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Analysis of the liver transcriptome in broiler chicken fed with dietary nucleotides and/or beta-glucan revealed enhancement in growth parameters, intestinal morphology, and some biochemical parameters

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ABSTRACT

The experimental protocol aimed to assess the influence of nucleotides and/or β-glucan on the production performance, growth-related gene expression in the broiler chickens' liver tissue, intestinal histomorphology, and some biochemical parameters. One hundred forty-four newly hatched chicks were categorized into four groups based on the supplements: a control group, a group supplemented with nucleotides (200 mg/kg diet), a group with β-glucan (1 g/kg diet), and a group with both nucleotides and β-glucan. The study's findings showed that, when compared to the control group, all dietary supplemented groups showed a substantial ($P < 0.05$) improvement in production parameters without having a negative impact on the general health of broilers. Additionally, the treatments positively impacted the expression of some genes associated with growth performance in the broiler chickens' liver tissue, such as the insulin-like growth factor 1 (IGF-1) and growth hormone receptor (GHR). The supplemented groups also demonstrated a significant ($P < 0.05$) enhancement in intestinal histomorphology, characterized by increased villi length, crypt depth, and goblet cell number. Furthermore, dietary supplementation of nucleotides and/or β-glucan led to a significant ($P < 0.05$) elevation in total protein and albumin levels, with no significant effect on globulin, AST, and ALT. The conclusion can be drawn that adding nucleotides along with β-glucan to the diet led to enhancements in growth performance, the expression of genes related to growth (GHR and IGF-1), intestinal histomorphology, and certain biochemical parameters (specifically total protein and albumin).

Introduction

"Raise Without Antibiotic," "Antibiotic Free," and "No Antibiotic Ever" are new trends in production strategies in the poultry industry (Marshall and Levy, 2011). For many years, antibiotics have been utilized as growth enhancers. Due to lingering contamination and the growth of resistant pathogenic bacteria, most antibiotics used in animal production have been outlawed. This has serious consequences for the continued effectiveness of these necessary medications (Xiong *et al*., 2015; Zou *et al*., 2019). Consequently, developing other approaches to maintain animal performance and shield animals from the illness, primarily utilizing high-condition animals, feeding procedures, and nutrition (Amer *et al*., 2020, 2021b).

Nucleotides are a natural alternative that can benefit the diet of young animals by providing them with bioactive substances (Sauer *et al*., 2009). It is considered a fundamental component of nucleic acids and consists of a nitrogen base, a pentose sugar, and one or more phosphate groups (Carver and Allan Walker, 1995; Devresse, 2000). Cells can obtain nucleotides from three different sources: de novo nucleotide synthesis, the salvage pathway, and dietary nucleotide supplementation (Hess and Greenberg, 2012). De novo synthesis is a process that consumes a lot of energy in the form of ATP (Carver and Allan Walker, 1995). The salvage pathway, which recycles approximately 90% of purine bases, is believed to rely on the availability of free bases and demands less energy compared to de novo synthesis (Cosgrove, 1998). Nucleotides that are supplied externally are necessary for tissues that have limited capacity for de novo synthesis, such as intestinal mucosa, erythrocytes, leucocytes, lymphocytes, and hematopoietic cells in the bone marrow (Sanderson and He, 1994; Gil and Uauy, 1995).

Nucleotides play a crucial role in energy metabolism, co-enzyme formation, and cellular defense mechanisms (Dawood *et al*., 2018). They are recognized as highly functional nutrients with significant potential to enhance broiler chicken productivity, particularly in challenging conditions (Salah *et al*., 2019). The nucleotides' adding in the diet has the capacity to enhance nutrient absorption from the small intestine by increasing villi height. Moreover, it has been shown that higher nucleotide levels effectively reduce the amount of dangerous bacteria in excreta, such as Clostridium perfringens and Escherichia coli (Abd-El-Wahab *et al*., 2019).

Beta-glucan is one of the most widely embraced prebiotics and exhibits a diverse range of biological functions, such as boosting immune activity, providing resistance against infections, and regulating glucose levels (Xiong *et al*., 2015). It is an integral structural component found in the cell walls of various sources, encompassing fungi, bacteria, algae, and yeast. Introducing β-glucan into diets has demonstrated enhancements in feed conversion efficiency and growth rates (Levine *et al*., 2018). Studies indicate that introducing yeast-derived components into the diets of broilers has a positive influence on parameters such as body weight (BW), feed conversion ratio (FCR), and body weight gain (BWG), and feed intake (FI). This is accomplished by promoting beneficial microflora in the gastrointestinal tract and contributing to the gut's development (Muthusamy *et al*., 2011; Ghosh *et al*., 2012). Elevated villus height and an extreme villus height to crypt depth ratio have been linked to chick diets supplemented with β-glucan as opposed to unsupplemented chicks (Morales-López *et al*., 2009; Shao *et al*., 2013). Adding β-glucan to the diet significantly increases the goblet cells' number in the jejunum of chicks (Shao *et al*., 2013), indicating that β-glucans contribute to the well-being of the gas-

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trointestinal tract (Kim and Ho, 2010; Lourenço *et al*., 2015). Thus, this study's aim was to measure the impact of dietary supplementation with nucleotides, either alone or in combination with β-glucan, on factors such as growth-related gene expression, intestinal histomorphology, growth performance, and some biochemical parameters.

Materials and methods

Under the guidance of the Institutional Animal Care and Use Committee, this study was done at Benha University's Faculty of Veterinary Medicine's Animal Wealth Development Department, with approval number BUFVTM 03-12-22.

Additives

Nucleotides (Ohly-GO® Nucleo): an enzymatically hydrolyzed yeast extract derived from Saccharomyces cerevisiae was provided by Ohly-GMbH, Germany.

Beta-glucan (AletaTM): a source of 1,3-beta glucans derived from alga (Euglena gracilis), with a concentration greater than 50% of 1,3-beta glucans, was provided by Kemin Industries, Belgium). Both nucleotides and β-glucan were added on top at doses of 200 mg/kg diet and 1 g/kg diet, respectively.

Experimental design and feeding program

One day old healthy broiler chicks (cobb 500) of both sexes, totaling 144 with an overall body weight of 46.76 ± 0.145 g were obtained from a profitable hatchery. The broiler chicks were randomly allocated to four dietary regimen groups. each comprising 36 birds; within each group, the birds were further divided into three replicates, with each replicate consisting of 12 birds. According to supplements, Group 1 was provided with the control diet, Group 2 was supplemented with the control diet plus nucleotides (200 mg/kg diet), Group 3 was given the control diet plus β-glucan (1 g/kg diet), and Group 4 was administered the control diet with nucleotides (200 mg/kg diet) plus β-glucan (1 g/kg diet).

The chicks were housed in a hygienic, well-ventilated environment. Ten broilers per square meter would be the stocking density. The chamber floor was bedded with fresh, clean, deep litter made of 5-cm-thick wood shavings that would be regularly replaced. Each partition received one feeder and one waterer. During the trial, the chicks had complete access to food and water. A lighting schedule of 23 hours of light and 1 hour of darkness was implemented to minimize the chicks' activity. The initial temperature in the house was 35°C, gradually decreasing by 2°C per week until reaching 24°C by the end of the experiment. Windows and negative-pressure fans were used to provide ventilation. All chicks were immunized against prevalent viral diseases, including Newcastle, bursal disease, and infectious bronchitis. The chicks were provided with a nutritionally balanced diet in accordance with the recommended nutrient levels for the Cobb 500 broiler strain, as shown in Table 1. The experimental feeding regimen consisted of three stages: starter for the first 0 to 10 days, grower for days 11 to 24, and finisher for days 25 to 35.

Assessment of growth performance

Initially, each bird's weight was measured individually to document their initial body weight. Subsequently, BWG, BW, and FI were assessed on a weekly basis throughout the experiment. The feed conversion ratio for the experimental chicks was computed following the method described by Wagner *et al*. (1983).

"FCR= " ("FI (g)"/"bird/week")/("BWG (g) "/"bird/week")

Table 1. Physical and chemical composition (%) of the experimental groups; starter, grower, and finisher diets.

Ingredients	Stage		
	Starter	Grower	Finisher
	(%)	(%)	(%)
Yellow corn	53.54	57.36	59.04
Soya bean meal-46	33.1	32.9	30.6
Corn gluten meal	4	0.5	0.5
Soya oil	2.5	3.5	4.5
Di calcium phosphate	2.28	1.85	1.64
Wheat Bran	2	1.5	1.3
Limestone	0.95	0.83	1
L-Lysine	0.31	0.25	0.22
DL-Methionine	0.28	0.28	0.21
Vit&. Min. Mixture	0.3	0.3	0.3
Sodium chloride	0.26	0.28	0.29
Sodium bicarbonate	0.24	0.2	0.16
Choline chloride	0.11	0.08	0.09
L -Threonine	0.06	0.1	0.08
Antimycotoxin	0.05	0.05	0.05
Antioxidant	0.01	0.01	0.01
Anticlostridia	0.01	0.01	0.01
Chemical composition			
ME (Kcal \ Kg diet)	2,953.35	3,024.39	3,103.39
Crude protein %	22	20	19
Crude fat %	5.04	5.99	7.01
Crude fiber %	2.35	2.34	2.28
Lysine %	1.32	1.24	1.15
Methionine %	0.62	0.57	0.49
Methionine + cysteine %	0.98	0.9	0.81
Threonine %	0.86	0.84	0.78
Tryptophan %	0.25	0.24	0.22
Calcium %	0.99	0.84	0.85
Available phosphorus %	0.5	0.42	0.38
Chloride %	0.25	0.25	0.25
Sodium %	0.17	0.17	0.16
Potassium %	0.86	0.85	0.81

Premix supplies the following: Vitamin D (5000 IU), Vitamin A (13000 IU), Vitamin E (80 mg), Vitamin B1 (3 mg), Vitamin K3 (3 mg), Vitamin B2 (9 mg), Vitamin B6 (3 mg), Niacin (60 mg), Vitamin B12 (0.02 mg), Pantothenic acid (15 mg), Biotin (0.15 mg), Folic acid (2 mg), Iron (40 mg), Copper (15 mg), Zinc (100 mg), Manganese (100 mg), Iodine (1 mg) and Selenium (0.3 mg) per 1 kg diet.

Gene expression quantification

Sample collection

On the 35th day of the experiment, thirty-six birds were randomly chosen and euthanized for sample collection. Liver samples were obtained from each bird and stored at -80°C for subsequent analysis.

RNA extraction

Total RNA extraction was carried out utilizing GENEzol™ Reagent (Geneaid-Taiwan Biotech Ltd.) in compliance with the guidelines provided by the manufacturer. Using a rotor Tissue Ruptor (Qiagen, GmbH, Germany), 50 mg of tissue was homogenized with 750 µl of GENEzol™ solution in a sterile collection tube.

RNA quantification using spectrophotometry

The purity and concentration of RNA were settled via measuring the absorbance in a nanodrop spectrophotometer (BMG Lab Tec.GmbH, Germany). The pure RNA A260/A280 ratio is between 1.8 and 2.0. The primer sequences generated using the NCBI Primer-BLAST Software are shown in Table 2.

cDNA and Real-Time PCR Analysis

Synthesis of cDNA was conducted using the ABT H-minus cDNA synthesis kit (Applied Biotechnology Co. Ltd., Egypt) under the manufacturer's guidelines. Applied Biosystem (USA) 7500 Fast Real-time PCR was utilized to conduct a quantitative real-time PCR assessment, and 10 µL of ABT 2X qPCR Mix SYBR (Applied Biotechnology Co. Ltd., Egypt) was utilized. The housekeeping gene selected to normalize the target genes was β-actin. Target genes were amplified utilizing the following thermocycling protocol: 95°C for 3 min; 45 cycles of 95°C for 15 sec, 50–60°C for 30 sec, and 72°C for 30 sec, accompanied by melting curve analysis. The $2^{-\Delta\Delta ct}$ approach was utilized to validate relative gene expression (Livak and Schmittgen, 2001).

Histopathology and morphometric evaluation of intestinal villi absorptive capacity

Sampling

For each treatment group, a 2.5 cm section from the jejunum, duodenum, and ileum had been collected and flushed with physiological saline. The fixation of the tissues was done by immersion in 10% neutral-buffered formalin for three days. After that, following a series of alcohol washes, the samples were dehydrated, and paraffin embedded. After slicing serial 5-µm longitudinal sections with a Leica rotary microtome (RM 2145, Leica Microsystems, Wetzlar, Germany), hematoxylin and eosin (H&E) staining was conducted (Bancroft and Gamble, 2008).

The morphometric assessment of the intestinal villi

By means of Image J analysis software (National Institutes of Health, MD, USA), a histomorphometric analysis was conducted. Villus height (VH), villus width (VW), and crypt depth (CD) were evaluated following the approach defined by Amer *et al*. (2022a). The quantity of goblet cells per millimeter (mm2) of surface area was used to compute the density of goblet cells (GC).

Biochemical blood analysis

On the 35th day, five birds were randomly chosen from each group. Approximately 3 ml of blood was gathered from the wing vein of each bird. The blood was placed in an Eppendorf tube for serum separation (3000 rpm; 15 min; 4ºC). The sera were kept at -20ºC until biochemical analysis. The assay for serum total protein was conducted by the colorimetric technique outlined by Weichselbaum, (1946). The method used to determine serum albumin levels was detailed by Doumas *et al*. (1971). The determination of globulin levels involved the subtraction of plasma albumin from plasma total protein. Additionally, the serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured according to the procedure established by Reitman and Frankel (1957).

Statistical analysis

The statistical analysis was done utilizing SPSS version 21. To compare different groups, one-way ANOVA and Duncan's post hoc tests were implemented (SPSS, Chicago, IL, USA). The homogeneity and normality of variances were assessed utilizing the Shapiro-Wilk W test. The data was subjected to a statistical analysis employing the mean ± standard error and a significance level of $P < 0.05$.

Results

Growth performance

Table 3 displays the results of supplementing the diet with nucleotides and/or β-glucan, encompassing BW), FI, BWG, and FCR as they pertain to the growth performance of broilers. Throughout the experimental period, the outcomes exhibit a statistically significant enhancement ($P < 0.05$) in both BW and BWG, particularly in the group that received nucleotides with β-glucan, followed by the nucleotides group, and finally the β-glucan group, in comparison to the control group. Based on the findings, the inclusion of nucleotides and/or β-glucan in the diet led to a statistically significant reduction in feed intake ($P < 0.05$) relative to the control group for all weeks except the 2nd week, where the nucleotides plus β-glucan group and control group exhibited a significantly higher FI than the nucleotides and β-glucan group. In relation to FCR, the data indicate a statistically significant decrease ($P < 0.05$) in FCR when nucleotides and/or β-glucan were supplemented throughout all weeks, with the exception of the third week, during which the β-glucan group demonstrated a significantly greater FCR ($P < 0.05$) than both the nucleotides group and the nucleotides with β-glucan group.

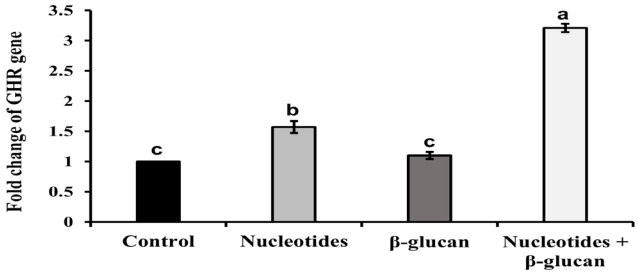

Fig. 1. The impact of supplementing the diet with nucleotides and/or β-glucan on gene expression of GHR. Columns containing distinct letters represent statistically significant results with p value < 0.05.

Gene expression of growth-related genes in liver tissues

The findings regarding the influence of dietary supplementation with nucleotides and/or β-glucan on the expression of growth-related genes

Table 2. Primers utilized for qRT-PCR.

Gene name	Primer Sequence (5′ - 3′)	Expected product size	Annealing temperature	References
β-actin	F- ACCCCAAAGCCAACAGA R- CCAGAGTCCATCACAATACC	136	60°C	Gasparino *et al*. (2018)
GHR	F- AACACAGATACCCAACAGCC R- AGAAGTCAGTGTTTGTCAGGG	145	60°C	
IGF-1	F- CACCTAAATCTGCACGCT R- CTTGTGGATGGCATGATCT	140	60°C	

(GHR and IGF-1) are illustrated in Figs. 1 and 2. The findings demonstrate a statistically significant increase (P < 0.5) in the expression of the GHR in the nucleotides with β-glucan group, followed by the nucleotides group, as opposed to the β-glucan and control groups. On the contrary, the expression of IGF-1 was significantly elevated (P < 0.05) in all groups that received dietary supplementation when compared to the control group.

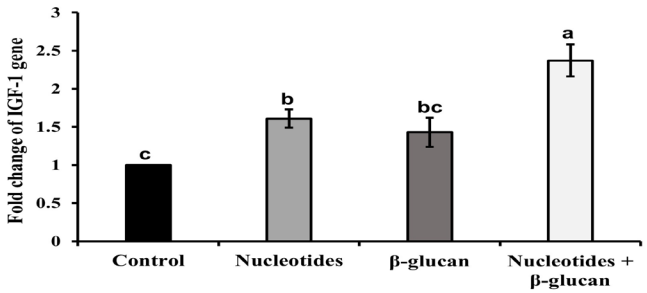

Fig. 2. The impact of supplementing the diet with nucleotides and/or β-glucan on gene expression of IGF-1. Columns containing distinct letters represent statistically significant results with p value < 0.05.

Intestinal histomorphology

The results of supplementing the diet with nucleotides and/or β-glucan on intestinal histomorphology are illustrated in Table 4. Villi length significantly (P < 0.05) elevated in the three portions of the intestine (jejunum, duodenum, and ileum). In the duodenum, villi width (VW) significantly (P < 0.05) elevated in the nucleotides with the β-glucan group and the nucleotides group when compared to the β-glucan group and the control group. In the jejunum, VW significantly (P< 0.05) elevated in the nucleotides group and the β-glucan group compared to the nucleotides with the β-glucan group and the control group. Furthermore, VW in the ileum significantly elevated (P < 0.05) in all dietary- supplemented groups compared to the control group. Crypt depth (CD) in the duodenum and jejunum experienced a significant (P < 0.05) elevation in the nucleotides with the β-glucan group and the nucleotides group when compared to the β-glucan group and the control group. In the ileum, CD significantly (P < 0.05) elevated in all dietary-supplemented groups compared to the control group. Additionally, GC significantly (P < 0.05) elevated in all dietary supplemented groups compared to the control group.

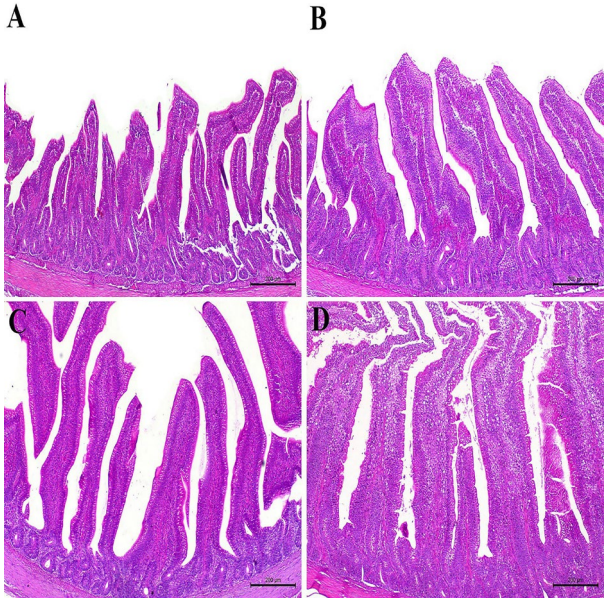

Fig. 3. Photomicrograph of small intestine (duodenum) from chickens subjected to various experimental diets; (A) basal diet, (B) nucleotides, (C) β-glucan, and (D) nucleotides with β-glucan. (A) showing normal intestinal villi, (B) showing an elevation of intestinal villi length, (C) demonstrating an elevation the length of intestinal villi, and (D) viewing marked rise the length of intestinal villi, H&E, X50, bar= 200 µm

Table 3. The impact of supplementing the diet of broiler chicken with nucleotides and/or β-glucan on their growth performance as measured by BW, BWG, FI, and FCR.

	Parameter	Control	Nucleotides	β-glucan	Nucleotides + β-glucan
BW (g)	Initial weight	$46.00^{a}\pm1.04$	$46.97^{a}\pm0.17$	$46.40^{a}\pm0.46$	$47.67^{a}\pm0.60$
	1 week	$149.61^{b}\pm2.86$	$159.58^{ab}\pm3.34$	$155.83^{ab}\pm3.09$	$163.39^{a}\pm2.46$
	2 weeks	$368.23^{c}\pm2.22$	$383.95^{b}\pm2.04$	$375.42^{c}\pm2.67$	$406.50^{a}\pm3.31$
	3 weeks	$792.09^{c}\pm4.98$	$821.61^{b}\pm3.43$	$801.07^{c}\pm6.01$	$851.61^{a}\pm5.92$
	4 weeks	$1267.6^{d}\pm4.82$	$1348.97^{b}\pm3.1$	$1321.42^{c}\pm2.09$	$1411.11^{a}\pm1.28$
	5 weeks	$1891.04^{d}\pm2.96$	$2099.99^{b}\pm2.08$	$2047.78^{c}\pm2.99$	$2170.78^{a}\pm2.25$
BWG (g)	1 week	$103.61^{b}\pm2.57$	$112.61^{ab}\pm3.51$	$109.44^{ab}\pm2.76$	$115.72^{a}\pm1.98$
	2 weeks	$218.63^{b}\pm4.88$	$224.36^{b}\pm5.36$	$219.58^{b}\pm3.39$	$243.11^{a}\pm5.76$
	3 weeks	$423.86^{b}\pm4.93$	$437.67^{ab}\pm4.96$	$425.65^{b}\pm7.85$	$445.11^{a}\pm2.75$
	4 weeks	$475.51^{c}\pm8.53$	$527.36^{b}\pm2.13$	$520.35^{b}\pm4.69$	$559.5^{a}\pm4.82$
	5 weeks	$623.44^{c}\pm7.72$	$751.01^{a}\pm4.05$	$726.36^{b}\pm5.08$	$759.67^{a}\pm2.69$
	Total BWG	$1845.04^{d}\pm3.54$	$2053.01^{b}\pm2$	$2001.38^{c}\pm3.3$	$2123.11^{a}\pm2.37$
FI (g)	1 week	$183.33^{a}\pm1.22$	$167.61^{b}\pm4.67$	$171.37^{ab}\pm2.84$	$152.56^{c}\pm6.01$
	2 weeks	$393.31^{a}\pm4.11$	$359.72^{b}\pm8.3$	$349.23^{b}\pm2.44$	$383.38^{a}\pm8.90$
	3 weeks	$650.67^{a}\pm4.79$	$592.54^{b}\pm3.66$	$651.11^{a}\pm4.37$	$601.35^{b}\pm5.70$
	4 weeks	$867.99^{a}\pm6.57$	$804.45^{c}\pm8.06$	$832.22^{b}\pm4.21$	$794.78^{c}\pm2.36$
	5 weeks	$1076.67^{a}\pm2.40$	$1063.67^{b}\pm2.6$	$1069.33^{ab}\pm3.48$	$1029^{c}\pm3.06$
	Total FI	$3171.98^{a}\pm1.89$	$2987.99^{c}\pm14.99$	$3073.26^{b}\pm11.66$	$2961.06^{c}\pm14.84$
FCR	1 week	$1.77^{a}\pm0.06$	$1.49^{bc}\pm0.01$	$1.57^{b}\pm0.06$	$1.32^{c}\pm0.07$
	2 weeks	$1.80^{a}\pm0.06$	$1.60^{b}\pm0.03$	$1.59^{b}\pm0.03$	$1.58^{b}\pm0.05$
	3 weeks	$1.54^{a}\pm0.01$	$1.35^{b}\pm0.02$	$1.53^{a}\pm0.03$	$1.35^{b}\pm0.01$
	4 weeks	$1.83^{a}\pm0.02$	$1.53^{c}\pm0.02$	$1.60^{b}\pm0.02$	$1.42^{d}\pm0.01$
	5 weeks	$1.73^{a}\pm0.02$	$1.42^{c}\pm0.00$	$1.47^{b}\pm0.01$	$1.35^{d}\pm0.01$
	Total FCR	$1.72^{a}\pm0.00$	$1.46^{c}\pm0.01$	$1.54^{b}\pm0.01$	$1.39^{d}\pm0.01$

Mean ± standard error is used to present the data. When two or more distinct letters appear in a row, the mean value is statistically significant at P < 0.05.

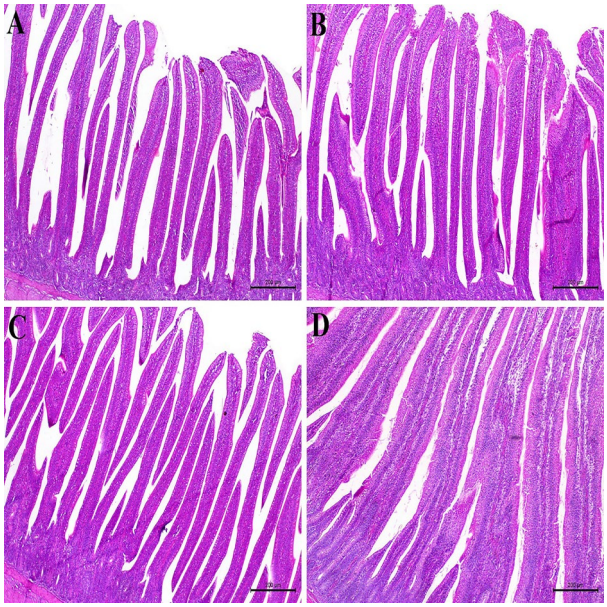

Fig. 4. Photomicrograph of small intestine (jejunum) from chickens subjected to various experimental diets; (A) basal diet, (B) nucleotides, (C) β-glucan, and (D) nucleotides with β-glucan. (A) showing normal intestinal villi, (B) demonstrating an increase of intestinal villi length, (C) showing an elevation the length of intestinal villi, and (D) indicating marked rise the length of intestinal villi, H&E, X50, bar= 200 µm

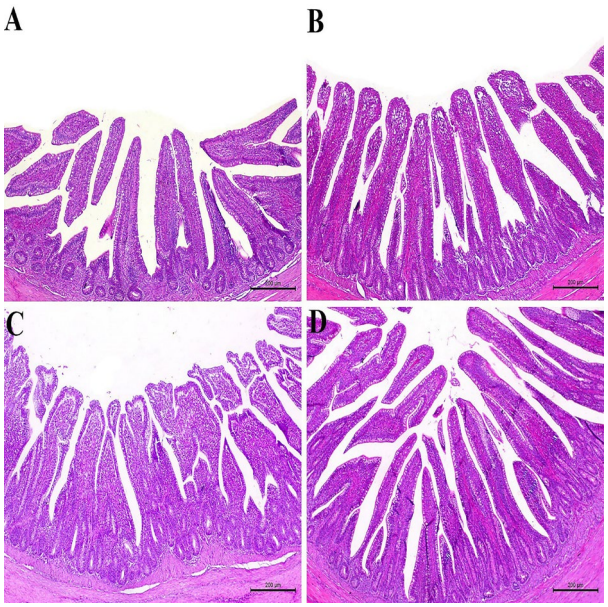

Fig. 5. Photomicrograph of small intestine (ilium) from chickens subjected to various experimental diets; (A) basal diet, (B) nucleotides, (C) β-glucan, and (D) nucleotides with β-glucan. (A) showing normal intestinal villi, (B) showing an elevation of intestinal villi length, (C) showing an elevation the length of intestinal villi, and (D) demonstrating marked rise the length of intestinal villi, H&E, X50, bar= 200 µm

Biochemical analysis

Table 5 presents the outcomes of incorporating nucleotide and/or β-glucan supplements into the diet on biochemical factors such as albumin, total protein, globulin, AST, and ALT. The findings indicate that the groups receiving dietary supplementation exhibited a significant ($P < 0.05$) elevation in both total protein and albumin compared to the control group. Nonetheless, no significant ($P > 0.05$) differences were noticed in globulin, AST, and ALT levels across the various treatment groups.

Discussion

Adding nucleotides and/or β-glucan to the diet of broiler chickens has been observed to enhance various aspects of growth performance, involving BWG, FI, BW, and FCR. This finding aligns with previous studies, such as Jung and Batal (2012) emphasized the importance of incorporating nucleotides in the diet to maintain optimal growth performance,

Table 4. The impact of supplementing the diet of broiler chicken with nucleotides and/or β-glucan on intestinal morphometric measures.

	Control	Nucleotides	β-glucan	Nucleotides + β-glucan
Duodenum				
Villi length (µm)	$428.37^{d}\pm2.45$	$541.78^{c}\pm3.93$	$636.3^{b}\pm2.92$	$873.51^{a}\pm3.25$
Villi width (µm)	$85.11^{c}\pm1.01$	$119.35^{a}\pm1.88$	$87.85^{c}\pm1.53$	$104.86^{b}\pm2.30$
Crypt depth (µm)	$93.53^{c}\pm3.40$	$111.36^{b}\pm2.26$	$132.46^{a}\pm6.04$	$148.08^{a}\pm7.69$
Goblet cells	$205.24^{c}\pm2.26$	$329.1^{b}\pm2.88$	$333.81^{b}\pm4.21$	$390.2^{a}\pm2.39$
Jejunum				
Villi length (µm)	$685.05^{c}\pm4.04$	$857.33^{b}\pm3.26$	$872.3^{b}\pm5.86$	$1260.7^{a}\pm6.45$
Villi width (µm)	$61.73^{b}\pm1.83$	$78.3^{a}\pm2.98$	$72.76^{a}\pm3.91$	$63.37^{b}\pm2.21$
Crypt depth (µm)	$161.24^{b}\pm2.20$	$171.13^{b}\pm3.79$	$188.18^{a}\pm4.29$	$199.09^{a}\pm5.36$
Goblet cells	$338.11^{d}\pm3.08$	$460.58^{c}\pm1.36$	$512.92^{b}\pm7.84$	$687.23^{a}\pm6.84$
Ileum				
Villi length (µm)	$383.00^{d}\pm4.23$	$494.24^{c}\pm4.04$	$568.72^{b}\pm5.33$	$780.2^{a}\pm5.45$
Villi width (µm)	$71.48^{c}\pm3.94$	$96.5^{b}\pm4.00$	$97.78^{b}\pm6.46$	$127.86^{a}\pm1.73$
Crypt depth (µm)	$72.53^{d}\pm2.59$	$87.88^{c}\pm2.34$	$104.13^{b}\pm3.15$	$128.02^{a}\pm3.52$
Goblet cells	$165.62^{d}\pm8.15$	$219.7^{c}\pm3.35$	$251.68^{b}\pm5.56$	$357.55^{a}\pm3.80$

Mean ± standard error is used to present the data. When two or more distinct letters appear in a row, the mean value is statistically significant at $P < 0.05$.

Table 5. The impact of supplementing the diet of broiler chicken with nucleotides and/or β-glucan on biochemical parameters.

	Control	Nucleotides	β-glucan	Nucleotides+ β-glucan
Total protein (g/dl)	$2.09^{b}\pm0.32$	$3.00^{ab}\pm0.25$	$3.34^{a}\pm0.36$	$3.71^{a}\pm0.35$
Albumin (g/dl)	$1.04^{b}\pm0.08$	$1.72^{a}\pm0.08$	$1.66^{a}\pm0.18$	$1.52^{a}\pm0.09$
Globulin (g/dl)	$1.05^{b}\pm0.31$	$1.27^{b}\pm0.20$	$1.68^{ab}\pm0.25$	$2.19^{a}\pm0.29$
AST (IU/l)	$306.93^{a}\pm2.19$	$313.67^{a}\pm2.49$	$307.8^{a}\pm5.07$	$317.27^{a}\pm4.12$
ALT (IU/l)	$18.59^{a}\pm0.51$	$22.36^{a}\pm0.74$	$20.87^{a}\pm1.06$	$22.61^{a}\pm1.87$

Mean ± standard error is used to present the data. When two or more distinct letters appear in a row, the mean value is statistically significant at $P < 0.05$.

especially under environmental stress conditions. In a study done by Salah *et al.*, (2019), it was shown that including yeast nucleotides into the bird's diet resulted in enhanced FCR, BWG, and overall BW. These positive results were linked to improved access to nucleotides for intestinal cell growth, which in turn improved digestive enzyme activity, digestion, and nutrient absorption. Additionally, Kreuz etal. (2020) showed that dietary nucleotides supplementation improved BW, daily weight gain, and FCR during the pre-challenge phase. Similarly, Khedr *et al.*, (2020) noted that groups receiving a diet supplemented with nucleotides exhibited enhancements in BW and BWG from day zero to slaughtering age. Gopi *et al*. (2023) concluded that dietary nucleoside supplementation improved broiler performance with enhanced cellular and humoral immunity.

Other studies also corroborate these results, Zhang *et al*. (2008) revealed that addition of β-glucans can improve average daily gain and FCR. Moon *et al*. (2016) suggested that β-glucan could serve as a potential antibiotic alternative, enhancing the survival and performance of broilers. Zhang *et al*. (2020) demonstrated that β-1,3-glucan supplementation could enhance body weight gain. However, Pelícia *et al*. (2010) found no significant effects on broiler performance with nucleotide additions to feed. Amer *et al*. (2022b) illustrated that β-glucan had non-significant effects on bird growth.

The findings indicated a notable increase in the expression of growth-related genes, including IGF-1 and GHR, in groups supplemented with dietary additives as compared to the control group. The GHR in broilers plays a crucial role in their production parameters, particularly in development (Al-Kelabi *et al*., 2019). Additionally, the comprehensive development and growth of birds are intricately linked to the cascades of the "hypothalamus-hypophyseal cascade" (Lin *et al*., 2012). Chicken IGF-1 has been recognized as a potential biological candidate gene accountable for body composition, growth, fat deposition, and metabolic processes in chickens, as documented by Kadlec *et al*. (2011). From the neurohypophysis, growth hormone and somatostatin are secreted, playing a dual role in regulating and balancing the pituitary and growth hormone (GH). Through the bloodstream, the circulated growth hormone reaches the liver where it binds to the GH receptor (GHR) on the surface of the liver cells, started signaling pathways that encourage the synthesis of IGFs. These IGFs stimulate cell division and proliferation as they travel via the bloodstream to various bodily regions (Pierce *et al*., 2005). Consequently, the increased expression of growth-related genes through dietary supplementation may have influenced the growth performance observed in this study. This aligns with the results of Rady *et al*. (2023) who noted a significant upregulation ($P < 0.05$) of IGF expression in liver tissue with the addition of nucleotides. The transcription levels of the insulin like growth factor 1 receptor (IGF1R) gene in the muscles of chicks treated with β-glucan demonstrated an elevation compared to those in the control chicks (ElSawy *et al.* 2014).

Evaluation of intestinal morphology and histology is deemed crucial, as it can impact the absorption of dietary nutrients and serves as a primary defense barrier against pathogens, as noted by Zhang *et al*. (2015). Increased villus height has been linked to enhanced nutrient digestion and absorption, as emphasized by Amer *et al.* (2021a). A deeper crypt exhibits faster tissue turnover, possibly reflecting a greater demand for new tissue, as recommended by Fasina and Olowo (2013). The present study reveals a substantial increase in intestinal histomorphology in groups supplemented with dietary additives, characterized by heightened villi length, crypt depth, and goblet cells. This result is in line with the observations of Gao *et al*. (2008) who noted that the addition of yeast nucleotides in diets improved intestinal villi length, leading to a broader surface area and elevated digestive enzyme activity, leading to improved nutrient absorption and digestibility. Abd-El-Wahab *et al.* (2019) observed increased nutrient absorption in Japanese quails with dietary yeast nucleotides.

These results are corroborated by additional research, Fasina and Olowo (2013) reported morphometric variations in the ileum and jejunum after dietary supplementation with β-glucan. Additionally, Ding *et al*. (2019) observed that chicks fed β-glucan had higher goblet cell density and crypt depth. Moreover, Amer *et al*. (2022b) observed enhanced intestinal histomorphology, typified by elongated villi and reduced crypt depth, in birds given diets enriched with β-glucan. Bar-Dagan *et al*. (2023) asserted that β-glucan supplementation benefits GC number and VH in both the ileum and the jejunum.

The outcomes of adding nucleotides and/or β-glucan to the diet demonstrated a noteworthy improvement ($P < 0.05$) in total albumin and protein concentrations in the groups receiving dietary supplementation in comparison to the control group. Nevertheless, no statistically significant difference ($P > 0.05$) was identified in the concentrations of globulin, AST, and ALT among the groups. The findings got from this study align with those noted by Villavan *et al*. (2021) in which groups that were fed nucleotides exhibited a notable rise in both total protein and albumin. There was no statistically significant variations in the AST and ALT activity between broilers fed a basal diet and those supplemented with β-glucan according to ElSawy *et al*. (2014).

Conclusion

Based on the results obtained, supplementing the diet with nucleotides and/or β-glucan at doses of 200 mg/kg and 1 g/kg respectively, significantly enhanced growth performance (BW, BWG, FI, and FCR), upregulated the expression of growth-related genes such as GHR and IGF1, improved intestinal histomorphology (villi length, crypt depth, and goblet cells), and increased biochemical indicators such as total protein and albumin. Comparing the globulin, AST, and ALT levels between the groups, no significant difference ($P > 0.05$) was noted.

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Conflict of interest

The authors declare that they have no conflict of interest.

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