Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Biochemical Studies on the Effects of Collagen and Nucleotides on Growth of Broiler chickens

Ibtehal Hashem Mahmoud Soliman*, Magdy M.Youssef and Manar Refaat*

Chemistry Department, Faculty of Science, Mansoura University, Egypt.

Abstract

THIS STUDY was designed to evaluate the effect of collagen and nucleotides as feed additives alone or in a combination on the growth performance, biochemical, hematological parameters, immune, antioxidant status and intestinal histomorphology in broiler chickens. A total of 60 one-day-old unsexed broiler chicks (Ross 308) were divided into four groups, each group included 15 chicks fed with different formulated diets for a period of 35 days. G1 was the control group and was fed basal diet only, G2 was fed basal diet with nucleotides (0.5 g/Kg feed), G3 was fed basal diet with collagen (100 mg/kg feed) and G4 was fed basal diet with a combination of nucleotide 0.5 g/Kg feed and collagen 100 mg/Kg feed. The obtained results showed that birds in the G2, G3 and G4 groups had significantly ($p<0.05$) higher body weight than control group. Nucleotides and collagen significantly improved feed conversion ratios in all treatments. Addition of nucleotides and collagen to broiler diets significantly ($p<0.05$) increased the blood levels of total leucocytes, erythrocytes, hemoglobin and hematocrit, also, increased the serum level of total proteins mainly globulin, uric acid, creatinine, triglycerides, GPx, SOD and Newcastle virus antibody titer, while decreased cholesterol, high-density lipoproteins, low-density lipoproteins, MDA and total antioxidant capacity. Nucleotides and nucleotides with collagen groups showed an improvement in intestinal histomorphology. Inclusion of collagen to broiler chickens diet significantly ($p<0.05$) increased the level of serum thrombocytes. In conclusion, the results demonstrated an enhancement in growth performance, hematological parameters, immunity, antioxidant status and intestinal histomorphology of broiler chickens by providing their diet with collagen and nucleotides.

Keywords: Broiler chickens; nucleotides; collagen; growth performance; immunity; intestinal histomorphology.

Introduction

Birds' immune systems, growth rates and performance have all taken a hit as a result of the last few decades of selective breeding. Chickens have a high mortality rate and are extremely vulnerable to several viral and metabolic diseases in recent years. Antibiotics were commonly used to boost poultry productivity and growth [1]. However, these antibiotics now leave harmful residues in animals and humans and have led to the development of resistant bacteria. As a result, there is a rising interest in finding alternatives, such as feed additives like nucleotides and collagen which boost growth and immunity [1]. An essential part of animals' physiological processes involves nucleotides, a class of bioactive substances characterized by low molecular weight and intracellular molecules [2]. Numerous methods exist for synthesizing nucleotides, including as the salvage pathway and endogenous anabolism(de novo synthesis). Intestinal injury, fast growth and development, immunosuppression, stress, exposure to bacterial and viral infections, increasing heat or bad weather, reduced protein intake and other conditions are marked by an increased demand for nucleotides making external dietary sources essential in these cases [3]. Livestock frequently have yeast extract or the pure material added to their diets [4]. Research has demonstrated that adding nucleotides to poultry feed greatly improves their antioxidant status [5]. Weight increase, feed intake and FCR were all enhanced when nucleotides were added to the diet of broilers [6–8]. Adding nucleotides to poultry diets made their immune systems react more strongly and more quickly to common immunizations [9]. In both the extracellular matrix and the connective tissues of animals, collagen is one of the most prevalent fibrous structural proteins [10]. The primary amino acids that make up collagen are glycine, proline, and hydroxyproline. Poultry health, growth performance and increased collagen synthesis all depend on getting enough of these amino acids [11]. According

*Corresponding authors: 1.Ibtehal H. M. Soliman ebtihal.ammar1993@gmail.com, Tel.: 01111515043
2.Manar Refaat, E-mail: manar refaat@mans.edu.eg Tel.: 002 01002932760
(Received 11 November 2024, accepted 07 January 2025)
DOI: 10.21608/EJVS.2025.335440.2485

to Tsiagbe et al. [12], methionine is crucial for young broilers to keep their growth rate up, but it's also required at a higher rate to keep their immune response strong and their antioxidant status improved. Methionine deficiency reduces immunological function and has limited impact on antibody-forming cells according to Aschkenasy [13]. Additionally, glycine is an essential amino acid of collagen that protects against a number of disorders and works as an anti-inflammatory agent [14]. The antioxidant enzyme glutathione peroxidase catalyzes the detoxification of hydrogen peroxides, avoiding oxidative stress damage; glycine is also involved in glutathione production and serves as a substrate (hydrogen donor) or co-factor for this enzyme. According to study adopted by Khomich et al., [15], glutathione triggers the host immune system to control innate immunity. Supplementing the diet of broiler chickens with glycine enhanced their ability to absorb fat, produce mucin, acquire weight and skeletal muscle, depose collagen and maximize feed efficiency [16]. As an antioxidant, proline reduces oxidation stress within biological cells by scavenging oxidants, such as free radical species. Subsequently, post-weaning pigs who receive dietary supplements containing the collagen precursor's glycine and proline have an increase in intestinal villus height, an improvement in nutritional absorption, an increase in body weight, and an increase in collagen production [17]. In addition to its role as an anti-oxidative molecule and regulator of cellular redox events, hydroxyproline is involved in the synthesis of glycine in various animal tissues and helps maintain the structure of connective tissues. The control of gene expression to enhance oxidant clearance is another important function of hydroxy proline [18].

Material and Methods

Animal Management:

Sixty healthy unsexed one-day-old Ross 308 chicks, each with an initial average body weight of 43.5 g, were procured from a local commercial supplier and reared in cages within a controlled environmental chamber. A constant lighting regimen was implemented for the entire study duration. The temperature is maintained at 34°C to 36°C for the initial five days, subsequently decreased by 2°C weekly until it attains 24°C to 26°C. Upon arrival on day 0, all chicks were individually weighed, wing-tagged and categorized into four groups, each comprising fifteen chicks. The feeding trial lasted for 35 days. All chicks were provided a crumble diet during the starter and grower phases, followed by a pellet diet until the conclusion of the study period, during which feed and water were available ad libitum. At seven days of age, chicks were vaccinated for Newcastle disease using Nobilis ND Clone 30 by eye drop, provided by MSD Animal Health, Boxmeer, The Netherlands.

Experimental Design and Diet:

According to Aviagen [19], four experimental diets were developed to meet the nutritional needs of broiler chicks. These diets were divided into three phases and are displayed in Table 1. To prevent rancidity and lipid oxidation, the diet was stored in a cool and dry environment. Each of the four food treatments was administered to a different group of 15 chicks; three sets of five chicks will make up each group. Group 1 served as a control and received simply a basal diet. Group 2 received a basal diet with nucleotides at a rate of 0.5g/kg feed. Group 3 received a basal diet with collagen at a rate of 100 mg/kg feed. Group 4 received a basal diet with a mix of nucleotides and collagen at a rate of 0.5 g/kg and100mg/kg feed respectively. The prepared feed's collagen is sourced from bovine collagen that has been refined using enzymatic hydrolysis; it has a crude protein content of around 94.5 percent. We sourced our nucleotides from Nucleoforce. ® livestock for veterinary (unrestricted nucleotides derived from dried yeast extract).

Growth performance parameters:

Growth performance was assessed by recording body weight (BW) and daily feed consumption all over the trail period. Feed Intake (FI), feed conversion ratios (FCR), body weight gain (BWG) and average day gain (ADG) were calculated. On the last day of experimentation (35 d), all birds were slaughtered [5].

Data collection:

The body weight of the experimental birds was documented initially and subsequently on a weekly basis. Feed intake: The feed was administered once daily, and the leftover feed was collected and weighed the next day. The feed conversion ratio (FCR) for each treatment group was determined by dividing the mean weekly total feed intake by the mean weekly total body weight gain (FI/BWG) [6].

Collection of serum and blood samples and biochemical analysis:

Blood and serum were collected on 35 d from the wing vein in non-heparinized tubes. Serum was collected by centrifugation at 3000 rpm for 10 minutes and stored at (− 20°C) to analysis time. Whole blood count was performed immediately after blood collection and not stored. Serum tests were measured spectrophotometrically by fully automated chemical analyzer (Mindray BS-240) according to manufacturer's instructions, thrombocytes count, hemoglobin and hematocrit levels of non-coagulated blood samples were measured using (Diagon D-Cell 60) automatic blood analyzer, RBCs and total leukocyte count were calculated manually using hemocytometer. The serum was analyzed for Total cholesterol [20], Triglyceride [21], Serum high-density lipoprotein-cholesterol (HDL-C) [22], Serum

low-density lipoprotein-cholesterol (LDL-C) [23], total serum protein [24], albumin [25] and globulin [26].

Uric acid [27], and creatinine [28]. Total bilirubin was also determined [29], AST and ALT [30]. The serum level of total antioxidant capacity (TAC) was also measured [31] and total antioxidant capacity was determined with colorimetric kit (Biodiagnostic Company, Dokki, Giza, Egypt). Serum tests ALT, AST, albumin, triglyceride and total cholesterol were performed by using commercial kits (Shenzhen Mindray Bio-Medical Electronics Co Ltd, China).

Super oxide dismutase (SOD) and glutathione peroxidase GPx) enzymes activity was assayed by an ELISA protocol using (CUSABIO Biotech Co., Ltd, Taxes, US) and (Nova Lifetech Limited Company, Hong Kong, China) kits respectively. Malondialdehyde (MDA) the biomarker of lipid peroxidation was also determined by ELISA protocol using (Nova Lifetech Limited Company, Hong Kong, China) kit. The optical densities in ELISA protocol were measured by using stat fax 4700 microplate reader. NDV vaccination titer was determined by HI test (Heam agglutination inhibition test) using reference antigen according to method described before [32].

Intestinal histopathology:

Three birds from separate experimental groups were randomly slain and their jejunums were fixed in 10% buffered formalin for histopathological analysis. After being dehydrated in increasing concentrations of alcohol, the specimens were clarified using methyl benzoate. They were then embedded in paraffin wax and sectioned at 3-5 µm thickness. Staining the sections with the Harris Hematoxylin and Eosin solution was done as previously described [33]. The intestine's structural morphology was determined using Image J software. This included measures such as villus height (VH), villous width (VW), crypt depth (CD), villous height to crypt depth (VH/CD), villous surface area and villous perimeter.

Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE):

Serum proteins were purified using SDS-PAGE according to protocol adopted by Laemmli, [34]. Serum samples were prepared and diluted to a concentration related to serum total proteins with sample buffer which contain the Bromphenol blue stain and SDS detergent. Standard protein markers were used to determine the molecular weight of the separated proteins.

Statistical analysis

The collected data was revised, coded, and tabulated using the Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Data were presented and suitable analysis was done according to the type of data obtained for each parameter. Mean and Standard deviation (± SD) were calculated for numerical data using the standard statistical formula [35]. The data was studied by ANOVA one-way classification to test the significance of difference between diverse treatment groups followed by Kruskal-Wallis 's test assess the statistical significance of the difference between more than two study group non parametric variables. A $p < 0.05$ value is considered significant at confidence interval 95%.

Results

Growth Performance:

The body weight significantly increased through the experimental period among all groups, nucleotides and collagen groups showed significantly higher body weight than control group and the highest increasing body weight was for nucleotides and collagen group which significantly surpassing all other groups (Table 2). Feed consumption was significantly reduced from (day 7-14) of trail in nucleotides and collagen groups and significantly increased in nucleotides and collagen group than control group. By the day (28-35) no significantly differences in feed consumption were observed among experimental groups except nucleotides group that has the highest value (Table 3). Regarding Feed Conversion Ratios among the study groups, (FCR) were significantly improved among all experimental groups than control group and the best result was for nucleotides with collagen group. In day 28, the nucleotides group exhibited a significantly higher FCR compared to the control, collagen, and nucleotides with collagen groups (Table 4). In terms of Body weight gain (BWG) among the study groups, the nucleotides with collagen group showed significantly higher body weight gains compared to the other groups from day (14-35). No significant differences were observed in nucleotides and collagen groups except from day (28-35) where both groups had significantly higher BWG than control group (Table 5). According to average daily gain (ADG) among the study groups, the nucleotides and collagen group showed significantly higher average daily gains compared to the other groups from day (14-35). No significant differences were observed in nucleotides and collagen groups except from day 28-35 where both groups had significantly higher ADG than control group (Table 6).

Serum biochemical and haematological parameters and antioxidant status:

In the comparison of haematological and biochemical parameters among the study groups, several significant differences were observed. The nucleotides and collagen group consistently showed significantly highest levels of leucocytes, RBCs, HB and HCT than other groups. Thrombocytes were

significantly higher in the collagen and nucleotides and collagen groups than control and nucleotides groups. Total Protein and Globulin levels were significantly increased in the nucleotides, collagen and nucleotides and collagen groups compared to the control group. For liver enzymes (AlT and AST) and albumin no significant differences were found among the groups. Total Bilirubin was significantly higher in the nucleotides and nucleotides and collagen groups compared to the control group. Uric Acid was significantly higher in the nucleotides and collagen group than in all other groups. Creatinine levels were highest in the collagen group and significantly lower in the control group. Cholesterol and HDL were significantly lower in the nucleotides and collagen group compared to the control group, while LDL was significantly lower in the nucleotides and collagen group compared to all other groups. TG significantly increased in nucleotides group than control group. TAC levels were significantly lower among the trail groups compared to the control group. Finally, ND antibody titer against new castle disease vaccination was significantly increased in all trail treatment groups than control group and the highest level was observed in collagen group compared to the other groups (Table 7).

In the comparison of antioxidant parameters among the study groups, significant differences were observed across all parameters. Dietary nucleotides and collagen supplementation significantly decreased lipid peroxidation by reducing malondialdehyde (MDA) level in serum samples. GPx and SOD antioxidant enzymes significantly increased in nucleotides and collagen groups and the highest levels were in nucleotides and collagen group which surpassing other groups (Table 8).

Histopathological Morphology:

The histopathological morphology changes of jejunum had been shown in Figure 1. The sections showed no structural damage in all groups when stained by H&E stain. Significant increases in villus height were observed in nucleotides, collagen and nucleotides and collagen groups comparing with the control group. In addition, we observed significant increases in villus width and crypt depth in collagen and nucleotides with collagen groups than the control group while crypt depth significantly decreased in nucleotides group. The ratio of villus height to crypt depth (VH/CD) was significantly increased among the nucleotides and nucleotides and collagen groups comparing with control group and significantly decreased in collagen group than other groups. Villous surface area and villus perimeter were significantly increased in nucleotides and collagen group compared to nucleotides, collagen and control groups (Table 9).

Sodium Dodecyl sulphate polyacrylamide gel Electrophoresis (SDS-PAGE):

In order to find the molecular weight of the purified chicken IgY antibodies, SDS-PAGE gel electrophoreses were employed. Figure 2 displays the proteins that have been purified. Using a standard protein marker, the mobility of the bands in SDS-PAGE were used to quantify the molecular weight of the serum protein. Figure 2 shows the estimated molecular weight of Immunoglobulin Y for the heavy chain, which is 65 KDa, and for the light chain, which is 25 KDa. Another band at around 38 kDa reveals the chicken IgY heavy chain, which is an alternate splicing variant.

Discussion

Growth Performance:

Supplementing broiler chickens' diets with nucleotides was thought to boost their immunological function, antioxidant status, gastrointestinal development and growth performance. Nucleotide supplementation increased BW, decreased feed intake and improved feed conversion ratio (FCR) of broilers across all treatments in our study. The results matched those of the previous studies [36, 37]. The previous findings [38, 39] found no significant difference in feed intake and weight increase, which contradicts our results. Supplementing with nucleotides boosted body weight gain and feed conversion ratio (FCR), according to Hassanein et al. [40], but had no effect on feed consumption. However, research out of Catholic University of Ecuador found that adding 1.5% NuPro to pre-starter diets significantly increased weight gain and feed efficiency. NuPro is a product rich in amino acids, glutamine, and nucleotides. The first three weeks after hatching in broilers show a magnifistic rate of development and a rapid growth, so, nucleotides may become conditionally essential [3]. The aforementioned positive effects may be due to the fact that amino acids like glutamine, aspartate, and glycine are available for de novo synthesis while taking nucleotide supplements; this allows for their utilization in growth while decreasing the energy needed for the salvage route. Prior study was reported that supplementation with 0.1% nucleotides to broilers can improve growth parameters [41], while others found no such effect on BW, weight gain and feed intake [42]. Our findings demonstrated that broiler chickens given collagen as a dietary supplement had a considerable improvement in FCR due to the reduction of feed intake so feed efficiency improved, as well as increases in BW, BWG and ADG. These findings are in line with those of Wu [43], who found that collagen is essential for the development of connective tissues in chickens. Supplementing the diet of broiler chickens with glycine enhanced feed efficiency in 21-35 day old chicks, fat absorption, mucin formation and muscle building, body weight gain and collagen synthesis and deposition in 5-21 day old chicks, respectively [16]. In order to keep their growth rate constant,

young broilers need methionine, according to research by Tsiagbe et al. [12]. For this reason, collagen plays a crucial function in the diets of chickens and other poultry by increasing the amount of muscle tissue and the rate of muscle growth, which in turn improves the birds' overall health [44]. Collagen synthesis and growth performance are hindered in young pigs [45] and poultry [11].

Biochemical and haematological Parameters:

Results showed that adding nucleotides and collagen significantly ($p<0.05$) raised leucocytes, RBC, HB, and HCT levels, with the combination group showing the highest values. Thrombocytes were significantly higher in the collagen and nucleotides and collagen groups than other groups. As an evidence for this result that collagen is one of the major activators of thrombocytes response after injury. Collagen is the only matrix protein which supports both thrombocytes adhesion and complete activation [46]. The first limiting essential amino acid that enhances growth performance and protein synthesis in chicken is methionine, which is a component of collagen [47]. Among its many functions, methionine is an important biological methyl donor for the methylation of DNA, RNA, and proteins [48]. The process of making proteins in eukaryotic organisms begins with the amino acid met, which is a precursor to homocysteine. This proves that methionine is crucial for the production and growth of all cells, including blood cells.

In comparison to the control group, the groups that received nucleotides, collagen, or a combination of nucleotides and collagen showed a significant increase ($p<0.05$) in total protein and globulin levels. These findings align a previous report [5], which found that adding nucleotides to serum significantly raised total protein and globulin levels. There were no notable variations observed across the groups when it came to albumin, liver enzymes (AST and AlT). The Control group had the lowest total bilirubin levels, while the nucleotides and nucleotides and collagen groups had considerably greater levels. Compared to the other groups, the nucleotides and collagen group had much greater urinary acid levels. Collagen and nucleotides both raised creatinine levels compared to the control group, with the former showing the greatest rise and this may contributed that creatinine an uric acid are byproducts of nucleotides and collagen metabolism, which are both rich in amino acids. The combination group, which had both nucleotides and collagen, had the most dramatic reduction in serum HDL, LDL, and cholesterol levels. Consistent with earlier research [40], this study found that broiler chickens had considerably lower cholesterol and LDL concentrations after receiving nucleotide supplementation and in contrast with [40] which revealed high concentrations of HDL. Yeast may help regulate blood cholesterol levels through de-conjugation of bile acids, which is why researchers as it was found that adding nucleotides produced by yeast lowered serum cholesterol levels [7]. Despite contradicting [40] and agreeing with [5], which also found that nucleotides substantially raised serum TG. Collagen hydrolysates impact lipid absorption and metabolism in rats and reduce blood triglycerides, according to [49], and this finding was in agreement with our results showing that collagen substantially reduced serum TG levels.

Immunology Function:

Nucleotide supplementation was found to raise broilers' serum IgY levels, which is the titer of humoral immunity against NDV vaccination, on day 35 of age, which is the last day of the trial. The effect of vaccination on humoral immunity is shown by the change in IgY levels in broilers given nucleotide or collagen. This finding is in line with numerous prior investigations that found that pigs and birds whose immune systems were supplemented with nucleotides produced more immunoglobulins [50]. Lymphocytes use a high number of nucleotides to produce immunoglobulins which are synthesized de novo by other organs (primarily the liver), to fast maintaining and production [51]. Additionally, our study revealed that broiler feed containing collagen increased leukocyte count, suggesting that collagen plays a significant role in animal immune response. This, in turn, suggests that methionine enhances T cell activation and development, which in turn impacts cell-mediated immune response [52]. Earlier studies hypothesized that broilers could benefit greatly from higher amounts of methionine, which would increase both antibody production and T-cell proliferation [53].

Antioxidant status:

Nucleotides and collagen, either separately or in combination, considerably improves broiler chickens antioxidant status, according to this research. Combining collagen with nucleotides produced the greatest benefits, which included a marked decrease in malondialdehyde (MDA) levels and an increase in glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity. There was some evidence that nucleotide supplementation could protect DNA from oxidative stress-induced damage and breaks. Consequently, nucleotides may mitigate oxidative stress in pigs as a consequence of a diet rich in polyunsaturated fatty acids (PUFA), so, animals will see a reduction in genotoxic stressors, which are oxidative pressures on immune cells, DNA, and RNA, as a result of their diet [54]. As a major component of collagen, proline reduces oxidation stress inside biological cells by acting as an antioxidant by scavenging free radical species. As an anti-oxidative chemical, Hyp controls cellular redox processes. The control of gene expression to enhance oxidant clearance is another important function of

Hyp [18]. Nucleotides and collagen, either singly or in combination, improved broiler chickens' antioxidant status. When collagen and nucleotides were added, total antioxidant capacity (TAC) dropped dramatically [40].

Histopathological Examination:

Intestinal morphology is greatly enhanced by dietary nucleotide supplementation, according to our study. This is shown by a decrease in villus crypt (VC), an increase in jejunal villus height (VH), and a decrease in villus width (VW). Both the surface area and perimeter of the villus were considerably enhanced by the addition of nucleotides and collagen. These findings were the same results that reported by [5, 40]. It appears from these results that yeast nucleotides play a significant role in the maturation of the intestines. The ratio of villus height to crypt depth was shown to decrease after dietary collagen considerably enhanced crypt depth. Intraintestinal morphology was enhanced by dietary collagen supplementation, which led to a marked increase in villus height (VH) and villus width (VW). Intestinal villus height and nutrient absorption are both likely to be improved by taking dietary supplements of the collagen precursors glycine and proline [55].

Sodium Dodecyl sulphate polyacrylamide gel Electrophoresis (SDS-PAGE):

Using SDS-PAGE, the proteins were purified [34]. The SDS-PAGE bands verified that the isolated protein was immunoglobulin Y; the heavy chain had a molecular weight of 65 KDa and the light chain 25 KDa. In addition, there is a variation at around 40 kDa for the chicken IgY heavy chain that alternatively splicing. Each group had a strong band, but the nucleotides with collagen group had the strongest. These findings corroborated those mentioned before, as IgY molecule has two heavy chains (H) with molecular weights of 67 to 70 kDa each and two light chains (L) with molecular weights of 25 kDa each [56]. One part of the light chains is constant (CL), and the other part is variable (VL). Due to its heavier chains' four constant domains (CH1-CH4) and an additional constant domain containing the related carbohydrate chains, IgY has a larger molecular weight of 180 kDa [57]. The SDS-PAGE method was used before [58] to isolate chicken IgY from egg yolk. They then measured the molecular weight of the purified protein, which they found to be around 65 kDa for the heavy chain and 27 kDa for the light chain. This strongly suggests that the protein that isolated is chicken IgY. Additionally, the chicken IgY heavy chain crystallization fragment Fc showed an alternatively spliced variant at around 40 kDa, which matched the findings that previously reported the presence of a novel protein band corresponding to 47.4 kDa in the culture of the recombinant pPIC9 ompA-Fc transformant as determined by SDS-PAGE [59].

Conclusion

Body weight, FI, FCR, BWG, and ADG were all significantly improved in the nucleotide and collagen groups compared to the control group in this study. The combination group also had the greatest results. Nucleotide and collagen fed birds consumed less food than control birds. Increased humoral immunity against ND vaccination and improved serum and hematological parameters were observed in birds given nucleotides and collagen. In addition to lowering MDA levels and raising GPx and SOD, collagen and nucleotides enhanced antioxidant status and intestinal histomorphology.

Acknowledgments

"We thank Allah who cause the reasons and ask him to bless our steps, to show us the path and fill our souls and minds with his light. We wish to express our scientific appreciation to all stuff members for their help in performing field work. Thanks for all study participants.".

Funding statement

This study didn't receive any funding support

Declaration of Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author's contributions

Ibtehal Hashem Mahmoud: Data curation, formal analysis, investigation, methodology, writing original draft. Magdy M.Youssef: Data curation, methodology, supervision, formal analysis, software, visualization, writing original draft, writing review and editing. Manar Refaat: Data curation, methodology, supervision, visualization, writing original draft, writing-review and editing.

Ethical of approval

The animal ethics declaration regarding the care and use of animals adhered to all relevant national and institutional regulations. All avian samples utilized in research received approval from the Animal Care and Use Committee of Mansoura University, Faculty of Veterinary Medicine, Mansoura, Egypt with the code number MU-ACUC (SC.MS.23.05.22).

TABLE 1. Composition of the experimental diet ingredients:

	Starter	Grower	Finisher
Feeding period(days)	0-10 days	11-24 days	25-35 days
Yellow corn	57.15	62.74	69.11
Corn gluten meal 60%	9.66	12.73	15.10
Soybean meal 46%	27.60	19.12	10.62
Oil	1.00	1.00	1.00
Ground limestone	1.85	1.65	1.59
mono-calcium phosphate	1.25	1.25	1.05
L-lysine	0.33	0.38	0.44
DL- methionine	0.20	0.13	0.09
L-threonine	0.06	0.03	0.03
Vitamin mineral premix*	0.30	0.30	0.30
Common salts	0.35	0.35	0.35
Sodium bicarbonate	0.10	0.10	0.10
Phytase	0.01	0.01	0.01
Anti-mycotoxins	0.20	0.20	0.20
Sum	100.00	100.00	100.00
Nutritive value			
CP	23.16	21.50	19.53
EE	3.56	3.74	3.94
ME	3000.38	3100.32	3200.30
Calcium	0.96	0.87	0.79
Available Phosphorous	0.45	0.44	0.40

TABLE 2. The effects of nucleotides and collagen on experimental groups according to body weight.

	Control	Nucleotides	Collagen	Nucleotides and Collagen
Day 0	43.53 ± 1.60^{a}	43.93 ± 1.79^{a}	43.33 ± 2.09^{a}	43.33 ± 2.32^{a}
Day 7	197.40 ± 37.66^{a}	203.73 ± 23.89^{a}	204.60 ± 11.65^{a}	209.67 ± 19.79^{a}
Day 14	521.87 ± 37.05^{a}	533.40 ± 45.61^{a}	537.80 ± 23.35^{a}	552.07 ± 37.11^{a}
Day 21	969.07 ± 62.13^{a}	990.53 ± 72.89^{a}	994.13 ± 47.71^{a}	1023.07 ± 67.57^{b}
Day 28	1581.33 ± 105.09^{a}	1617.27 ± 102.86^{a}	1622.00 ± 117.26^{a}	1670.67 ± 99.66^{a}
Day 35	1966.00 ± 147.83^{a}	2233.67 ± 116.33^{b}	2237.33 ± 291.53^{b}	2394.00 ± 221.89^{c}

*Mean with different superscript letters in the same rows are significantly different at (p <0.05).

TABLE 3. The effects of nucleotides and collagen on experimental groups according to feed intake:

	Control	Nucleotides	Collagen	Nucleotides and Collagen
	n=15	n=15	n=15	n=15
Day 7	183.12 ± 5.08[a]	180.07 ± 3.36[a,b]	178.41 ± 6.43[b]	184.15 ± 4.77[a,b]
Day 14	394.71 ± 13.54[a]	384.99 ± 97.14[a,b]	386.95 ± 24.06[b]	396.29 ± 12.04[a]
Day 21	626.86 ± 31.78[a]	622.38 ± 26.92[a]	619.98 ± 31.30[a]	631.37 ± 28.73[a]
Day 28	759.19 ± 53.48[a]	841.73 ± 81.33[b]	752.58 ± 33.17[a]	761.60 ± 58.31[a]
Day 35	1303.15 ± 84.66[a]	1326.34 ± 85.68[a]	1273.61 ± 67.46[a]	1313.40 ± 72.28[a]

Means within the same raw carrying different superscript letters are significantly at (p <0.05).

TABLE 4. Comparison between study groups according to feed conversion ratio:

	Control	Nucleotides	Collagen	Nucleotides and Collagen
	n=15	n=15	n=15	n=15
Day 7	0.98 ± 0.29[a]	0.90 ± 0.11[a]	0.87 ± 0.05[a]	0.89 ± 0.09[a]
Day 14	0.76 ± 0.07[a]	0.73 ± 0.20[a]	0.72 ± 0.05[a]	0.72 ± 0.05[a]
Day 21	0.65 ± 0.04[a]	0.63 ± 0.04[a]	0.62 ± 0.04[a]	0.62 ± 0.05[a]
Day 28	0.48 ± 0.04[a]	0.52 ± 0.05[b]	0.47 ± 0.05[a]	0.46 ± 0.03[a]
Day 35	0.67 ± 0.07[a]	0.60 ± 0.05[b]	0.58 ± 0.08[b]	0.55 ± 0.05[b]

Means within the same raw carrying different superscript letters are significantly at (p <0.05).

TABLE 5. Comparison between study groups according to body weight gain:

	Control	Nucleotides	Collagen	Nucleotides and collagen
	n=15	n=15	n=15	n=15
Day 7	153.87 ± 37.43[a]	159.80 ± 23.91[a]	145.87 ± 37.82[a]	166.33 ± 20.94[a]
Day 14	478.33 ± 36.81[a]	489.47 ± 46.12[a]	494.47 ± 24.36[a]	508.73 ± 37.41[b]
Day 21	925.53 ± 61.60[a]	946.60 ± 72.99[a]	950.80 ± 47.83[a]	979.73 ± 68.10[b]
Day 28	1537.80 ± 104.74[a]	1573.33 ± 103.37[a]	1578.67 ± 117.26[a]	1627.33 ± 100.00[b]
Day 35	1922.47 ± 147.65[a]	2189.73 ± 116.00[b]	2194.00 ± 292.39[b]	2350.67 ± 221.52[c]

Means within the same raw carrying different superscript letters are significantly at (p <0.05).

TABLE 6. Comparison between study groups according to average daily gain:

	Control	Nucleotides	Collagen	Nucleotides and Collagen
	n=15	n=15	n=15	n=15
Day 7	21.98 ± 5.35[a]	22.83 ± 3.42[a]	23.04 ± 1.82[a]	23.76 ± 2.99[a]
Day 14	34.17 ± 2.63[a]	34.96 ± 3.29[a]	35.32 ± 1.74[a]	36.34 ± 2.67[b]
Day 21	44.07 ± 2.93[a]	45.08 ± 3.48[a]	45.28 ± 2.28[a]	46.65 ± 3.24[b]
Day 28	54.92 ± 3.74[a]	56.19 ± 3.69[a]	56.38 ± 4.19[a]	58.12 ± 3.57[b]
Day 35	54.93 ± 4.22[a]	62.56 ± 3.31[b]	62.69 ± 8.35[b]	67.16 ± 6.33[c]

Means within the same raw carrying different superscript letters are significantly at (p <0.05).

TABLE 7. Comparison between study groups according to biochemical studies:

	Control	Nucleotides	Collagen	Nucleotides and Collagen
WBCs (thousand cells/cu mm)	188.72 ± 4.89[a]	195.35 ± 10.43[b]	199.78 ± 6.57[c]	211.31 ± 7.14[d]
RBCs (million cells/cu mm)	2.23 ± 0.11[a]	2.54 ± 0.27[b]	2.49 ± 0.11[c]	2.81 ± 0.27[d]
HB (grams per deciliter)	9.68 ± 0.34[a]	10.30 ± 1.15[ab]	11.00 ± 0.44[bc]	11.69 ± 1.19[c]
Thrombocytes (thousand cells/cu mm)	10.53 ± 1.88[a]	10.00 ± 2.93[a]	16.67 ± 5.49[b]	12.80 ± 2.86[c]
HCT (%)	27.05 ± 1.56[a]	31.52 ± 2.76[b]	30.63 ± 1.04[b]	34.63 ± 3.31[c]
AlT (units per liter)	2.45 ± 0.55[a]	2.74 ± 0.86[a]	2.65 ± 0.56[a]	2.45 ± 0.42[a]
AST (units per liter)	475.60 ± 78.50[a]	492.40 ± 54.91[a]	496.47 ± 98.78[a]	495.93 ± 74.22[a]
Total Bilirubin (milligrams per deciliter)	0.17 ± 0.08[a]	0.23 ± 0.09[b]	0.22 ± 0.06[ab]	0.25 ± 0.10[b]
Total Protein (grams per deciliter)	3.16 ± 0.15[a]	3.50 ± 0.17[b]	3.45 ± 0.19[b]	3.49 ± 0.24[b]
Albumin (grams per deciliter)	1.91 ± 0.08[a]	1.93 ± 0.06[a]	1.80 ± 0.10[b]	1.84 ± 0.12[b]
Globulin (grams per deciliter)	1.33 ± 0.10[a]	1.55 ± 0.16[b]	1.63 ± 0.23[b]	1.57 ± 0.40[b]
Uric Acid (milligrams per deciliter)	2.41 ± 0.35[a]	2.41 ± 0.27[a]	2.65 ± 0.69[a]	4.85 ± 0.96[b]
Creatinine (milligrams per deciliter)	2.45 ± 1.24[a]	4.26 ± 1.18[b]	4.89 ± 1.26[b]	3.47 ± 0.86[c]
Cholesterol (milligrams per deciliter)	120.15 ± 7.95[a]	115.27 ± 16.69[ab]	110.72 ± 6.28[b]	103.91 ± 8.28[c]
TG (milligrams per deciliter)	40.48 ± 4.36[a]	59.44 ± 7.16[b]	37.79 ± 10.06[a]	43.43 ± 5.82[a]
HDL (milligrams per deciliter)	39.44 ± 4.61[a]	36.37 ± 2.43[ab]	33.60 ± 7.04[b]	29.59 ± 1.83[c]
LDL (milligrams per deciliter)	73.53 ± 9.76[a]	67.43 ± 13.84[a]	72.02 ± 7.98[a]	58.16 ± 8.14[b]
TAC (millimoles/liter)	0.32 ± 0.04[a]	0.19 ± 0.05[b]	0.17 ± 0.05[b]	0.19 ± 0.04[b]
ND ab titer	2.67 ± 1.18[a]	3.73 ± 0.46[b]	4.53 ± 0.74[c]	3.93 ± 0.59[b]

Means within the same raw carrying different superscript letters are significantly at (p <0.05).

TABLE 8. Comparison between study groups according to antioxidant parameters:

	Control	Nucleotides	Collagen	Nucleotides and Collagen
MDA (mmol/ml)	5.5±1.2 [a]	5.1±1.2 [b]	4.7±1.1 [c]	4.4±1 [d]
SOD (U/ml)	61.7±6.5 [a]	66.4±6.9 [b]	71.2±7.2 [c]	76.4±7.9 [d]
GPx (U/ml)	71.4±6.7 [a]	76.5±6.8 [b]	82.6±7.7 [c]	88.6±8.8 [d]

Means within the same raw carrying different superscript letters are significantly at (p <0.05).

TABLE 9. Comparison between study groups according to histopathological examination.

	Control	Nucleotides	Collagen	Nucleotides and Collagen
VH (µm)	220.23 ± 16.28[a]	240.42 ± 18.79[b]	200.65 ± 9.63[c]	339.48 ± 8.00[d]
CD (µm)	40.10 ± 4.56[a]	35.27 ± 2.19[b]	59.43 ± 5.35[c]	50.30 ± 2.40[d]
VH/CD ratio	5.57 ± 0.82[a]	6.84 ± 0.70[b]	3.39 ± 0.32[c]	6.76 ± 0.35[d]
Villous Width (µm)	15.13 ± 0.93[a]	16.82 ± 1.03[b]	19.95 ± 1.64[c]	22.12 ± 1.57[d]
Villous Surface Area (µm)	500.60 ± 24.12[a]	496.57 ± 22.66[b]	551.63 ± 24.03[c]	795.55 ± 27.03[d]
Villous Perimeter (µm)	488.08 ± 23.30[a]	350.25 ± 26.85[c]	400.75 ± 27.90b	604.07 ± 24.51[d]

Means within the same raw carrying different superscript letters are significantly at (p <0.05).

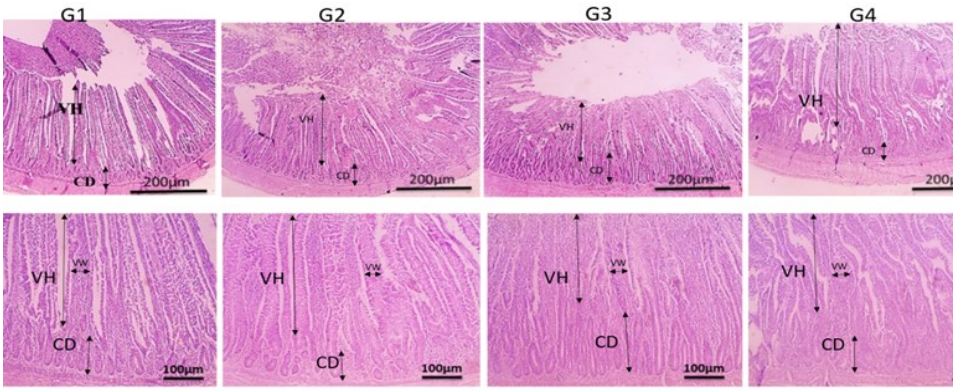

Fig.1. Microscopic picture of H&E stained sections from intestine from groups G1-G4 showing no structural damage. Marked increase of villous height is recognized in G4 supplemented with collagen+nucleotides (Magnification x:40 bar 200 and x:100 bar 100).

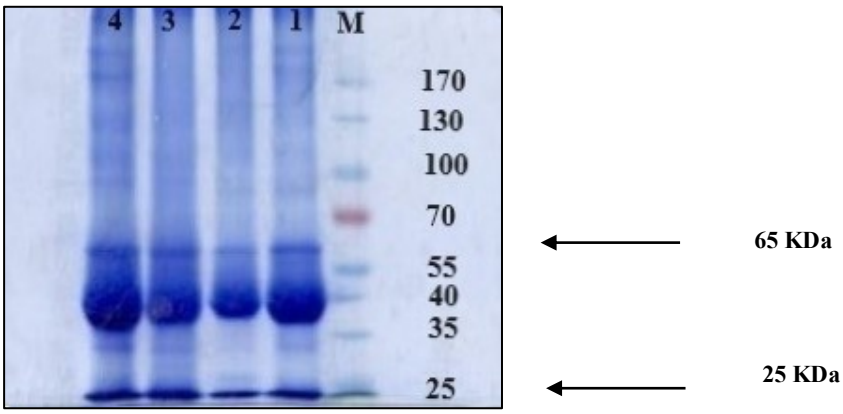

Fig. 2. Purified serum proteins by SDS-PAGE. M: protein marker, Lane1: Control group, Lane 2: Nucleotides group, Lane 3: Collagen group and Lane 4: Nucleotides and Collagen group.

References

1. Jaiswal, S.K., Chaturvedani, A.K., Raza, M., Dilliwar, L., Dhruw, K. and Sahu, V. Review: Natural growth promoters, alternative to antibiotic growth promoters on poultry. *International Journal of Science, Environment and Technology*, **6**, 254-259 (2017).
2. Superchi, P., Saleri, R., Borghetti, P., De Angelis, E., Ferrari, L., Cavalli, V. and Sabbioni, A. Effects of dietary nucleotide supplementation on growth performance and hormonal and immune responses of piglets. *Animals,* **6**(6), 902-908 (2012).
3. Hess, J. R. and Greenberg, N. A. The role of nucleotides in the immune and gastrointestinal systems: potential clinical applications. *Nutrition and Clinical Practices,* **27**(2), 281-294 (2012).
4. Alizadeh, M., Rodriguez-Lecompte, J. C., Yitbarek, A., Sharif, S., Crow, G. and Slominski, B. A. Effect of yeast-derived products on systemic innate immune response of broiler chickens following a lipopolysaccharide challenge. *Poultry Sciences,* **95**(10), 2266-2273 (2016b).
5. Rady, W. F., Sayed, A. B. N. and Abdel-Raheem, H. A. Effect of dietary supplementation of echinacea and nucleotides on productive performance, intestinal histomorphology and gene expression of broiler chickens. *Assuit Veterinary Medical Journal,* **69**(176), 141-155 (2023).
6. Salah, M., Suprijatna, E., Djauhari, M.L. and Dwi, Y.V. The effects of nucleotide supplementation on the productivity, immune response and meat quality of broiler chicken reared under different environmental conditions. *Livestock Research and Rural Development,* **31**(11), 174 (2019).
7. Rafique, K., Rahman, A. and Mahmood, M. Effect of dietary supplementation of different levels of saccharomyces cerevisiae on growth performance and hematology in broiler. *Indian Journal of Animal Research,* **54**(1), 59-64 (2020).
8. Zahran, R. H., Khader, N. E., & Ahmed, T. E. Effect of dietary nucleotide supplementation on broiler performance and economic efficiency. *Benha Veterinary Medical Journal,* **39**(1), 34-39 (2020).

9. Wu, C., Yang, Z., Song, C., Liang, C., Li, H., Chen, W. and Xie, Q. Effects of dietary yeast nucleotides supplementation on intestinal barrier function, intestinal microbiota, and humoral immunity in specific pathogen-free chickens. *Poultry Science,* **97**(11), 3837-3846 (2018).

10. Maurer, T., Stoffel, M. H., Belyaev, Y., Stiefel, N. G., Vidondo, B., Küker, S. and Balmer, J. Structural characterization of four different naturally occurring porcine collagen membranes suitable for medical applications. *PLoS One,* **13**(10), e0205027 (2018).

11. Baker, D.H. Advances in protein–amino acid nutrition of poultry. *Amino Acids,* **37**(1), 29-41.2009)).

12. Tsiagbe, V. K., Cook, M. E., Harper, A. E. and Sunde, M. L. Enhanced immune responses in broiler chicks fed methionine-supplemented diets. *Poultry Sciences,* **66**(7), 1147-1154 (1987).

13. Aschkenasy, A. Dietary Proteins and Amino Acids in Leucopoiesis: Recent Hematological and Immunological Data1. *World Review of Nutrition and Dietetics,* **21**, 151-197 (1975).

14. Wang, W., Wu, Z., Dai, Z., Yang, Y., Wang, J. and Wu, G. Glycine metabolism in animals and humans: implications for nutrition and health. *Amino Acids,* **45**, 463e477 (2013).

15. Khomich, O. A., Kochetkov, S. N., Bartosch, B. and Ivanov, A.V. Redox biology of respiratory viral infections. *Viruses,* **10**(8), 392 (2018).

16. Ospina-Rojas, I.C., Murakami, A.E., Oliveira, C.A.L. and Guerra, A.F.Q.G. Supplemental glycine and threonine effects on performance, intestinal mucosa development, and nutrient utilization of growing broiler chickens. *Poultry Sciences,* **92**(10), 2724-2731 (2013).

17. Wu, G., Bazer, F.W., Burghardt, R.C., Johnson, G.A., Kim, S.W., Knabe, D. A. and Spencer, T. E. Proline and hydroxyproline metabolism: implications for animal and human nutrition. *Amino Acids*, **40**, 1053-1063 (2011).

18. Phang, J. M., Liu, W. E. I., and Zabirnyk, O. Proline metabolism and microenvironmental stress. *Annual Reviews in Nutrition,* **30**(1), 441-463 (2010).

19. Aviagen, R. Ross 308 nutrition specifications. Scotland (UK), Aviagen.2019)).

20. Meiattini, F., Prencipe, L., Bardelli, F., Giannini, G. and Tarli, P. The 4-hydroxybenzoate/4-aminophenazone chromogenic system used in the enzymic determination of serum cholesterol. *Clinical Chemistry,* **24**(12), 2161-2165 (1978).

21. Wahlefeld, A.W. Triglycerides determination after enzymatic hydrolysis. In Methods of enzymatic analysis (pp. 1831-1835). Academic Press (1974).

22. Burstein, M. and Scholnick, H.R. Lipoprotein-polyanion-metal interactions. In Advanced Industry in Lipids Research (Vol. **11**, pp. 67-108). Elsevier (1973).

23. Friedewald, W. T., Levy, R. I. and Fredrickson, D.S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, **18**(6), 499-502 (1972).

24. Ohnishi, S. T. and Barr, J. K. A simplified method of quantitating protein using the biuret and phenol reagents. *Analitical Biochemistry,* **86**(1), 193-200 (1978).

25. Doumas, B. T., Watson, W. A., and Biggs, H. G. Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica Chimica Acta,* **31**(1), 87-96 (1971).

26. Doumas, B.T. and Biggs, H.G. Standard methods of clinical chemistry. *Academic Press Chicago*, **7**, 175-189 (1972).

27. Kageyama, N.A. direct colorimetric determination of uric acid in serum and urine with uricase-catalase system. *Clinica Chimica Acta*, **31**(2), 421-426 (1971).

28. Fossati, P., Prencipe, L. and Berti, G. Enzymic creatinine assay: a new colorimetric method based on hydrogen peroxide measurement. *Clinical Chemistry,* **29**(8), 1494-1496 (1983).

29. Puppalwar, P.V., Goswami, K. and Dhok, A. Review on "evolution of methods of bilirubin estimation". *IOSR Journal of Dental Medical Sciences,* **1**(3), 17-28 (2012).

30. Reitman, S. and Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology,* **28**(1), 56-63 (1957).

31. Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S. and Cosic, V. Method for the measurement of antioxidant activity in human fluids. *J. Clin. Pathol.,* **54**(5), 356-361 (2001).

32. Spackman, E. and Sitaras, I. Hemagglutination inhibition assay. *Ani. Influ. V.: Meth. Proto.,* 11-28 (2020).

33. Bancroft, J.D., Layton, C. and Suvarna, S.K. Theory and Practice of Hitological Techniques.7a Edição. Churchill Livingstone, New York, (2013).

34. Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* **227**(5259), 680-685 (1970).

35. Snedecor, G. W. and Cochran, W. G. Statistical methods. 8th Ed East West Press Pvt. Ltd., New Delhi, India, 313, (1994).

36. Rutz, F., Xavier, E.G., Anciuti, M.A., Roll, V.F.B. and Rossi, P. The role of nucleotides in improving broiler prestarter diets: the Brazilian experience (2007).

37. Saravanan, E., Soren, S. and Samanta, G. Replacement of fishmeal with yeast nucleotide on the performance in ducklings. *Indian Journal of Poultry Science,* **45**(1), 74-76 (2010).

38. Zavarize, K.C., Sartori, J.R., Pelícia, V.C., Pezzato, A.C. and Araujo, P.C. Desempenho de frangos de corte criados no sistema alternativo suplementados com L-glutamina e nucleotídeos. In Anais da Conferência APINCO (2007).

39. Pelícia, V.C., Sartori, J.R., Zavarize, K.C., Pezzato, A.C., Stradiotti, A.C., Araujo, P.C. and Madeira, L. A. Effect of nucleotides on broiler performance and carcass yield. *Brazilian Journal of Poultry Sciences,* **12**, 31-34 (2010).

40. Hassanein, I.I., Metwally, A.E. and Abd Elbaky, H.E.M. Effects of dietary nucleotides supplementation on the performance of broiler chicks. *Slovenian Veterinary Research,* **60**(Suppl., 25), 1575-2022 (2023).

41. Leung, H., Patterson, R., Barta, J.R., Karrow, N. and Kiarie, E. Nucleotide-rich yeast extract fed to broiler chickens challenged with Eimeria: impact on growth performance, jejunal histomorphology, immune system, and apparent retention of dietary components and caloric efficiency. *Poultry Sciences*, **98**, 4375-4383 (2019b).

42. Alizadeh, M.; Rodriguez-Lecompte, J. C.; Rogiewicz, A.; Patterson, R. and Slominski, B. A. Effect of yeast-derived products and distillers dried grains with solubles (DDGS) on growth performance, gut morphology, and gene expression of pattern recognition receptors and cytokines in broiler chickens. *Poultry Science,* **95**, 507-517 (2016a).

43. Wu, G. Dietary requirements of synthesizable amino acids by animals: a paradigm shift in protein nutrition. *Journal of Animal Science and Biotechnology,* **5,** 1-12 (2014).

44. Nurubhasha, R., Sampath Kumar, N. S., Thirumalasetti, S. K., Simhachalam, G., & Dirisala, V. R. Extraction and characterization of collagen from the skin of Pterygoplichthys pardalis and its potential application in food industries. Food science and biotechnology, **28**, 1811-1817(2019)..

45. Hou YongQing, H.Y., Yao Kang, Y.K., Yin YuLong, Y.Y. and Wu GuoYao, W.G. Endogenous synthesis of amino acids limits growth, lactation, and reproduction in animals (2016).

46. Kawamoto, Y., & Kaibara, M. (1990). Procoagulant activity of collagen. Effect of difference in type and structure of collagen. *Biochimica et Biophysica Acta (BBA)-General Subjects,* **1035**(3), 361-368.

47. Carew, L.B., McMurtry, J.P. and Alster, F. A. Effects of methionine deficiencies on plasma levels of thyroid hormones, insulin-like growth factors-I and-II, liver and body weights, and feed intake in growing chickens. *Poultry Sciences*, **82**(12), 1932-1938 (2003).

48. Kano, Y., Sakamoto, S., Kasahara, T., Kusumoto, K., Hida, K., Suda, K. and Takaku, F. Methionine dependency of cell growth in normal and malignant hematopoietic cells. *Cancer Research,* **42**(8), 3090-3092 (1982).

49. Saito, M., Kiyose, C., Higuchi, T., Uchida, N., and Suzuki, H. Effect of collagen hydrolysates from salmon and trout skins on the lipid profile in rats. *Journal of Agriculture and Food Chemistry*, **57**(21), 10477-10482 (2009).

50. Jang, Y.D., Kang, K.W., Piao, L.G., Jeong, T.S., Auclair, E., Jonvel, S., D'Inca, R. and Kim, Y.Y. Effects of live yeast supplementation to gestation and lactation diets on reproductive performance, immunological parameters and milk composition in sows. *Livestock Science*, **152**, 167-173 (2013).

51. Rudolph, F.B., Kulkarni, A.D., Fanslow, W.C., Pizzini, R.P., Kumar, S. and Buren, C.V. Role of RNA as a dietary source of pyrimidines and purines in immune function. Nutrition. **6**(1), 45-52 (1990).

52. Zhao, T. Lum, J.J. Methionine cycle-dependent regulation of T cells in cancer immunity. *Frontiers in Oncology,* **12**, 969563 (2022).

53. Bouyeh, M. Effect of excess lysine and methionine on immune system and performance of broilers. *Ann. Biol. Res,* **3**(7), 3218-3224(2012)..

54. Yamamoto, S., Wang, M.F., Adjei, A.A. and Ameho, C.K. Role of nucleosides and nucleotides in the immune system, gut reparation after injury, and brain function. *Nutrients,* **13**(4), 372-374 (1997).

55. Wu, G., Bazer, F. W., Burghardt, R. C., Johnson, G. A., Kim, S. W., Knabe, D. A., ... & Spencer, T. E. (2011). Proline and hydroxyproline metabolism: implications for animal and human nutrition. *Amino acids,* **40**, 1053-1063.

56. Leslie, G. A. and Clem, L. W. Phylogeny of immunoglobulin structure and function: III. Immunoglobulins of the chicken. Journal of Experemental Medicine, 130(6), 1337 (1969).

57. Michael, A., Meenatchisundaram, S., Parameswari, G., Subbraj, T., Selvakumaran, R. and Ramalingam, S. Chicken egg yolk antibodies (IgY) as an alternative to mammalian antibodies. *Indian Journal of Science and Technology,* **3**(4), 468-74 (2010).

58. Amro, W. A., Al-Qaisi, W. and Al-Razem, F. Production and purification of IgY antibodies from chicken egg yolk. *Journal of Genetic Engineering and Biotechnology.,* **16** (1), 99-103 (2018).

59. Dong, W., Zhang, H., Huang, H., Zhou, J., Hu, L., Lian, A. and Zhu, R. Chicken IgY Fc linked to Bordetella avium ompA and Taishan Pinus massoniana pollen polysaccharide adjuvant enhances macrophage function and specific immune responses. *Frontiers in Microbiology,* **7**, 1708 (2016).

دراسات كيميائية حيوية على تأثير الكولاجين والنيوكليوتيدات على نمو دجاج التسمين

ابتهال هاشم محمود سليمان، مجدي محفوظ يوسف، منار رفعت

قسم الكيمياء، كلية العلوم، جامعة المنصورة، مصر.

الملخص

تم إجراء هذه التجربة لدراسة تأثير الكولاجين والنيوكليوتيدات كاضافات أعلاف منفردة أو مجتمعة معًا على كلاً من: كفاءة النمو والأداء، القياسات الحيوية في مصل الدم، نسبة الإجهاد التأكسدي، الحالة المناعية والتركيب النسيجي للأمعاء في دجاج التسمين.

تمت الدراسة على عدد (60) دجاجة من سلالة روص 306 عمر 1 يوم، تم تقسيم الدجاج الى أربع مجموعات كل مجموعة تحتوي على عدد (15) كتكوت، تم تقديم أربع علائق تم تحضيرها خصيصًا حسب احتياجات السلالة، وتم تقديم العلائق كالآتي: المجموعة الاولى: المجموعة الضابطة وتم تقديم عليقة تحتوي على المكونات والاحتياجات الأساسية، المجموعة الثانية: العليقة الأساسية بالإضافة إلى 0.5 جرام نيوكليوتيدات لكل 1 كجم عليقة، المجموعة الثالثة: العليقة الأساسية بالإضافة إلى 100 ملجم كولاجين لكل 1 كجم عليقة، المجموعة الرابعة: العليقة الأساسية بالإضافة إلى 0.5 جرام نيوكليوتيدات و 100 ملجم كولاجين لكل 1 كجم عليقة، واستمرت الدراسة لمدة 35 يوماً.

تم تسجيل النتائج والملاحظات، وقد أظهرت الدراسة أن هناك زيادة معنوية في وزن الجسم والوزن اليومي المكتسب ومتوسط الوزن المكتسب في المجموعة الثانية والثالثة والرابعة مقارنة بالمجموعة الضابطة وأن أفضل النتائج كانت في المجموعة الرابعة، أيضاً أظهرت الدراسة انخفاض معنوي في قيمة العلف المستهلك وأيضا تحسن في نسبة التحويل الغذائي مقارنة بالمجموعة الضابطة. من النتائج اللتي لخصت إليها الدراسة أيضاً أن استهلاك النيوكليوتيدات والكولاجين في العلف أدى إلى زيادة عدد كريات الدم البيضاء والحمراء ونسب الهيموجلوبين والهيماتوكريت في الدم، أيضًا زيادة نسب الصفائح الدموية نتيجة لاستهلاك الكولاجين في العلف،تم تسجيل زيادة واضحة في البروتينات في مصل الدم، زيادة نسبة الإنزيمات المضادة للأكسدة كالجلوثيون بيروكسيديز و السوبر أوكسيد ديسميوتيز، وانخفاض نسبة المالونداي ألدهيد والذي تعد زيادة معدله دليلاً واضحًا على زيادة الإجهد التأكسدي داخل الخلايا الحية، انخفاض الكوليسترول الكلي والكوليسترول النافع عالي الكثافة وأيضا الضار منخفض الكثافة، تم تسجيل زيادة ملحوظة في تركيز الدهون الثلاثية. إضافة النيوكليوتيدات والكولاجين أدى إلى تحسين الاستجابة المناعية عن طريق زيادة انتاج الأجسام المناعية كنتيجة للتحفيز المناعي بالتحصين ضد فيروس النيوكاسل. إضافة النيوكليوتيدات والكولاجين إلى العلف أدى إلى التحسين من صحة الأمعاء والتركيب النسيجي لها حيث لم تظهر النتائج أي نخر أو تآكل في أي مجموعة عند صبغ الأنسجة ودراستها.

خلصت هذه التجربة إلى أن إضافة النيوكليوتيدات والكولاجين الى علف دجاج التسمين أدى إلى التحسن الملحوظ من أداء ونمودجاج التسمين وتقليل الإجهد التأكسدي في الخلايا الحية عن طريق زيادة انتاج الانزيمات المضادة للأكسدة، أيضًا تحسين الإستجابة المناعية وتحسين التركيب النسيجي وصحة الأمعاء.

الكلمات الدالة: التركيب النسيجي للأمعاء، الحالة المناعية، النمو والأداء، الكولاجين، النيوكليوتيدات، دجاج التسمين.