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Impact of dietary-nucleotides and Saccharomyces cerevisiae-derivatives on growth-performance, antioxidant-capacity, immune-response, small-intestine histomorphometry, caecal-*Clostridia*, and litter-hygiene of broiler-chickens treated with florfenicol

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ABSTRACT

Stress in poultry production is energy-demanding. Nucleotides and yeast cell-wall products are essential nutrients for broiler performance, gut function, and immune response. Antibiotics, like florfenicol, negatively affect the immune system. A total of 600 one-d-old broiler chickens (Cobb-500) were weighed and randomly allotted into four groups with three replicates each. The control group (G1) received the basal diet, G2 received a diet supplemented with a combination of nucleotides and *Saccharomyces cerevisiae* derivatives (250 g/Ton), G3 received the basal diet and medicated with florfenicol (25 mg/Kg body weight) in drinking water for 5 days, while G4 received a combination of nucleotides and *Saccharomyces cerevisiae*-derivatives (250 g/Ton) and medicated with florfenicol in drinking water. Growth performance criteria were recorded weekly. Blood, intestinal contents, small-intestine sections, and litter samples were collected to measure birds' performance, carcass yields, leukocytic counts, antioxidant capacity, antibody titres, phagocytic index, caecal *Clostridia*, intestinal histomorphometry, and litter hygiene. Nucleotide-supplemented groups (G2 and G4) revealed significant ($p \leq 0.05$) improvements in feed conversion, and body weight, but not for carcass yields in comparison to the control. Dietary nucleotides in G2 elevated blood total proteins, leucocytic count, antioxidant capacity, and phagocytic index, while they lowered blood lipids and litter moisture and nitrogen ($p \leq 0.05$). Dietary nucleotides in G4 ameliorated the immunosuppressive effect of florfenicol ($p \leq 0.05$) indicated in reducing caecal *Clostridia*, improving duodenal and ileal villi length, and increasing blood albumin and globulin levels, and phagocytosis%. Supplementing diets with nucleotides and yeast products has improved the immune system and provided a healthier gut for broilers.

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1. Background

Antibiotics have been used in the poultry industry to prevent and treat microbial infections in chickens. Additionally, adding antibiotics to drinking water during vaccination is commonly applied in poultry farms. There are insufficient data on the impact of antibiotics on the immune system of broiler chickens [1–3]. Further investigations are required to examine the effects of frequently applied antibiotics such as florfenicol on the performance and immune response of broilers. Florfenicol is an antibiotic which commonly has been used in poultry production and can effectively fight against a range of gram-negative and gram-positive bacteria [4]. The development of florfenicol aimed to create a safer alternative to chloramphenicol and thiamphenicol by substituting a sulfomethyl group for the nitro group attached to the benzene ring. This substitution was necessary to avoid the dangerous side effects that could be fatal in some cases [5]. In intensive farming, birds experience stressors like overcrowding, hot weather, vaccination, and bacterial and parasitic infections that can reduce their immunity and productivity. Recently, non-drug feed additives like yeast-derived products have gained popularity as an alternative to antibiotics and antibiotic-free manufacturing techniques. Evidence suggests that yeast-derived additives can improve the birds' immunological status, intestinal integrity, and growth parameters [6,7].

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Yeast nucleotides are often used in livestock and poultry diets to replace antibiotics and as valuable feed additives either in the form of yeast extracts or as pure constituents [8,9]. Nucleotides are essentially supplemented in poultry diets because the animal's ability for synthesis is insufficient in some conditions, such as fast development, stress, and illness [10]. In broilers, dietary nucleotide supplementation increased the body weight and carcass yield [11]. Furthermore, according to Bonato et al. [12], egg production, fertility, and hatchability improved by supplementing nucleotides to breeders' diets. Yeast-derived compounds contain high concentrations of nucleotides, mannan polysaccharides, and β1,3–1,6-glucans [13]. The dietary supplementation of yeast cell wall extracts (mannan polysaccharides and β1,3–1,6-glucans) improved broiler growth performance [14]. Yeast beta-glucans are natural polysaccharides that are safe to be taken orally [15]. These natural components, such as β-glucans and mannans, can potentially improve intestinal microflora and provide nutrients for the gut flora [11,16]. Several investigations have shown that yeast components, specifically *Saccharomyces cerevisiae*, can reduce caecal *Clostridia* count and promote the growth of beneficial bacteria [17]. Additionally, yeast-derived β-(1, 3/1, 6) glucans have an immune-strengthening effect by modulating the antimicrobial activity of macrophages [15].

The objective of this study was to assess the impact of dietary nucleotides and yeast-derived products from *Saccharomyces cerevisiae* on the growth rate, carcass yields, immunity, intestinal mucosa, *Clostridia*, and litter conditions of broiler chickens treated with florfenicol in their drinking water for 5 days.

2. Materials and methods

2.1. Ethical approval

All procedures for handling and treating birds at the Poultry and Animal Research Center of the Faculty of Veterinary Medicine were approved by the Cairo University Institutional Animal Care and Use Committee (Vet CU 09092023756).

2.2. Experimental design

The feeding trial was conducted for 33 days in the Poultry and Animal Research Center using deep litter floor pens in a semi-closed system house. In total, 600 hatch day-old Cobb-500 broiler chicks were involved in the trial. Birds were randomly assigned to four groups, each consisting of three replicates of 50 birds.

The experiment design was as follows: The G1 was the control group that received the basal diet [18] without any growth-promoting factors, as listed in Table 1. G2 was the bird group supplemented with 250 g NutriFix®/Ton of basal diet. G3 was the bird group that received the basal diet and was medicated for 5 days with florfenicol (25 mg/Kg body weight) in drinking water (from day 15 to day 20). G4 was the bird group supplemented with 250 g NutriFix®/Ton of basal diet and medicated for 5 days with florfenicol

Table 1. Feed ingredients and nutrient contents of broiler diets from 1 to 33 days of the experiment.

	Control basal diet (G1)		
Ingredients %	Starter	Grower	Finisher
Yellow corn	57.43	61.23	65.93
Corn gluten meal (60% CP)	3.00	2.00	2.00
Soybean meal (46% CP)	36.0	32.20	27.00
Soy-oil	0.00	1.20	2.0
Di-calcium phosphate	0.90	0.80	0.50
Limestone	1.40	1.30	1.25
Common salt	0.35	0.35	0.35
Sodium bicarbonate	0.15	0.15	0.15
DL-Methionine	0.20	0.20	0.20
L-Lysine HCl	0.20	0.20	0.25
UNIKE® Plus *	0.05	0.05	0.05
Phytase	0.01	0.01	0.01
Xylanase	0.01	0.01	0.01
Vitamin-Mineral premixes **	0.30	0.30	0.30
Total (%)	100	100	100
Chemical composition			
ME (Kcal/kg)	3010.06	3105.84	3201.51
Crude protein (%)	23.11	21.04	19.05
Crude fat (%)	2.57	3.83	4.73
Crude fibre (%)	2.36	2.30	2.22
Calcium (%)	1.02	0.95	0.86
Available phosphorus (%)	0.50	0.47	0.41

*Toxin binder (Adisseo, France).

**Vitamin-mineral premix per Kg of diet: 1200000 IU vit. A, 350,000 IU vit. D3, 4000 mg vit. E, 250 mg vit. B1, 800 mg vit. B2, 600 mg vit. B6, 3.2 mg vit. B12, 450 mg vit. K3, 4.5 g nicotinic acid, 1.5 g Ca pantothenate, 120 mg folic acid, 5 mg biotin, 55 mg choline chloride, 3 g Fe, 2 g Cu, 10 g Mn, 8 g Zn, 120 mg I, 40 mg Co.

Table 2. Composition of NutriFix® (Agromed GMBH Company, Austria).

Composition	Quantity
Brewer's yeast (*Saccharomyces cerevisiae*)	960 g
Mannan oligosaccharide	100 g
Beta glucan (1,3&1,6)	125 g
Diatomaceous earth	Up to 1 kg

(25 mg/Kg body weight) in drinking water (from day 15 to day 20).

NutriFix® (Agromed GMBH Company, Austria) is an optimized combination of Nucleotides, ß-(1–3/1–6)-glucans and mannans derived from *Saccharomyces cerevisiae*, as explained in Table 2. Florfenicol (98% N. L.T. on a dried basis; Pharma Swede-Egypt Company) is a novel broad-spectrum antibiotic, as it is a fluorinated derivative of thiamphenicol.

2.3. Sampling and measurements

2.3.1. Growth performance

Throughout the experiment, weekly body weights (BW), feed intake (FI), and daily mortality values were recorded for each replicate. Based on this data, the weekly body weight gain (BWG) and feed conversion ratio (FCR) adjusted for mortality were calculated. At the end of the 33-day trial, we estimated the European Production Efficiency Factor (EPEF) using the following formula: EPEF = (liveability (%) × live body weight (kg)/(age in days × FCR) × 100).

2.3.2. Carcass traits

On day 33, five birds were slaughtered from each replicate in each group. The weight of carcasses, breasts, thighs, drums, and abdominal fat were measured to determine their yield.

2.3.3. Intestinal histomorphometric examination

On day 33, intestinal sections (duodenum, jejunum, and ileum) were collected from five birds from each group and flushed with 0.9% saline solution, then preserved, and fixed for 48 h in neutral buffered 10% formalin [19]. A rotatory microtome was used to prepare 3–4 µm paraffin wax sections, which were deparaffinized, stained with haematoxylin and eosin (H&E), and examined under a light microscope [20]. The histomorphometry of villus height and crypt depth was implemented by a high-power lens (X 400). Histomorphometry was done through a computerized microscopic image analyser linked to a full HD microscopic camera (Leica Microsystems, Germany).

2.3.4. Blood biochemical indices

Blood was sampled from eight birds per group on the 33rd day (at slaughtering). These samples were centrifuged at 3,000 rpm for 15 min to obtain serum samples. Standardized kits manufactured by Spectrum-Germany were used to measure the serum levels of glucose, total proteins, albumin, total cholesterol, and triglycerides, following the manufacturer's instructions. The serum globulins were calculated by subtracting the albumin value from the total protein value.

2.3.5. Blood leukocytic count and H/L ratio

On day 33, the leukogram was measured using whole blood samples collected in EDTA tubes. The total leukocytic count was determined using Natt and Herrick diluent. The differential leukocytic count was performed manually using Giemsa stain [21–23]. The heterophil–lymphocyte ratio (H/L) was evaluated for the assessment of stress [24,25].

2.3.6. Serum total antioxidant capacity (TAC)

Serum TAC was determined using kits purchased from Biodiagnostic Co.-Egypt. The principle of the reaction is to calorimetrically determine the residual amount of hydrogen peroxide that is exogenously offered after the reaction with the antioxidants in serum samples [26].

2.3.7. Immunological phagocytic parameter

On days 27 and 33, heparinized blood samples were collected from five birds per group. From the heparinized blood, the polymorphonuclear cells (PMNC) were separated using a discontinuous gradient of Percoll (Pharmacia) rendered isotonic with 0.15 M of NaCl (solution referred to at 100%) according to Zagami [27]. Then, the Phagocytosis assay was developed according to Vujanovic et al. [28] with minor modifications. Then, 1×10^6 cells PMNCs were suspended in 400 ml Haemacel medium. The heat-inactivated yeast particles adjusted to a concentration of 6×10^6/ml and labelled with Neutral red (Sigma) were mixed. The mixture was incubated at 37° C for 1-h. After two washes with ice-cold 0.02% EDTA, any non-ingested yeast particles were removed. The phagocytic index (PI) was calculated by dividing the average number of yeast particles ingested by a single cell. The percentage of cells that ingested at least one yeast particle was referred to as the percent of phagocytosis (PP).

2.3.8. Vaccinal newcastle disease virus (NDV) immune status

The serum vaccinal immune status of the eight birds/group was evaluated through the haemagglutination inhibition (HI) test [29] on day 27 (after florfenicol medication) and day 33 of age. In 99-V-bottomed microwell plates, 25 µl from each serum sample were two-fold serially diluted and assessed against 25 µl of NDV antigen (commercial Lasota) standardized at

four haemagglutination units (4 HAU). After 20 min incubation at room temperature, each well received 25 µl of 1% chicken RBCs suspension. Readings of antibody titres were recorded as mean $\log_2$ HI titres.

2.3.9. Litter examination

On day 33, litter samples were collected from three different spots of each replicate pen, and the upper 7 cm was scraped and collected in sterile plastic bags [30,31]. Litter moisture was determined by hot-air oven through drying 10 g of each litter sample at 100 ± 5°C [30]. After 24–48 h, dry weights were obtained and subtracted from the initial values to get the moisture percentage. In addition, the litter's total nitrogen content was estimated through total Kjeldahl nitrogen [32].

2.3.10. Caecal clostridial count

On day 33, caecal contents from four slaughtered birds per group were collected for microbiological investigation. One gram of each sample was diluted in a 9 ml sterile saline solution and homogenized (10^{-1} dilution), followed by 10-fold serial dilutions till the dilution 10^{-12} [33]. Out of the last 3-dilutions, 100 µl were collected and distributed onto Reinforced Clostridial Agar plates (Oxoid Ltd, Basingstoke, Hants, UK) and incubated for 24–48 h at 37°C under anaerobic conditions. Finally, the clostridial colonies were enumerated, and the counts were expressed as mean $\log_{10}$ CFU/g caecal content.

2.3.11. Statistical analysis

The statistical analysis was implemented by PASW Statistics Software from SPSS Inc., Chicago, IL, USA, Version 18.0. Results were reported as means ± pooled standard errors of the means (SEM). The one-way ANOVA test was applied to compare the means of control and test groups, and then Tukey's test was applied to determine post hoc comparisons. The 0.05 level was used as the significance level (*P*-value). Boxplots were produced through R (Version 3.6.1, R Foundation for Statistical Computing) by applying "ggplot2" and "ggpubr" packages [34,35].

3. Results

3.1. Productive performance

By the end of the study (day 33), the birds that received a combination of yeast cell wall extracts and nucleotides (G2 and G4), as well as those treated with florfenicol in water (G3), showed significant improvement in body weight (BW), body weight gain (BWG), and feed conversion ratio (FCR) compared to the control group ($p < 0.05$). Moreover, there was a slight improvement in the European Production Efficiency Factor (EPEF) in all groups except the control. However, there was no significant difference in BW, BWG, and FCR between the groups that fed on the yeast bioactive product (G2 and G4) and the group that received florfenicol in water (G3). Additionally, all groups had similar mortality rates (Tables 3 and 4).

3.2. Carcass traits

Table 5 presents the impact of yeast nucleotide dietary supplementation on carcass traits. The results indicated that adding yeast bioactive product (a combination of yeast cell wall extracts and nucleotides) did not significantly affect the dressing, breast, drumstick, or thigh yields compared to the control group.

3.3. Histomorphometry of the intestine

Results in Table 6 showed that the length of the intestinal villi of the duodenum, jejunum, and ilium increased significantly in the group that was given florfenicol (G3) compared to the control group. Additionally, there was a significant increase in villi length in both the duodenum and ileum in the group that was given florfenicol and NutriFix® (G4) compared to the control group. The NutriFix® group (G2) also showed a significant increase in the ileal villi length compared to the control. The duodenum in groups (G3) and (G4) had significantly increased villus crypt ratios compared to the control group. Moreover, the villus crypt ratio in the ileum showed a significant increase in the combination group (G4) compared to the control group.

Birds experienced positive micromorphological changes in their gut due to increased villus height and the villus height-to-crypt-depth ratio. These changes enhance the small intestine's capacity to digest and absorb nutrients effectively. When small intestine sections of poultry fed with florfenicol were examined, the presence of lymph nodules in the lamina propria of the duodenum and jejunum was noticed. However, the addition of NutriFix® supplement along with florfenicol resulted in a significant increase in the lymphoid tissue of the lamina propria of the jejunum only.

3.4. Blood biochemical parameters

Table 7 shows a significantly higher serum glucose level in G3 than in other groups. In addition, serum total proteins and albumin levels were significantly elevated in G2 and G4 compared to the control group and G3. While in G2 sera, cholesterol and triglycerides were significantly declined compared to other groups.

3.5. Leukogram and H/L ratio

Results presented in Table 8 revealed immunosuppression associated with a significant reduction of

Table 3. Influence of dietary Nutrifix® on performance parameters of broiler chickens.

	Body weight (g)					Weight gain (g)					Feed intake (g)					FCR (g/g)				
Groups	D 7	D 14	D 21	D 28	D 33	D 7	D 14	D 21	D 28	D 33	D 7	D 14	D 21	D 28	D 33	D 7	D 14	D 21	D 28	D 33
G1	198	547	1105	1576	1905[b]	152	349	558	471	329	147	471	682	861	731	0.97	1.35	1.22	1.84	2.30
G2	200	565	1144	1700	2059[a]	154	365	580	556	359	148	470	724	893	721	0.96	1.29	1.25	1.63	2.13
G3	210	560	1163	1681	2059[a]	164	350	603	518	378	166	479	730	864	715	1.02	1.37	1.21	1.69	1.89
G4	205	572	1162	1664	2058[a]	159	367	591	502	394	150	459	726	895	744	0.94	1.25	1.23	1.79	1.90
SEM[1]	3.42	6.72	10.07	22.36	21.88	3.42	6.15	8.84	17.54	18.09	4.37	3.60	13.14	10.13	14.63	0.02	0.02	0.02	0.06	0.12
P-value	0.667	0.665	0.123	0.216	**0.001**	0.667	0.691	0.369	0.435	0.679	0.395	0.297	0.606	0.543	0.928	0.347	0.278	0.971	0.635	0.644

[ab]Mean values with different superscripts in the same column indicate significant differences (Tukey's test; $p \leq 0.05$).

(G1), Control group (basal diet); (G2), basal diet +250 g NutriFix®/Ton of feed; (G3), basal diet + florfenicol (25 mg/Kg body weight) in drinking water for 5 days; (G4), basal diet +250 g NutriFix®/Ton of feed + florfenicol (25 mg/Kg body weight) in drinking water for 5 days.

FCR, Feed Conversion Ratio (g of feed/g of weight gain).

[1]SEM:Pooled standard error of means.

Table 4. Influence of dietary Nutrifix® on cumulative growth performance parameters of broiler chickens (days 1–33).

Groups	Body weight (g)	Daily gain (g)	Feed intake (g)	FCR (g/g)	EPEF	Mortality (%)
G1	1905[b]	56[b]	2892	1.56[a]	332	10.00
G2	2059[a]	61[a]	2956	1.47[b]	389	10.00
G3	2059[a]	61[a]	2954	1.47[b]	392	9.33
G4	2058[a]	61[a]	2974	1.48[b]	386	10.00
SEM[1]	21.88	0.66	22.13	0.01	9.32	1.38
P-value	**0.001**	**0.001**	0.643	**0.003**	0.129	0.998

[a,b]Mean values with different superscripts in the same column indicate significant differences (Tukey's test; $p \leq 0.05$).
(G1), Control group (basal diet); (G2), basal diet +250 g NutriFix®/Ton of feed; (G3), basal diet + florfenicol (25 mg/Kg body weight) in drinking water for 5 days; (G4), basal diet +250 g NutriFix®/Ton of feed + florfenicol (25 mg/Kg body weight) in drinking water for 5 days.
FCR, Feed Conversion Ratio (g of feed/g of weight gain).
EPEF: European Production Efficiency Factor= (liveability × live weight (kg)/(age in days × FCR) × 100.
[1]SEM:Pooled standard error of means.

Table 5. Influence of dietary Nutrifix® on Carcass traits of broiler chickens.

Groups	Dressing (%)	Breast (%)	Thigh (%)	Drum (%)	Abdominal fat (%)
G1	71.36	33.50	25.52	12.85	1.18
G2	73.86	32.25	25.47	12.89	1.42
G3	72.89	33.81	25.00	12.81	0.93
G4	73.07	33.28	24.68	13.07	1.17
SEM[1]	0.41	0.35	0.21	0.12	0.15
P-value	0.180	0.446	0.454	0.891	0.710

(G1), Control group (basal diet); (G2), basal diet +250 g NutriFix®/Ton of feed; (G3), basal diet + florfenicol (25 mg/Kg body weight) in drinking water for 5 days; (G4), basal diet +250 g NutriFix®/Ton of feed + florfenicol (25 mg/Kg body weight) in drinking water for 5 days.
[1]SEM:Pooled standard error of means.

Table 6. Effect of diet supplementation with NutriFix® on the histomorphometry of the small intestine of broiler chickens medicated with florfenicol.

	Duodenum			Jejunum			Ileum		
Groups	Villi length	Crypt depth	V/C	Villi length	Crypt depth	V/C	Villi length	Crypt depth	V/C
G1	1702.78[b]	114.91	14.88[b]	1053.19[b]	88.23[b]	12.23[a]	580.94[b]	76.51[b]	7.61[b]
G2	1566.86[c]	102.91	15.25[b]	905.83[c]	132.15[a]	6.85[b]	785.78[a]	110.14[a]	7.13[b]
G3	1888.57[a]	105.37	17.92[a]	1428.85[a]	123.11[a]	11.65[a]	840.13[a]	106.42[a]	7.91[ab]
G4	1989.42[a]	105.49	19.10[a]	1035.29[b]	109.71[b]	9.44[ab]	854.59[a]	100.89[a]	8.48[a]
SEM[1]	44.84	6.01	0.87	43.63	6.18	0.82	26.54	3.90	0.29
P-value	**<0.0001**	0.241	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.002**

[a,b,c]Mean values with different superscripts in the same column indicate significant differences (Tukey's test; $p \leq 0.05$).
(G1), Control group (basal diet); (G2), basal diet +250 g NutriFix®/Ton of feed; (G3), basal diet + florfenicol (25 mg/Kg body weight) in drinking water for 5 days; (G4), basal diet +250 g NutriFix®/Ton of feed + florfenicol (25 mg/Kg body weight) in drinking water for 5 days.
[1]SEM:Pooled standard error of means.
V/C: Villus crypt ratio.

Table 7. Effect of diet supplementation with NutriFix® on blood biochemical indices of broiler chickens medicated with florfenicol.

	Protein profile				Lipid profile		
Groups	Total protein (g\dl)	Albumin (g\dl)	Globulin (g/dl)	A/G ratio	Cholesterol (mg\dl)	TAG (mg\dl)	Glucose (mg/dl)
G1	3.99[b]	1.82[b]	2.10[ab]	0.89	154.62[a]	55.75[a]	228.25[b]
G2	4.55[a]	2.36[a]	2.24[ab]	1.08	137.75[b]	41.13[b]	234.25[b]
G3	3.46[c]	1.85[b]	1.57[b]	1.21	160.50[a]	49.87[a]	256.50[a]
G4	4.43[a]	2.19[a]	2.51[a]	1.00	159.00[a]	52.75[a]	242.13[ab]
SEM[1]	0.87	0.05	0.67	0.05	2.66	1.43	2.80
P-value	**0.0001**	**0.0001**	**0.030**	0.125	**0.004**	**0.0001**	**0.001**

[a,b,c]Mean values with different superscripts in the same column indicate significant differences (Tukey's test; $p \leq 0.05$).
(G1), Control group (basal diet); (G2), basal diet +250 g NutriFix®/Ton of feed; (G3), basal diet + florfenicol (25 mg/Kg body weight) in drinking water for 5 days; (G4), basal diet +250 g NutriFix®/Ton of feed + florfenicol (25 mg/Kg body weight) in drinking water for 5 days.
[1]SEM:Pooled standard error of means.

TLC and lymphocytes as well as significant elevation in heterophils %, H/L ratio, and monocytes % in the group that received florfenicol in the water when compared to other groups at $p \leq 0.05$. On the other hand, NutriFix® enrichment in the diet after florfenicol administration showed a significant immune-modulatory role through amelioration of TLC, heterophils %, lymphocytes %, and H/L ratio when compared to the florfenicol group at $p \leq 0.05$.

3.6. Total antioxidant capacity (TAC)

The data presented in Figure 1 showed a significant increase in serum total antioxidant capacity in the

Table 8. Effect of diet supplementation with NutriFix® on total and differential leucocytic counts of broiler chickens medicated with florfenicol.

Groups	TLC	Heterophiles (%)	Lymphocytes (%)	Monocytes (%)	Eosinophils (%)	Basophils (%)	H/L ratio
G1	22.16[a]	17.00[b]	71.16[a]	8.83[ab]	2.50	0.67	0.24[b]
G2	23.16[a]	18.17[b]	69.33[ab]	8.85[b]	3.00	0.66	0.26[b]
G3	18.17[b]	24.00[a]	61.00 [c]	11.16[a]	3.50	0.68	0.39[a]
G4	20.17[a]	19.00[b]	67.83[b]	9.83[ab]	2.50	1.00	0.28[b]
SEM[1]	0.47	0.71	0.88	0.36	0.18	0.14	0.02
P-value	**0.0001**	**0.0001**	**0.0001**	**0.024**	0.120	0.801	**0.0001**

[a,b,c]Mean values with different superscripts in the same column indicate significant differences (Tukey's test; $p \leq 0.05$).
(G1), Control group (basal diet); (G2), basal diet +250 g NutriFix®/Ton of feed; (G3), basal diet + florfenicol (25 mg/Kg body weight) in drinking water for 5 days; (G4), basal diet +250 g NutriFix®/Ton of feed + florfenicol (25 mg/Kg body weight) in drinking water for 5 days.
[1]SEM:Pooled standard error of means.

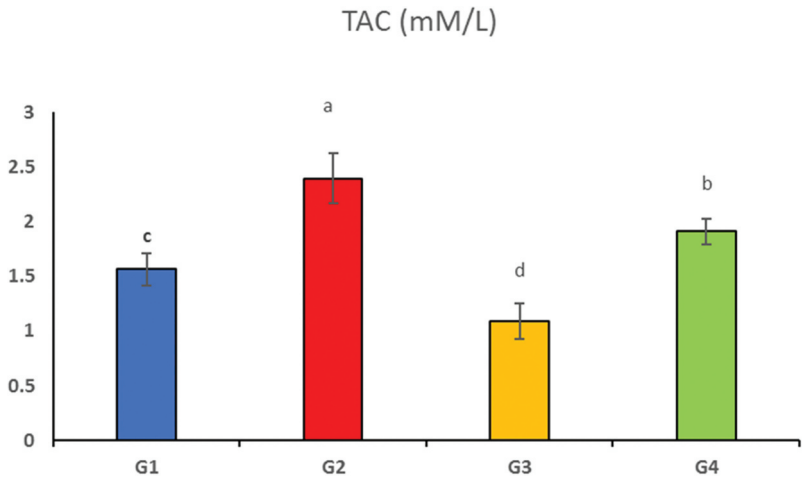

Figure 1. Effect of diet supplementation with Nutrifix on serum total antioxidant capacity (TAC) of broiler chickens (day 33). (G1), control group (basal diet); (G2), basal diet +250 g NutriFix®/Ton of feed; (G3), basal diet + florfenicol (25 mg/Kg body weight) in drinking water for 5 days; (G4), basal diet +250 g NutriFix®/Ton of feed + florfenicol (25 mg/Kg body weight) in drinking water for 5 days. [a,b,c,d] mean values with different letters on the same columns indicate significant differences (Tukey's test; $p \leq 0.05$).

group that received a diet enriched with NutriFix®, either alone or after being administered with florfenicol, when compared to the control group and the group that received only florfenicol, respectively ($p = 0.0001$).

3.7. Phagocytic assay

NutriFix® supplemented group (G2) showed a 111% average increase in phagocytosis% at different ages compared to the control, and 98% (day 27) and 108% (day 33) more increases than florfenicol (G3) group ($p < 0.0001$). Also, phagocytic index (PI) reported 48% and 71% increases in NutriFix® supplemented birds (G2) at 27- and 33-days of age, respectively, compared to control, and 85% (day 27) and 117% (day 33) compared to florfenicol (G3) group ($p < 0.0001$). On the other hand, the florfenicol (G3) group showed a significantly lower phagocytic index (−20.5%) than the control. However, supplementing the broiler diet with NutriFix® raised phagocytic activity in florfenicol-subjected birds (G4), where phagocytosis % recorded 52% and 56% increases at 27- and 33-days of age, and PI reported 16% and 24% elevated levels at 27- and 33-days of age over control ($p < 0.0001$) (Table 9).

3.8. Vaccinal newcastle disease virus (NDV) HI titres

On day 27, NDV antibody titres in birds' sera of G4 reported the highest mean HI titres of 4.00 ± 0.00 $\log_2$, but with no significance compared to the other groups ($p = 0.077$). On day 33, the highest NDV antibody titres were recorded in sera of G2 and G4, which received NutriFix® dietary supplement, regardless of florfenicol medication. However, those elevated titres were not significant compared to the control (G1) and florfenicol (G3) groups ($p = 0.126$), Table 10.

Table 9. Effect of diet supplementation with NutriFix® on the phagocytic assay of broiler chickens medicated with florfenicol.

	Phagocytosis %		Phagocytic Index (PI)	
Groups	Day 27	Day 33	Day 27	Day 33
G1	27.67^{c}	31.33^{c}	5.10^{c}	5.43^{c}
G2	58.33^{a}	66.00^{a}	7.57^{a}	9.30^{a}
G3	29.33^{c}	31.67^{c}	4.07^{d}	4.27^{d}
G4	42.00^{b}	49.00^{b}	5.93^{b}	6.73^{b}
SEM[1]	3.82	4.37	0.39	0.57
P-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

[a,b,c,d]Mean values with different superscripts in the same column indicate significant differences (Tukey's test; $p \leq 0.05$).

(G1), Control group (basal diet); (G2), basal diet +250 g NutriFix®/Ton of feed; (G3), basal diet + florfenicol (25 mg/Kg body weight) in drinking water for 5 days; (G4), basal diet +250 g NutriFix®/Ton of feed + florfenicol (25 mg/Kg body weight) in drinking water for 5 days.

[1]SEM:Pooled standard error of means.

Table 10. Effect of diet supplementation with NutriFix® on NDV vaccinal antibody titres of broiler chickens medicated with florfenicol.

	Mean HI titre (log_2)	
Groups	Day 27	Day 33
G1	3.67	4.50
G2	3.33	6.33
G3	3.00	5.00
G4	4.00	6.40
SEM[1]	0.15	0.35
P-value	0.077	0.126

(G1), Control group (basal diet); (G2), basal diet +250 g NutriFix®/Ton of feed; (G3), basal diet + florfenicol (25 mg/Kg body weight) in drinking water for 5 days; (G4), basal diet +250 g NutriFix®/Ton of feed + florfenicol (25 mg/Kg body weight) in drinking water for 5 days.

[1]SEM:Pooled standard error of means.

3.9. Caecal clostridial count

On day 33, caecal clostridial counts (Figure 2) of G4 received NutriFix® dietary supplement and medicated with florfenicol reported the lowest mean of 5.78 ± 0.40 log_{10}, compared to control (G1) 9.00 ± 1.53 log_{10}/g, G2 (9.51 ± 0.51 log_{10}/g), and G3 (8.69 ± 0.88 log_{10}/g). No significant differences were indicated between the diverse groups ($p = 0.127$).

3.10. Litter moisture and nitrogen percentages

On day 33, the NutriFix® (G2; $33.70 \pm 2.25\%$) and control (G1; $33.78 \pm 5.99\%$) groups reported the lowest levels of litter moisture % (Figure 3). Higher values were recorded for groups medicated with florfenicol; either with or without dietary NutriFix® supplementation. However, NutriFix® supplementation in G4 medicated with florfenicol aided in reducing litter moisture ($46.47 \pm 4.07\%$) to levels lower than G3 that received florfenicol alone ($53.40 \pm 1.36\%$). Significant differences were indicated between the experimental groups ($p = 0.029$), as indicated in Figure 3. The lowest litter nitrogen content was recorded in NutriFix® group (G2; 1.88%) compared to other groups (G1 = 2.02%; G3 = 2.72%; and G4 = 2.38%).

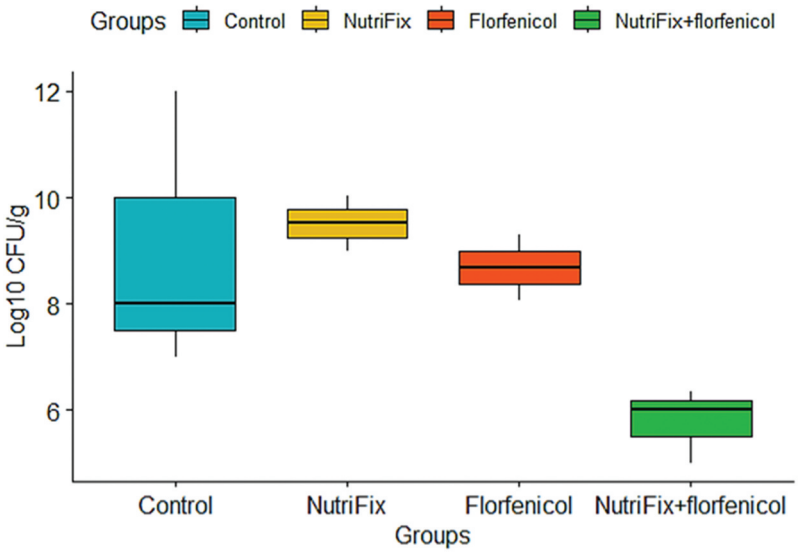

Figure 2. Effect of diet supplementation with Nutrifix® on the caecal clostridial count of broiler chickens (day 33). Control (G1), control group (basal diet); NutriFix (G2), basal diet +250 g NutriFix®/Ton of feed; Florfenicol (G3), basal diet + florfenicol (25 mg/Kg body weight) in drinking water for 5 days; NutriFix+ florfenicol (G4), basal diet +250 g NutriFix®/Ton of feed + florfenicol (25 mg/Kg body weight) in drinking water for 5 days.

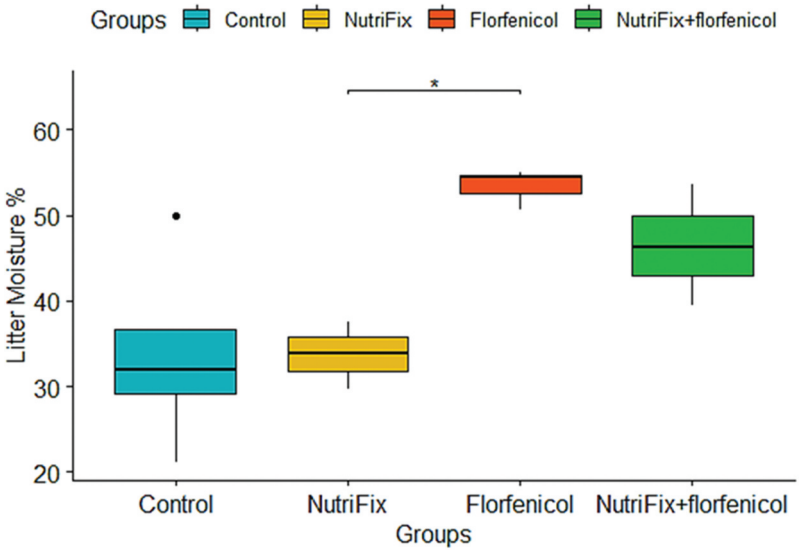

Figure 3. Effect of diet supplementation with nutrifix® on litter moisture % of broiler chickens (day 33). Control (G1), control group (basal diet); NutriFix (G2), basal diet +250 g NutriFix®/Ton of feed; Florfenicol (G3), basal diet + florfenicol (25 mg/Kg body weight) in drinking water for 5 days; NutriFix+ florfenicol (G4), basal diet +250 g NutriFix®/Ton of feed + florfenicol (25 mg/Kg body weight) in drinking water for 5 days. Asterisk (*) indicates significance at $p \leq 0.05$.

4. Discussion

In poultry farming, antibiotic medications are extensively used without an adequate understanding of their potential side effects on birds' immunity. Some antibiotics, such as salinomycin, cyclophosphamide, tilmicosin, enrofloxacin, and florfenicol, have immunosuppressive effects on the immune response of birds [1]. Furthermore, the usage of antibiotics in poultry production undergoes severe criticism due to food safety public issues of antibiotic residues and the development of antimicrobial resistance [17]. Also, antibiotics are commonly used as dietary growth promoters in the poultry industry. Growth-promoting substances, such as antibiotics, coccidiostats, exogenous enzymes, antioxidants, probiotics, and prebiotics, are non-nutrient additives that are added to nutritionally balanced diets so that birds will utilize nutrients, grow, have enhanced gut health, and exhibit a competent immune system [17]. Probiotics are microbes, like *Streptococcus, Lactobacillus*, and yeast *(Saccharomyces cerevisiae)*, that improve the intestinal microbiota of the birds and exert positive health effects. The bioactive compounds of yeast, such as beta-1,3;1,6 glucans, mannans, and nucleotides, have been shown to improve intestinal health, feed efficiency, growth, immunity, and gut morphology in chickens [11,14,17]. Nucleotides are low-molecular-weight bioactive agents that are either endogenously synthesized or dietary-supplemented.Nucleotides are essential for the physiological processes and immunological modulation in birds [11]. Dietary nucleotides are provided to birds as yeast extract or pure material.

4.1. Growth performance

In the current study, the growth performance parameters (BW, BWG, and FCR) were significantly improved in broiler chickens fed yeast-derived components (beta-1,3;1,6 glucans, mannans, and nucleotides) and birds treated with florfenicol in water compared with the control group. Previous studies have discussed that under challenging field conditions, supplementing yeast derivatives and antibiotics in diets protected the intestinal tract from the colonization of pathogens, enhanced FCR, and improved birds' health [8,10,36–38]. In addition, NutriFix® contains high concentrations of nucleotides, which may contribute to improved performance since yeast nucleotides have been shown to increase chicken growth in terms of average daily gain and FCR and are beneficial for broilers immunity and nutrient utilization [11,39,40]. The duration of florfenicol treatment, which was only for five successive days, was insufficient to induce weight differences. According to Hassanin et al. [41], oral florfenicol administration to birds possibly maintains the mucosal surfaces intact, and the increase in the florfenicol dose may cause a short-term deleterious effect on the BW of birds.

4.2. Carcass traits

In the current study, numerical increases in dressing% were observed in bird groups that received dietary yeast-derived supplements (beta-1,3;1,6 glucans, mannans, and nucleotides). However, no significant improvements in carcass attributes were

recorded between the experimental groups. According to certain studies, dietary yeast-derived products boosted the yields of broiler chicken carcasses, including the weights of the breast, thigh, and drumstick [6,36]. However, numerous research [42,43] showed no significant variation in carcass characteristics. Several studies have indicated that one likely reason is that the exogenic nucleotides are absorbed in the intestinal tract and moved to immunologic organs as bursa, where they enhance broiler immunity [44].

4.3. Intestinal histomorphometry

Birds treated with florfenicol and supplemented with dietary NutriFix® achieved the highest villi length in both the duodenum and ileum. NutriFix® showed better histomorphometry indices in the ileum compared to other intestinal sections. Nucleotides are mainly absorbed in the upper region of the small intestine [44]. Exogenous dietary nucleotides aid in the proliferation of enterocytes during periods of growth and the high demand for protein and DNA synthesis, accelerate intestinal mucosa recovery and enhance the activity of brush-border enzymes [44,45]. Wu et al. [11] reported a considerable rise in ileal villus heights in chickens fed 0.1% dietary yeast nucleotides. In the current experiment, villi crypts of jejunum and ileum showed significant depths in birds that received yeast derivatives and exogenous nucleotides. Alizadeh et al. [8] discussed that villi crypts are the factories that renew the villi, and they reported that birds that received 0.025 and 0.05% exogenous dietary nucleotides showed deeper jejunal villi crypts where most of the nutrient absorption takes place. Khalifeh et al. [1] mentioned that oral treatment with florfenicol maintained the intactness of intestinal mucosal surfaces.

4.4. Blood biochemical parameters

Serum biochemical parameters of birds supplemented with dietary NutriFix® revealed significant elevations in serum proteins, albumin, and globulins while lowering cholesterol and triglyceride levels. Those findings could be attributed to the role of dietary inclusion of yeasts (*S. cerevisiae*) in the enhancement of nutrient bioavailability and boosting the enzymatic activity such as alanine aminotransferase, aspartate aminotransferase, creatine phosphokinase, and lactate dehydrogenase, which promote food utilization [46]. Fathima et al. [36] stated that dietary yeast is a rich source of protein, minerals, and vitamins and meets the amino acid demands of broilers, which could replace soybean and fish meals. Furthermore, NutriFix® supplementation showed significant hypolipidemic action. Kannan et al. [47] supported these findings, as they reported that yeast-nucleotide supplementation to poultry lowered cholesterol levels due to the ability of the yeast to regulate serum cholesterol via bile acid de-conjugation. However, the group treated with florfenicol reported a significant elevation in glucose concentration, which suggested stress, as a previous report indicated that heat-stressed chicks showed increased glucose levels [48]. Additionally, florfenicol lowered blood proteins, albumin, and globulin, which correlates with Khalifeh et al. [1] and Hassanin et al. [41], who stated that the oral administration of 30 mg of florfenicol per kg body weight downregulated the immunoglobulins in chickens. Results agreed with Cao et al. [49], who discussed that florfenicol suppresses protein production when administered in high doses.

4.5. Leukogram and H/L ratio

The present study found a significant decrease in the number of lymphocytes and an increase in the percentage of neutrophils in the florfenicol-treated group. Previous studies have suggested that florfenicol can alter lymphocyte subsets and impact the immune response in chickens, which is consistent with Klaudia and Alina's [50] findings. Recent studies have shown that probiotics do not always need to be alive to benefit one's health [51]. In fact, "postbiotics", "paraprobiotics", and "metabiotics" have been newly developed. Paraprobiotics refer to non-living microbial cells or cell fragments that are immunologically active and thus beneficial to the host. Postbiotics are the soluble substances released by probiotic microbes [36]. The results of the current study suggest that supplementing with NutriFix® can significantly improve the immunosuppressive effects of florfenicol by enhancing several parameters of the leukogram. It has been known since 1964 that mannans produced from yeast cell walls have immunogenic properties [52]. Additionally, β-glucans can stimulate the innate immune system, which leads to lymphocyte activation and stimulation of the adaptive immune system [36,53].

The Heterophil/Lymphocyte ratio (H/L) reveals the immunity and robustness of poultry. Considering longevity, immunological response, and resistance to infection and heat stress [54], chickens with low H/L ratios outperform birds with high H/L ratios [55], which is consistent with results in the current study that showed a higher H/L ratio in the florfenicol-treated group, and supplementation with NutriFix® significantly normalized the H/L ratio.

4.6. Total antioxidant capacity (TAC)

In this study, the groups that received diets enriched with yeast derivatives, mannans, and glucans (NutriFix®) reported significant elevations of serum

TAC. Superoxide and hydroxide free radicals and reactive oxygen species are known for their ability to generate degenerative diseases and immune disorders [56,57]. Yeast beta-glucans exhibit the ability to scavenge free radicals. Similarly, *S. cerevisiae* mannans displayed high antioxidant activity in the Križková et al. [57] study. The enzymatic and nonenzymatic antioxidant expressions in chicken, such as glutathione and superoxide dismutase, are also upregulated by yeast cells. As a result, they protect the integrity of proteins, nucleic acids, and cell membrane structure and function [56]. To prevent oxidative stress in poultry, yeast cells, and yeast-derived products increase the activity of antioxidant enzymes, lower the level of reactive oxygen species, and lower lipid peroxidation [36].

4.7. Phagocytic assay

The phagocytic index is the ability of white blood cells to engulf and destroy bacteria. The current results indicated that florfenicol inhibited the phagocytic activity of immune cells, but NutriFix® potentiated the phagocytic activity of leukocytes and ameliorated the immune-suppressive effect of florfenicol medication. These findings agreed with Paape et al. [58], who found that florfenicol significantly inhibited the in-vitro phagocytic activity of blood neutrophils in bovine. Results also resembled those of Attia et al. [59], who stated that stressors cause a reduction in phagocytic activity in broilers. Dietary supplements of nucleotides and yeast derivatives can help mitigate the negative impacts of florfenicol, improve broiler's immune systems, and enhance macrophage phagocytosis even under stressful conditions [59,60]; however, their molecular pathways of this modulation are almost unidentified.

4.8. NDV-HI titres

NDV-HI antibody titres in this study indicated elevated antibody titres in groups that received NutriFix® either with or without florfenicol medication. The effect of florfenicol as an antibiotic on the immune system of chickens is still debatable. Previous research pointed to a significant down-regulating effect of florfenicol on humoral immune response (HI antibody titre) [1]. Another study reported lower NDV-HI titres in groups treated with florfenicol (30 mg/Kg BWt) compared to the non-treated birds [41]. In a different trial, florfenicol administration to chicks at 50 mg/Kg feed did not result in a noticeably reduced immunological response to the Newcastle disease virus vaccination [49]. Dietary nucleotides influenced the immunoglobulin response and increased HI antibody titres and immune response of birds, as reported in previous studies [60,61]; however, the mechanism is still unclear.

4.9. *Caecal* clostridia *count*

Birds supplemented with NutriFix® and medicated with florfenicol exhibited the lowest caecal clostridial counts. Studies have shown that *Saccharomyces cerevisiae* yeast extracts and 0.1% nucleotide supplementation can lower *Clostridia* levels [62–64]. Wu et al. [11] have reported that dietary nucleotides increase the number of goblet cells in the intestinal mucosa, which increases the mucus secretion that protects intestinal epithelium. Also, Wu et al. [11] documented the upregulation of MUC2 and TFF2 gene expressions in birds supplemented with yeast nucleotides, which enhance intestinal mucosal immunity. Additionally, feeding yeast nucleotides to birds promoted intestinal beneficial bacteria and enhanced the intestinal barriers [11]. On the other hand, florfenicol causes thinning of the protective mucus coating of the intestine. Furthermore, it could significantly reduce the abundance of beneficial intestinal bacteria such as *Lactobacillus*, which block the pathogen attachment sites and resist pathogens such as *Clostridia* [65].

4.10. Litter moisture and nitrogen percentages

The study found that NutriFix® supplementation resulted in the most hygienic litter conditions, while the florfenicol-medicated group had the lowest litter quality. Those results are compatible with previous research findings [64] that the *Saccharomyces cerevisiae* yeast extracts supplemented group exhibited high litter quality with reduced moisture and nitrogen contents. Dietary probiotics such as *Bacillus*, yeast products, and nucleotides increase nitrogen utilization, lower its excretion in bird droppings, improve environmental quality, and lower feeding costs [64,66,67]. In a recent study, Wang et al. [68] administered florfenicol in drinking water at different doses to broilers for 5-days and found that doses greater than 0.15 g/L induced significant kidney damage and proved the renal toxic effect of florfenicol on chicks. This finding could explain the high moisture content of the florfenicol-treated group, as the affected kidney function led to an increase in excreted urine.

5. Conclusion

NutriFix® dietary supplementation efficiently enhanced weekly body weights, weight gains, and FCR, raised dressing and drum yields, enhanced protein and lipid blood parameters, elevated the phagocytic activity, and raised the NDV vaccinal antibody titres even when birds medicated with florfenicol. Additionally,

NutriFix® supplementation increased total leucocytic count, augmented the serum antioxidant capacity (TAC), and reduced litter moisture and nitrogen. On the other hand, the leucocytic profile in the florfenicol-medicated group indicated immunosuppression. Dietary NutriFix® supplied to florfenicol medicated birds showed a significant immune modulatory role, lowered caecal *Clostridia* counts, and increased duodenal and ileal villi length, crypt depth, and V/C ratio.

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Author contributions

ARAA, AMG, KNEF, and TMA; Implemented the experimental design, and diet formulations. TA, EI, and BM; Performance parameters and carcass characteristics. EI; Litter examinations, microbiological testing, and serological analysis. SAO; microbiological testing and phagocytic index. NHH; Blood biochemical analysis, leukocytic counts, and total antioxidant capacity. EMM; Histopathological examination. EI, BM, NH, EMM, and TMA; Formal analysis, data curation, writing, and original draft preparation. ARAA, AMG, KNEF, and EI; reviewing and final editing. All authors read and approved the final manuscript.

Data availability statement

The generated data of the current study are offered on a reasonable request.

References

[1] Khalifeh MS, Amawi MM, Abu-Basha EA, et al. Assessment of humoral and cellular-mediated immune response in chickens treated with tilmicosin, florfenicol, or enrofloxacin at the time of Newcastle disease vaccination. Poult Sci. 2009;88(10):2118–2124. doi: 10.3382/ps.2009-00215

[2] Foroutankhah M, Toghyani M, Landy N. Evaluation of *Calendula officinalis* L.(marigold) flower as a natural growth promoter in comparison with an antibiotic growth promoter on growth performance, carcass traits and humoral immune responses of broilers. Anim Nutr. 2019;5(3):314–318. doi: 10.1016/j.aninu.2019.04.002

[3] Landy N, Kheiri F, Faghani M. Evaluation of cottonseed bioactive peptides on growth performance, carcase traits, immunity, total antioxidant activity of serum and intestinal morphology in broiler chickens. Ital J Anim Sci. 2020;19(1):1375–1386. doi: 10.1080/1828051X.2020.1844085

[4] Vancutsem PM, Babish JG, Schwark WS. The fluoroquinolone antimicrobials: structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. Cornell Vet. 1990;80(2):173–86.

[5] Skolimowski IM, Knight RC, Edwards DI. Molecular basis of chloramphenicol and thiamphenicol toxicity to DNA in vitro. J Antimicrob Chemother. 1983;12(6):535–542. doi: 10.1093/jac/12.6.535

[6] Araujo LF, Bonato M, Barbalho R, et al. Evaluating hydrolyzed yeast in the diet of broiler breeder hens. J Appl Poult Res. 2018;27(1):65–70. doi: 10.3382/japr/pfx041

[7] Pascual A, Pauletto M, Giantin M, et al. Effect of dietary supplementation with yeast cell wall extracts on performance and gut response in broiler chickens. J Animal Sci Biotechnol. 2020;11(1):1–1. doi: 10.1186/s40104-020-00448-z

[8] Alizadeh M, Rodriguez-Lecompte JC, Rogiewicz A, et al. 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. Poult Sci. 2016;95(3):507–17. doi: 10.3382/ps/pev362

[9] Anwar MI, Muhammad F, Awais MM, et al. A review of β-glucans as a growth promoter and antibiotic alternative against enteric pathogens in poultry. World's Poul Sci J. 2017;73(3):651–61. doi: 10.1017/S0043933917000241

[10] Leung H, Yitbarek A, Snyder R, et al. Responses of broiler chickens to *Eimeria* challenge when fed a nucleotide-rich yeast extract. Poult Sci. 2019;98(4):1622–33. doi: 10.3382/ps/pey533

[11] Wu C, Yang Z, Song C, et al. Effects of dietary yeast nucleotides supplementation on intestinal barrier function, intestinal microbiota, and humoral immunity in specific pathogen-free chickens. Poult Sci. 2018;97(11):3837–46. doi: 10.3382/ps/pey268

[12] Bonato MA, Araújo LF, Santos GD, et al. Nucleotide supplementation in the diet of broiler breeders and their effect on hatchability and subsequent progeny performance. Poult Sci. 2014;93:230.

[13] Klis FM, Mol P, Hellingwerf K, et al. Dynamics of cell wall structure in *Saccharomyces cerevisiae*. FEMS Microbiol Rev. 2002;26(3):239–56. doi: 10.1111/j.1574-6976.2002.tb00613.x

[14] Zhang AW, Lee BD, Lee SK, et al. Effects of yeast (*Saccharomyces cerevisiae*) cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. Poult Sci. 2005;84(7):1015–21. doi: 10.1093/ps/84.7.1015

[15] Stier H, Ebbeskotte V, Gruenwald J. Immune-modulatory effects of dietary yeast beta-1, 3/1, 6-D-glucan. Nutr J. 2014;13(1):38. doi: 10.1186/1475-2891-13-38

[16] Faustino M, Durão J, Pereira CF, et al. Mannans and mannan oligosaccharides (MOS) from *Saccharomyces cerevisiae*–A sustainable source of functional ingredients. Carbohydr Polym. 2021;272:118467. doi: 10.1016/j.carbpol.2021.118467
[17] Khalid AH, Ullah KS, Naveed S, et al. Effects of spray dried yeast (*Saccharomyces cerevisiae*) on growth performance and carcass characteristics, gut health, cecal microbiota profile and apparent ileal digestibility of protein, amino acids and energy in broilers. Trop Anim Health Prod. 2021;53(2):1–1. doi: 10.1007/s11250-021-02684-5
[18] NRC, Nutrient requirements of poultry. 9th ed. National Research Council. National Academy of Sciences: Washington, 1994.
[19] Murwani R, Kusumanti E, Naumova EN. *Areca catechu L.* and *anredera cordifolia* (Ten) Steenis supplementation reduces faecal parasites and improves caecal histopathology in laying hens. Int J Vet Sci Med. 2022, 31;10(1):52–63. doi: 10.1080/23144599.2022.2090732
[20] Bancroft JD, Gamble M. Theories and practice of histological techniques. New York, London And Madrid: Churchil Livingstone. 2013;7(12):2768–2773.
[21] Barrett KE, Barman S. Ganong's review of medical physiology (25th). USA: McGraw-Hill Education. 2016.
[22] Lewis SM, Bain BJ, Bates I. Dacie and Lewis Practical Haematology. 10th ed. USA: Elsevier; 2006.
[23] Natt MP, Herrick CA. A new blood diluent for counting the erythrocytes and leucocytes of the chicken. Poult Sci. 1952;31(4):735–8. doi: 10.3382/ps.0310735
[24] Cotter PF. An examination of the utility of heterophil-lymphocyte ratios in assessing stress of caged hens. Poult Sci. 2015;94(3):512–7. doi: 10.3382/ps/peu009
[25] Gross WB, Siegel HS. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Dis. 1983;1(4):972–979. doi: 10.2307/1590198
[26] Koracevic D, Koracevic G, Djordjevic V, et al. Method for the measurement of antioxidant activity in human fluids. J Clin Pathol. 2001;54(5):356–361. doi: 10.1136/jcp.54.5.356
[27] Zagami F. Phagocytic index: a valid method of phagocytosis study. Aggiornamenti di Medicina E Chirurgia. 1993;11(5):469–479.
[28] Vujanovic NL, Arsenijevic NN, Vlajic M. Modulation of polymorphonuclear neutrophyl motility and monocyte phagocytosis by factor released from human primary breast cancer tissue. Period Biol. 1986;88(Suppl 1):53Á.
[29] WOAH. Newcastle disease (infection with Newcastle disease virus). Chapter 3.3.14 (version adopted in May 2021). 12th ed. Paris, France: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals; 2023.
[30] Dumas MD, Polson SW, Ritter D, et al. Impacts of poultry house environment on poultry litter bacterial community composition. PloS One. 2011;6(9):e24785. doi: 10.1371/journal.pone.0024785
[31] Lopes M, Roll VF, Leite FL, et al. Quicklime treatment and stirring of different poultry litter substrates for reducing pathogenic bacteria counts. Poult Sci. 2013;92(3):638–44. doi: 10.3382/ps.2012-02700
[32] Jackson ML. Soil chemical analysis, pentice hall of India Pvt. Ltd, New Delhi, India. 1973;498:151–154.
[33] Esmaeilipour O, Moravej H, Shivazad M, et al. Effects of diet acidification and xylanase supplementation on performance, nutrient digestibility, duodenal histology and gut microflora of broilers fed wheat based diet. Br Poult Sci. 2012;53(2):235–44. doi: 10.1080/00071668.2012.681771
[34] Wickham H. 2016. ggplot2: elegant graphics for data analysis. New York: Springer-Verlag. ISBN 978-3-319-24277-4: https://ggplot2.tidyverse.org
[35] Kassambara A Ggpubr: "ggplot2" based publication ready plots. R package version 0.4. 0. 438. 2020. https://CRAN.R-project.org/package=ggpubr.
[36] Fathima S, Shanmugasundaram R, Sifri M, et al. Yeasts and yeast-based products in poultry nutrition. J Appl Poult Res. 2023;16(2):100345. doi: 10.1016/j.japr.2023.100345
[37] Santin E, Maiorka A, Macari M, et al. Performance and intestinal mucosa development of broiler chickens fed diets containing *Saccharomyces cerevisiae* cell wall. J Appl Poult Res. 2001;10(3):236–44. doi: 10.1093/japr/10.3.236
[38] Yang Y, Iji P, Kocher A, et al. Effects of mannanoligosaccharide on growth performance, the development of gut microflora, and gut function of broiler chickens raised on new litter. J Appl Poult Res. 2007;16(2):280–8. doi: 10.1093/japr/16.2.280
[39] Wang Z, Cerrate S, Coto C, et al. Evaluation of Nupro® yeast product in diets for broilers. Int J of Poul Sci. 2009;8(6):515–520. doi: 10.3923/ijps.2009.515.520
[40] Gao L, Xie C, Liang X, et al. Yeast-based nucleotide supplementation in mother sows modifies the intestinal barrier function and immune response of neonatal pigs. Anim Nutr. 2021;7(1):84–93. doi: 10.1016/j.aninu.2020.06.009
[41] Hassanin O, Abdallah F, Awad A. Effects of florfenicol on the immune responses and the interferon-inducible genes in broiler chickens under the impact of *E. coli* infection. Vet Res Commun. 2014;38(1):51–58. doi: 10.1007/s11259-013-9585-7
[42] Yalçın S, Yalçın S, Eser H, et al. Effects of dietary yeast cell wall supplementation on performance, carcass characteristics, antibody production and histopathological changes in broilers. Kafkas Univ Vet Fak Derg. 2014;20(5):757–764. doi: 10.9775/kvfd.2014.11088
[43] Fonia N, Singh CB, Singh DV, et al. Effect of nucleotides supplementation on carcass traits and meat composition of thigh and breast muscles of broiler chicken. India Journ Poul Scien. 2019;54(3):213–216. doi: 10.5958/0974-8180.2019.00035.7
[44] Hess JR, Greenberg NA. The role of nucleotides in the immune and gastrointestinal systems: potential clinical applications. Nut in Clin Prac. 2012;27(2):281–294. doi: 10.1177/0884533611434933
[45] Hawkes JS, Gibson RA, Roberton D, et al. Effect of dietary nucleotide supplementation on growth and immune function in term infants: a randomized controlled trial. Eur J Clin Nutr. 2006;60(2):254–264. doi: 10.1038/sj.ejcn.1602310
[46] Al-Afifi SH, Manal I, Bayomi AS. Biochemical effects of *Saccharomyces cerevisiae* (biological product) and hydrated sodium calcium alimono-silicate" HSCAS"(chemical compound) as anti-aflatoxin in chicken rations. Anim Health Res J. 2018;6(1):19–38.
[47] Kannan M, Karunakaran R, Balakrishnan V, et al. Influence of prebiotics supplementation on lipid

profile of broilers. Int J Poult Sci. 2005;4(12):994–7. doi: 10.3923/ijps.2005.994.997
[48] Khan WA, Khan A, Anjuman A, et al. Effects of induced heat stress on some biochemical values in broiler chicks. Int J Agric Biol. 2002;4:74–75.
[49] Cao J, Li K, Lu X, et al. Effects of florfenicol and chromium (III) on humoral immune response in chicks. Asian Australas J Anim Sci. 2004;17 (3):366–370. doi: 10.5713/ajas.2004.366
[50] Klaudia C, Alina W. The influence of enrofloxacin, florfenicol, ceftiofur and *E. coli* LPS interaction on T and B cells subset in chicks. Vet Res Commun. 2015;39(1):53–60. doi: 10.1007/s11259-015-9632-7
[51] Aguilar-Toalá JE, Garcia-Varela R, Garcia HS, et al. Postbiotics: an evolving term within the functional foods field. Trends Food Sci Technol. 2018;75:105–114. doi: 10.1016/j.tifs.2018.03.009
[52] Domer JE. Candida cell wall mannan: a polysaccharide with diverse immunologic properties. Crit Rev Microbiol. 1989;17(1):33–51. doi: 10.3109/10408418909105721
[53] Ali MF, Driscoll CB, Walters PR, et al. β-glucan–activated human B lymphocytes participate in innate immune responses by releasing proinflammatory cytokines and stimulating neutrophil chemotaxis. J Immunol. 2015;195(11):5318–5326. doi: 10.4049/jimmunol.1500559
[54] Lentfer TL, Pendl H, Gebhardt-Henrich SG, et al. H/L ratio as a measurement of stress in laying hens–methodology and reliability. Br Poult Sci. 2015;56 (2):157–63. doi: 10.1080/00071668.2015.1008993
[55] Thiam M, Wang Q, Barreto Sanchez AL, et al. Heterophil/Lymphocyte ratio level modulates *salmonella* resistance, cecal microbiota composition and functional capacity in infected chicken. Front Immunol. 2022;13:816689. doi: 10.3389/fimmu.2022.816689
[56] Wang T, Cheng K, Yu CY, et al. Effects of a yeast-derived product on growth performance, antioxidant capacity, and immune function of broilers. Poult Sci. 2021;100(9):101343. doi: 10.1016/j.psj.2021.101343
[57] Križková L, Ďuračková Z, Šandula J, et al. Antioxidative and antimutagenic activity of yeast cell wall mannans in vitro. Mutat Res Genet Toxicol Environ Mutagen. 2001;497(1–2):213–22. doi: 10.1016/S1383-5718(01)00257-1
[58] Paape MJ, Miller RH, Ziv G. Effects of florfenicol, chloramphenicol, and thiamphenicol on phagocytosis, chemiluminescence, and morphology of bovine polymorphonuclear neutrophil leukocytes. J Dairy Sci. 1990;73(7):1734–44. doi: 10.3168/jds.S0022-0302(90)78850-9
[59] Attia YA, Al-Harthi MA, El-Shafey AS, et al. Enhancing tolerance of broiler chickens to heat stress by supplementation with vitamin E, vitamin C and/or probiotics. Annals of Animal Science. 2017; 17 (4):1155–1169.
[60] Gil A. Modulation of the immune response mediated by dietary nucleotides. Eur J Clin Nutr. 2002;56(3): S1–4. doi: 10.1038/sj.ejcn.1601475
[61] Kamel NF, Hady MM, Ragaa NM, et al. Effect of nucleotides on growth performance, gut health, and some immunological parameters of broiler chicken exposed to high stocking density. Livestock Sci. 2021;253:104703. doi: 10.1016/j.livsci.2021.104703
[62] Thanissery R, McReynolds JL, Conner DE, et al. Evaluation of the efficacy of yeast extract in reducing intestinal *Clostridium perfringens* levels in broiler chickens. Poult Sci. 2010;89(11):2380–2388. doi: 10.3382/ps.2010-00879
[63] Mohamed FF, Hady MM, Kamel NF, et al. The impact of exogenous dietary nucleotides in ameliorating *Clostridium perfringens* infection and improving intestinal barriers gene expression in broiler chicken. Vet Anim Sci. 2020;10:100130. doi: 10.1016/j.vas.2020.100130
[64] Ismael E, Ismail EM, Khalefa HS, et al. Evaluation of *Saccharomyces cerevisiae* yeast fermentate and xylanase in reduced energy diet fed to broiler chicken. Int J Vet Sci. 2022;11(2):141–150.
[65] Yun S, Guo Y, Yang L, et al. Effects of oral florfenicol on intestinal structure, function and microbiota in mice. Arch Microbiol. 2020;202(1):161–169. doi: 10.1007/s00203-019-01731-y
[66] Lin LU, Liao XD, Luo XG. Nutritional strategies for reducing nitrogen, phosphorus and trace mineral excretions of livestock and poultry. J Integr Agric. 2017;16 (12):2815–2833. doi: 10.1016/S2095-3119(17)61701-5
[67] Elleithy EMM, Bawish BM, Kamel S, et al. Influence of dietary *bacillus coagulans* and/or *Bacillus licheniformis*-based probiotics on performance, gut health, gene expression, and litter quality of broiler chickens. Trop Anim Health Prod. 2023;55(1):38. doi: 10.1007/s11250-023-03453-2
[68] Wang X, Han C, Cui Y, et al. Florfenicol induces renal toxicity in chicks by promoting oxidative stress and apoptosis. Environ Sci Pollut Res. 2021;28(1):936–46. doi: 10.1007/s11356-020-10550-4