Synergetic Action of Dietary Nucleotides and Phytobiotics as Entero-Hepatic Tonic, Immune Modulators, and Growth Promoters in Broiler Chickens

Mustafa Bastamy[1], Nasser Khedr[2], Ismail A. Raheel[3], Ahmed R. Elbestawy[4], Mervat A. Abdel-Latif[5], Ahmed Orabi[6*]

1. Department of Poultry Diseases, Faculty of Veterinary Medicine, Cairo University, Egypt.
2. Department of Nutrition and Clinical Nutrition, Faculty of Veterinary Medicine, Benha University, Egypt.
3. Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Beni-Suef University, Egypt
4. Department of Bird and Rabbit Diseases, Faculty of Veterinary Medicine, Menoufia University, Egypt
5. Department of Nutrition and Veterinary Clinical Nutrition, Faculty of Veterinary Medicine, Damanhour University, Egypt
6. Department of Microbiology, Faculty of Veterinary Medicine, Cairo University, Egypt.

* Corresponding author: Ahmed Orabi, e-mail address: ahmed_orabi@cu.edu.eg

1. Abstract

The global poultry production industry is currently facing significant challenges due to the complete or gradual removal of in-feed antibiotics. One of the most impactful diseases under these conditions is necrotic enteritis (NE), which causes considerable economic losses by compromising intestinal wall permeability and liver health in broiler chickens. The present study investigates the efficiency of dietary nucleotides and phytobiotics feed supplementation on broiler intestinal to alleviate (NE and their effects on gut, liver, growth performance, and immunity parameters. Four groups (Gs) of broiler chickens were allocated as G1; nucleotides and phytobiotics from day one till the end of the experiment with a dose of 200 g and 250 g/ton feed, respectively and non-challenged with *Clostridium perfringens* (*C. perfringens*), G2; nucleotides and phytobiotics treated as G1 and challenged with *C. perfringens*, while respectively G3 and G4 were control non-treated, challenged and non-challenged groups, The results revealed that *C. perfringens* challenge in treated chickens had little effects on liver functions, growth performance, immunity parameters, and gut integrity genes (Occuldin, JAM, and MUC) which indicated the beneficial effects of continuous feed supplementation with nucleotides and phytobiotics in broilers.

Key words: Broiler Chickens, Gut Integrity, Liver Health, *C. perfringens*.

2. Introduction

Extensive research has been directed toward identifying alternatives to antibiotics that can maintain low mortality and high productivity, while also protecting the environment and consumer health [1]. Several strategies have shown varying degrees of success in mimicking the growth-promoting effects of antibiotics. These include the use of probiotics, prebiotics, organic acids, and exogenous enzymes [2].

Little is known about dietary nucleotides and their role in performance, intestinal development, inflammation, and nitrogen nutrient utilization in

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.406300.1049

stressed birds. Nucleotides are the basic units that make up nucleic acids DNA and RNA involved in many biological and physiological processes, and also act as a source of cellular energy. They affect the immune system and involve in small intestinal development. [7]. The internal synthesis of nucleotides from amino acid precursors is not always adequate to supply requirements during growth and stress, so the dietary nucleotide supplementation of broiler chickens may be important to maintain maximum growth and performance when birds are exposed to stress conditions [3].

Liver is a major metabolic organ for the vital physiological functions, including biosynthesis, detoxification, clearance, and host defense. Recently, liver damage has become a common disorder with more risk factors such as exposure to xenobiotics, malnutrition, and chronic diseases [4]. immune suppression and environmental stress combined mainly with toxigenic *Clostridium perfringens* challenge may lead to physical and performance changes in broiler birds [5]. *Clostridium perfringens* is the causative agent of clinical necrotic enteritis (NE)which is usually a disease of broilers, but occasionally in young broiler breeders and layers kept on litter bedding. However, the subclinical form of NE induces focal intestinal mucosal ulcers and necrosis in liver [6]. Type A α-toxigenic strains of *C. perfringens* are the predominant type which isolated from chickens have NE [7]. Recently, a new toxin "*net B*", was identified in *C. perfringens* type A strains of avian origin. The importance of *net B* in avian necrotic enteritis was demonstrated in an experimental chicken model; a *net B* mutant strain failed to cause the disease, but virulence was restored when a functional *net B* gene was reintroduced into the mutant [8]. Therefore, the current study designed to study the effects of nucleotides and phytobiotics on broiler chickens that challenged with *C. perfringens* on performance, immunity and enterohepatic integrity.

3. Materials and Methods

3. 1. Ethical Statement

All experimental procedures were approved by the Animal Care Committee of Beni-Suef University, Egypt (approval number: 022-488-2).

3. 2. Experimental Design

The effect of a dietary nucleotides source, *Agaricus bisporus* plus yeast (Nucleoregen®, MN Trade Industrial Inc., 10th of Ramadan city, Egypt) which consisted from 30% nucleotides and a phytobiotics (Phytococc®, MN Trade Industrial Inc., 10th of Ramadan city, Egypt) which consisted from herbal extracts mixed with prebiotics and probiotics on performance, immunity, and gastrointestinal tract health parameters was studied. A total 120 one-day-old male Arbor Acres broiler chicks with initial weight about 40 g were randomly allocated and divided equally into 4 groups, with 3 replicates of 10 birds/ group. Feed and water were supplied *ad libitum*. Vaccination program against avian influenza subtypes H5N1 and H9N2, Newcastle disease, infectious bronchitis, and infectious bursal disease viruses was carefully applied. Group 1 (G1) was supplemented with nucleotides and phytobiotics with a dose of 200 g and 250 g/ton feed, respectively from a day-old till 35-days-old and non-challenged. G2 had been treated with nucleotides and phytobiotics as G1 and challenged orally using crop gavage with a fresh preparation of toxigenic *C. perfringens* type-A avian isolate (GenBank accession number of MW925054) on days 18, 19, 20, and 21 of age. The concentration of

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.406300.1049

daily inoculum of *C. perfringens* was 3 × 10^8 Colony Forming Unit (CFU)/ml/bird [9,10]. G3 was reared as "control negative", non-treated and non-challenged, whileG4 "control positive" was non-treated and challenged. Basal starter, grower, and finisher diets were supplied according to recommended levels of Arbor Acres.

3.3. Growth Parameters

At 15, 28, and 35 days of age, the feed intake (FI) (g/chick), average body weights, body weight gain (BWG), and feed conversion ratio (FCR) were recorded.

3.4. Gastrointestinal Tract Parameters

The length of intestine, diameter in the ileum center, and the intestinal lesion scoring (scale from 0-4) [12were measured in five birds/group at 35th day of age. Also, the enteric lactic acid bacteria was evaluated on De Man–Rogosa–Sharpe broth and *C. perfringens* was detected on tryptose sulfite-cycloserine agar supplemented by D-cycloserine and egg yolk emulsion under anaerobic incubation at 37°C [13].

3.5. Immune Parameters

Serum samples (*n*=3) from each chicken group were collected at 15, 25, and 35 days of age and screened for determining antibody titers against ND, AI-H5N1 and AI-H9N2 viruses vaccines [14] using hemagglutination inhibition (HI) test and the titre of immunoglobulin (Ig) A was monitored using chicken IgA Enzyme Linked Immuno-sorbent Assay (ELISA) kit (Bethyl Laboratories Inc., Montgomery, TX, USA) [15]. On the 35th day old, five birds/group were chosen to examine the carcass characteristics, weight of immune organs, intestinal parameters, intestinal microbiota and immune response [11].

3.6. Liver Health Parameters

Serum enzymatic activities of alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (AKP), and gamma glutamyl transferase (γ-GT) were determined in serum samples (*n*=3) collected at 15, 25, and 35 days of age using the corresponding kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) [16].

3.7. Gut Genes Expression

Intestinal samples from each group (*n*=3) were obtained at 15 and 30 days of age and examined using real time-PCR methods to determine changes in some genes expression (Table 2) in enterocytes. QuantiTect SybrGreen master mix (Qiagen, Germany) were utilized. Their cycling circumstances were carried out at 95°C/5min, 40 cycles of 95°C/10s, 60°C/30s, and 72°C/1min. RT-qPCR results were then analyzed using comparative cycle threshold (CT) [17].

3.8. Statistical Analysis

Statistical analysis was performed using SPSS version 20 by applying Analysis of Variance (ANOVA). Significant differences between groups were determined using Tukey's post hoc test at a significance level of $p \leq 0.05$. RT-qPCR data were analysed using GraphPad Prism version 5.

4. Results

The effects of dietary Nucleoregen® and Phytococc® supplementation on growth and performances are recorded in table (3). The results revealed a potent significant impact on BWG in G1 and G2, (333 and 329 g/day) at 15-days-old and (995 and 984 g/day) at 25-days and (1848 and 1813

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.406300.1049

g/day) at 35-days-old compared to G3, while G4 recorded a significant decrease in BWG (303, 806, and 1214 g/d) at 15, 25, and 35 days, of age respectively ($p \leq 0.05$). on the other hand, FI and FCR recorded 2056 and 2106 g, and 1.24 and 1.36 at 35-days-old in G1 and G2, respectively compared to G3 and G4 ($p \leq 0.05$) although G2 was challenged with *C. perfringens*. Table (1) shows the positive impact of Nucleoregen® and Phytococc® on the carcass quality as the dressing% was 82% in G1, 77% and 70% in G2 and G3, respectively compared to a significant loss of the carcass quality in G4 (58%).

The results of liver enzymes in (Table 4) revealed that chickens of both G1 and G2 had normal liver enzymes with a significant increase of ALT, AST, AKP, and γ-GT at 35-days-old (1.69, 22.07, 525.67, and 26.97, respectively) after challenge in comparison with high level of these enzymes at G3 (1.7, 27.4, 569 and 31.6) and G4 (1.76, 34.2, 753.3 and 36.13) at 35 days old.

Humoral and mucosal immune responses after vaccination against ND, AI-H5N1 and AI-H9N2 viruses' vaccines showed a significant higher HI titer in G1 and G2 compared to G3 and G4 especially at 25- and 35-days-old ending with 7 and 6-6.3 Log_2 HI titers for ND, AI-H5N1 and AI-H9N2 at 35-days-old. IgA titers of mucosal immunity determined by ELISA in figure (1). which revealed high titer in G2 (277 pg/ml) at 35 days of age while G1 from (254.6 pg/ml) at the same age compared to significant lower titers in the other 2 groups (124.3 pg/ml) at the same age as indicated The gut integrity genes, (Occuldin, MUC2, and JAM) in figure (2) explains the impact of dietary nucleotides and phytobiotics supplements on at 35-days-old, the γ-CT of all 3 genes arranged as 24.6, 26, and 20 for G1; 27, 24 and 22.6 in G2 in comparison with 28.3, 26 and 28.3 in G3 and 29.6, 28.3 and 35 in G4, respectively which indicated the low gene expression in G2 compared to G1.The gut microflora modulation was reported in Figure (3). *Lactobacillus* counts were 3.4 and 2.6 log_{10} and 5.3 and 5.9 log_{10} in G1 and G2 at 15 and 35–days-old, respectively without aa significant difference. This significant increase of *Lactobacillus* counts in both G1 and G2 was met with a significant decrease in *C. perfringens* counts. However, G3 and G4 showed a significant decrease in *Lactobacillus* and increase in *C. perfringens* counts with increasing the age of chickens.

5. Discussion

The intestinal wall comprises four distinct layers; the mucosa, submucosa, muscularis, and serosa, forming a highly protective structure. The term "intestinal barrier" refers specifically to the wall's protective role, while "intestinal permeability" describes a quantifiable trait that reflects how well this barrier is functioning [18]. Maintaining gut health is essential for achieving efficient and economically viable growth in poultry, particularly within high-intensity broiler farming systems. The gastrointestinal tract plays a central role in metabolic regulation by controlling the absorption and utilization of nutrients. However, intestinal health can be compromised by various factors, including pathogens, environmental stress, and suboptimal management practices. These challenges can hinder nutrient absorption and ultimately reduce the productivity of the birds.

Nucleotides and phytobiotics contribute to the development and maturation of the gastrointestinal tract which is the primary site for nutrient absorption and digestion. Their stimulatory effects on gut growth have

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.406300.1049

been associated with improvements in overall growth performance [19]. Our results indicated a potent significant impact on BWG, FI and FCR in G1 and G2 at 15, 25, and 35 –days-old compared to G3 and G4 ($p \leq 0.05$) although G2 was challenged with *C. perfringens*. These results were agreed with Madrigal *et al.* [20] who reported that the yeast components significantly improved feed utilization and BWG in broiler chickens.

Necrotic enteritis, caused by *C. perfringens*, is recognized as one of the most economically diseases affecting the broiler industry [21,22]. It leads to substantial financial losses by impairing growth performance and increasing mortality rates in poultry [23]. Therefore, nutritionists are exploring natural alternatives to antibiotic growth promoters to mitigate productivity losses and support intestinal health. Among these strategies is applying bioactive compounds, such as dietary nucleotides in chicks [24]. The observed reduction in *C. perfringens* infection in the treated and challenged chickens was attributed to the beneficial effects of nucleotide supplementation on gut health parameters, such as increased villus height, villus-to-crypt (V/C) ratio, mucosal thickness, and goblet cell count. Notably, an increase in goblet cells specialized secretory epithelial cells located in the intestine—supports mucus production, forming a gel-like protective layer over the epithelial surface, and contributing to defense against bacterial invasion. Furthermore, nucleotides may promote the growth of beneficial gut microbiota, such as *Lactobacillus and Bifidobacterium* species, while reducing populations of harmful enterobacteria [25,26,27]. As structural units of nucleic acids and proteins, nucleotides are low molecular weight compounds considered "semi-essential nutrients" [28]. They are essential for cellular replication and support key physiological functions in poultry [29]. Therefore, they play a critical role in protein synthesis, cell mitosis, lipid metabolism, hematopoiesis, immunity [27], and gut health [25,26]. In the present study the gut microflora showed significant increase of *Lactobacillus* counts in both G1 and G2, along with a significant decrease in *C. perfringens* counts compared to a significant decrease in *Lactobacillus* counts and a significant increase in *C. perfringens* counts with increasing age of chickens in G3 and G4.

The positive impact of nucleotides and phytobiotics on the carcass quality was recorded in table (1) as the dressing % in G1 was 82%, while in G2 was 77% and 70% in G3 compared to the significant loss of the carcass quality in G4 (dressing% of 58). Additionally, nucleotides act as the main component in cell metabolism which are essential for maintenance and repair of muscle and hepatic tissue [30].

Additionally, nucleotides may stimulate the intestinal immune system, leading to increased production of IgA in chickens [31]. IgA plays a crucial role in mucosal immunity by binding to antigens and preventing them from penetrating the mucosal barrier, thereby reducing the risk of infection and tissue damage [32]. However, in the present investigation there were significant differences in IgA titers of mucosal immunity with higher titers in chickens of G2 (277 pg/ml) than G1 (254.6 pg/ml) at 35 days old compared to significant lower titers in the other 2 groups (124.3 pg/ml) at the same age. Also, the humoral immune responses to ND, AI-H5N1 and AI-H9N2 viruses' vaccines recorded significant higher HI titers in G1 and G2 compared to G3 and G4 especially at 25- and 35-days-old ending with 7 and 6-6.3 Log_2 HI titers for ND, AI-H5N1 and AI-H9N2 at 35-days-

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.406300.1049

old. This indicates the immuno-stimulatory effects of both nucleotides and phytobiotics which is a favorable advantage over antibiotics in the control of NE.

Furthermore, the reduced intestinal lesion scores of chickens supplemented with dietary mixtures suggested a significant mitigation of the adverse *C. perfringens* adverse effects. This improvement maybe attributed to enhanced intestinal barrier function—specifically through the upregulation of key markers such as Occludin, JAM, and MUC2 which collectively contributed to better growth performance in challenged birds. The proposed mechanism underlying this effect involves the role of nucleotides in gene regulation. As the fundamental building blocks of genetic material, nucleotides support gene expression, RNA transcription, and mRNA synthesis, thereby facilitating protein production and cellular structure formation [33]. *MUC2*, a critical component of the mucus layer, serves both protective and regenerative functions in the intestinal tract [34], and is widely recognized as an indicator of gut health in poultry and other animals [35]. Occludin and JAM are specialized proteins that comprise the tight junctions structural barriers that regulate intestinal permeability and restrict the passage of macromolecules [36,37]. Disruption of these tight junctions can result in increased permeability to luminal antigens and facilitate bacterial translocation [38]. Although data on tight junction protein expression in *C. perfringens*-infected poultry is limited, Liu et al. [39] reported reduced expression of the Occludin gene in the small intestines of infected birds.

Dietary nucleotides have also been shown to support the growth and differentiation of the gastrointestinal tract [40], likely by enhancing the function of intestinal epithelial cells [41]. Additionally, emerging evidence suggests that nucleotide supplementation may boost innate immune responses and increase host resistance against toxin-induced damage in chickens [42].

The effect of nucleotides and Phytobiotics supplements on liver function showed that chickens of both G1 and G2 had normal liver enzymes with a significant increase in ALT, AST, AKP, and γ-GT at 35 days old after challenge in comparison with high level of these enzymes at G3 and G4 at 35 days old due to liver cells destruction.

6. Conclusions

The Dietary supplementation of broiler chickens with Nucleoregen® and Phytococc® at 0.2%, and 0.25%, respectively noticeably attenuated the negative effects of *C. perfringens* challenge in terms of improving the growth performance, intestinal health, liver integrity, and immune functions with a significant reduction in *C. perfringens* counts and intestinal lesion score, which indicated the beneficial effects of continuous feed supplementation with nucleotides and phytobiotics in broilers.

Conflict of interest: Nothing to declare

7. References

1. Mehdi, Y., Letourneau-Montiminy, M., Gaucher, M., Chorfi, Y., Rouissi, T., Brar, S.K., Cote, C., Ramirez, A.A. and Godbout, S. (2019). Use of antibiotics in broiler production: Global impacts and alternatives. *Anim. Nutr.*, **4**,170-178.
2. Yadav, S. and Jha, R. (2019). Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.406300.1049

poultry. *J. Anim. Sci. Biotechnol.,* **10**(2), 1-11

3. Hoffmann, K. (2007). The use of Nucleotides in animal feed. *Feed mix*, **6**, 25–27.
4. Malhi, H. and Gores, G.J. (2008). Cellular and molecular mechanisms of liver injury. *J. Gastroenterol,* **134**, 1641–1654 .
5. Barbara, A.J., Trinh, H.T., Glock, R.D. and Songer, J.G. (2008). Necrotic enteritis-producing strains of Clostridium perfringens displace non-necrotic enteritis strains from the gut of chicks. *Vet. Microbiol*., **126**, 377 – 382.
6. Gholamiandehkordi, A.R., Ducatelle, R., Heyndrickx, M., Haesebrouck, F. and Van Immerseel, F. (2006). Molecular and phenotypical characterization of Clostridium perfringens isolates from poultry flocks with different disease status. *Vet. Microbiol.*, **113**, 143 – 152.
7. Timbermont, L., Lanckriet, A., Gholamiandehkordi, A.R., Pasmans, F., Martel, A., Haesebrouck, F., Ducatelle, R. and Van Immerseel, F. (2009). Origin of Clostridium perfringens isolates determines the ability to induce necrotic enteritis in broilers. *Comp. Immunol. Microbiol. Infect. Dis.*, **32**, 503–512.
8. Keyburn, A.L., Boyce, J.D., Vaz, P., Bannam, T.L., Ford, M.E., Parker, D., Di Rubbo, A., Rood, J.I., and Moore, R.J. (2008). NetB, a new toxin that is associated with avian necrotic enteritis caused by Clostridium perfringens. *PLoS Pathog.*, **4**(2), e26.
9. Thanissery, R., McReynolds, J.L., Conner, D.E., Macklin, K. S., Curtis, P. A. and Fasina, Y.O. (2010). Evaluation of the efficacy of yeast extract in reducing intestinal Clostridium perfringens levels in broiler chickens. *Poult. Sci.,* 89, 2380–2388.
10. Bastamy, M., Raheel, I., Ellakany, H., Samir, A., Hamoud, M., Khalifa, R.A., Ibrahim, S., Abd El Rahman, S., and Orabi, A. (2021). Insight of Prevalence, Toxin Typing and Antimicrobial Susceptibility of Egyptian Clostridium perfringens Isolates Recovered from Broiler, Layer and Breeder Chicken Flocks. *Int. J. Vet. Sci*., **11**, 110-116.
11. Shariatmadari, F. (2012). Plans of feeding broiler chicken. *Worlds Poult. Sci. J.*, **68**(3), 21-31.
12. Dahiya, J.P., Hoehler, D., Wilkie, D., Van Kessel, A. and Drew, M. (2005). Dietary glycine concentration affects intestinal Clostridium perfringens and lactobacilli populations in broiler chickens. *Poult. Sci.*, **84**, 1875e85.
13. Eisgruber, H., Schalch, B., Sperner, B. and Stolle, A. (2000). Comparison of four routine methods for the confirmation of *Clostridium perfringens* in food. *Int. J. Food Microbiol.,* **57**, 135–140.
14. Merino-Guzmán, R., Latorre, J.D., Delgado, R. and et al. (2017). Comparison of total immunoglobulin A levels in dif- ferent samples in Leghorn and broiler chickens. *Asian. Pacific J. Trop. Biomed*., **7**(2),116–120.
15. Capua I. and Alexander D.J. (2004). Avian influenza: recent developments. *Avian Pathol.,* **33**, 393–404.
16. Tong Xing, Xiaona Pan, Lin Zhang and Feng Gao. (2021). Hepatic Oxidative Stress, Apoptosis, and Inflammation in Broiler Chickens with Wooden Breast Myopathy. *Front. physiol.*, 14 April.
17. Chen, H., Yan, F.F., Hu, J.Y., Wu, Y., Tucker, C.M., Green, A.R. and Cheng, H.W. (2017). Immune Response of Laying Hens Exposed to

30 ppm Ammonia for 25 Weeks. *Int. J. Poult. Sci.*, **16** (4), 139-146.
18. Bischoff, S.C., Barbara, G., Buurman, W., Ockhuizen, T., Schulzke, J. D., Serino, M. and et al. (2014). Intestinal permeability - a new target for disease prevention and therapy. *BMC Gastroenterol*. **14**, 189.
19. Yegani, M. and Korver, D. (2008). Factors Affecting Intestinal Health in Poultry. *Poult. Sci.,* **87**(10), 2052–2063.
20. Madrigal, S.A., Watkins, S.E., Skinner, J.T., Adams, M.H., Waldroup, A.L., and Waldroup, P.W. (1993). Effect of an active yeast culture on performance of broilers. *Poult. Sci.*, **72,** 87-87.
21. Young, R. and Craig. A. (2001). The use and misuse of antibiotics in UK agriculture-Part 3. Residues of dangerous drugs in intensively produced chicken meat and eggs. Soil Association, Bristol, UK.
22. McDevitt, R.M., Brooker, J.D., Acamovic, T. and Sparks, N.H.C. (2006). Necrotic enteritis; a continuing challenge for the poultry industry. *Worlds. Poult. Sci. J.,* **62**, 221–247.
23. Beltran-Alcrudo, D., Cardona, C., McLellan, L., Reimers, N., and Charlton, B. (2008). A persistent outbreak of ulcerative enteritis in Bobwhite quail (Colinus virginianus). *Avian Dis.*, **52** (3), 531–536.
24. Sauer, N., Bauer, E. and Mosenthin, R. (2009). Dietary nucleotides: Potential contenders as feed additive for monogastrics. Proceedings of the 18th international scientific symposium on nutrition of domestic animals (pp. 173–184). Radenci, Slowenia.
25. Martinez-Puig, D., Manzanilla, E. G., Morales, J., Borda, E., Pérez, J. F., Pineiro, C. and Chetrit, C. (2007). Dietary nucleotide supplementation reduces occurrence of diar- rhoea in early weaned pigs. *Livest. Sci*., **108**, 276–279.
26. Sauer, N., Eklund, M., Roth, S., Rink, F., Jezierny, D., Bauer, E. and Mosenthin, R. (2012). Short-term effect of dietary yeast nucleotide supplementation on small intestinal enzyme activities, bacterial populations and metabolites and ileal nutrient digestibilities in newly weaned pigs. *J. Anim. Physiol. Anim. Nutr.,* **96**, 700–708.
27. Hung, I.F. (2015). Theses and Dissertations–Animal and Food SciencesLexington: University of Kentucky.
28. Carver, J. (2007). Dietary nucleotides: Effects on the immune and gastrointestinal systems. *Acta Paediatr*, **88**, 83–88.
29. Superchi, P., Saleri, R., Borghetti, P., De Angelis, E., Ferrari, L., Cavalli, V., Amicucci, P., Ossiprandi, M.C. and Sabbioni, A. (2012). Effects of dietary nucleotide supplementation on growth performance and hormonal and immune responses of piglets. *Animal*, **6**, 902–908.
30. Grimble, G.K. and Westwood, O.M.R. Nucleotides. In M. E. Gershwin, J. B. German, & C. L. Keen (Eds.). (2000). Nutrition and immunology: Principles and practice (pp. 135–144). Totowa, NJ: Humana Press, Inc ISBN 978-1-59259-709-3.
31. Gao, J., Zhang, H.J., Yu, S.H., Wu, S.G., Yoon, I., Quigley, J., Gao, Y.P. and Qi, G.H. (2008). Effects of yeast culture in broiler diets on performance and immunomodulatory functions. *Poult. Sci.*, **87**, 1377–1384.
32. Kulkarni, R.R., Parreira, V.R., Jiang, Y.F. and Prescott, J.F. (2010). A live oral recombinant Salmonella enterica serovar Typhimurium vaccine expressing Clostridium perfringens antigens confers protection against

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.406300.1049

necrotic enteritis in broiler chickens. *Clin. Vaccine Immunol*., **17**, 205–214.

33. Hernandez, C.E., Santamatilde, K.J., McCreath, A.M., Cervera, I., Diez, D., Ortiz-Masia, N., Martinez, S., Calatayud, Esplugues, J.V. and Barrachina, M.D. (2009). Induction of trefoil factor (TFF) 1, TFF2 and TFF3 by hypoxia is mediated by hypoxia inducible factor-1: Implications for gastric mucosal healing. *Br. J. Pharmacol.*, **156**, 262–272.
34. Zeinali, S., Ghazanfari, S. and Ebrahimi, M.A. (2017). Mucin2 gene expression in the chicken intestinal goblet cells are affected by dietary essential oils. *Bulg. J. Agric. Sci.,* **23**, 134–141.
35. Li, C., Guo, S., Gao, J., Guo, Y., Du, E., Lv, Z. and Zhang, B. (2015). Maternal high-zinc diet attenuates intestinal inflammation by reducing DNA methylation and elevating H3K9 acetylation in the A20 promoter of offspring chicks. *J. Nutr. Biochem*., **26** (2), 173–183.
36. Schneeberger, E.E. and Lynch, R.D. (2004). The tight junction: a multifunctional complex. *Am. J. Physiol. Cell Physiol.*, **286** (6), C1213-C1228.
37. Ballard, S.T., Hunter, J.H. and Taylor A.E. (1995). Regulation of tight-junction permeability during nutrient absorption across the intestinal epithelium. *Annu. Rev. Nutr*., **15,** 35-55.
38. Ulluwishewa, D., Anderson, R.C., McNabb, W.C., Moughan, P.J., Wells, J.M. and Roy, N.C. (2011). Regulation of tight junction permeability by intestinal bacteria and dietary components. *J. Nutr.*, **141**(5), 769-776.
39. Liu, D., Guo, S. and Guo, Y. (2012). Xylanase supplementation to a wheat-based diet alleviated the intestinal mucosal barrier impairment of broiler chickens challenged by Clostridium perfringens. *Avian Pathol.*, **41**(3), 291-298 .
40. Carver, J. and Walker W.A. (1995). The role of nucleotides in human nutrition. *J. Nutr. Biochem.,* **6**(2), 58-72.
41. Seifert, R. and Schultz, G. (1989). Involvement of pyrimidinoceptors in the regulation of cell functions by uridine and by uracil nucleotides. *Trends Pharmacol. Sci.,* **10**(9), 365-369.
42. Frankic, T., Pajk, T., Rezar, V., Levart, A. and Salobir, J. (2006). The role of dietary nucleotides in reduction of DNA damage induced by T-2 toxin and deoxynivalenol in chicken leukocytes. *Food Chem. Toxicol.,* **44** (11), 1838-1844.

Table (1): Experiment dietary protocol

Group number	Dose/Treatment	Age of treatment/day	Challenged with *net B* toxin *C. perfringens* local isolates registered at GenBank MW925054
1	200 g Nucleoregen®	1-35 old	Nil
2	250 g Phytococc®	1-35 old	Yes
3	Control negative	-	Nil
4	Control positive	-	Yes

Table 2: RT-PCR primers used in the study

Gene	Primer	Function
MUC	F: AAACAACGGCCATGTTTCAT	Mucin production
	R: GTGTGACACTGGTGTGCTGA	
TGO	F-ACGGCAAAGCCAACATCTAC	Tight junction occluding
	R-ATCCGCCACGTTCTTCAC	
JAM-2	F-AGACAGGAACAGGCAGTGCT	Junction adhesion molecules
	TCCAATCCCATTTGA GGCTA	

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.406300.1049

Table 3: Impact of dietary Nucleoregen® and Phytococc® supplementation on performance

Items	Experimental groups				*p-value*
	1	2	3	4	
BWG, g/day					
d 15	333±2.89[a]	329.33±4.33 [a]	301.67±6.89[b]	303±3.61 [b]	0.001
d 25	995±2.89 [a]	984±2 [a]	808±10 [b]	806.67±5.61 [b]	0.001
d 35	1848.33±7.27 [a]	1813.33±9.28 [a]	1306.33±13.42 [b]	1214.33±11.26[c]	0.001
FI, g/day					
d 15	350±2.89 [a]	348.33±4.41 [a]	346.67±4.41 [a]	353.67±1.86[a]	0.574
d 25	1154±4 [a]	1157.67±1.45 [a]	1164.33±3.48 [a]	957.33±3.18 [b]	0.001
d 35	2056±4.64 [a]	2106.67±9.82 [a]	2185.33±3.71 [a]	1770.67±8.79 [b]	0.001
FCR					
d 15	1.05±0.01 [b]	1.06±0.01 [b]	1.15±0.02 [a]	1.17±0.01	0.001
d 25	1.16±0.01 [b]	1.18±0.01 [b]	1.44±0.02 [a]	1.19±0.05 [b]	0.001
d 35	1.24±0.01[c]	1.36±0.01[c]	1.67±0.02 [a]	1.46±0.05 [b]	0.001
Dressing%	82±0.58 [a]	77±1 [b]	70±1.16[c]	58.67±1.76[d]	0.001

Means with different superscripts within the same row are significantly different ($p \leq 0.05$). IBW, initial body weight; FBW, final body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.406300.1049

Table 4: Impact of dietary Nucleoregen® and Phytococc® supplementation on liver functions

Items	Experimental groups				*p-value*
	G 1	G 2	G 30	G 4	
ALT					
d 15	1.54±0.03^{b}	1.58±0.01^{ab}	1.65±0.02^{a}	1.62±0.03^{ab}	0.047
d 25	1.59±0.03^{c}	1.65±0.01^{bc}	1.69±0.02^{ab}	1.75±0.01^{a}	0.001
d 35	1.7±0.01^{a}	1.69±0.02^{a}	1.70±0.03^{a}	1.76±0.01^{a}	0.08
AST					
d 15	14.47±0.89^{a}	20.4±0.1^{a}	25.33±0.69^{a}	27.2±0.85^{a}	0.067
d 25	21.3±0.06^{b}	21.6±0.06^{b}	26.37±0.28^{b}	31.8±0.22^{a}	0.001
d 35	21.37±0.09^{b}	22.07±0.82^{b}	27.4±0.51^{b}	34.2±0.79^{a}	0.001
AKP					
d 15	436.33±8.45^{a}	447.33±7.22^{a}	459±7.09^{a}	460.67±4.37^{a}	0.119
d 25	459.67±4.17^{d}	507.67±9.94^{c}	550.67±8.69^{b}	655.13±13^{a}	0.001
d 35	477.67±5.21^{d}	525.67±3.18^{c}	569±6.08^{b}	753.33±5.3^{a}	0.001
GT					
d 15	24.47±0.15^{b}	24.53±0.12^{b}	26.2±0.25^{a}	25.83±0.32^{a}	0.001
d 25	25.33±0.09^{d}	26.5±0.47^{c}	28.23±0.22^{b}	35.63±0.09^{a}	0.001
d 35	26.13±0.18^{c}	26.97±0.27^{c}	31.63±0.67^{b}	36.13±0.23^{a}	0.001

Means with different superscripts within the same row are significantly different ($p \leq 0.05$).
ALT: alanine amino transferase, AST: aspartate amino transferase, AKP: alkaline phosphatase, γ-GT: gamma glutamyl transferase

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.406300.1049

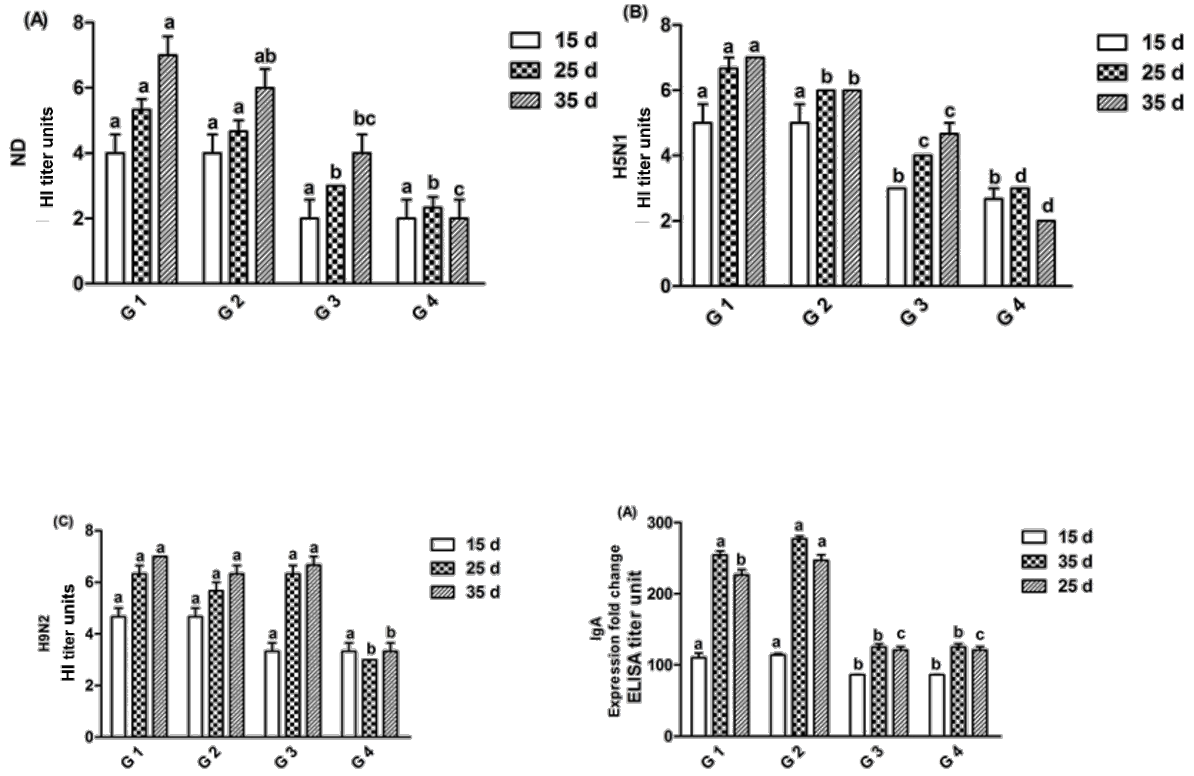

Fig (1): Impact of dietary Nucleoregen® and Phytococc® supplementation on immune response.

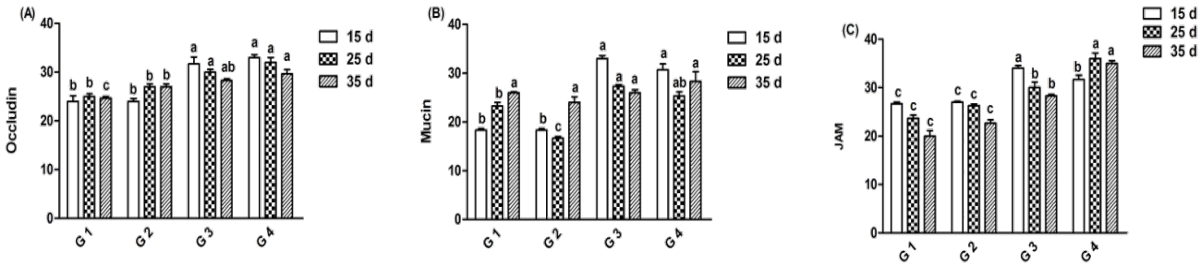

Fig (2): Impact of dietary Nucleoregen® and Phytococc® supplementation on gut integrity genes

Online ISSN: 2537-1045
Print ISSN: 1110-1423
DOI: 10.21608/vmjg.2025.406300.1049

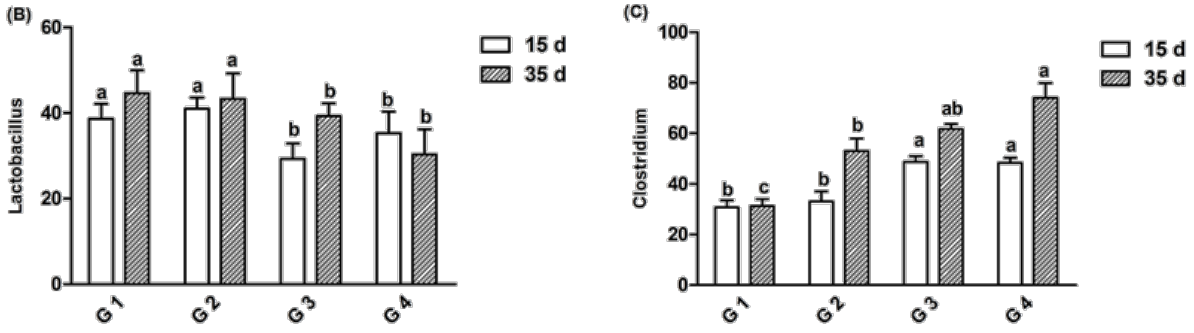

Fig (3): Impact of dietary Nucleoregen® and Phytococc® supplementation on gut microflora.