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Role of Egyptian powdered date palm kernels and probiotics in alleviating degradation induced by Doxycycline misuse in broilers

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ABSTRACT

Date palm kernel powder (DPKP) and *Saccharomyces cerevisiae* yeast (SCY) enhance poultry health and meat production. Antibiotic misuse in broilers has negative consequences. To show the ability of DPKP and SCY feed additives in alleviating degradation induced by doxycycline misuse in broilers. Five groups of fifteen chicks started on the ninth day and lasted until 42 days of their life. At the end, all birds were weighed, muscles were collected after euthanasia. and the meat samples was frozen at -20°C for residue measurement. Another part was refrigerated at 4±1°C for microorganism detection, and biochemical indices were tested on 0, 3rd, 7th, and 9th days after refrigeration. The Doxycycline-treated group showed a non-significantly higher mean total colony count, increased mean Doxycycline residue by 100%, 60.63%, 65.7%, and 100%, and decreased mean end body weight by 11.38%, 21.2%, 20.25%, and 12.3% compared to the control, Doxycycline (Doxy) + DPKP, Doxy + SCY, and SCY-treated groups, respectively ($p < 0.001$). Nonetheless, the Doxy + DPKP, Doxy + SCY, and SCY-treated groups showed significant increases ($p < 0.001$). Compared to the Doxy-treated group, all treated groups had lower *Enterobacteriaceae* counts ($p < 0.001$). The psychoactive count greatly increased in the Doxy + SCY and SCY groups, dramatically decreased in the Doxy-treated group when compared to the control and Doxy + DPKP-treated groups. *Staph* count was higher in the Doxy-treated groups than in the control. *Staphylococcus aureus* or *Pseudomonas aeruginosa* were not isolated. On the third day of refrigeration, the Doxy-treated group had higher TVBN and pH than the other treated groups. It could be concluded that SCY and DPKP reduce doxycycline degradation in broiler feeds while maintaining meat quality.

Introduction

The delicious flavor, low-fat content, high nutritional value, and inexpensive production costs have led consumers to turn to poultry meat as a source of protein to avoid the global incidence of cardiovascular disease. Due to its high protein and moisture content, high pH, and vulnerability to chemical and microbiological deterioration, poultry meat has a short shelf life of around five days in the refrigerator (Kerry *et al*., 2006). Meat and meat products derived from poultry are particularly prone to spoiling. Any change to a food product that makes it undesirable to the consumer's senses is considered spoilage (Gram *et al*., 2002).

Therefore, extending the shelf life of raw chicken flesh in the food industry is a main challenge that calls for innovative preservation strategies (Dainty *et al*., 1985). Meat components, such as sugars and free amino acids, are consumed by microbes, which cause organoleptic degradation and the release of unwanted volatile compounds (Can, 2011). Lipid oxidation and microbiological contamination are the causes of food quality loss and decreased shelf life, which also contribute to sensory deterioration (Fernandez-Lopez *et al*., 2004). Fatty acids reduce color and oxidative shelf life by increasing the degree of unsaturation (Sampels *et al*., 2004). An enhanced thiobarbituric acid (TBA) assay with antioxidant protection was used to quantify malondialdehyde in fat to assess the oxidative rancidity of fresh, frozen, and cooked chicken breast and leg meat (Abd El-Kader, 1996).

The fat content of beef makes it more prone to lipid oxidation, which can lead to abnormal flavor, taste, color, texture, and lower nutritional value.

Bacteria can be suppressed or eliminated by antibiotics (Olafsdottir *et al*., 1997; Yilmaz, 1998). Encourage veterinarians and chicken farmers to employ them to improve growth, feed efficiency, and control disease (Abdel-Mohsein *et al*., 2015). Although antibiotics have increased the productivity of chicken production and enhanced the health and welfare of chickens, they have also left behind residues (Donoghue, 2003). Approximately 80% of animals that produce food are frequently given tetracyclines, β-lactams, aminoglycosides, lincosamides, macrolides, and sulfonamides (Lee *et al*., 2001).

One of the most widely used yeast species and a great adsorbent is *S. cerevisiae*. Moreover, vitamin B complexes and proteins are highly concentrated in *S. cerevisiae*. Immune-stimulating substances found in *S. cerevisiae* include β-glucan and mannan-oligosaccharides. It has been demonstrated that this strain of yeast boosts immunity and promotes development (Celik *et al*., 2000; Çelýk *et al*., 2003).

Additionally, by preserving intestinal biostructure, it offers additional health benefits and possesses antimicrobial properties (Wang and Gu 2010; Zhang *et al*., 2016). In addition to absorbing mycotoxin, probiotics prevent some intestinal infections, provide a variety of nutrients, enhance intestinal metabolism in chickens (Zarei *et al*., 2018), and enhance both systemic and local immunity (Korver, 2012; Wang *et al*., 2017). Because manno-oligosaccharides can stop pathogen germs from colonizing broiler intestines, they have been selected as antibiotic substitutes (Fernandez *et al*., 2000). Animal immune systems have long been thought to be strengthened by manno-oligosaccharides (MOS) (Lyons, 2002).

Although palm kernel meals contain dietary fiber, particularly β-mannan, it is not suited for poultry diets due to its grittiness. It may replace commercial manno-oligosaccharides as a prebiotic to support the health and immunity of chickens, according to numerous recent studies (Fernandez *et al*., 2002). Because they are indigestible, mannose-based carbohydrates in palm kernel meal, including β-mannan or MOS, may ferment in the caeca. This facilitates the growth of beneficial microbes. This mode of action is based on the fact that non-pathogenic bacteria, such as Bifidobacterium sp., are encouraged to thrive by the mannose-based carbohydrate in palm kernel meal (Fernandez *et al*., 2002).

An increased concentration of lactic acid is produced by fermentation in the caeca (Okumura *et al*., 1994), which prevents the colonization of pathogenic organisms, such as *Salmonella*e. It has been demonstrated that feeding four-week-old broilers palm kernel meal lowers the quantity

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of dangerous bacteria in their stomachs (Fernandez *et al*., 2002). Dietary MOS from palm kernel meal may also attract microbes away from intestinal binding sites. It has been demonstrated that mannan oligosaccharides have receptor sites for the fimbriae of *Salmonella* sp. and *E. coli*, which causes them to be eliminated as the digesta flows out (Spring *et al*., 2000). Birds become less susceptible to these infections as a result of a decrease in the colonization of bacteria in that organ.

Sundu *et al*. (2006)) Propose that the efficacy of palm kernel meal as a prebiotic could be enhanced by employing mannan-degrading enzymes to transform β-mannan into manno-oligosaccharides and mannose. By adding palm kernel meal to the diet, birds' immune systems are strengthened, harmful bacteria are reduced, and the amount of nonpathogenic bacteria in the intestine is increased, all of which improve chicken health and productivity. Date kernels may enhance the health and productivity of chickens. To show the ability of DPKP and SCY feed additives in alleviating degradation induced by doxycycline misuse in broilers.

Materials and methods

Ethical approval

The study was approved before it started by the Institutional Animal Care and Use Committee (ARC-IACUC), Animal Health Research Institute, Agriculture Research Center, Egypt, with the numbers ARC, AHRI, 29 and 24.

Antibiotic

Doxycycline (DOXYDAD @) is an antibiotic. Each gram of the water-soluble powder, made by DADvet Firm, a company with its headquarters in Naour, Oman, Jordan, contains 200 mg of doxycycline HCL or 185 mg of doxycycline base. For broilers, 0.5 gram per litter is the recommended dosage.

Probiotics

Saccharomyces cerevisiae is a probiotic with living yeast cells (20 billion CFU/g), active dry yeast (*Saccharomyces cerevisiae* Sc 47 (Allgau vet German. Allgau yeast, Germany). (Maksimović *et al*., 2022) recommend a dose of 2.5 billion CFU per kg of feed.

Date palm kernel powder

Date palm kernel from New Valley Governorate in Egypt was pulverized. (Tareen *et al*., 2017) employed a 4% dosage in the ration.

Bird rearing and application of doxycycline, probiotics and date palm kernel powder

Seventy-five one-day-old Ross broiler chicks purchased from Ommat Arab Poultry Breeder Company in Giza, Egypt. At Animal Health Research Institute, Beni-Suef branch, Obour, Beni- Suef City, For three days in a row, they were incubated for 12 hours on Sacrolyte (Manufactured by UNIVET Company, Ireland), gram per liter of water, and then for an additional 12 hours on PANFLOR (Florfenicol 10%, Marcyrl Company, Cairo, Egypt) antibiotics, milliliter per liter of water (mL/L). With a relative humidity of 50%, the ideal temperature range for raising chicks was 35 to 37°C, which was progressively lowered until 25 to 23°C on the 42nd day.

Their typical diet consisted of yellow maize, fish meal, soybean meal, corn oil, gluten, dicalcium phosphate, crushed limestone, and sodium chloride. The first meal for a chick between the ages of 0 and 19 days included the following: A diet consisting of 3050 calories, 0.65% D-L-methionine, 1.49% L-lysine, 1.06% calcium, 0.36% total phosphorus, 23% crude protein, 4.41% crude fat, and 2.42% crude fiber. D-L-methionine 0.55%, L-lysine 1.19%, calcium 1.06%, total phosphorus 0.35%, crude protein 21%, crude fat 5.4%, crude fiber 2.4%, RE (Kcal/Kg) diet 3115, and a calorie/protein ratio of 148.33 were all included in the growth ration for chicks aged 20–35 days. Crude protein (17%), crude fat (6.4%), crude fiber (2.35%), and the calorie/protein ratio made up the previous week's ration. On the eighth day, the ration was split into five equal groups of fifteen chicks each. The ration was purchased from Hi-Feed Company for Feed Manufacturing, Poultry, and Animal Investment in Beni-Suef, Egypt.

Until the 42nd day of the trial, a control group was given water and a standard ration, Doxycycline treated group received water with 0.5 g/l doxycycline and a standard ration. Doxy + DPKP group, received water with doxycycline by 0.5 g/l and ration mixed with 4% date palm kernel powder until the end of the experiment; Doxy + SCY group, received water with doxycycline by 0.5 g/l and ration mixed with SC probiotics at 2.5 billion colony forming units per kilogram (CFU/Kg); SCY-Treated group received ration mixed with SC probiotics at 2.5 billion CFU/kg. Each bird was weighed and then thigh and breast muscles were collected after euthanasia.

Poultry meat examination

Collection of meat samples

Thigh and breast muscles were collected after euthanasia. and 5 g from each sample of the meat was frozen at -20°C for residue measurement. Another part of 500g of each sample was divided into 4 parts and refrigerated at 4±1°C for microorganism detection, and biochemical indices which were tested on 0, 3rd, 7th, and 9th days after refrigeration.

Physicochemical analysis

According to Waghamare *et al*. (2020), the obtained thigh and breast muscle samples were frozen at -20 degrees until doxycycline antibiotic residue was examined using HPLC Agilent 1200 at the Animal Health Research Institute, Reference laboratory for veterinary quality control on poultry production. According to the Egyptian Organization for Standardization and Quality Control (ES, 2005), another portion of the collected thigh and breast muscles was chilled at 4.0±1.0°C and examined for maintaining quality indices by measuring pH (ES, 2006c), total volatile basic nitrogen (ES, 2006a), and thiobarbituric acid reactive substances (ES, 2006b).

Microbiological Examination

After the samples were chilled for one day at 4 degrees Celsius, 25 grams of each sample were combined with 225 milliliters of 0.1% peptone water (SIGMA, Saint Louis, Missouri, USA) in a sterile blender jar for one to two minutes. For testing, further decimal serial dilutions were made. Total number of *Enterobacteriaceae* PGUA agar (Merck K GaA, Darmstadt, Germany) (ISO, 2001), total colony Standard Plate Count Agar (Oxoid Ltd., Hampshire, England, UK), total psychotropic counts Plate Count Agar (Oxoid Ltd., England, UK), and total *Staph*ylococci by Baired Parker Agar (Merck K GaA, Darmstadt, Germany), according to FAO (1992) and USDA (2011), respectively. *Staphylococcus aureus* count (USDA, 2011), wherein the suspected colonies were examined morphologically (Cruickshank *et al*., 1975), followed by biochemical identification (MacFaddin, 2000), testing for catalase activity, oxidase, growth at 10% NaCl, detection of Arginine Decarboxylase (ADH), bile esculent, mannitol, hemolysis, coagulase, thermostable nuclease test "D-Nase activity" (Lachia *et al*., 1971), and sugar fermentation. for *Pseudomonas aeruginosa* isolation 0.1 ml from the previously prepared dilution was spread on pseudomonas agar base (oxoid, UK, CM559) containing cetrimide, cephaloridine, and fucidin supplements(SR103: Oxoid, UK) and incubated for 24-48 hour at 37°C (Roberts and Greenwood, 2003),

Statistical analysis

Graph Pad InStat software (version 3, ISS-Rome, Italy) was used to do preliminary statistical analysis. Groups of data were compared using one-way analysis of variance (ANOVA) and the Tukey-Kramer (TK) Multiple Comparison post-test, unless otherwise noted. Tables and figures present the data as Mean±standard error (SEM). Significant values were defined as $p < 0.05$.

Results

On the zero day of refrigeration, the Doxy-treated group had a greater mean total volatile basic nitrogen level than the control and Doxy plus DPKP groups. TVBN at zero day was considerably lower in the Doxy plus SCY and SCY treated groups ($p < 0.001$) than in the DOXY treated group. When compared to other treatment groups, TVBN in the Doxy-treated group grew significantly ($p < 0.001$) on the third, seventh, and eighth days of refrigeration (Table 1).

Table 1. Total volatile basic nitrogen concentration mg/100g in different groups and on different days.

Treatment groups	Day of chilled storage at (4±1° c)			
	0 day	3rd day	7th day	9th day
Control	11.67±0.62^{ab}	12.97±0.23^{a}	16.62±0.45^{a}	20.74±0.34^{a}
Doxy-treated	13.37±0.41^{ab}	17.00±0.45^{b}	22.33±0.75^{b}	23.5±0.57^{b}
Doxy + DPKP treated	11.01±0.72^{ab}	12.96±0.52^{a}	17.02±0.24^{a}	20.76±0.47^{a}
Doxy + SCY-treated	10.67±0.93^{a}	12.77±0.34^{a}	16.67±0.41^{a}	20.76±0.62^{a}
SCY-treated	8.77±0.20^{c}	11.13±0.41^{a}	15.13±0.17^{a}	18.13±0.4^{c}

Means±standard errors of a minimum of three replicates (n≥3) are used to represent the data. High significant variations between means are shown by different tiny letter superscripts (a, b and c) in the same column (p <0.001).

The concentration of thiobarbituric acid-reactive compounds decreased significantly ($p < 0.001$) in the Doxy-treated group during all refrigeration days compared to the other treated group (Table 2).

Table 2. Thiobarbituric acid reactive substances (TBA) levels mg/kg in different groups and on different storage days.

Treatment groups	Day of chilled storage			
	0 day	3rd day	7th day	9th day
Control	0.24±0.012^{a}	0.475±0.077^{a}	0.803±0.042^{a}	0.89±0.052^{a}
Doxy-treated	0.023±0.009^{b}	0.037±0.012^{b}	0.055±0.019^{b}	0.057±0.02^{b}
Doxy +DPKP treated	0.24±0.012^{a}	0.25±0.012^{ab}	0.43±0.035^{ab}	0.525±0.056^{a}
Doxy +SCY-treated	0.206±0.008^{a}	0.43±0.11^{a}	0.52±0.14^{a}	0.525±0.056^{a}
(SCY-treated	0.187±0.013^{a}	0.226±0.018^{ab}	0.39±0.10^{b}	0.48±0.11^{b}

Means±standard errors of a minimum of three replicates (n≥3) are used to represent the data. High significant variations between means are shown by different tiny letter superscripts (a, b and c) in the same column (p <0.001).

The Doxy-treated group had a greater mean hydrogen potential (pH) at 0 days of refrigeration than the control, Doxy plus DPKP, Doxy plus SCY, and SCY-treated groups. The pH of the Doxy-treated group increased significantly ($p < 0.001$) compared to the other treatment group on the third, seventh, and eighth days of refrigeration (Table 3).

Table 3. Hydrogen potential (pH) changes in different groups on different storage days.

Treatment groups	Day of chilled storage			
	0 day	3rd day	7th day	9th day
Control	5.94±0.026^{ab}	5.79±0.058^{a}	6.19±0.018^{a}	6.36±0.017^{a}
Doxy-treated	6.06±0.069^{ab}	6.23±0.058^{b}	6.54±0.041^{b}	6.73±0.03^{b}
Doxy +DPKP treated	5.91±0.07^{ab}	5.99±0.046^{c}	6.37±0.065^{ab}	6.48±0.014^{c}
Doxy + SCY-treated	5.88±0.057^{ab}	5.99±0.015^{c}	6.32±0.05^{ab}	6.45±0.017^{a}
SCY-treated	5.74±0.061^{a}	5.89±0.012^{ac}	6.18±0.08^{a}	6.34±0.011^{a}

Means±standard errors of a minimum of three replicates (n≥3) are used to represent the data. High significant variations between means are shown by different tiny letter superscripts (a, b and c) in the same column (p <0.001).

Compared to the Doxy plus DPKP, Doxy plus SCY, and SCY treatment groups, the Doxy treatment group had a significantly higher mean total colony count ($p < 0.001$). When compared to the other treated groups, the mean *Enterobacteriaceae* counts in the Doxy treatment group were considerably higher ($p < 0.001$). When compared to the control and Doxy plus DPKP groups, the mean psychotropic count significantly decreased ($p < 0.001$) with Doxy treatment. When compared to the Doxy plus DPKP and Doxy plus SCY treatment groups, the mean total *Staph* count significantly decreased (P < 0.001) after Doxy treatment (Table 4).

The mean end body weight showed a significant decrease (p <0.001) in the Doxy-treated group in comparison with Control, Doxy plus DPKP, Doxy plus SCY, and SCY-treated groups Figure 1.

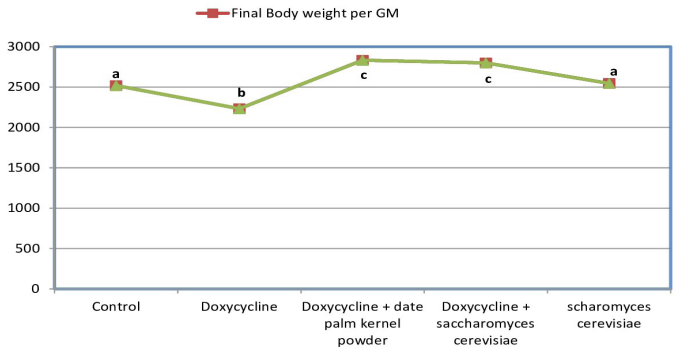

Figure 1. Body weight in various chicks' groups.
Means±standard errors of a minimum of three replicates (n≥3) are used to represent the data. High significant variations between means are shown by different tiny letter superscripts (a, b and c) in the same column (p <0.001).

The Doxycycline residue level in the Doxy-treated group showed a highly significant increase (p <0.001) in comparison with Control, Doxy plus DPKP, Doxy plus SCY, and SCY-treated groups Figure 2.

Table 4. Total colony, *Enterobacteriaceae*, psychotropic and *Staph*ylococcus sp. counts in different groups.

Treatment groups	Count per Colony Forming Unit per Gram (CFU/g)			
	Total Colony	Total *Enterobacteriaceae*	Total *Psychotropic*	Total *Staph*
Control	$6.3\times10^{5}\pm6.6\times10^{4a}$	$3\times10^{5}\pm5.67\times10^{4a}$	$3.3\times10^{5}\pm3.3\times10^{4a}$	$3.\ 6\times10^{5}\pm3.63\times10^{4a}$
Doxy-treated	$7.4\times10^{5}\pm8.7\times10^{4a}$	$5.6\times10^{5}\pm3.3\times10^{4b}$	$7\times10^{4}\pm5.7\times10^{3b}$	$5.3\times10^{5}\pm3.3\times10^{4a}$
Doxy + DPKP treated	$1.1\times10^{5}\pm1.3\times10^{4b}$	$3.3\times10^{5}\pm3.3\times10^{4a}$	$1.6\times10^{5}\pm8.8\times10^{3c}$	$1.5\times10^{6}\pm3.3\times10^{4b}$
Doxy + SCY-treated	$1.46\times10^{5}\pm3.3\times10^{3b}$	$5.4\times10^{4}\pm5.7\times10^{3c}$	$5\times10^{4}\pm5.7\times10^{3b}$	$1.16\times10^{6}\pm1.6\times10^{5b}$
SCY-treated	$3.3\times10^{4}\pm3.2\times10^{3b}$	$1.3\times10^{3}\pm3.3\times10^{2c}$	$3\times10^{4}\pm5.7\times10^{3b}$	$5\times10^{5}\pm5.7\times10^{4a}$

Means±standard errors of a minimum of three replicates (n≥3) are used to represent the data. High significant variations between means are shown by different tiny letter superscripts (a, b and c) in the same column (p <0.001).

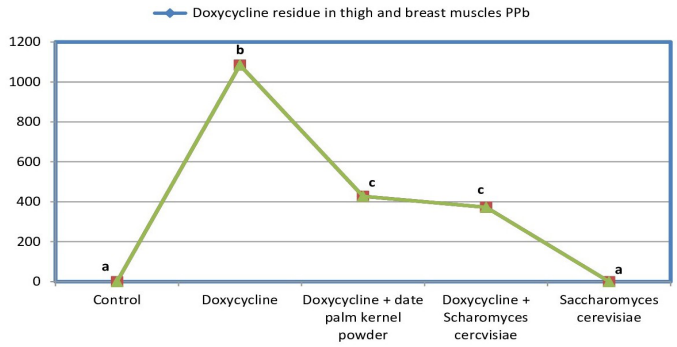

Figure 2. Doxycycline residue in breast and thigh muscles of different groups. Means±standard errors of a minimum of three replicates (n≥3) are used to represent the data. High significant variations between means are shown by different tiny letter superscripts (a, b and c) in the same column (p <0.001).

Discussion

Antibiotic residues had economic challenges and health risks. Meat and its products are excluded when antibiotic levels exceed the maximum residual limit. When compared to the control and other treated groups, the mean body weight of the doxycycline-treated group significantly decreased (Figure 1), and Doxy plus SCY and Doxy plus date palm kernel powder showed a substantially ameliorative effect.

Furthermore, Figure 2 showed that the Doxy-treated group had more Doxycycline residue than the control and other treatment groups; these results were in line with those of Gajda *et al*. (2014) and Gbylik-Sikorska *et al*. (2016). Although the Doxy + DPKP and Doxy + SCY groups had significantly less doxycycline residue than the Doxy group, this is in accordance with the results of Fadwa *et al*. (2022), who found that SCY reduced residue in boiler muscles and increased body weight.

Devegowda *et al*. (1996 and Shetty and Jespersen (2006)) reported that a modified SC mannan-oligosaccharide derivative produced 95% MT binding and that SC binds more than 77% of additional mycotoxins into the chicken diet. The maximum residual limits (MRL) for doxycycline in poultry set by the European Union are 100 ng/g of muscle. Antibiotic residues in poultry meat are most frequently caused by uncontrolled usage, disregard for label instructions, and inadequate withdrawal time (Cetinkaya *et al*., 2012). Therefore, a brief withdrawal period may be necessary for groups receiving probiotic and date kernel treatments. TVBN in Table 1 reveals a rise in the doxy-treated group starting on the first day, which increased dramatically on the third day and remained higher until the ninth day; the high residual content may be connected to the Doxy-treated group samples' spoiling early. Additionally, Table 3 demonstrates an early pH elevation in the doxy-treated group on the third day. This could be due to increased partial proteolysis, which raises free alkaline groups (Lyon *et al*., 1984). According to Cai *et al*. (2014), basic compounds such as ammonia, primary amines, and secondary amines are produced by aerobic microbes on meat surfaces, raising the pH concentration of chicken fillet samples.

The Doxy-treated group had the lowest TBA levels Table 2, which may be related to their decreased body weight or fat content. Chicken cutups, which are more prone to rancidity due to their high unsaturated fatty acid content, can quickly develop a rancid flavor when stored in a refrigerator or freezer (Ang, 1988).

On the other hand, TVBN and pH were lower in the Doxy plus Saccharomyces and Doxy + date palm kernel groups compared with the Doxy group, while TBA was lower than in the control. Both the Saccharomyces-treated group and the bacterial findings in Table 4 showed the best outcomes. This could be connected to the fact that manno-oligosaccharides, or mannose, are found in both yeast and date palm kernels and have been shown by Lyons (2002) and Oyofo *et al*. (1989) to strengthen animal immune systems. Immune-boosting substances found in *S. cerevisiae* include β-glucans and mannanoligosaccharides. It has been demonstrated that this strain of yeast boosts immunity and promotes development (Celik *et al*., 2000; Çelýk *et al*., 2003).

By preserving intestinal biostructure, it also possesses antimicrobial properties and offers further health advantages (Wang and Gu, 2010; Zhang *et al*., 2016). Along with enhancing local and systemic immunity, they also improve intestinal metabolism in chickens, release various nutrients, suppress some intestinal infections, and improve overall performance (Zarei *et al*., 2018).

Although palm kernel meal contains a high dietary fiber, particularly β-mannan, it is not suitable for poultry diets due to its grittiness. According to numerous recent studies, it may replace commercial manno-oligosaccharides as a prebiotic to support the health and immunity of chickens (Fernandez *et al*., 2002).

The doxy treated group had a significantly higher total colony count than the Doxy plus DPKP, Doxy plus SCY, and SCY groups (p < 0.001). Compared to the other treated groups, the Doxy treated group had significantly higher mean *Enterobacteriaceae* counts (p < 0.001). Compared to the control and Doxy plus DPKP groups, Doxy therapy resulted in a significant decrease (p < 0.001) in mean psychoactive count. Doxy treatment resulted in a considerably lower mean total *Staph* count (P < 0.001) compared to the Doxy plus DPKP and Doxy plus SCY groups (Table 4). Tetracyclines can alter the composition and variety of intestinal microorganisms by preferentially targeting particular kinds of bacteria while ignoring others, resulting in an excess of pathogens and significant dysbiosis (Baran *et al*., 2023). Although the muscles of healthy birds are sterile, the digestive tract, lungs, skin, and feathers contain a range of microbiot as that infect carcasses before and after slaughter (Rouger *et al*., 2017). Videnska *et al*. (2013) and Green *et al*. (2022), found a higher prevalence of Enterobacterial in the fecal microbiota of chickens receiving tetracycline therapy.

Wang *et al*. (2021) discovered that probiotic meal treatment improves intestinal microbiota, which impacts immunological function, tract digestibility, anti-oxidative capabilities, and broiler development performance, all of which are negatively impacted by antibiotics. This is because the germs that contaminate the birds' muscles after slaughter reflect their gut microorganisms. According to Mountzouris *et al*. (2010), probiotic dietary supplementation boosts Bifidobacterium and Lactobacillus concentrations while significantly reducing Escherichia coli populations in the caecum. enhances caecal microbial activity (Mountzouris, *et al*., 2007). Increasing Lactobacillus and Bifidobacterium levels in the fecal environment while decreasing *Salmonella*, Clostridium perfringens, and *E. coli* levels (Yang, *et al*., 2012).

Furthermore, introducing xylanase and β-glucanase to the caeca of broiler chickens affects the microbiota and may lower populations of potentially dangerous *Enterobacteriaceae* (Jozefiak *et al*., 2010). Probiotic therapy dramatically alters the general composition of the fecal bacterial community, as shown in Kristensen *et al*. (2016). Wang *et al*. (2021) found that probiotics improved the gut microbiota richness and the relative abundances of Bacteroidetes and Proteobacteria in broiler caeca. In the areas of metabolizing dietary nutrients, generating SCFA from indigestible carbohydrates, creating vitamins and amino acids, and controlling metabolism, intestinal microbiota are recognized to have physiological impacts on a host (Kamada *et al*., 2012). Cheng *et al*. (2014) claim that probiotics improve production outcomes by preserving the digestive system's physiological microbiota, regulating the human immunological response, and reducing the chance of infection with harmful bacteria. Probiotics have varying effects on broilers. Probiotic feeding may improve broiler growth performance for two reasons: immune regulation and gut microbiota balance. Probiotics lower intestinal pH by producing short-chain fatty acids, which inhibits the growth of harmful bacteria and preserves the equilibrium between beneficial and harmful bacteria. Both broiler growth and intestinal health depend on these activities (Yaqoob *et al*., 2022).

Broiler performance can be enhanced by probiotics through immunomodulation, adhesion site competition, antimicrobial agent production, and the diversity and stability of the gut flora (Lutful, 2009; Hu *et*

al., 2017). Probiotics have been shown in a growing number of studies to improve the body's capacity to absorb nutrients, digest food, and combat free radicals. They also reduce cell apoptosis, which ultimately enhances intestinal health and broiler performance (Bai *et al*., 2017; Wang *et al*., 2018). Previous research showed that birds fed yeast products had reduced levels of *E. coli*. This could be explained by the development of enzymes that degrade bacterial toxins and the eradication of competing pathogens (Ghosh *et al*., 2012).

Date kernel powder, which possesses antibacterial and antioxidant properties, can be used to preserve the quality and safety of meat while increasing its shelf life (Habib and Ibrahim, 2011; Perveen *et al*., 2012). Elhadef *et al.* (2023) investigated the effect of date palm seed ethanolic extract (DSEE) concentrations of 0.156%, 0.312%, and 0.624% on the chemical stability and microbiological quality of chicken breast meat held in the refrigerator for 14 days. DSEE greatly inhibited microbial growth and reduced lipid/protein oxidation processes, suggesting that it could be effective for preserving chicken flesh.

Conclusion

In broiler feeds, SCY and DPKP prevent doxycycline degradation while maintaining meat quality. Both the microbiological count and the low concentration of thiobarbituric acid-reactive compounds in the Doxy-treated group need further investigation. Further research is required to explain the impact of combining SCY and date palm kernel powder with the broiler ration.

Conflict of interest

The authors have no conflict of interest to declare.

References

Abdel-Mohsein, S.H., Mahmoud, M.A., Ibrahim, A., 2015. Tetracycline Residues in Intensive Broiler Farms in Upper Egypt: Hazards and Risks. J. World's Poult. Res. 5, 48-58.

Abd El-Kader, Z.M., 1996. Lipid Oxidation in chicken as affected by cooking and frozen storage. Nahrung. 40, 21-42.

Ang, C.Y., 1988. Comparison of broiler tissues for oxidative changes after cooking and refrigeration storage. J. Food Sci. 53, 1072-1075.

Bai, K., Huang, Q., Zhang, J., He, J., Zhang, L., Wang, T., 2017. Supplemental effects of probiotic *Bacillus subtilis* fmbJ on growth performance, antioxidant capacity, and meat quality of broiler chickens. Poult Sci. 96, 74 –82.

Baran, A., Kwiatkowska, A., Potocki, L., 2023. Antibiotics and bacterial resistance-a short story of an endless arms race. Int. J. Mol. Sci. 24, 5777.

Cai, L., Wu, X., Dong, Z., Li, X., Yi, S., Li, J., 2014. Physicochemical responses and quality changes of red sea bream (*Pagrosomus major*) to gum arabic coating enriched with ergothioneine treatment during refrigerated storage. Food Chemistry. 160, 82– 89.

Can, Ö.P., 2011. Evaluation of the Microbiological, Chemical and Sensory Quality of Carp Processed by the Sous Vide Method. World Academy of Science, Engineering and Technology, 80.

Celik, I., Oguz, H., Demet, O., Donmez, H.H., Boydak, M., Sur, E., 2000. Efficacy of polyvinylpolypyrrolidone in reducing the immunotoxicity of aflatoxin in growing broilers. British Poultry Science 41, 430-439.

Çelýk, K., Denlý, M., Savas, T., 2003. Reduction of toxic effects of aflatoxin B1 by using baker yeast (*Saccharomyces cerevisiae*) in growing broiler chicks' diets. Revista brasileira de zootecnia 32, 615-619.

Cetinkaya, F., Yibar, A., Soyutemiz, G.E., Okutan, B., Ozcan, A., Karaca, M.Y., 2012. Determination of tetracycline residues in chicken meat by liquid chromatography-tandem mass spectrometry. Food Addit Contam., Part B, 5, 45-49.

Cheng, G., Hao, H., Xie, S., Wang, X., Dai, M., Huang, L., Yuan, Z., 2014. Antibiotic alternatives: The substitution of antibiotics in animal husbandry? Front. Microbiol. 5, 217.

Dainty, R.H., Edwards, R.A., Hibbard, C.M., 1985. Time course of volatile compounds formation during refrigerated storage of naturally contaminated beef in air. J. Appl. Bacteriol., 59, 303-309.

Devegowda, G., Aravind, B.I.R., Morton, M.G., 1996. *Saccharomyces cerevisiae* and mannan oligosaccharides to counteract aflatoxicosis in broilers. In Proceedings of Australian poultry science symposium Sydney 103, 106.

Donoghue, D.J., 2003. Antibiotic residues in poultry tissues and eggs: Human health concerns. Poultry Science, 82, 618-621.

Egyptian organization for standardization and Quality control, 2005. Standard specification No. 1090 for chicken meat.

Egyptian Standard (ES), 2006a. Methods of analysis and testing for meat and meat products. Determination of total volatile nitrogen (TVB-N). Egyptian organization for standardization EOS NO 63-9 (TVB –N).

Egyptian Standard, (ES), 2006b. Methods of analysis and testing for meat and meat products. Determination of thiobarbituric acid (TBA). Egyptian organization for standardization EOS NO 63-10 (TBA).

Egyptian Standard, (ES), 2006c. Methods of analysis and testing for meat and meat products. Determination of hydrogen ion concentration (pH). Egyptian organization for standardization EOS NO 63-11 (PH).

Elhadef, K., Chaari, M., Akermi, S., Nirmal, N. P., Mousavi Khaneghah, A., Abdelkafi, S., Michaud, P., Ali, D. S., Mellouli, L., Smaoui, S., 2023. Production of functional raw chicken meat by incorporation of date palm seed extract: an assessment of microbiological, chemical and sensory properties. J. of Food Measurement and Characterization 17, 5117-5133.

Fadwa, F.M., Eman, A.A., Ali, M.A., Nada, I.H.A., 2022. Control of Aflatoxin Residues in Broiler Chicken Using *Saccharomyces cerevisiae* Fortified Ration. Journal of Advanced Veterinary Research.12, 409-414.

FAO, 1992. Manual of Food Quality Control, Food and Agriculture Organization of the United Nations, Roma. Microbiological analysis part 4, 131.

Fernandez, F., Hintaon, M., Van, G.B., 2002. Dietary mannan oligosaccharides and their effect on chicken caecal microflora in relation to *Salmonella* Enteritidis colonization. Avian Pathology 31, 49-58.

Fernandez, F., Hintaon, M., Vangils, B., 2000. Evaluation of the effect of mannan oligosaccharides on the competitive exclusion of *Salmonella* Enteritidis colonization in broiler chicks. Avian Pathology 29, 575-581.

Fernandez-Lopez, J., Zhi, N., Aleson-Carbonell, L., Perez-Alvarez, J.A., Kuri, V., 2004. Antioxidant and antibacterial activities of natural extracts: application in beef meatballs. Meat Science 69, 371-380.

Gajda, A., Posyniak, A., Tomczyk, G., 2014. LC-MS/MS analysis of doxycycline residues in chicken tissues after oral administration. Bull Vet Inst Pulawy/58.573-579.

Gbylik-Sikorska, M., Posyniak, A., Śniegocki, T., Sell, B., Gajda, A., Tomczyk, G., Żmudzki, J., 2016. Effect of doxycycline concentrations in chicken tissues as a consequence of permanent exposure to enrofloxacin traces in drinking water. J. Vet. Res. 60, 293-299.

Ghosh, T.K., Haldar, S., Bedford, M.R., Muthusami, N., Samanta, I., 2012. Assessment of yeast cell wall as replacements for antibiotic growth promoters in broiler diets: Effects on performance, intestinal histo-morphology and humoral immune responses. J. Anim. Physiol. Anim. Nutr. 96, 275-284.

Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B., Givskov, M., 2002. Food spoilage-interactions between food spoilage bacteria. Int. J. Food Microbiol. 78, 79-97.

Greene, G., Koolman, L., Whyte, P., Burgess, C., Lynch, H., Coffey, A., Lucey, B., O'Connor, L., Bolton, D., 2022. Effect of doxycycline use in the early broiler production cycle on the microbiome. Frontiers in Micro. 13, 885862.

Habib, H.M., Ibrahim, W.H., 2011. Effect of date seeds on oxidative damage and antioxidant status in vivo. J. Sci. Food Agric. 91, 674-1679.

Hu, S., Wang, L., Jiang, Z., 2017. Dietary additive probiotics modulation of the intestinal microbiota. Protein Peptide Lett 24, 382–387.

ISO 21528-3, 2001. International Organization for Standardization Microbiology of Food and Animal Feeding Stuffs-Horizontal method for the detection and enumeration of *Enterobacteriaceae*- Colony count technique. Geneva.

Jozefiak, D., Rutkowski, A., Kaczmarek, S., Jensen, B.B., Engberg, R.M., Hojberg, O., 2010. Effect of β-glucanase and xylanase supplementation of barley-and rye-based diets on caecal microbiota of broiler chickens. Br Poultry Sci 51, 546–557.

Kamada, N., Kim, Y.G., Sham, H.P., Vallance, B.A., Puente, J.L., Martens, E.C., Nunez, G., 2012. Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. Science 336, 1325–1329.

Kerry, J.P., O'Grady, M.N., Hogan, S.A., 2006. Past, current and potential utilisation of active and intelligent packaging systems for meat and muscle- based products: A review. Meat Science, 74, 113– 130.

Korver, D.R., 2012. Implications of changing immune function through nutrition in poultry. Animal Feed Science and Technology 173, 54-64.

Kristensen, N.B., Bryrup, T., Allin, K.H., Nielsen, T., Hansen, T.H., Pedersen, O., 2016. Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials. Genome Med. 8, 52.

Lachia, R., Genigeogis, C., Hoeprich, P., 1971. Meta chromatie agar- diffusion methods for detecting *Staph*ylococcal nuclease activity. Appl. Microbiol. 21, 585-587.

Lee, H.J., Lee, M.H., Ruy, P.D., 2001. Public health risks: chemical and antibiotic residues. Asian Australasian Journal of Animal Science 14, 402413.

Lutful, K.S.M., 2009. The role of probiotics in the poultry industry. Int J Mol Sci 10, 3531–3546.

Lyon, C.E., Hamm, D., Thomson, J.E., 1984. The effect of holding time added salt on pH and Functional properties of chicken meat. J. poult. Sci. 8, 1952- 1956.

Lyons, T.P., 2002. Navigating from niche markets to mainstream: A feed industry Kakumei. Proceedings of Alltech's 16th Anuual Asia Pacific Lecture Tour, pp. 1-16.

MacFaddin, J.F., 2000. Biochemical tests for identification medical bacteria. Warery Press Inc., Baltimore, Md.

Maksimović, Ž., Starčević, M., Šefer, D., Janjić, J., Radovanović, A., Radulović, S., Perić, D., Marković, R., 2022. Effects of adding different dosages of *Saccharomyces cerevisiae* in diet on growth performance, carcass characteristics, intestinal morphology, and gut micro flora of broilers. Kafk as Univ Vet Fak Derg 28, 461-468.

Mountzouris, K.C., Tsitrsikos, P., Palamidi, I., Arvaniti, A., Mohnl, M., Schatzmayr. G., Fegeros. K., 2010. Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. Poult. Sci. 89, 58-67.

Mountzouris, K.C., Tsirtsikos, P., Kalamara, E., Nitsch, S., Schatzmayr, G., Fegeros, K., 2007. Evaluation of the efficacy of a probiotic containing *Lactobacillus, Bifidobacterium, Enterococcus, and Pediococcus* strains in promoting broiler performance and modulating cecal microflora composition and metabolic activities. Poult Sci. 86, 309–317.

Okumura, J., Furuse, M., Kawamura, T., Toyoshima, K., Sugawara, M., Suzuki, T., Seo, G., Soga, H., 1994. Effects of glucooligosaccharides and bacteria on egg production rate and caecal bacteria population in the chicken. Japanese Poultry Science 31, 189-194.

Olafsdottir, G., Martinsdottir, E., Oehlenschlager, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie, I,M., Henehan, G., Nielsen, J., Nilsen, H., 1997. Methods to evaluate fish freshness in research and industry. Trends in Food Sci. Technol. 8, 258-265.

Oyofo, B.A., Deloach, J.R., Corrier, D.E., Norman, J.O., Ziprin, R.L., Mollenhauser, H.H., 1989. Effects of carbohydrates on *Salmonella* typhimurium colonisation in broiler chickens. Avian Diseases 33, 531– 534.

Perveen, K., Bokhari, N.A., Soliman, D.A., 2012. Antibacterial activity of Phoenix dactylifera L. leaf and pit extracts against selected gram negative and Gram positive pathogenic bacteria. J. Med. Plants Res. 6, 296-300.

Roberts D., Greenwood, M., 3rd. London, UK: Blackwell publishing: Ltd: 2003. Practical food microbiology: pp. 273-274.

Rouger, A., Tresse, O., Zagorec, M., 2017. Bacterial contaminants of poultry meat: sources, species, and dynamics. Microorganisms 5, 50.

Sampels, S., Pickova, J., Wiklund, E., 2004. Fatty acids, antioxidant and oxidation stability of processed reindeer meat. Meat Science 67, 523-532.

Şenyuva, H., Özden, T., Sarica, D.Y., 2000. High performance liquid chromatographic determination of oxytetracycline residue in cured meat products. Turkish J. Chem. 24, 395-400.

Shetty, P.H., Jespersen, L., 2006. *Saccharomyces cerevisiae* and lactic acid bacteria as potential mycotoxin decontaminating agents. Trends in Food Science Technology 17, 48-55.

Spring, P., Wenk, C., Dawson, K.A., Newman, K.E., 2000. The effects of dietary mannan oligosaccharide on caecal parameters and the concentration of enteric bacteria in the caeca of *Salmonella* challenged broiler chicks. Poultry Science 79, 205-211.

Sundu, B., Kumar, A., Dingle, J., 2006. Palm kernel meal in broiler diets: effect on chicken performance and health. World's Poultry Science Journal 62 (2).

Tareen, M.H., Wagan, R., Siyal, F.A., Babazadeh, D., Bhutto, Z.A., Arain, M.A., Saeed, M., 2017. Effect of various levels of date palm kernel on growth performance of broilers, Veterinary World 10, 227-232.

USDA, 2011. Quantitative Analysis of Bacteria in Foods as Sanitary Indicators January 2011.

Videnska, P., Faldynova, M., Juricova, H., Babak, V., Sisak, F., Havlickova, H., Rychlik, I., 2013. Chicken faecal microbiota and disturbances induced by single or repeated therapy with tetracycline and streptomycin. BMC Vet. Res. 9, 30.

Waghamare, R.N., Paturkar, A.M., Vaidya, V.M., Zende, R.J., Kumar, A., Bedi, J.S., 2020. Screening of

Antimicrobial Residues and Confirmation of Doxycycline in Samples Collected from Chicken Farms and Processing Units Located Around Mumbai, India. Indian Journal of Animal Research 54, 1415-1421.

Wang, Y., Heng, C., Zhou, X., Cao, G., Jiang, L., Wang, J., Li, K., Wang, D., Zhan, X., 2021. Supplemental *Bacillus subtilis* DSM 29784 and enzymes, alone or in combination, as alternatives for antibiotics to improve growth performance, digestive enzyme activity, anti-oxidative status, immune response and the intestinal barrier of broiler chickens. British Journal of Nutrition 125, 494–507.

Wang, Y., Wang, Y., Xu, H., Mei, X., Gong, L., Wang, B., Li, W., Jiang, S., 2018. Direct-fed glucose oxidase and its combination with B. amyloliquefaciens SC06 on growth performance, meat quality, intestinal barrier, antioxidative status, and immunity of yellow-feathered broilers. Poult. Sci. 97, 3540–3549.

Wang, Y., Sun, J., Zhong, H., Li, N., Xu, H., Zhu, Q., Liu, Y., 2017. Effect of probiotics on the meat flavor and gut microbiota of chicken. Scientific Reports 7, 1-13.

Wang, Y., Gu, Q., 2010. Effect of probiotic on growth performance and digestive enzyme activity of Arbor Acres broilers. Research in Veterinary Science 89, 163-167.

Yang, C., Cao, G.T., Ferket, R.R., Liu, T.T., Zhou, L., Zhang, L., Xiao, Y.P., Chen, A.G., 2012. Effects of probiotic, Clostridium butyricum, on growth performance, immune function, and cecal microflora in broiler chickens. Poult. Sci. 91, 2121–2129.

Yaqoob, M.U., Wang, G., Wang, M., 2022. An updated review on probiotics as an alternative of antibiotics in poultry- A review. Anim Biosci 35, 1109-1120.

Yilmaz, I., 1998. Farkli ambalajlama yontemi ve depolama sicakliginin Tekirdag koftesinin bazi mikrobiyolojik, fiziksel ve kimyasal ozellikleri uzerine etkilerinin belirlenmesi. Doktora Tezi. T. Uni. Zir. Fak. Tekirdag, pp: 204.

Zarei, A., Lavvaf, A., Motamedi, M.M., 2018. Effects of probiotic and whey powder supplementation on growth performance, microflora population, and ileum morphology in broilers. Journal of Applied Animal Research 46, 840-844.

Zhang, L., Zhang, L., Zhan, X.A., Zeng, X., Zhou, L., Cao, G., Chen, A.G., Yang, C., 2016. Effects of dietary supplementation of probiotic, Clostridium butyricum, on growth performance, immune response, intestinal barrier function, and digestive enzyme activity in broiler chickens challenged with Escherichia coli K88. Journal of Animal Science and Biotechnology 7, 1-9.