percent survivability was recorded in the T_1, T_2 and T_3 groups whereas T_4 recorded 97.60 percent survivability.

In the third period (P3), 100 percent survivability was recorded in the T_1 and T_2 groups whereas T_3 and T_4 recorded 97.60 percent survivability. During the fourth period (P4), 100 percent survivability was recorded in the T_4 group whereas the survivability of T_1, T_2 and T_3 was 85.20, 97.60 and 95.20 percent, respectively. In the fifth period (P5), 100 percent survivability was recorded in the T_1 group whereas T_2, T_3 and T_4 recorded 95.20 percent survivability.

During the sixth period (P6), 100 percent survivability was recorded in the T_1, T_2 and T_4 groups whereas T_3 recorded 97.60 percent survivability. Overall survivability (P1-P6) of T_1, T_2, T_3 and T_4 groups were 85.20, 90.20, 85.20 and 90.20 percent, respectively.

Table 5: Mean (±SE) survivabilityof White Leghorn layers in different dietary treatment groups, percent

Period	T_1 (Control)	T_2 (0.5g/kg nucleotide)	T_3 (0.75g/kgnucleotide)	T_4 (1g/kgnucleotide)
P1	100.00±0.00	97.60±2.40	100.00±0.00	100.00±0.00
P2	100.00±0.00	100.00±0.00	100.00±0.00	97.60±2.40
P3	100.00±0.00	100.00±0.00	97.60±2.40	97.60±2.40
P4	85.20±4.71	97.60±2.40	95.20±2.94	100.00±0.00
P5	100.00±0.00	95.20±2.94	95.20±2.94	95.20±2.94
P6	100.00±0.00	100.00±0.00	97.60±2.40	100.00±0.00
Overall	85.20 ± 4.71	90.20 ± 4.65	85.20 ± 7.22	90.20 ± 4.65

4. Discussion

4.1. Body weight

The results revealed that body weight and body weight gain at the 40th week of age were significantly ($p<0.01$) higher in the T_3 and T_4 groups compared to the T_1 and T_2 groups. These findings suggest that the inclusion of nucleotides at 0.75 and 1 mg/kg levels in the diet influences body weight gain in White Leghorn layers indicating a positive influence on growth performance.

The findings of the current study were consistent with previous research emphasizing the importance of nucleotide supplementation on final body weight gain in poultry species. Khedr *et al*. (2020) [9], Mohamed *et al*. (2020) [14], Rafique *et al*. (2020) [18] and El-Rahman *et al*. (2023) [3] observed significant improvements in the final body weight of broiler birds supplemented with different levels of nucleotides. Similarly, body weight gain was significantly improved as reported by Fathi *et al*. (2012) [4], Prakash *et al*. (2013) [16], Prakash *et al*. (2016) [17] and Daneshmand *et al*. (2017) [2] in nucleotide-supplemented groups. The current study's nucleotide inclusion levels match the effective ranges cited in previous studies including Leung *et al*. (2019) [13] and Salah *et al*. (2019) [19] which showed positive impacts on body weight.

Kocher *et al*. (2010) [11] and Pelicia *et al*. (2010) [15] also demonstrated that nucleotide supplementation accelerated growth rate enabling chicks to reach market weight faster. Similarly, Kanmanee *et al*. (2022) [8] found that laying hens supplemented with red yeast from 22 to 60 weeks had significantly higher final body weights. Further, studies by Daneshmand *et al*. (2017) [2], Kreuz *et al*. (2020) [12], Tembhurne *et al*. (2020) [20] and Villavan *et al*. (2021) [23] suggested that nucleotides contributed to faster intestinal development and villi maturation enhancing nutrient absorption and promoting superior growth performance.

The observed benefits in this experiment may be because dietary nucleotides play a crucial role in supporting the growth of avian species by reducing the energy expenditure associated with de novo nucleotide synthesis which usually consumes amino acids like glutamine, aspartate and glycine. Grimble (1996) [5] reported that de novo synthesis requires about 1 percent of glutamine flux suggesting that exogenous nucleotide supply may spare glutamine for other essential physiological functions. Therefore, reducing reliance on de novo synthesis allows amino acids and the energy conserved to be redirected toward metabolic functions essential for overall growth and development (Guo *et al*., 2017; Jung and Batal, 2012) [6, 7].

However, not all studies report significant effects of nucleotides on weight gain. Trairatapiwan *et al*. (2017) [22] found no notable impact on broiler weight gain and Kidd *et al*. (2013) [10] observed no improvement in weight gain among yeast-extract-supplemented broiler breeder hens. Similarly, Yalcin *et al*. (2014) [24] reported no significant differences in Hy-Line Brown laying hens fed with yeast cell walls. These discrepancies may result from differing experimental conditions, bird age and breed, dietary compositions, growth stages or nucleotide dosages.

The collective evidence suggests that nucleotide supplementation at the levels of 0.75 and 1 g/kg in the diet is beneficial for optimizing avian growth by improving nutrient absorption, ultimately enhancing growth performance. The current study's results are consistent with much of the existing literature supporting nucleotides as effective growth promoters.

4.2. Survivability

The study found no significant differences in the survivability of layer birds across the treatment groups, with all groups maintaining high survivability rates throughout the experimental period. This indicates that nucleotide supplementation does not adversely affect the survival of layer birds. High survivability rates across all groups suggest that nucleotide is well tolerated and does not pose any risk to the overall health and survival of the birds. These results are consistent with previous findings by Wang *et al*. (2009) who noticed insignificant differences in the mortality of birds fed with nucleotides in different age groups. Similarly, Pelicia *et al*. (2010) [15], Kidd *et al*. (2013) [10], Alizadeh *et al*. (2016) [1] and Khedr *et al*. (2020) [9] also reported no significant effect on mortality with various levels of nucleotide supplementation. Thanissery *et al*. (2010) [21] also observed no significant impact on mortality rates in broiler birds supplemented with nucleotide.

In conclusion, the study demonstrates that nucleotide supplementation does not negatively impact the survivability of layer birds, as all treatment groups maintained high survival rates throughout the experimental period. These findings indicate that nucleotides are well tolerated by the birds and do not pose any health risks related to the mortality of the birds.

5. Conclusion

This study demonstrated that dietary supplementation with nucleotides positively influenced the growth performance of White Leghorn layers, particularly when included at levels of 0.75 g/kg (T_3) and 1.0 g/kg (T_4) of feed. Birds in these groups exhibited significantly higher body weights and weight gain at the 40th week of age than those fed the basal diet (T_1) or basal diet with 0.5 g/kg nucleotide supplementation (T_2). The overall survivability percentages were slightly higher in T_2 and T_4 groups than in T_1 and T_3, the differences were not consistently significant across all experimental periods. The findings suggest that incorporating nucleotides at 0.75-1.0 g/kg in the diet of White Leghorn layers is a practical and effective strategy to improve productivity in commercial layer operations under standard management conditions.

6. Conflict of Interest

Not available.

7. Financial Support

Not available.

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