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Antioxidant capacity in broiler chicken enhanced by nucleotides and/or beta-glucan, resulting in increased antioxidant-related gene expression plus enzymes and improved meat quality.

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ABSTRACT

The current protocol investigated how nucleotides and/or β-glucan affect antioxidant-related gene expression, hepatic tissue lipid peroxidation in chickens, and meat quality. The newly hatched chicks were divided into four groups (36 birds pre group) based on the nutritional supplements they received: the first was a control group, the second was supplemented with 200 mg nucleotides per kg of diet, the third was supplemented with 1-gram β-glucan per kilogram of diet, and the fourth was complemented with both nucleotides and β-glucan. On day 35, three chickens per replicate were slaughtered to evaluate the antioxidant-related gene expression, the antioxidant enzymes, and meat quality. The results demonstrated that the antioxidant-related gene expression was substantially ($P < 0.05$) upregulated in the fourth broiler group with the addition of nucleotides with β-glucan compared to the other experimental groups. Furthermore, it was detected that the supplements enhanced antioxidant-related enzyme activity in broiler liver tissue, reducing malondialdehyde (MDA) as an indicator of lipid peroxidation. Moreover, the study's results indicated that the two supplements significantly ($P < 0.05$) affected tenderness, water-holding capacity (WHC), and cook loss. However, there were no statistically significant variations observed in drip loss, PH, and color (lightness and redness); nevertheless, in comparison to the control group, all dietary-supplemented groups demonstrated a notably reduced yellowness value ($P < 0.05$). It can be concluded from the findings that the inclusion of nucleotides in conjunction with β-glucan in the diet resulted in improvements in the expression of some genes linked to antioxidants (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)), antioxidant-related enzymes with decreased MDA, and meat quality characteristics including cook loss, yellowness value of meat color, water holding capacity (WHC), and tenderness.

1. INTRODUCTION

Recently, due to intensive breeding, broiler chickens have been subjected to several stimuli, including immunologic and oxidative stress (Zhang et al., 2020). Oxidative damage arises when an imbalance occurs between the system that generates free radicals and the system that counteracts them (Huang and Ahn, 2019). Reduced growth performance, compromised antioxidative defense, and inferior meat quality are consistent outcomes of oxidative damage (Chen et al., 2020). The liver is tasked with clearing hazardous pathogens and producing, secreting, metabolizing, and detoxifying macromolecules (Lalor and Adams, 2002).
Consequently, functional feed additives featuring safe and significant properties, such as enhancing the antioxidant defenses of broilers and alleviating the stress caused by oxidation, have garnered growing interest over several decades (Wang et al., 2022).
Yeast-derived products have been utilized for decades as natural feed additives for animals. The digestive function, growth, and antioxidant defenses of animals were all enhanced through the supplementation of yeast cell walls (Li et al., 2016; Wang et al., 2017).
Nucleotides contribute to RNA and DNA synthesis, serve as coenzyme components, generate cellular energy, and facilitate the action of hormones (Carver, 1999). They are endogenously synthesized and significantly influence the growth and development of cells that experience frequent replacement, including those found in the immune or gastrointestinal systems. Nevertheless, exogenous nucleotides may be semi-essential under specific conditions (e.g., stress, immune suppression, rapid growth, and

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inadequate nutrition) when the endogenous supply of nucleotides are insufficient to optimize energy utilization and immune system function (Jung and Batal, 2012). Furthermore, the addition of nucleotides notably elevated the levels of antioxidant enzymes, including glutathione peroxidase (GPx) and superoxide dismutase (SOD) (Rady et al., 2023).

Beta-glucan is a naturally occurring antioxidant abundant in barley, oats, fungi, microbes and algae (Du et al., 2019). The administration of β-glucan as a supplement to broilers can enhance growth performance and ameliorates stress caused by oxidation and inflammation induced by *E. coli* (Fadl et al., 2020). The stress level experienced by birds during their growth, transportation, handling before slaughter, and processing substantially impacts the quality of the flesh. β-glucan in the diet can decrease stress caused by oxidation in birds during their growth phases, which means it can significantly affect the quality of their meat (Moon et al., 2016). Hence, this research endeavor was intended to evaluate the influence of nucleotides and/or β-glucan on the expression of genes associated with antioxidants in liver tissue, liver antioxidant enzymes, and flesh quality in broilers.

2. MATERIAL AND METHODS

This work was conducted at the Department of Animal Wealth Development, Faculty of Veterinary Medicine, Benha University. An approval with reference number (BUFVTM 03-12-22) was obtained to ensure appropriate ethical standards from the Committee for the Care and Use of Institutional Animals.

2.1 Experimental design and management

A commercial hatchery supplied a set of 144 male and female broiler chicks (Cobb 500) that were healthy and just one day old. On average, these chicks weighed 46.76 ± 0.15 g. They were subdivided into four dietary-supplemented groups, each consists of three replicates that comprised of twelve birds per replicate. The experimental period was extended for 5 weeks, from October 23rd to November 27th, 2022.

Group 1 was administered the diet that served as a control.

Group 2 was given a control diet supplemented with +200 mg nucleotides per kg of diet (Ohly-GO®Nucleo, Ohly-GMbH, Germany), which is an enzymatically hydrolyzed yeast extract sourced from Saccharomyces cerevisiae.

Group 3 was administered a control diet supplemented with 1-gram β-glucan per kilogram of diet (Aleta™, Kemin Industries, Belgium), a product sourced from the algae *Euglena gracilis* that contains 1,3-beta glucans in concentrations exceeding 50%.

Group 4 had a control diet supplemented with nucleotides and β-glucan.

Environmental and hygiene standards were held constant for every chick during its upbringing. A fresh and clean floor was made of wood shavings. Food and water were supplied ad libitum. As illustrated in table (1), a nutritionally balanced diet was administered to the chicks, adhering to the recommended nutrient levels for the Cobb 500 broiler strain. The nutrition schedule was divided into three stages during the experimental period: starter (days one to ten), grower (days eleven to twenty-four), and finisher (days twenty-five to thirty-five).

2.2. Expression of antioxidant-associated genes in hepatic tissue

2.2.1. RNA isolation and cDNA formation

Following the manufacturer's directions, total RNA was extracted using GENEzol™ Reagent (Geneaid Biotech Ltd., Taiwan). Approximately 50 mg of liver tissue was pulverized using a rotor Tissue Ruptor (Qiagen GmbH, Germany) within a sterile collecting receptacle filled with 750 µl of GENEzol™ solution.

The absorbance of RNA was utilized to ascertain its integrity and concentration using a nanodrop spectrophotometer (BMG Lab Tec. GmbH, Germany). The A260:A280 ratio of pure RNA ranged from 1.8 to 2.0. The cDNA synthesis procedure was executed employing the ABT H-minus cDNA synthesis reagent (Applied Biotechnology Co. Ltd., Egypt) following the specific protocols laid forth by the manufacturer.

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

Diet formulation for 10 kg diet	Stage		
Ingredients	Starter	Grower	Finisher
Yellow corn	5.346	5.727	5.897
Soya bean meal-46	3.31	3.290	3.06
Corn gluten meal	0.4	0.05	0.050
Soya oil	0.25	0.35	0.45
Di calcium phosphate	0.228	0.185	0.164
Wheat Bran	0.2	0.15	0.13
Limestone	0.095	0.083	0.1
L-Lysine	0.031	0.025	0.022
DL-Methionine	0.028	0.028	0.021
Vit&. Min. Mixture	0.03	0.03	0.03
Sodium chloride	0.026	0.028	0.029
Sodium bicarbonate	0.024	0.02	0.016
Choline chloride	0.011	0.008	0.009
L -Threonine	0.006	0.01	0.008
Antimycotoxin	0.005	0.005	0.005
Antioxidant	0.001	0.001	0.001
Anticlostridia	0.001	0.001	0.001
Chemical composition			
ME (Kcal \ Kg diet)	2,953.35	3,024.39	3,103.39
Crude protein %	22.00	20.00	19.00
Crude fat %	5.04	5.99	7.01
Crude fiber %	2.35	2.34	2.28
Digested lysine %	1.20	1.12	1.04
Digested methionine %	0.59	0.54	0.47
Digested methionine + cysteine %	0.88	0.82	0.73
Digested threonine %	0.74	0.73	0.67
Digested tryptophan %	0.22	0.21	0.20
Calcium %	0.99	0.84	0.85
Available phosphorus %	0.50	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

2.2.2. Analysis by quantitative real-time PCR

The primer sequences, detailed in table (2), were generated using the NCBI Primer-BLAST software. A quantitative real-time PCR evaluation was conducted using a 7500 Fast Real-time PCR manufactured by Applied Biosystems (USA) and 10 µL of ABT 2X qPCR Mix SYBR (Applied Biotechnology Co. Ltd., Egypt). As the endogenous reference gene was employed to normalize the target genes, β-actin was chosen. The following thermocycling protocol was used to amplify target genes: 95°C for three minutes; 45 cycles of 50–60°C for thirty seconds; 95°C for fifteen seconds; and 72°C for thirty seconds; a melting curve analysis was then performed. The $2^{-\Delta\Delta ct}$ technique was employed to ascertain the relative expression of genes (Livak and Schmittgen, 2001).

2.3. Determination of liver antioxidants

Liver tissue samples were homogenized using an electrical homogenizer placed on ice with a volume of ten milliliters of cold phosphate buffer with a pH of 7.4. After 30 minutes of centrifugation at 5000 rpm, the homogenized tissues were separated. The supernatant was removed for measurement of the activities of SOD following Nishikimi et al., (1972), GPx as measured by Weissman (1976), CAT

according to Aebi, (1984), and MDA according to Uchiyama and Mihara, (1978).

2.4. Evaluation of the physico-chemical characteristics of chicken breast flesh

Table 2 The primers employed for qRT-PCR.

Gene name	Primer Sequence (5′ - 3′)	Accession number	Expected product size	Annealing temperature	References
β-actin	F- ACCCCAAAGCCAACAGA R- CCAGAGTCCATCACAATACC	NM_205518.1	136	60°C	(Gasparino et al., 2018)
CAT	F- ACTGGTGCTGGCAACCC R- ACGTGGCCCAACTGTCAT	NM_001031215	57	60°C	(El-Naggar et al., 2019)
SOD	F- CGGGCCAGTAAAGGTTACTGGAA R-TGTTGTCTCCAAATTCATGCACATG	NM_205064.1	83	60°C	
GPx	F- GCGACTTCCTGCAGCTCAACGA R- CGTTCTCCTGGTGCCCGAAT	GQ502186.2	99	60°C	(Li and Sunde, 2016)

CAT: catalase, SOD: superoxide dismutase, GPx: glutathione peroxidase

The muscles were chilled at 4°C for twenty-four hours before being reviewed for characteristics including cook loss, color (L*, a*, and b*), tenderness, PH, WHC, and drip loss.

2.4.1. Color and pH

The color of chicken meat was assessed using the method suggested by Al-Hijazeen et al., (2016). Colors were represented using CIE (L*) values for lightness, (a*) values for redness, and (b*) values for yellowness. PH was determined by penetrating an electrode through three distinct locations in the chickens' pectoralis major muscles using a calibrated pH meter (Jenway 3510 PH- meter, Cole-Parmer, Staffordshire, United Kingdom) according to Özbek et al., (2020).

2.4.2. Water holding capacity, cooking loss, drip loss, and tenderness.

The technique by Tarnauceanu and Pop (2016) involved compressing chicken meat over filter paper, weighing 0.5g, and then setting it on a 7-centimeter-diameter piece of filter paper that had been dried and weighed. In between the two glass platters was the paper containing the meat. Five minutes of labor were performed at a burden of 2.25 kilograms. The percentage of water holding capacity (WHC) was calculated by weighing the wet filter paper and dividing the weight of the released water by the weight of the meat tested.

Cooking loss was determined according to Honikel (1998).

Cooking loss (%) = (Weight before cooking - Weight after cooking) / Weight before cooking) × 100

A drip loss analysis was conducted using the method used by Demirok et al. (2013). Drip loss (%) $= \frac{(F- C) \times 100}{C}$.

Where F= Weight of the fresh sample and C= Weight of the chilled sample.

A texture analyzer was used to assess the tenderness of six sliced rectangular samples of cooked meat (Cavitt et al., 2004).

2.5. Statistical analysis

The normality and homogeneity of variances were assessed using the Shapiro-Wilk W test. To compare various groups, SPSS version 21 was applied for analysis (SPSS, Chicago, IL, USA), employing one-way ANOVA and Duncan's post hoc tests. $P < 0.05$ was used as the criterion of significance, which utilized the mean ± standard error.

3. RESULTS

3.1. Expression of genes linked to antioxidants in hepatic tissue.

The expression of genes associated with antioxidants as affected by nucleotides and/or β-glucan supplementation were illustrated in Figs. (1-3). The findings revealed a significant increase in the expression of genes linked to antioxidants, inluding CAT, SOD, and GPx genes, in liver tissue in groups supplemented with nucleotides and β-glucan as opposed to other groups.

Pectoral muscles (n = 3 per replicate) were used in the current study to examine the physicochemical attributes of chicken carcasses.

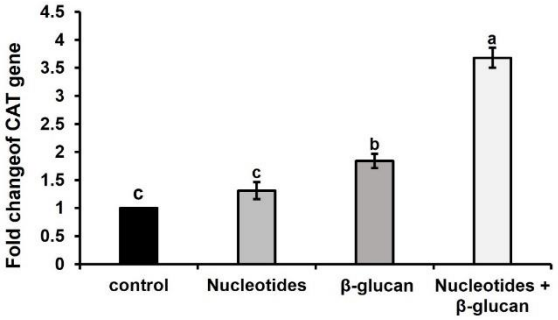

Fig. 1: Impact of nucleotides and/or β-glucan dietary supplements on CAT gene expression. Columns with different litters indicate statistically significant values with P-value.

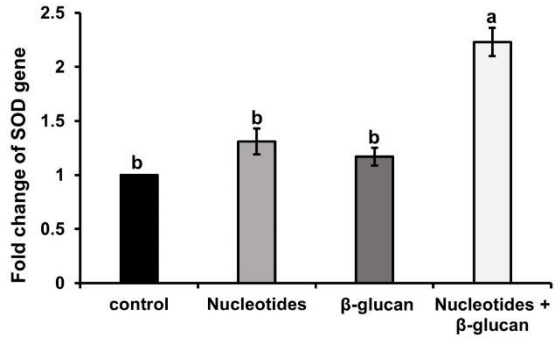

Fig. 2 Impact of nucleotides and/or β-glucan dietary supplements on SOD gene expression. Columns with different litters indicate statistically significant values with P-value.

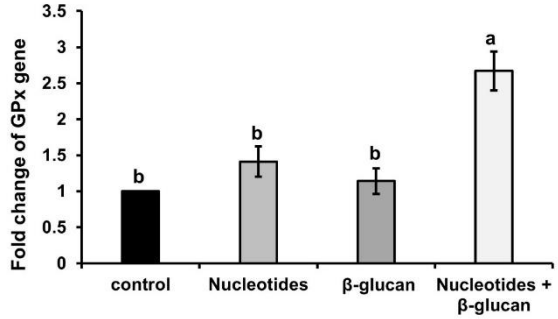

Fig. 3 Impact of nucleotides and/or β-glucan dietary supplements on GPx gene expression. Columns with different litters indicate statistically significant values with P-value.

3.2. Antioxidant enzymes in liver tissues

Table (3) showed the influence of nucleotides or β-glucan individually or in combination on antioxidant enzymes (GPx, SOD, and CAT) and MDA. There was a substantial ($P< 0.05$) enhancement of SOD, GPx, and CAT in these groups when compared with the control group. On the other hand, a substantial ($P< 0.05$) decrease in MDA was apparent when compared with the control group.

3.3. Meat quality

The outcomes of the influence of groups whose diets included supplements on the meat characteristics

(color, PH, WHC, cook loss, drip loss, and tenderness) are displayed in Table 4. This result significantly ($P < 0.05$) affected tenderness, WHC, and cook loss in the groups that received nutritional supplements compared to the control group. There was no discernible change ($P > 0.05$) in drip loss, PH, and color (L*) and (a*), while the (b*) value was noticeably lower in dietary-supplemented groups than in control ($P < 0.05$).

Table 3 The impact of incorporating nucleotides and/or β-glucan into the diet on the activity of liver antioxidant enzymes.

	Control	Nucleotides	β-glucan	Nucleotides + β-glucan
CAT (U/gm)	3.27±0.5^{c}	5.44±0.62^{b}	9.03±0.21^{a}	10.8±0.96^{a}
GPx (U/gm)	6691.96±190.24^{c}	7158.84±224.35^{bc}	7722.99±302.83^{b}	8997.19±106.88^{a}
SOD (U/gm)	522.14±41.68^{c}	676.23±26.76^{b}	671.99±26.04^{b}	948.95±15.87^{a}
MDA (nmol/gm)	201.77±12.24^{a}	146.81±13.42^{b}	115.12±2.39^{bc}	91.16±7.82^{c}

Data is expressed as means ± standard error. Different letters of the mean values in the same row are statistically significant ($P < 0.05$). CAT: catalase, GPx: glutathione peroxidase, SOD: superoxide dismutase, MDA: Malondialdehyde.

Table 4 The impact of incorporating nucleotides and/or β-glucan into the diet on meat quality.

	Control	Nucleotides	β-glucan	Nucleotides + β-glucan
Color				
L* (lightness)	60.17±0.8^{a}	60.6±0.67^{a}	61.2±0.76^{a}	61.77±0.69^{a}
a* (redness)	4.8 ±0.5^{b}	5.9 ±0.35^{ab}	5.33±0.41^{ab}	6.4 ±0.32^{a}
b* (yellowness)	14.2±0.5^{a}	13.33±0.43^{ab}	12.87±0.35^{b}	12.27±0.23^{b}
PH	5.74±0.05^{a}	5.66±0.04^{ab}	5.63±0.04^{ab}	5.57±0.03^{b}
WHC %	60.97±0.18^{c}	70.5±0.23^{bc}	80.03±0.23^{ab}	80.4 ±0.26^{a}
Cooking loss %	20.57±0.43^{a}	19.6 ±0.4^{ab}	19.3±0.23^{b}	18.97±0.26^{b}
Drip loss %	1.27±0.09^{a}	1.13±0.09^{ab}	1.07±0.03^{ab}	0.97±0.03^{b}
Tenderness	4.43±0.24^{c}	5.00±0.32^{bc}	5.67±0.24^{ab}	6.10±0.17^{a}

Data is expressed as means ± standard error. Different letters of the mean values in the same row are statistically significant ($P < 0.05$).

4. DISCUSSION

Several physiological functions and biochemical pathways rely on the reactive oxygen species (ROS) that the body usually produces due to metabolism (Cheng et al., 2020). Extreme ROS production can harm biological macromolecules within cells, like nucleic acids and proteins, leading to tissue damage, adverse health issues, and growth production (Bai et al., 2018). CAT, GPx, and SOD are enzymes that initiate the detoxification process of free radicals shortly after they are formed (Wang et al., 2022). The current results indicated a notable elevation in the expression of genes linked to antioxidants (SOD, CAT, as well as GPx) in Figs. 1, 2, and 3. This outcome was accepted by Wang et al. (2021), who stated that the expression of genes associated with antioxidants such as SOD1 and GPx1 is regulated by yeast culture alongside an enzyme-hydrolyzed yeast cell wall comprised of β-glucan, crude protein, and mannan-oligosaccharide.

According to the results in Table (3), as compared to the control, the dietary-supplemented groups exhibited a substantial increase in enzymes associated with antioxidants (SOD, CAT, and GPx) and a decrease in MDA. This outcome is in line with the findings of Li et al. (2016), those who noticed that on day 21, SOD activities were more significant in the ileum of broilers given a diet containing yeast cell wall powder, and on day 42, MDA levels were lower. Supplementing quails with yeast as a source of nucleotides elevated their serum SOD (Abd El-Wahab et.al., 2019). Incorporating yeast-derived products into a supplementation regimen can boost the activity of enzymes linked to antioxidants and related gene expression (El-Murr et al., 2019). The levels of SOD and GPx enzymes were considerably elevated with the addition of nucleotides (Rady et al., 2023). Additionally, the oxidative condition of broiler chickens was improved by elevating GSH and decreasing MDA after being administered β-glucan, according to Abd El-Tawab et al. (2019). Structural components of β-glucans may have an impact on the activities of enzymes associated with antioxidants, particularly SOD and CAT, which are recognized as crucial in protecting cells from stress caused by oxidation (He et al., 2021).

When comparing the control group to the groups that received nutritional supplements, the outcomes of meat quality in table (4) are accepted by Chiofalo et al. (2011) and Salah et al. (2019), those who reported that the meat of the nucleotide-supplemented group had no effects on color or PH. In addition, the lack of yellowing in breast meat after β-glucan therapy was illustrated by Zhang et al. (2012), perhaps due to reduced abdomen fat. A substantial decrease in cooking loss was noticed when β-glucan was added to the diet (Cho et al., 2013; Zhang et al., 2020).

5. CONCLUSIONS

Based on the results of the current research, adding nucleotides and/or β-glucan to the diet significantly increased the expression of antioxidant genes (GPx, CAT, and SOD). They are crucial in preventing oxidation-related damage because of their capacity to neutralize superoxide and free radicals. As gene regulators, they can similarly control the expression of antioxidant enzymes produced internally. The additives improved the antioxidant enzymes' activity. They reduced the peroxidation of lipids in broilers' hepatic tissue, resulting in enhanced meat quality characteristics such as cook loss, tenderness, (b*) value of meat color, and WHC.

6. REFERENCES

1. Abd El-Tawab, A., Elnaggar, O., Elsissi, A. 2019. The impact of β glucan on the Immune Response of Broiler Chickens Vaccinated with NDV and AI H9V Vaccines. Benha Veterinary Medical Journal, 36,2, 100–108. https://doi.org/10.21608/bvmj.2019.13775.1021.
2. Abd El-Wahab, A.; Mahmoud, R.;Marghani, B. and Gadallah, H. 2019. Effects of Yeast Addition to the Diet of Japanese Quails on Growth Performance, Selected Serum Parameters and Intestinal Morphology as well as Pathogens Reduction. Pakistan Veterinary Journal, 40,02, 219–223. https://doi.org/10.29261/pakvetj/2019.125.
3. Aebi, H. 1984. Catalase in vitro. Methods in Enzymology, 105, 121–126. https://doi.org/10.1016/s0076-6879,8405016-3.
4. Al-Hijazeen, M., Lee, E., Mendonca, A., Ahn, D. 2016. Effect of Oregano Essential Oil ,Origanum vulgare subsp. hirtum on the Storage Stability and Quality Parameters of Ground Chicken Breast Meat. Antioxidants, 5,2, 18. https://doi.org/10.3390/antiox5020018.
5. Bai, K., Feng, C., Jiang, L., Zhang, L., Zhang, J., Zhang, L., Wang, T. 2018. Dietary effects of Bacillus subtilis fmbj on growth performance, small intestinal morphology, and its antioxidant capacity of broilers. Poultry Science, 97,7, 2312–2321. https://doi.org/https://doi.org/10.3382/ps/pey116.
6. Carver, J. 1999. Dietary nucleotides: effects on the immune and gastrointestinal systems. Acta Paediatrica, 88,s430, 83–88. https://doi.org/10.1111/j.1651-2227.1999.tb01306.x.

7. Cavitt, L. C., Youm, G. W., Meullenet, J. F., Owens, C. M., Xiong, R. 2004. Prediction of Poultry Meat Tenderness Using Razor Blade Shear, Allo-Kramer Shear, and Sarcomere Length. Journal of Food Science, 69,1, 11–15. https://doi.org/10.1111/j.1365-2621.2004.tb17879.x.
8. Chen, Y., Gu, Y., Zhao, H., Zhang, H., Zhou, Y. 2020. Effects of graded levels of dietary squalene supplementation on the growth performance, plasma biochemical parameters, antioxidant capacity, and meat quality in broiler chickens. Poultry Science, 99,11, 5915–5924. https://doi.org/10.1016/j.psj.2020.08.042.
9. Cheng, Y., Chen, Y., Li, J., Qu, H., Zhao, Y., Wen, C., Zhou, Y. 2020. Dietary β-sitosterol regulates serum lipid level and improves immune function, antioxidant status, and intestinal morphology in broilers. Poultry Science, 99,3, 1400–1408. https://doi.org/https://doi.org/10.1016/j.psj.2019.10.025.
10. Chiofalo, B., Lo Presti, V., Savoini, G., D'Alessandro, E., Chiofalo, V., Liotta, L. 2011. Nucleotides in broiler chicken diet: effect on breast muscles quality. Czech Journal of Food Sciences, 29,4, 308–317. https://doi.org/10.17221/327/2010-CJFS.
11. Cho, J. H., Zhang, Z. F., Kim, I. H. 2013. Effects of single or combined dietary supplementation of β-glucan and kefir on growth performance, blood characteristics and meat quality in broilers. British Poultry Science, 54,2, 216–221. https://doi.org/10.1080/00071668.2013.777691.
12. Demirok, E., Veluz, G., Stuyvenberg, W. V., Castañeda, M. P., Byrd, A., Alvarado, C. Z. 2013. Quality and safety of broiler meat in various chilling systems. Poultry Science, 92,4, 1117–1126. https://doi.org/10.3382/ps.2012-02493.
13. Du, B., Meenu, M., Liu, H., Xu, B. 2019. A Concise Review on the Molecular Structure and Function Relationship of β-Glucan. International Journal of Molecular Sciences, 20,16, 4032. https://doi.org/10.3390/ijms20164032.
14. El-Murr, A. elhakeem I., Abd El Hakim, Y., Neamat-Allah, A. N. F., Baeshen, M., Ali, H. A. 2019. Immune-protective, antioxidant and relative genes expression impacts of β-glucan against fipronil toxicity in Nile tilapia, Oreochromis niloticus. Fish & Shellfish Immunology, 94, 427–433. https://doi.org/https://doi.org/10.1016/j.fsi.2019.09.033.
15. El-Naggar, K., El-Kassas, S., Abdo, S. E., Kirrella, A. A. K., Al wakeel, R. A. 2019. Role of gamma-aminobutyric acid in regulating feed intake in commercial broilers reared under normal and heat stress conditions. Journal of Thermal Biology, 84, 164–175. https://doi.org/10.1016/j.jtherbio.2019.07.004.
16. Fadl, S. E., El-Gammal, G. A., Sakr, O. A., Salah, A. A. B. S., Atia, A. A., Prince, A. M., Hegazy, A. M. 2020. Impact of dietary Mannan-oligosaccharide and β-Glucan supplementation on growth, histopathology, E-coli colonization and hepatic transcripts of TNF-α and NF- ϰB of broiler challenged with E. coli O78. BMC Veterinary Research, 16,1, 204. https://doi.org/10.1186/s12917-020-02423-2.
17. Gasparino, E., Del Vesco, A. P., Khatlab, A. S., Zancanela, V., Grieser, D. O., Silva, S. C. C. 2018. Effects of methionine hydroxy analogue supplementation on the expression of antioxidant-related genes of acute heat stress-exposed broilers. Animal, 12,5, 931–939. https://doi.org/10.1017/S1751731117002439.
18. He, T., Mahfuz, S., Piao, X., Wu, D., Wang, W., Yan, H., Ouyang, T., Liu, Y. 2021. Effects of live yeast ,Saccharomyces cerevisiae as a substitute to antibiotic on growth performance, immune function, serum biochemical parameters and intestinal morphology of broilers. Journal of Applied Animal Research, 49,1, 15–22. https://doi.org/10.1080/09712119.2021.1876705.
19. Honikel, K. O. 1998. Reference methods for the assessment of physical characteristics of meat. Meat Science, 49,4, 447–457. https://doi.org/10.1016/S0309-1740,9800034-5.
20. Huang, X., Ahn, D. U. 2019. Lipid oxidation and its implications to meat quality and human health. Food Science and Biotechnology, 28,5, 1275–1285. https://doi.org/10.1007/s10068-019-00631-7.
21. Jung, B., Batal, A. B. 2012. Effect of dietary nucleotide supplementation on performance and development of the gastrointestinal tract of broilers. British Poultry Science, 53,1, 98–105. https://doi.org/10.1080/00071668.2012.659654.
22. Lalor, T., Adams, D. 2002. The liver: a model of organ-specific lymphocyte recruitment. Expert Reviews in Molecular Medicine, 4,2, 1–15. https://doi.org/10.1017/S1462399402004155.
23. Li, J.-L., Sunde, R. A. 2016. Selenoprotein Transcript Level and Enzyme Activity as Biomarkers for Selenium Status and Selenium Requirements of Chickens ,Gallus gallus. PLOS ONE, 11,4, e0152392. https://doi.org/10.1371/journal.pone.0152392.
24. Li, X. H., Chen, Y. P., Cheng, Y. F., Yang, W. L., Wen, C., Zhou, Y. M. 2016. Effect of yeast cell wall powder with different particle sizes on the growth performance, serum metabolites, immunity and oxidative status of broilers. Animal Feed Science and Technology, 212, 81–89. https://doi.org/10.1016/j.anifeedsci.2015.12.011.
25. Livak, K. J., Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. Methods, 25,4, 402–408.
26. Moon, S. H., Lee, I., Feng, X., Lee, H. Y., Kim, J., Ahn, D. U. 2016. Effect of Dietary Beta-Glucan on the Performance of Broilers and the Quality of Broiler Breast Meat. Asian-Australas J Anim Sci, 29,3, 384–389. https://doi.org/10.5713/ajas.15.0141.
27. Nishikimi, M., Appaji Rao, N., Yagi, K. 1972. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research Communications, 46,2, 849–854. https://doi.org/https://doi.org/10.1016/S0006-291X,7280218-3.
28. Özbek, M., Petek, M., Ardıçlı, S. 2020. Physical quality characteristics of breast and leg meat of slow- and fast-growing broilers raised in different housing systems. Archives Animal Breeding, 63,2, 337–344. https://doi.org/10.5194/aab-63-337-2020.
29. Rady, W. F., Sayed, A.B. N., Abdel-raheem, H. A. 2023. Effect of dietary supplementation of echinacea and nucleotides on productive performance, intestinal histomorphology and gene expression of broiler chickens. Assiut Veterinary Medical Journal, 69,176, 141–155.
30. Salah, M., Suprijatna, E., Djauhari, M. L., Y B I Vitus, D. 2019. The Effects of Nucleotide Supplementation on the Productivity, Immune Response and Meat Quality of Broiler Chicken Reared Under Different Environmental Conditions. Livestock Research for Rural Development, 31,11, 1-14.
31. Tarnauceanu, G. ,FRUNZĂ, Pop, C. 2016. Water holding capacity of meat from rabbits ,Belgian Giant breed. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Animal Science and Biotechnologies, 73,1, 1–2. https://doi.org/10.15835/buasvmcn-asb:11590.
32. Uchiyama, M., Mihara, M. 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analytical Biochemistry, 86,1, 271–278. https://doi.org/10.1016/0003-2697,7890342-1.
33. Wang, S., Zhao, X., Liu, Q., Wang, Y., Li, S., Xu, S. 2022. Selenoprotein K protects skeletal muscle from damage and is required for satellite cells-mediated myogenic differentiation. Redox Biology, 50, 102255. https://doi.org/10.1016/j.redox.2022.102255.
34. Wang, T., Cheng, K., Yu, C., Tong, Y., Yang, Z., Wang, T. 2022. Effects of yeast hydrolysate on growth performance, serum parameters, carcass traits, meat quality and antioxidant status of broiler chickens. Journal of the Science of Food and Agriculture, 102,2, 575–583. https://doi.org/10.1002/jsfa.11386.
35. Wang, T., Cheng, K., Yu, C. Y., Li, Q. M., Tong, Y. C., Wang, C., Yang, Z. Bin, Wang, T. 2021. Effects of a yeast-derived product on growth performance, antioxidant capacity, and immune function of broilers. Poultry Science, 100,9, 101343. https://doi.org/10.1016/j.psj.2021.101343.
36. Wang, X., Tsai, T. C., Walk, C. L., Wilcock, P., Maxwell, C. V. 2017. 219 Effect of yeast cell wall ,YCW inclusion rate on growth performance in nursery pigs. Journal of Animal Science, 95,suppl_2, 105–105. https://doi.org/10.2527/asasmw.2017.12.219.
37. Weissman, S. M. 1976. Red Cell Metabolism. A Manual of Biochemical Methods. 2nd Edition. The Yale Journal of Biology and Medicine, 49, 3, 310–311.
38. Zhang, H., Chen, Y., Chen, Y., Li, Y., Jia, P., Ji, S., Zhou, Y., Wang, T. 2020. Dietary pterostilbene supplementation attenuates intestinal damage and immunological stress of broiler chickens challenged with lipopolysaccharide. Journal of Animal Science, 98,1, skz373. https://doi.org/10.1093/jas/skz373.
39. Zhang, S., Ou, J., Luo, Z., Kim, I. H. 2020. Effect of dietary β-1,3-glucan supplementation and heat stress on growth performance, nutrient digestibility, meat quality, organ weight, ileum microbiota, and immunity in broilers. Poultry Science,

99,10, 4969–4977. https://doi.org/10.1016/j.psj.2020.06.036.
40. Zhang, Z. F., Zhou, T. X., Ao, X., Kim, I. H. 2012. Effects of β-glucan and Bacillus subtilis on growth performance, blood profiles, relative organ weight and meat quality in broilers fed maize–soybean meal based diets. Livestock Science, 150,1–3, 419–424. https://doi.org/10.1016/j.livsci.2012.10.003.