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Amelioration of doxycycline side effects in broiler chickens using probiotic and date kernels powder

Ghada H. Ali[1], Salwa M. Hafez[2*], Asmaa M. Abd-Elrahman[1]

[1]*Biochemistry, Nutritional Deficiency Diseases and Toxicology Department, Animal Health Research Institute, Beni-Suef Lab. (AHRI), Agriculture Research Center (ARC), Egypt.*
[2]*Food Hygiene Department, Animal Health Research Institute, Beni-Suef Lab (AHRI), Agriculture Research Center (ARC), Egypt.*

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*Correspondence:

Corresponding author: Salwa M. Hafez
E-mail address: salwamohamed201477@ahri.gov.eg

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ABSTRACT

Antibiotic administration in broiler farms is common, especially in developing countries. Doxycycline, a tetracycline antibiotic used in poultry farms effectively prevents and treats avian diseases as well as alters gut microbiota stability and composition. Researchers are now interested in the digestive tract microbiota. It has long been believed that the metabolic processes and gut microbial ecology influence how well broilers perform. This study intended to ascertain: the effect of doxycycline administration on broilers' performance metrics, serum lipid and protein profiles, meat quality, and intestinal microbiome and health. The possible protective impact of *Saccharomyces cerevisiae* probiotic and date kernel powder was evaluated. Sixty, one-day-old Ross broiler chicks were reared and split on the 8th day into 4 equal groups. G1 (Control group); fed on a balanced ration all over the experiment (42 days). G2 (Doxycycline group); fed on a balanced ration and doxycycline drug 0.5g/L in drinking water. G3 (Doxycycline+Sc group); fed on a balanced ration supplemented with *Saccharomyces cerevisiae* (Sc) probiotic 2.5 billion CFU/kg ration and doxycycline drug 0.5g/L in drinking water. G4 (Doxycycline+dk group); fed on a balanced ration supplemented with 4% date kernel powder (dk) and doxycycline drug 0.5g/L in drinking water. The findings showed that doxycycline administration adversely affected broilers' performance, serum lipid and protein profiles, meat quality, and intestinal microbiome and health of broilers. Supplementation with *Saccharomyces cerevisiae* probiotic and date kernel powder effectively ameliorated doxycycline adverse effects in broilers.

Introduction

The poultry industry has expanded rapidly throughout the past few decades and is now a significant economic activity in many countries, especially considering the increase in global poultry consumption. This industry faces several challenges to produce functional and safe food for consumers (climatic conditions, microbial load, and stress during rearing) causing an imbalance in the intestinal microflora and impairment of nutrient absorption leading to disturbing the normal functioning of the birds and increased mortality resulting in serious economic losses (Grashorn, 2010).

A common broad-spectrum tetracycline antibiotic in farming is doxycycline (Blau *et al*., 2019). Microbial content and balance necessary for metabolism and bird growth are reduced, and avian illnesses in broilers are efficiently prevented and treated by antibiotics (Maki *et al*., 2019; Haberecht *et al*., 2020). Furthermore, being growth promoters, antibiotics are applied to prevent and treat avian diseases. Nonetheless, they have been prohibited as growth promoters in the US since 2017 and the EU since 2006 (Roth *et al*., 2019).

Abuse of antibiotics is the most potent factor that can cause changes in the intestinal microbial content (Gawey and Czaja, 2017). The lack of beneficial microbes, the proliferation of pathogens, and the decline in the intestinal flora variety are the three types of dysbiosis; usually, these three processes co-occur (DeGruttola *et al*., 2016). Tetracyclines preferentially target some bacterial species while mostly ignoring others, changing the gut microbiome's diversity and composition. Serious dysbiosis may result from the overabundance of opportunistic infections brought on by this (Baran *et al*., 2023).

Probiotics are applied as feed supplements for poultry as they have advantageous effects on broilers performance, better feed utilization, modification of the intestinal microbes and pathogen inhibition, certain haemato-biochemical parameters, immunomodulation, and reduction incidence of diseases (Islam *et al*. 2004; Kabir *et al*. 2005; Lee *et al*., 2011; Allen *et al*., 2013). Intestinal flora alteration, growth enhancement of lactic acid producing bacteria, inhibition of intestinal pathogen proliferation and improved digestion and absorption of nutrients are some of the methods by which probiotics enhance efficacy of feed conversion (Yeo *et al*., 1997).

Egypt is the world's top producer of dates, which represents 17.7% of global production, therefore being strategically important (FAO, 2014). About 6–12% of date fruit is made up of date kernels, which are inexpensive, readily available and high in energy. Poultry productivity, feed expenses and feed absorption were all greatly enhanced by the dietary addition of date kernel powder (Abdollahi *et al*., 2016; Tareen *et al*., 2017; Sholichatunnisa *et al*., 2022). Date kernels can act as a prebiotic modulating the gut ecosystem, regulating the intestinal bacterial population by binding to pathogenic bacteria and blocking bacterial lectin, improving gut development and overall broiler health (Canibe and Jensen, 2003). This study aimed to determine the possible protective impact of *Saccharomyces cerevisiae* probiotic and date kernel powder to eliminate the side effects of doxycycline administration on broilers' performance metrics, serum lipid and protein profiles, meat quality, and intestinal microbiome and health.

Materials and methods

Ethical Approval

The Institutional Animal Care and Use Committee (ARC-IACUC), Animal Health Research Institute, Agriculture Research Center, Egypt, authorized the experimental design and procedures for this study (ARC, AHRI, 128, 24).

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Doxycycline drug

Doxydad (water-soluble powder of doxycycline HCL 200 mg/g) produced by DADvet, Jordan, is applied in a dose of 0.5g/L of drinking water.

Probiotic

Allgäu Yeast (active dry yeast "20 billion" CFU/g of *Saccharomyces cerevisiae* Sc 47) produced by AllgäuVet, German, is applied in a dosage of 2.5 billion CFU/kg feed (Maksimović, *et al*., 2022).

Date kernel powder

Local date kernel powder was prepared according to Wahini (2016) and added to the ration with 4% (Tareen *et al*., 2017)

Experimental birds, diet and design

One day old Ross broiler chicks (n.= 60) were presented from Ommat Arab Poultry Breeder Company, Giza, Egypt. Chicks were reared under optimum conditions of temperature, humidity, and ventilation. The chicks got ad libitum water and feed. On the 8th day, chicks (190±10 g.) were distributed into four equal groups up to end of the experiment (42 days).
G1 (Control group): fed on a balanced ration all over the experiment.
G2 (Doxycycline group): Fed on a balanced ration and doxycycline drug 0.5g/L in drinking water.
G3 (Doxycycline+Sc group): Fed on a balanced ration supplemented with *Saccharomyces cerevisiae* (Sc) probiotic 2.5 billion CFU/kg ration and doxycycline drug 0.5g/L in drinking water.
G4 (Doxycycline+dk group): Fed on a balanced ration supplemented with 4% date kernel powder (dk) and doxycycline drug 0.5g/L in drinking water.

Growth Performance data

Birds and feed were weighed every two weeks for growth performance parameters (body weight, feed intake, body weight gain, and feed conversion ratio).

Sampling

Blood samples

On the 42nd day of the experiment, blood specimens were drawn via wing vein puncture for serum separation. The serum was separated by centrifugation for 10 minutes at 2900 r.p.m. to determine the lipid profile (cholesterol, total glycerides, and high-density lipoproteins) and protein profile (total proteins and albumin), the serum was stored in a deep freeze at -20°C.

Meat samples

After obtaining blood samples, birds were euthanized based on the recommendations of the Animal Ethics Committees. The samples of thigh and breast muscles were collected under strict aseptic conditions for total counts of fungi and aerobic bacteria and for determination of moisture percentage.

Intestine samples

Intestinal content (cecum content) was aseptically collected for microbiological analysis (total counts of aerobic bacteria, *Enterobacteriaceae*, and *Staphylococci*, as well as *Staphylococcus aureus* and *Salmonella* isolation and identification) and for determination of pH values.

Ilium specimens were cleaned with sterile physiological saline to remove any depressants and kept in RNA protector for 24 hours then maintained in a deep freeze at -20°C until assessing of occluding gene expression by rt-PCR.

Biochemical analysis

Serum cholesterol, triglycerides, high-density lipoproteins, total proteins, and albumin levels were estimated following the procedure described by Young (2001) using commercial kits from SPINREACT, located in Santa Coloma, Spain.

Moisture content of raw and cooked meat was measured by Hot air oven method (AOAC, 1980). pH of cecum content was determined according to the AOAC guidelines (AOAC, 2000).

Microbial analysis

Preparation of the samples (ICMSF, 1978) involved aseptically weighing and blending 25g of meat samples with 225mL of buffered peptone water in a sanitized jar and aseptically weighing and mixing 1g of cecum content samples with 9mL of buffered peptone water in a sanitized test tube. From the original dilution, ten-fold serial dilutions were prepared for microbiological analysis.

Total fungal count (FAO, 1992), total aerobic bacterial count (USDA, 2011), total *Enterobacteriaceae* count (ISO, 2001), total *Staphylococci* count and *Staphylococcus aureus* count (USDA, 2011). *Staphylococcus aureus* isolation and identification (ICMSF, 1978; MacFaddin, 2000). *Salmonella* isolation and identification (ICMSF, 1978; Andrews and Hammack, 1998) were carried out.

Molecular analysis

Real-time qPCR was applied to assess mRNA expression levels of occludin gene in the intestine of broilers. Broiler intestinal samples were used to extract and purify total RNA using the QIAamp RNeasy Mini kit proce-

Table 1. Ingredient composition* of broilers' basal diet and nutrient levels.

Nutrient levels	Starter diet 23% (1−19 d)	Grower diet 21% (20−35 d)	Finisher diet 17% (36−42 d)
Crude protein	23%	21%	17%
Crude fat	4.41%	5.42%	6.43%
Crude fiber	2.42%	2.40%	2.35%
D-L-Methionine	0.65%	0.55%	0.42%
L-lysine	1.49%	1.19%	1%
Calcium	1.06%	1.06%	1.06%
Total phosphorous	0.36%	0.35%	0.33%
Representative energy (Kcal/Kg diet)	3050	3115	3265
Calorie/Protein ratio	132.61	148.33	186.57

*Ingredients: Yellow corn, fish meal, Gluten, limestone, soybean meal, corn oil, Di-calcium phosphate, Na Cl

dure (Qiagen GmbH, Hilden, Germany). Using a Stratagene MX3005P machine (Agilent Technologies, Inc., Santa Clara, CA) and a QuantiTect SYBR Green RT-PCR Kit (Qiagen GmbH, Germany). The proper gene-specific primers for assessing the expression are GAGCCCAGACTACCAAAGCAA as Forward primer sequence (5′-3′) and GCTTGATGTGGAAGAGCTTGTTG as Reverse primer sequence (5′-3′) supplied from Metabion (Germany). The stratagene MX3005P software was applied for determination of amplification curves and control values, $(2^{-\Delta\Delta ct})$ technique interpreted data of gene expression (Yuan *et al*., 2006).

Statistical analysis

Graph Pad InStat software (version 3, ISS-Rome, Italy) was applied for statistical analysis. Data groups were compared using one-way analysis of variance (ANOVA) and the Tukey-Kramer (TK) Multiple Comparison post-test. Microbial counts are declared as Log_{10} CFU/g. The data are presented as mean ± standard error (SEM) in tables and figures. SE. $P \leq 0.05$ values were significant, while $P \leq 0.001$ values were highly significant.

Results

Results of performance data (body weight, feed intake, weight gain, and FCR) of broilers of all groups are summarized in Table 2. Doxycycline administration (G2) resulted in a high significant decrease in the final body weight and weight gain and a significant increase in FCR of broilers' entire cycle in comparison to the control (G1). The addition of *Saccharomyces cerevisiae* probiotic (G3) and date kernel powder (G4) improved such results and returned to be within normal compared with the control. Supplementation with *Saccharomyces cerevisiae* probiotic and date kernel powder decreased the feed intake of broilers' entire cycle in comparison to the control.

Broilers of the doxycycline group (G2) showed a significant elevation of serum cholesterol and a notable increase of serum triglycerides and high-density lipoprotein values, on the contrary, serum total proteins and albumin values showed a high significant decrease in comparison to G1 (Table 3). The dietary addition of *Saccharomyces cerevisiae* probiotic and date kernel powder decreased serum cholesterol levels and triglycerides, on the contrary, values of high-density lipoproteins, total proteins, and albumin increased in comparison to the doxycycline group. A significant increase in high-density lipoproteins was found in G3 and G4 in comparison to G1.

Meat analysis results are seen in Table 4, microbiological analysis of broilers' meat samples revealed an increase in total fungal count in the doxycycline group (G2) in comparison to G1. The dietary addition of *Saccharomyces cerevisiae* probiotic and date kernel powder (G3 and G4) improves such results and the total fungal count returns to be within normal control. The total aerobic bacterial count of broilers of G2 group shows a numerical increase in comparison to the control, on the contrary,

Table 2. Performance data of broilers of all groups.

	Groups			
Parameters	G1	G2	G3	G4
8- 14 days				
Body weight(g)	538.75±11.1^{a}	482.86±8.92^{b}	491.66±8.33^{c}	445.71±2.97^{b}
Feed intake(g)/bird	650±5.77^{a}	650±2.88^{a}	700±8.66^{b}	590±5.77^{b}
Weight gain(g)/bird	493.75±3.61^{a}	437.86±7.01^{b}	446.66±4.82^{a}	400.71±11.55^{b}
FCR	1.32±0.05	1.48±0.04	1.57±0.08	1.47±0.07
15-28 days				
Body weight(g)	1346.36±26.3	1434.29±48.15	1370.90±32.12	1356.29±46.97
Feed intake(g)/bird	1470±11.55^{a}	1440±5.77^{b}	1290±5.77^{c}	1310±8.66^{c}
Weight gain(g)/bird	807.61±4.04^{a}	951.43±5.77^{b}	879.24±5.77^{d}	910.58±2.89^{c}
FCR	1.82±0.08^{a}	1.51±0.05^{b}	1.47±0.04^{c}	1.44±0.03^{c}
29-42 days				
Body weight(g)	2550±35.36^{a}	2087.14±24.76^{b}	2502.5±88.64^{a}	2442±57.74^{a}
Feed intake(g)/bird	1870±17.32^{a}	1860±11.55^{a}	1770±11.54^{c}	1970±14.43^{b}
Weight gain(g)/bird	1203.64±13.28^{a}	652.85±6.93^{b}	1131.6±17.90^{d}	1085.71±17.32^{c}
FCR	1.55±0.09^{a}	2.85±0.12^{b}	1.56±0.08^{a}	1.81±0.11^{a}
Entire period (1-42 day)				
Body weight(g)	2550±35.36^{a}	2087.14±24.76^{b}	2502.5±88.64^{a}	2442±57.74^{a}
Feed intake(g)/bird	3990±14.43^{a}	3950±28.87^{a}	3760±23.09^{b}	3870±17.32^{c}
Weight gain(g)/bird	2505±14.44^{a}	2042.14±12.72^{b}	2457.5±11.55^{a}	2397±13.28^{c}
FCR	1.59±0.06^{a}	1.94±0.03^{b}	1.53±0.04^{a}	1.61±0.05^{a}

The data is displayed as Mean ± Standard Error of the Mean (SEM). Means within the same row carrying different superscript letters are significantly different ($P \leq 0.05$). ($n \geq 3$)
G1: control group; G2: doxycycline group; G3: doxycycline + *Saccharomyces cerevisiae* group; G4: doxycycline + date kernel powder group.

Table 3. Biochemical parameters of broilers of all groups.

Groups	Cholesterol (mg⁄dl)	Triglycerides (mg⁄dl)	High-density lipoproteins (mg⁄dl)	Total proteins (g⁄dl)	Albumin (g⁄dl)
G1	157±11.24^{a}	131.67±17.17	70.33±4.49^{a}	4.10±0.19^{a}	2.22±0.07^{a}
G2	228.33±8.82^{b}	165±19.14	81.5±1.71^{a}	2.38±0.12^{b}	1.35±0.12^{b}
G3	192.66±9.60^{a}	138.33±14.26	86.33±4.91^{b}	4.05±0.09^{a}	1.89±0.17^{c}
G4	183.33±12.35^{a}	156.33±18.85	86±2.31^{b}	3.80±0.11^{a}	1.79±0.03^{c}

The data is displayed as Mean ± Standard Error of the Mean (SEM). Means within the same column carrying different superscript letters are significantly different ($P \leq 0.05$). ($n \geq 3$)
G1: Control group; G2: Doxycycline group; G3: Doxycycline + *Saccharomyces cerevisiae* group; G4: Doxycycline + date kernel powder group.

both G3 and G4 groups show a highly significant decrease in comparison to the control. Moisture % values didn't show significance among groups.

Results of cecum content analysis are shown in Table (5), microbiological analysis of broilers' cecum content showed a highly significant increase in total aerobic bacterial count in the doxycycline group (G2) in comparison to G1. The dietary addition of *Saccharomyces cerevisiae* probiotic (G3) and date kernel powder (G4) decreased these results to be within normal control. Total *Enterobacteriaceae* count showed a significant elevation in G2 group in comparison to G1 group, supplementation of *Saccharomyces cerevisiae* probiotic and date kernel powder decreased these results to be within normal control. Total *Staphylococci* count shows a significant increase in G2, G3, and G4 groups in comparison to G1. *Staphylococcus aureus* and *Salmonella* were not isolated in any group. Values of pH of broilers' cecum content revealed that doxycycline didn't alter cecum content pH, on the other hand, pH values of broilers of G3 and G4 were high significantly and significantly decreased, respectively in comparison to the control.

Occludin gene expression was significantly downregulated in the gut of broilers in the doxycycline group in comparison to the control group. While the dietary addition of probiotic *Saccharomyces cerevisiae* and date kernel powder enhanced the expression of the occludin gene in comparison to the doxycycline group (Fig. 1).

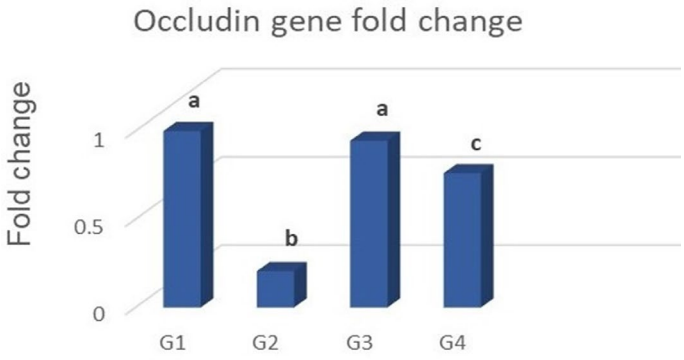

Fig. 1. Occludin gene fold change of broilers of all groups.Mean values with different superscript (a, b, c) are significantly different ($p \leq 0.05$).
G1: Control group; G2: Doxycycline group; G3: Doxycycline + *Saccharomyces cerevisiae* group; G4: Doxycycline + date kernel powder group.

Discussion

Because of its importance to health and productivity, microbiota in broiler digestive tract has attracted researchers' attention in recent years. Intestinal microbes play an essential role in nutrients releasing from feed, immunostimulant and intestinal health promotor. Breed, gender, age, feed type, antibiotic use, and housekeeping for broilers constitute some of the aspects that affect the microbial content of the broilers' gut (Clavijo and Flórez, 2018; Khasanah *et al*., 2024).

Doxycycline is a broad-spectrum tetracycline antibiotic. In addition to suppressing infections and influencing the microbial balance and content which are vital for immunity, metabolism and bird growth, antibiotics are successfully applied to therapy of avian diseases (Blau *et al*., 2019; Maki *et al*., 2019; Haberecht *et al*., 2020; Greene *et al*., 2022).

Broilers administrated doxycycline showed a decline in the body weight gain and the final body weight and an increase of FCR of the entire cycle in comparison to the control, which agrees with Madubuike *et al*. (2020), who stated that broilers' live weight, weight gain and feed conversion efficiency are all negatively impacted when doxycycline is administered early in life. Allama *et al*. (2012) stated that the tissue will turn protein into energy if the energy and protein do not match predetermined standards. Systemic energy metabolism is negatively impacted by dysbiosis resulted from antibiotic treatment and its effect on host energy metabolism is the consequence of a co-occurring alteration in the content of the intestinal microbes and the metabolism of steroids (Praharaj *et al*., 2015; Le Roy *et al*., 2019).

The performance metrics of broilers were improved by adding the probiotic *Saccharomyces cerevisiae* and the date kernel powder to their diet. According to studies by Ezema and Ugwu (2014); Ogbuewu *et al*. (2019) and He *et al*. (2021), adding *Saccharomyces cerevisiae* to the diet of broilers enhanced their intestinal morphology, nutrient digestibility and growth performance. Both their direct nutritional effects (absorption of nutrients, fermentation of fibers, production of bile acids, and elimination of free radicals) and their capacity to modulate intestinal microbiomes may contribute to the beneficial effects of probiotics (Shareef and Al-Dabbagh, 2009; Cisek and Binek, 2014; Daniel *et al*., 2018).

In agreement with Habibi *et al*. (2013); Macelline *et al*. (2017) and Rybarczyk *et al*. (2023), the addition of *Saccharomyces cerevisiae* to the broiler diet led to a reduction of feed consumption, which was clarified by Koenen *et al*. (2004) who found that broilers' lower nutrient absorption led to higher feed intake to meet their nutritional needs. Consequently, broiler feed intake decreased as a result of the feed's effective nutrient utilization.

El-Far *et al*. (2016); Tareen *et al*. (2017) and Attia *et al*. (2021) mentioned that using of date kernel in poultry diets at levels up to 5-10%

Table 4. Meat analysis of broilers of all groups.

Groups	Microbiology (log_{10} cfu/g.)		Moisture %
	Total fungal count	Total aerobic bacterial count	
G1	3.748±0.01^{a}	3.838±0.01^{a}	71.47±0.74
G2	3.793±0.01^{b}	3.857±0.01^{a}	72.07±1.05
G3	3.729±0.01^{a}	3.724±0.00^{b}	70.00±0.81
G4	3.744±0.01^{a}	3.746±0.00^{b}	69.60±0.99

The data is displayed as Mean ± Standard Error of the Mean (SEM). Means within the same column carrying different superscript letters are significantly different (P≤ 0.05). (n≥3)
G1: Control group; G2: Doxycycline group; G3: Doxycycline + *Saccharomyces cerevisiae* group; G4: Doxycycline + date kernel powder group.

Table 5. Cecum content analysis of broilers of all groups.

Groups	Microbiology (log_{10} cfu/g.)			pH value
	Total aerobic bacterial count	Total *Enterobacteriaceae* count	Total *Staphylococci* count	
G1	6.713±0.01^{a}	5.609±0.01^{a}	4.509±0.02^{a}	7.63±0.07^{a}
G2	6.920±0.02^{b}	5.725±0.03^{b}	4.693±0.05^{b}	7.42±0.13^{a}
G3	6.788±0.03^{a}	5.627±0.01^{a}	4.684±0.02^{b}	7.06±0.05^{b}
G4	6.790±0.01^{a}	5.629±0.02^{a}	4.677±0.02^{b}	7.19±0.09^{c}

The data is displayed as Mean ± Standard Error of the Mean (SEM). Means within the same column carrying different superscript letters are significantly different (P≤ 0.05). (n≥3)
G1: Control group; G2: Doxycycline group; G3: Doxycycline + *Saccharomyces cerevisiae* group; G4: Doxycycline + date kernel powder group.

positively affects productive performance (body weight, feed intake and FCR), probably as a reason of the nutritional value of crude fiber. The dietary addition of date kernel powder significantly reduced the feed intake of the entire cycle of broilers, this agrees with Tareen *et al*. (2017) who pointed out that the addition of 3% and 4% levels of date palm kernel in broilers' feed for 42 days significantly reduced the feed consumption in comparison to the control. In their guts, broilers may effectively digest their diet and absorb nutrients. According to Abdollahi *et al*. (2016), adding date kernel meal to the diet, regardless of the addition degree, improved the digestibility of fat and starch, which could be responsible for the impact of date kernel powder on broiler feed consumption.

Hypercholesterolemia and hypertriglyceridemia associated with doxycycline administration coordinate with Kalavathy *et al*. (2008); Li *et al*. (2019) and Shittu *et al*. (2022) as they stated that oxytetracyclines administration caused an increase in serum lipids in broilers. Doxycycline administration disrupts lipid metabolism in murine preadipocytes (Lei *et al*., 2023). Total proteins value is an important parameter for the diagnosis of diseases associated with metabolic disorders, hypoproteinemia seen in the doxycycline group agrees with Slyamova *et al*. (2016), who mentioned that tetracycline administration resulted in a reduction of serum total proteins of broilers indicating a protein metabolism disturbance. Doxycycline negative impacts on body weight, weight gain and FCR alongside serum proteins of broilers could be attributed to its impact on gut microbiota and energy and protein metabolism.

Saccharomyces cerevisiae probiotic decreased serum cholesterol levels and triglycerides while it increased values of high-density lipoproteins this coordinates with Al-Zuhairi *et al*. (2014); He *et al*. (2021) and Soren *et al*. (2024). Al-Zuhairi *et al*. (2014) stated that *Saccharomyces cerevisiae* in addition to laying hens' diet reduced serum and egg cholesterol levels, triglycerides, and increased values of high-density lipoproteins. *Saccharomyces cerevisiae* probiotic effect could be explained as it improves lipid metabolism and inhibits cholesterol oxidation, in addition to their role in organic acids production which causes the reduction of triglycerides and elevation of high-density lipoproteins (Yeo and Kim, 1997; Paryad and Mahmoudi, 2008; He *et al*., 2021). Supplementation with *Saccharomyces cerevisiae* probiotic increased serum total proteins and albumin levels concurring with Al-Zuhairi *et al*., 2014. Its direct nutritional effects (absorption of nutrients, fermentation of fibers, and production of bile acids) or its ability to modify gut microbiomes may be the cause of its beneficial impacts (Shareef and Al-Dabbagh, 2009; Cisek and Binek, 2014; Daniel *et al*., 2018).

The dietary addition of date kernel powder decreased serum cholesterol levels, this agrees with Roziqin *et al*. (2024), who stated that date pit flour in broilers' feed caused a reduction of carcass cholesterol. Fiber presence in feed causes a reduction of cholesterol, triglycerides, and increased values of high-density lipoproteins as it decreases cholesterol absorption and subsequently wasted through excreta, also, fiber fermentation generates short-chain fatty acids such as acetic, propionic and butyric acids which reduce triglycerides and increase high-density lipoproteins (Yeo and Kim, 1997; Paryad and Mahmoudi, 2008; Cohn *et al*., 2010). The addition of date kernel powder increased serum total proteins and albumin levels compared to the doxycycline group, probably due to its nutritional value as it is abundant in protein, fat, and fibers as stated by Al-Farsi and Lee (2008). As well as its beneficial effect on feed digestion and nutrient absorption inside the gut. According to Sholichatunnisa *et al*. (2022), adding date seed flour to broilers' feed considerably altered the apparent digestibility of protein, demonstrating potent protein utilization.

Although the muscles of birds in healthy living condition are sterile, the digestive tract, lungs, skin and feathers harbor a variety of microbiotas that contaminate corpses both during and after slaughter (Rouger *et al*., 2017). Tetracyclines can change the content and diversity of the intestinal microbes via selectively some species of bacteria despite ignoring others, this can result in excess of pathogens leading to serious dysbiosis (Baran *et al*., 2023).

Supplementation of probiotics and prebiotics improves the microbial intestinal content and raises the quality of poultry meat. They also optimize the equilibrium of microbes in the poultry hosts' digestive systems and provide protection from harmful bacteria and pathogens (Wang *et al*., 2018; Jha *et al*., 2020). Because date kernel powder has antibacterial and antioxidant properties, it may be used to preserve the quality and safety of meat while extending its shelf life (Habib and Ibrahim, 2011; Perveen *et al*., 2012).

Microbiological analysis of the doxycycline group meat samples revealed an increase in total fungal count (statistical) and total aerobic bacterial count (numerical) compared to the control. Microbiological analysis of cecum content samples of the doxycycline group revealed an increase in total aerobic bacterial count, total *Enterobacteriaceae* count, and total *Staphylococci* count in comparison to the control. The growth of total *Enterobacteriaceae* count in cecum content analysis in the doxycycline group in comparison to the control agrees with Videnska *et al*. (2013) and Green *et al*. (2022) who recorded a raised prevalence of Enterobacteriales in the fecal microbiota of chickens that received tetracycline therapy.

The dietary addition of *Saccharomyces cerevisiae* probiotic and date kernel powder caused a reduction of total aerobic bacterial count (in meat and cecum content) in comparison to the control group. The supplementation of *Saccharomyces cerevisiae* probiotic decreased the total aerobic bacterial count and the total *Enterobacteriaceae* count concurs (Mountzouris *et al*., 2010; Maksimović, *et al*., 2022; Soren *et al*., 2024). In comparison to the control birds, *Saccharomyces cerevisiae* dietary supplementation showed a significant decline in total *E. coli*, pathogenic *E. coli*, and ESBL-producing *Enterobacteriacea* of intestinal microbes (Mountzouris *et al*., 2010; Soren *et al*., 2024). Prior investigations found that birds fed yeast products had lower levels of *E. coli*. This could be explained by the production of enzymes that break down bacterial toxins and the competitive pathogens elimination (Ghosh *et al*., 2012).

Elhadef *et al*. (2023) researched the impact of date palm seed ethanolic extract (DSEE) at 0.156%, 0.312%, and 0.624% on the chemical stability and microbiological quality of chicken breast meat stored in a refrigerator for 14 days. DSEE significantly slowed down microbial growth and reduced lipid/protein oxidation processes, indicating that it may be useful for preserving chicken meat.

Doxycycline administration didn't alter cecum content pH values, while the dietary addition of *Saccharomyces cerevisiae* and date kernel powder caused a reduction of intestinal pH values. Such results agree with Kamel *et al*. (1981); Ciurescu *et al*. (2021); Maksimović *et al*. (2022) and Sholichatunnisa *et al*. (2022). By producing a variety of organic acids, including lactic and acetic acid, *Saccharomyces cerevisiae* lowers the pH of the intestine, forming an acidic condition that eventually inhibits pathogens (Ogbuewu *et al*., 2019).

Date kernel flavonoid compounds and high carbohydrate content affect the intestinal acidity level producing a low pH value by increasing the proliferation of lactic acid producing bacteria (Widodo *et al*., 2015).

Both endothelial and epithelial cells include tight junction proteins. Claudins and occludin are the two main protein types that form tight junctions, and both are vital for maintaining the gut epithelial cells' viability and barrier function, which permits nutrient absorption (Tsukita *et al*., 2019). According to Alberts *et al*. (2015), occludin is a highly conserved protein that controls functional permeability in birds. Many variables, including nutrition, stress, intestinal microbiota, illness, infections, toxins and drugs, may alter intestinal permeability (Bischoff *et al*., 2014).

According to earlier research, gene expression of tight junctions may be a gastrointestinal biomarker of intestinal barrier performance because disorder of tight junction proteins may result in decrease of nutrients absorption, increase of luminal antigens permeability, prolonged inflammation and tissue disruption (Peterson and Artis, 2014; Chen *et al*., 2015; Celi *et al*., 2019).

In coordination to Duan *et al*. (2024), broilers in the doxycycline

group showed a down-regulation of intestinal occludin gene expression in comparison to the control group. The disrupted gut mucosa and the reduced mRNA expression levels of tight junction proteins (ZO-1, Occludin, and Claudin-3) in juvenile groupers were among the intestinal health effects of tetracycline, they noted.

The expression of occludin gene is enhanced by the dietary inclusion of the probiotic *Saccharomyces cerevisiae* and the date kernel powder in comparison to the doxycycline group. The enhancement of occludin gene expression accompanied with the addition of *Saccharomyces cerevisiae* coordinates with the findings of Rahimi *et al*. (2009) and Wu *et al*. (2022), who declared that probiotic supplementation increased the occludin mRNA expression in the broilers' intestine. The modulatory role of butyrate, a short-chain fatty acid, on intestinal barrier integrity determined by tight junction protein expression in chickens was assessed by Mátis *et al*. (2022), they found that butyrate provides advantages in enhancing intestinal barrier integrity and performance metrics of broilers. It is hypothesized that up-regulation of occludin gene expression caused by date kernel powder resulted from impact of butyrate fatty acid produced by fermentation of date fibers in the large intestine.

Conclusion

Dysbiosis of broilers' gut microbiota due to doxycycline administration results in adverse effects on broilers' performance metrics, serum lipid and protein profiles, quality of meat and intestinal health of broilers. Supplementation with *Saccharomyces cerevisiae* probiotic and date kernel powder are effective in amelioration of such adverse effects.

Conflict of interest

The authors have no conflict of interest to declare.

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