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Unleashing the Potential of Nucleotide Supplementation on Broiler Health and Performance

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ABSTRACT

The potential benefits of nucleotide supplementation may include an increase in the length of intestinal villi, promotion of nutrient absorption, enhanced weight gain (Yu, 1998), and the rapid turnover of intestinal cells (enterocytes) following damage caused by stress or pathogens. 120-day-old broiler chicks were randomly allocated to 4 experimental groups, each consisting of six replicates of five chicks. The standard broiler diets (Con) were formulated. The ConA: Con+ antibiotic growth promoter. In the N5 and N10 treatments, the antibiotic was replaced with nucleotide @ 5 and 10 mg/L in the drinking water, respectively. The individual body weight and feed intake of the broilers were recorded weekly, and subsequently, the feed-to-gain ratio and production efficiency factor were determined. A metabolic trial was conducted at the end of the experiment to evaluate nutrient utilization. At the end of the trial (42ndday), 02 birds from each replicate were sacrificed to assess carcass traits, nutrient composition of breast muscle, and intestinal morphology. The comprehensive performance evaluation of broilers over the six-week period indicated a significant enhancement ($p<0.05$) in overall performance with the N5 broiler diet. The results further demonstrated a noteworthy improvement ($p<0.05$) in protein digestibility among broilers fed nucleotide supplementation, particularly in the N5 group, signifying a substantial increase in protein utilization. Notably, nucleotide supplementation did not exert any discernible effect on the nutrient composition of the meat. Consequently, based on the findings of this study, it can be inferred that the supplementation of nucleotides at a concentration of 5 mg/L significantly ($p<0.05$) contributed to improved growth performance, enhanced intestinal morphology, and increased intestinal capacity for nutrient absorption in broilers and could be regarded as a viable alternative to antibiotic growth promoters.

KEYWORDS: Antibiotic growth promoter, Broiler, Histo-morphometerical analysis, Nucleotide, Nutrient utilization.

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INTRODUCTION

Nucleotides play essential roles in both physiological and biochemical functions, participating in the encoding and decoding of genetic information, regulating energy metabolism, facilitating cell signaling, and serving as crucial components of coenzymes, allosteric effectors, and cellular agonists in terrestrial animals. These low molecular weight biological molecules are particularly vital as constituents of nucleic acids—Deoxyribonucleic acid (DNA) and Ribonucleic acid (RNA).

Comprising three major components—a sugar, a nitrogen base, and one or more phosphate groups—nucleotides undergo cleavage of the phosphate group by alkaline phosphatase and nucleosidases in the small intestine, resulting in the formation of nucleosides (Carver and Walker, 1995). Remarkably, over 90% of dietary, endogenous, purine, and pyrimidine nucleotides are absorbed into the enterocytes, where they undergo rapid degradation into uric acid and allantoin.

Unlike mammals, poultry can synthesize nucleotides *de novo*; however, it is now believed that the capacity for nucleotide production, particularly in young animals, may not suffice to meet their needs. For this reason, nucleotides are considered "semi" or "conditionally" essential nutrients in animals, especially during periods of stress, rapid growth, health challenges, high stocking

densities, and the replacement or removal of antibiotics (Themburne et al., 2020). In response to the need to mitigate losses in poultry production and the search for natural alternatives to antibiotic growth promoters (AGPs), nucleotides have emerged as promising candidate. Given these considerations, the present experiment was conducted to investigate the effects of nucleotide supplementation on the growth performance and intestinal morphology of broilers.

MATERIALS AND METHODS

Study was approved by Institutional Animal Ethical Committee (IAEC) vide D.No.23/IAEC/Vety/2022 dated 20.05.2022 which was affiliated from CPCSEA, Ministry of Animal Husbandry, India. The experimental design involved the random allocation of one hundred and twenty day-old broiler chicks into four experimental groups, each consisting of 6 replicates with 5 chicks per replicate. Standard isonitrogenous and isocaloric broiler diets (Con) were formulated according to commercial chick feed specifications (2011) for three growth stages: Pre-starter (0-14 days, 22.5% CP and 3000 kcal ME/kg diet), Starter (15-28 days, 21.0% CP and 3125 kcal ME/kg diet), and Finisher (29-42 days, 19.50% CP and 3250 kcal ME/kg diet). The dietary treatment ConA was identical to Con but included an antibiotic growth promoter. In groups N5 and N10, the antibiotic was substituted with nucleotide at concentrations of 5 and 10 mg/L in the drinking water, respectively.

Growth Parameters:

Weekly measurement of bodyweights and feed consumptions was recorded. Accordingly, Feed to gain ratio (F:G) was calculated. Similalry, Production efficiency factor (PEF) as calculated by following formula [Pelicia et al., 2010].

$$PEF = \frac{daily\ weight\ gain\ (kg) \times livability\ (\%)}{Feed\ to\ gain\ ratio\ (FCR)} \times 100$$

Nutrient utilization or nutrient digestibility

To know the utilization of nutrients (DM, CP and EE) from different diets metabolic trial was conducted on all the experimental birds at 6th week of the experiment. During collection period quantity of feed offered and leftover were taken daily. The excreta of each replicate were collected quantitatively at every 24 hours period for 3 days.

Nutrient composition of meat

At the conclusion of the 42-day trial, birds were sacrificed to assess carcass traits. Simultaneously, samples from the breast muscle (two from each replicate) were obtained for analysis of proximate composition, including dry matter, crude protein, and ether extract. These analyses were conducted using AOAC (2012).

Carcass traits

To study the carcass traits, birds were slaughtered and after complete bleeding, weight was recorded. The weight was again recorded after manual defeathering using hot water (50-55°C). The dressed weight was then recorded as follows:

$$Dressed\ wt = Live\ wt - Wt\ loss\ as\ blood, head, feather, shank\ and\ wing\ tips$$

$$Eviscerated\ weight = Dressed\ weight - weight\ of\ viscera$$

$$Drawn\ wt = Eviscerated\ weight + weight\ of\ giblet$$

Various processing losses (% of live weight) such as blood, head, feathers, shank, separable fat and wing tips were also recorded.

The organs (liver, heart, gizzard, spleen and pancreas) and weight of lymphoid organs (bursa of fabricius, spleen and thymus), collected replicate wise at the time of slaughter and their weights (% of dressed weight) were recorded.

Examination of intestinal morphology structure

Duodenum and ileo-jejunum samples were obtained for histo-morphometric analysis. Tissue samples measuring 3-4 mm were collected from the duodenum and ileo-jejunum, fixed in 10% formalin, and processed using the paraffin embedding method. Sections, approximately 4-5 µm thick, were cut and stained with haematoxylin and eosin to reveal the general histoarchitecture. Out of 12 samples of

duodenum and ileo-jejunum from each treatment, total of six intact and well-oriented villi were selected, resulting in 15 measurements for each sample and 60 measurements per replicate. The evaluated gastrointestinal morphological variables included intestinal wall thickness, villus height, crypt depth, the ratio of villus height to crypt depth, and goblet cells. Measurements were taken from each bird and then averaged to derive a mean value for each variable per treatment (n = 6).

Villus height was measured from the top of the villus to the top of the lamina propria, while crypt depth was measured from the base upward to the transition region between the crypt and the villus. Neutral goblet cells were identified through H and E staining. This detailed histo-morphometric analysis aimed to provide a comprehensive understanding of the structural aspects of the duodenum and ileo-jejunum in response to the experimental treatments.

Statistical Analysis

The statistical analysis of the data was performed using analysis of variance (ANOVA) with a Completely Randomized Design (CRD), which was conducted using SPSS version 20.0.

RESULTS AND DISCUSSION

Performance of broilers

The results of the current study clearly reveal a distinct trend of increased live weight and weight gain in broilers supplemented with nucleotides. Notably, the group receiving nucleotide at a concentration of 5 mg/L in drinking water (N5) exhibited the highest and significantly greater live weight and weight gain compared to other groups ($p<0.05$). This observation underscores the positive impact of nucleotide supplementation on the overall growth and weight gain of broilers, suggesting its potential as an effective nutritional intervention in poultry production.

Moreover, minimal feed consumption was observed in broilers fed the N5 diet with nucleotides at 5 mg/L, while diets ConA, N5, and N10 were statistically similar to each other but lower than those fed the Con diet.

Table 1. Performance of broilers in different treatment groups

	Treatments					
Weekly	Con	ConA	N5	N10	SEM	*p-value*
Performance						
Live weight	2672.70^{c}	2738.66^{b}	2839.80^{a}	2727.13^{b}	14.34	0.00
Average weight gain	2616.22^{c}	2682.50^{b}	2783.67^{a}	2670.73^{b}	14.36	0.00
Average FI§	4476.26^{a}	4291.74^{b}	4291.57^{b}	4325.20^{b}	23.85	0.01
Average F:G§§	1.71^{a}	1.60^{b}	1.54^{c}	1.62^{b}	0.01	0.00
PEF§§§	270.80^{b}	316.00^{a}	339.00^{a}	307.50^{ab}	8.03	0.01
Nutrient Utilization (%)						
DM	70.05	70.47	72.90	71.43	2.01	0.96
CP	71.58^{b}	72.58^{ab}	75.10^{a}	73.09^{ab}	0.49	0.06
EE	82.63	82.62	83.37	82.71	1.37	0.99
Nutrient Composition of breast muscle (%)						
DM	24.51	24.55	24.97	24.02	0.47	0.94
CP	19.11	19.13	20.95	19.26	1.19	0.95
EE	5.72	5.48	5.77	5.43	0.23	0.95
Lymphoid organs weight (% of live weight)						
Spleen	0.11^{b}	0.12^{ab}	0.14^{a}	0.12^{ab}	0.00	0.03
Thymus	0.60^{c}	0.63^{bc}	0.84^{a}	0.78^{ab}	0.03	0.02
Bursa of fabricius	0.23	0.21	0.25	0.20	0.00	0.76
Economics of broiler production						
Feed cost Rs/kg	44.02	44.06	44.17	44.32	0.00	-
Feed cost Rs/kg body weight gain	75.30^{a}	70.48^{b}	69.40^{b}	74.54^{a}	0.63	0.00

Means of row with different superscript showing significant difference ($p<0.05$)

At the conclusion of the experiment, a more favorable feed-to-gain ratio was observed in broilers fed the N5 diet, followed by N10 diet which was statistically similar to ConA (Table 01). The production efficiency factor was lower in broilers fed the basal diet (ConA) and highest in those fed the N5 diet containing nucleotides at 5 mg/L. Although the groups ConA, N5, and N10 were statistically similar, they were numerically lower than those fed the N5 diet. Overall, these findings suggest that nucleotide supplementation, particularly at a concentration of 5 mg/L, positively influences weight gain, feed consumption, feed-to-gain ratio, and production efficiency factor in broilers, highlighting its potential as a beneficial nutritional component in poultry diets.

This detailed excerpt offers a comprehensive overview of various studies emphasizing the positive effects of nucleotide supplementation on broiler diets. Thembhurne et al. (2020) observed improved live weight with graded levels of nucleotide rich yeast supplementation. The stimulation of brush border enzyme activity may enhance digestion and absorption, promoting better growth (Villavan et al., 2021). Nucleotide availability may lead to the proliferation of intestinal cells, thereby improving digestion and absorption (Salah et al., 2019). Sheik et al. (2021) noted a numerical reduction in cumulative feed intake with nucleotide supplementation. Jung and Batal (2012) reported no significant difference in feed intake, suggesting variability in results due to management practices, nucleotide sources, and environmental conditions. Sampath et al. (2021) observed an improved feed-to-gain ratio with yeast hydrolysates, highlighting the potential benefits of enhanced intestinal health and increased enzyme activity. Nucleotide supplementation was associated with a numerically increased production efficiency factor (Pelicia et al., 2010; Sheik et al., 2021).

Nutrient Utilization

No notable trend was observed in dry matter and ether extract utilization with dietary nucleotide supplementation in broilers, a significant influence on crude protein utilization was apparent. Specifically, among all the diets, the highest crude protein utilization was recorded in broilers supplemented with nucleotide at a level of 5 mg/L in drinking water (N5). Conversely, the lowest crude protein utilization was noted in the group assigned to the Con diet. This indicates that nucleotide supplementation, particularly at the 5 mg/L level, markedly enhances the utilization of dietary crude protein by broilers, demonstrating its potential to positively influence nutrient utilization in poultry diets.

Improved crude protein digestibility was observed with nucleotide supplementation. Nucleotide supplementation did not significantly influence ether extract utilization (Srinivas et al., 2018; Ahiwe et al., 2020). Balanced microbial populations may play a role in altered metabolism and enhanced digestive enzyme activity.

Weight of lymphoid organs

The study results indicate an enhancement in the weight of lymphoid organs, specifically the spleen and thymus, in broilers supplemented with nucleotides at a concentration of 5 mg/L in drinking water (Table 1). However, the bursa of Fabricius did not exhibit a significant change in weight with nucleotide supplementation. This implies that nucleotide supplementation, particularly at the specified concentration, positively influences the development and weight of certain lymphoid organs in broilers. The spleen and thymus, which play vital roles in the immune system, showed increased weight, potentially indicating a beneficial impact on the immune response of broilers receiving nucleotide supplementation. The absence of a significant change in the bursa of Fabricius may suggest that the effects of nucleotide supplementation are organ-specific within the avian immune system.

Increased spleen weight was noted with yeast RNA supplementation (Deng et al., 2005; Sampath et al., 2021), suggesting potential immunomodulatory effects. Nucleotide deprivation may impact the T-cell cycle and immune responses.

Nutrient composition of meat (breast muscle)

The study data on the proximate composition of broiler meat, specifically dry matter, crude protein, and ether extract, as influenced by the supplementation of different levels of nucleotides is presented in Table 1. The results indicate that there were no significant differences ($p>0.05$) in the nutrient composition of meat among broilers fed basal diets supplemented with various levels of nucleotides compared to those assigned the basal diet alone.

Histo-morphometrical analysis of intestine

The results of the current study, presented in Table 2, indicate that broilers fed basal diets supplemented with varying levels of nucleotide did not show significant ($p>0.05$) effects on duodenum and ileo-jejunum wall thickness, crypt depth, villus height: crypt depth ratio, and goblet cells per field. However, a significant effect was observed on villus height in both the duodenum and ileo-jejunum sections of the intestine for broilers receiving nucleotide at 5mg/L in drinking water (N5).

The intestine serves as the primary site for maximum nutrient absorption in broilers. The villi of the small intestine play a crucial role in this process by projecting into the intestinal cavity, significantly increasing the surface area available for absorption and facilitating the addition of digestive secretions. It is noteworthy that villi are most abundant at the beginning of the small intestine and gradually decrease in number toward the end of the tract.

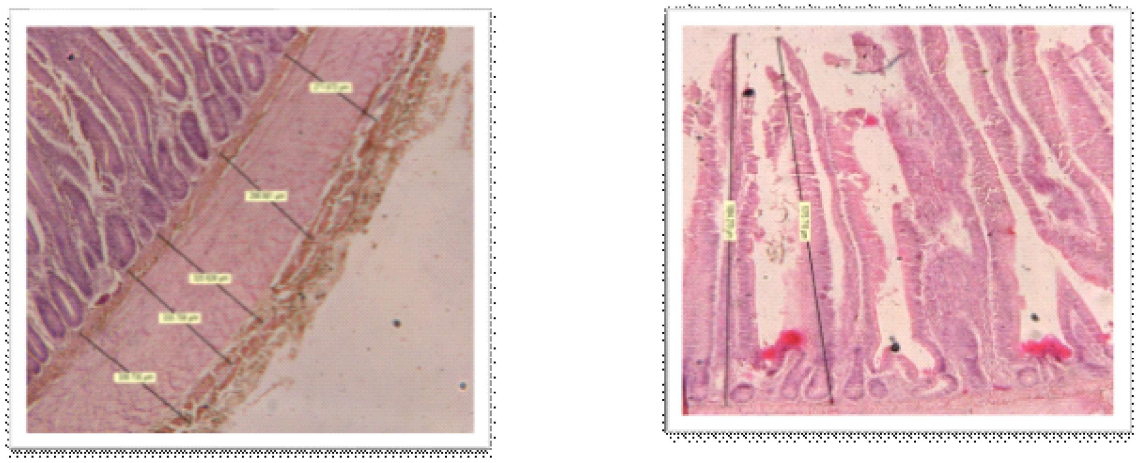
The significant effect on villus height in both the duodenum and ileo-jejunum sections suggests that nucleotide supplementation, particularly at the concentration of 5mg/L, positively influences the structural aspects of the small intestine in broilers. This may enhance nutrient absorption efficiency, highlighting the potential benefits of nucleotide supplementation in poultry diets.

Improved villus height was recorded with nucleotide supplementation, aiding in enhanced nutrient absorption (Daneshmand et al., 2017; Lin et al., 2022). *Saccharomyces cerevisiae* supplementation also improved villus height and the villus height to crypt depth ratio (Lin et al., 2022).

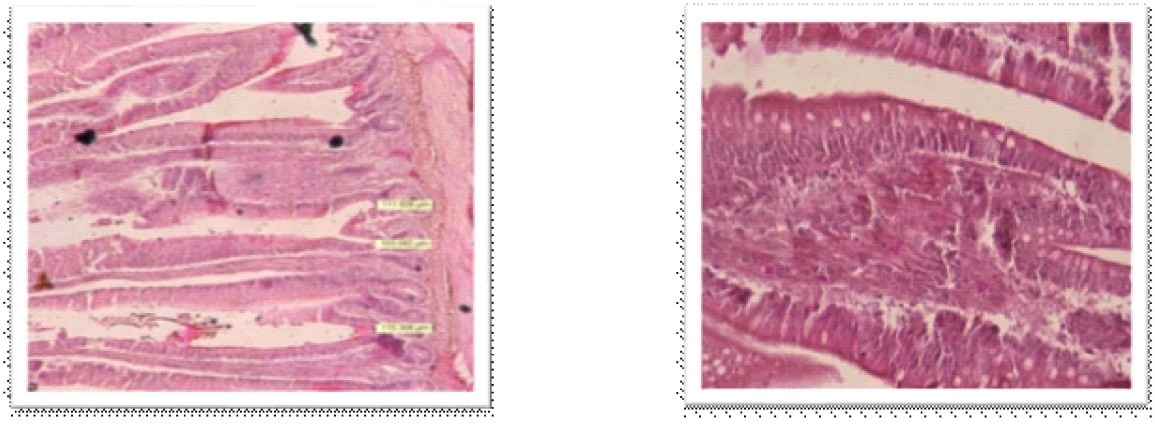
Table 2. Microscopic histological parameters in different segment of small intestine in different treatment groups

Treatment	Con	ConA	N5	N10	SEM	*p-value*
Intestinal wall thickness (µm)						
Duodenum	355.93	354.93	351.48	352.27	11.04	0.99
Ileo-jejunum	330.23	328.92	325.22	325.99	7.20	0.99
Villous height (µm)						
Duodenum	1434.92^{b}	1439.32^{b}	1545.92^{a}	1448.31^{b}	19.10	0.05
Ileo-jejunum	1122.93^{b}	1131.44^{b}	1217.27^{a}	1163.96^{ab}	14.70	0.04
Cyrpt depth (µm)						
Duodenum	115.09	115.38	117.13	116.14	5.18	0.99
Ileo-jejunum	109.92	109.54	110.53	111.36	5.39	1.00
Villous height/Cyrpt depth ratio						
Duodenum	12.82	12.66	13.33	12.65	0.51	0.97
Ileo-jejunum	10.30	10.39	11.39	10.73	0.55	0.92
Goblet cells per field						
Duodenum	9.51	9.62	9.96	9.67	0.21	0.92
Ileo-jejunum	9.50	9.53	9.69	9.62	0.15	0.98

Means of row with different superscript showing significant difference ($p<0.05$)



A B

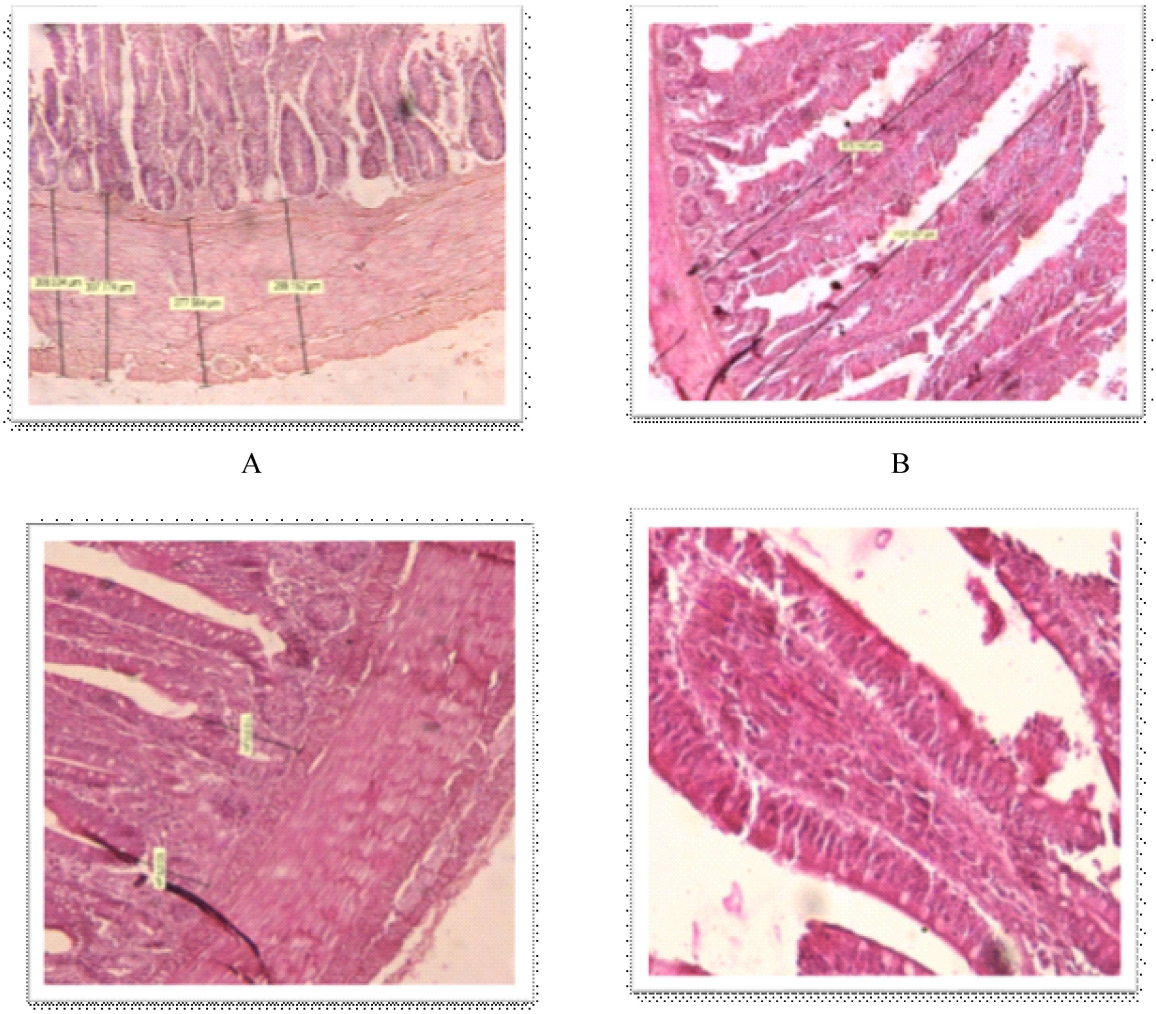


C D

Fig.1. Photomicrograph of duodenum

(A) Intestinal wall thickness, H&E×500 (B) Villus height, H&E×500

(C) Crypt depth, H&E×500 (D) Goblet cells per field, H&E×200



A B

C D

Fig.2. Photomicrograph of ileo-jejunum showing

(A) Intestinal wall thickness, H&E×500 (B) Villus height, H&E×500

(C) Crypt depth, H&E×500, (D) Goblet cells per field, H&E×200

The results presented in Table 3 concerning carcass yield, expressed as a percentage of live body weight, indicate that the eviscerated weight of broilers was significantly affected when fed diets supplemented with nucleotides. However, no significant differences were observed in dressing yield and drawn weight among the various treatment groups. Specifically, the eviscerated weight, expressed as a percentage of live weight, was significantly higher in broilers receiving nucleotide at a concentration of 5mg/L in drinking water. Broilers fed diets ConA and N10 were statistically comparable to those on the N5 diet. These findings suggest that nucleotide supplementation, particularly at the 5mg/L level, significantly impacts the eviscerated weight of broilers, thereby influencing the overall carcass yield in terms of live weight. The absence of significant differences in dressing yield and drawn weight indicates that these specific parameters were not notably affected by the nucleotide supplementation at the studied concentrations.

Table 3.Carcass yields (% of live weight) and serum biochemical parameters of broilers in different treatment groups

	Treatments					
Parameters	Con	ConA	N5	N10	SEM	*p-value*
Carcass weights (% of live weight)						
Dressed weight	77.89	80.21	80.48	79.35	0.48	0.23
Eviscerated weight	67.99^{b}	70.06^{ab}	71.05^{a}	69.26^{ab}	0.49	0.13
Drawn weight	76.55	75.99	77.52	75.60	0.43	0.48
Organ weights (% of dressed weight)						
Heart	0.62	0.57	0.65	0.62	0.02	0.54
Liver	2.00	2.26	2.31	2.28	0.16	0.92
Gizzard	2.31	2.05	2.35	2.34	0.08	0.61
Pancreas	0.22	0.23	0.25	0.24	0.01	0.57
Giblet	7.88	7.39	8.03	7.99	0.23	0.79
Processing losses (% of live weight)						
Blood	2.90	2.77	2.28	2.19	0.14	0.20
Feather	4.18	3.50	4.58	5.26	0.30	0.23
Head	2.14	2.09	2.32	2.10	0.04	0.26
Appendages	4.38	3.94	3.72	3.84	0.21	0.77
Separated fat	1.40	0.90	1.12	1.01	0.09	0.24
Serum biochemical parameters						
Total protein (g/dl)	2.69^{c}	2.72^{bc}	3.06^{a}	2.84^{b}	0.03	0.00
Albumin (g/dl)	1.26^{c}	1.27^{bc}	1.43^{a}	1.33^{b}	0.01	0.00
Globulin(g/dl)	1.43^{c}	1.45^{bc}	1.63^{a}	1.51^{b}	0.01	0.00
Total triglycerides (mg/dl)	49.51	48.95	46.97	48.53	0.43	0.19
Total cholesterol (mg/dl)	158.06^{a}	155.64^{a}	117.64^{b}	126.62^{b}	5.00	0.00
HDL (mg/dl)	88.84^{b}	94.17^{ab}	99.20^{a}	94.53^{ab}	1.26	0.02
LDL (mg/dl)	51.28^{a}	50.62^{a}	45.42^{b}	49.42^{a}	0.61	0.00
ALT/SGPT (U/L)	19.46	19.45	17.96	18.60	0.39	0.49
AST/SGOT (U/L)	167.43	165.02	163.17	162.95	1.18	0.52

Means of row with different superscript showing significant difference ($p<0.05$)

Organs weights

The findings of the current study indicate that birds supplemented with varying levels of nucleotide did not exhibit any significant effects compared to those fed the basal diet. This suggests that the supplementation of nucleotide at different levels did not lead to statistically significant differences in the measured parameters within the study, as detailed in Table 3.

The relative weights of organs, including the liver, gizzard, and abdominal fat, did not exhibit significant changes with nucleotide supplementation (Sampath et al., 2021; Sheik et al., 2021; Ciza et al., 2019; Pelicia et al., 2010).

Processing losses

The findings of the current study, as shown in Table 3, indicate that diets supplemented with nucleotide at 5mg/L and 10mg/L had no significant effect on processing losses. Specifically, factors such as blood, feathers, head, appendages, and separated fat, expressed as a percentage of live weight, did not show statistically significant differences among the various groups. This suggests that the supplementation of nucleotide at these specific concentrations did not lead to notable variations in the measured processing losses compared to the control or other treatment groups in the study.

Blood biochemical indices

The results presented in Table 3 indicate that dietary supplementation of nucleotides, particularly in the N5 group, has a significant effect on certain serum parameters. Specifically, compared to the control group (Con), the N5 group showed a significant decrease in serum concentrations of total cholesterol and LDL cholesterol. Additionally, serum concentrations of total proteins, albumin, and globulin significantly increased in the N5 group ($p<0.05$).

However, no significant effect was observed in total triglyceride levels, nor in the values of SGOT/AST (aspartate aminotransferase) and SGPT/ALT (alanine aminotransferase) across the dietary supplementation of nucleotides among the different groups.

These findings suggest that dietary supplementation with nucleotides, particularly at the specified concentration, may have a favorable impact on lipid profiles by decreasing total cholesterol and LDL cholesterol. Additionally, the increase in total proteins, albumin, and globulin indicates potential positive effects on the overall protein profile in the N5 group. The lack of significant effects on triglyceride levels and liver enzymes (SGOT/AST and SGPT/ALT) suggests that nucleotide supplementation may not have a notable impact on these parameters in the context of this study.

Nucleotide supplementation influenced HDL cholesterol levels (Daneshmand et al., 2017). Monosodium glutamate supplementation significantly impacted total protein, albumin, globulin, total cholesterol, HDL cholesterol, and LDL cholesterol levels (Ciza et al., 2019). Proximate analysis of breast meat did not reveal significant effects from nucleotide supplementation (Majdeddin et al., 2018; ChioFalo et al., 2011). Studies comparing nucleotide supplementation with other additives (e.g., dry yeast) demonstrated significant improvements (Abd El Latif, 2022; Sampath et al., 2021). Proposed mechanisms include altered metabolism, increased digestive enzyme activity, and balanced microbial populations.

CONCLUSION

The conclusion drawn from the current experiment indicates that nucleotide supplementation had a significant positive impact on various aspects of broiler performance and physiology. Nucleotide supplementation led to a notable improvement in growth performance parameters, including body weight, weight gain, feed intake, feed-to-gain ratio, and production efficiency factor. It positively influenced nutrient utilization, Immune function and carcass yield. The cumulative findings indicate that nucleotide supplementation, of 5mg/L can exert significant growth-promoting effects on commercial broilers.

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