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Biochemical and Functional Characterization of Kombucha tea or Probiotic bacteria and Their Preservative Action on Chicken Meat

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ABSTRACT

Antibiotic residues in chicken meat pose significant health risks, including direct toxicity, antibiotic resistance, gut microbiota disruption and mutagenesis. The aim of this study is to detect the antibiotic residues in meat samples of chicken, evaluate the thermal stability of these residues, and investigate if the kombucha can be used as a growth promoter instead of veterinary antibiotics. Meat samples of total 41 chickens were collected randomly from various slaughterhouses in Cairo. Qualitative analysis using the Agar diffusion method was performed and quantitative analysis for positive samples were estimated by LC–MS/MS. The thermal stability of antibiotics was tested by boiling at 100°C for 30 minutes and freezing for 4 weeks at -18°C. Antibiotic residues were detected in 16%, 5%, and 5% of total samples for chlortetracycline, ciprofloxacin, and sulphadiazine, respectively, and in 11% for other antibiotics. Out of the 41 samples, 19 (46%) were violative, exceeding the Maximum Residue Limits set by the European Union. Boiling for 30 minutes could effectively eliminate antibiotic residues but freezing for 4 weeks at -18°C did not. Kombucha demonstrated growth-promoting effects in chickens, with the best performance observed when adding 80 ml per 1000 ml of drinking water.

Keywords: Antibiotic residues; Chicken meat; Detection LC-MS/MS; Growth-promoting antibiotics; kombucha.

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INTRODUCTION

The chicken meat sector is considered the most dynamic sector; its nutritional value is high so that it's considered a rich source of animal protein that has a great taste and widespread popularity among both children and adults. Growth rotation is a different advantage of farming chicken, especially chicken, over other animals. This advantage permits repeated generations in one the year. There's a quick return of investment (ROI) in chicken, also the feed conversion efficiency (FCE) is high **(Hussein *et al.* 2016 & Darko *et al.* 2017).**

Antibiotics are a form of antimicrobial medication extensively utilized in both food-producing animals and human medicine. Most modern classes of drugs used in human medicine are also employed in food-producing animals. The resistant germs appearance is the negative impact of the antibiotic misuse **(Page, and Gautier, 2012 & Chantziaras *et al.* 2014).**

Farmers generally use growth promoters (GPs) to promote growth and prevent infections, as well as to boost the overall efficiency, feed conversion efficiency improvement, and product quality improvements. These growth promoters are added to chicken feed supplements and drink water or induced in the form of injection to increase chicken nutrition. Various growth promoters are utilized in chicken feed additives including antibiotics, probiotics, prebiotics, exogenous enzymes, antioxidants, herbs and other substances.

Since 1950, antibiotics have been incorporated into animal feed in the United States of America as chicken GPs, and this approach has been adopted globally **(Dibner and Richards, 2005).** GPs are utilized as feed supplements to enhance feed efficiency conversion in food animals, particularly during the initial stages of rearing, feed conversion efficiency improvement and improving the production quality. GPs could reduce growth-suppressing metabolites caused by microbes, and hence, promote chicken growth. These GPs also increase the levels of Gut amino acids and reduces stress and mortality in chicken. Most of the antibacterial products are utilized in sub-therapeutic quantities throughout the animal's lifespan, with consideration given only to the withdrawal period before slaughter **(Lorençon *et al.* 2007).**

The utilization of GPs in chickens alleviated Antibiotics impose selection pressure on bacteria, resulting in a rapid change in the antibiotic resistance profile of intestinal bacteria **(Wang *et al.* 2020).**

The primary reasons for the existence of harmful antibiotic remnant in chicken meat include unregulated administration, non-compliance with label instructions, or insufficient withdrawal periods **(Donoghue, 2003 & Cetinkaya *et al.* 2012**).To ensure food safety, the antibiotic remnant in foods is determined by applying different methods.

The high concentrations of veterinary medicines residues in food havea negative impact on human health, this encourages the Codex Aliment Arius Commission (CAC) to justified maximum residue limits for about 59 veterinary drugs **(Codex Aliment Arius Commission, 2017**). Maximum Residue Limit (MRL) denotes the highest residue concentration of veterinary medicines residues (stated in mg/kg or µg/kg) that the society can consider as allowable in or on food without posing any significant health hazard. (**Mc Glinchey *et al.* 2008**).

Kombucha is made through the process of fermenting tea sweetened with sugar and bacterial and yeast cellulose matrix, including metabolites like vitamins, minerals, as well as, organic acids, polyphenols, and other bio-active constituents **(Neffe-Skocinska *et al.* 2017, Villarreal-Soto *et***

***al.* 2018, and Jakubczyk *et al.* 2020)**. Hence, Kombucha may be viewed as a viable option for substituting growth-promoting antibiotics.

This study was conducted 1) to study the antibiotic remnants present in chicken meat samples, 2) to study the thermal processing effect on these remnants, and 3) to assess the impact of Kombucha on the growth of chickens.

MATERIALS AND METHODS

Study area

This study started from August 2021 to May 2022 in the lab of microbiology in Air force specialized hospital, in cooperation with the department of food analysis , QCAP , Dokki ,Giza for antibiotics residues detection in accordance with Food Safety and Inspection Services (FSIS) and United States Department of Agriculture (USDA) (2011).

Estimation of antibiotics residues present in chicken meat samples

A: Microbiological methods

The overall amounts of 41 chicken samples were examined. After melting the Muller-Hinton agar medium at 100°C, it was left and cooled at 55 °C and then poured for inoculation into Petri dishes. Inoculation applied with *B. subtilis* which was used as an indicator for the presence of antibiotics. The agar diffusion method was performed by preparing a well on the agar using a sterile cork borer, the latest was impregnated with chicken meat homogenized with 1ml of sterilized water and placed on the well and the Petri dishes were then left for 24h incubation at 37°C. The inhibition zones diameters of the *B. subtilis* around chicken samples were measured by using a vernier caliper that is precise to 0.1 mm.

a) The zone results within the range of 1-2 mm are considered suspicious.
b) The zone results below 1mm will be categorized negative.
c) The zone results recorded 2mm or more would be considered positive.

B: Chemical methods

Samples were homogenized using an Ultra Turrax after the addition of EDTA–Na_n solution for 2-3 minutes, followed by shaking 1 minute. Subsequently, the sample underwent centrifugation for 10 minutes at 5000 rpm. Nitrogen stream was used to evaporate the resulting supernatant. Following their dissolution in 10 mL of citrate buffer (0.3 M, pH 4.0), the dried residuals were added to the solid phase SPE cartridge. The sample was then activated with 5 mL methanol (HPLC grade), 5 mL of HCl 0.5 N, and 5 mL of de-ionized water). Washing the SPE has been done using 5 mL of methanol (5%) + 5 mL of de-ionized water. Drying the SPE cartridge has been done using air under reduced pressure. SPE cartridge elution was done using 10 mL methanol (HPLC grade), and then evaporated until dryness at 38°C. After re-dissolving the dried residue in 1mL the mobile phase, injection using 20 µL was applied into the LC-MS/MS system (**Romero-Gonzalez *et al.*, 2007).**

Thermal stability of antibiotics under boiling and freezing treatments

The raw samples that tested positive were selected for thermal stability assessment, and subsequently, the processed and raw samples were carried out for the thermal processing.

a. Cooking (Boiling): 15-20 grams of samples that showed positive results for antibiotics residues were put through a strainer, boiled at 100°C for 30 min in a 100ml water bath. After that, the samples were left to cool and LC–MS/MS was used for antibiotic residues examination.

b. Freezing: Samples with positive results for antibiotics residues were kept frozen at (-18°C) and stored for 4 weeks, and then they were examined for antibiotic residue detection by using LC–MS/MS.

Investigating the antimicrobial effect of the combined Probiotic and Kombucha on pathogenic bacteria

The bacterial suspensions underwent testing for turbidity increase to the McFarland standard 0.5 (1.5×108 cfu/ml) before being transferred to Mueller-Hinton (MH) broth (Difco™, USA) and subsequently swabbed onto MHA agar media (Difco™, USA). Using a sterile cork borer, three wells 10 mm in diameter were applied on the agar plate. 10µl of each probiotic (Bifidobacterium spp. and *lactobacillus* spp.) 10µl of kombucha were spilled to punch wells in the plates. The plates have been incubated at 37 °C for 24 hours. The diameters of inhibition zones around the well have been measured using calipers that are precise to 0.1 mm.

Use Antimicrobial agents (kombucha) to prevent the risk of using antibiotics

A. Chickens and housing

The amount of 15 chickens were housed completely random, every treatment involved chickens housed on wood shavings as bedding, with access to hanging water bottles and feeders. In the first day after arrival, the lighting schedule was continuous for 24h. After that, the chickens were housed under a 23 h light and kept only for 1 h dark.

B. Kombucha preparation

All equipment used for kombucha production was sterilized by autoclave to avoid contamination. For one liter kombucha preparation, tea was mixed with white sugar (1.5g tea: 70g white sugar). Tea was brewed for 10 minutes within boiled water. Sugar was added to the tea solution after removing tea mash. The sweet tea was left at room temperature until cooling to the degree of 25 °C, and then moved into a glass jar. The liquid culture of previously fermented kombucha (Kombucha starter that was needed to start the fermentation of kombucha) was included as the starter within the same of 10% (v/v). Following that, a 24 g symbiotic culture of bacteria and yeast (SCOBY) was added, then covered with clean cotton dressing and then stored under sterile and aseptic conditions. The kombucha was fermented through incubation for 10 days at 25 °C then, it was filtered and used.

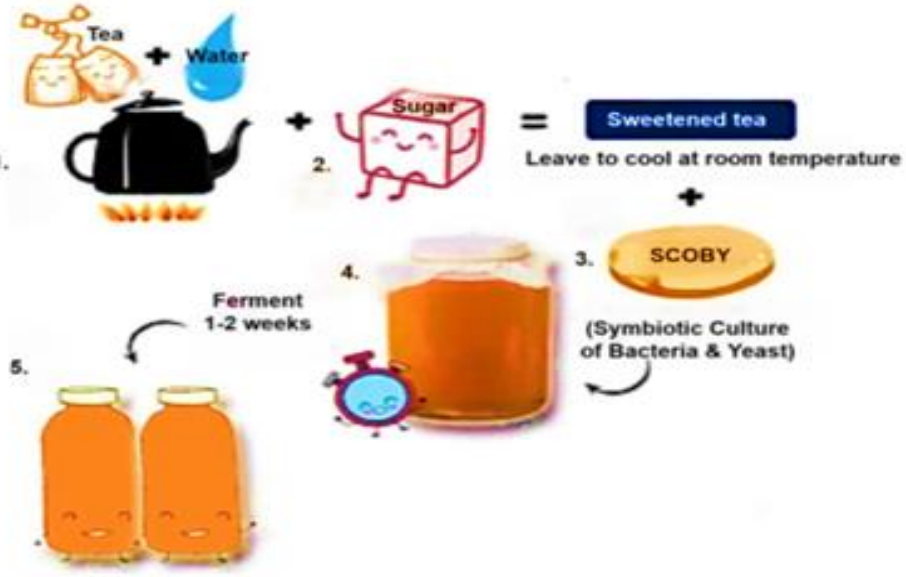

Figure 1. kombucha tea Preparation and fermentation

C. Treatments

The kombucha was included in the drinking water provided to chicken, so all chickens got the same feed. To fulfill all of the nutrient requirements, basal diets were developed according to the rearing standards for chickens. The three treatments were "Control (Tap water only), antibiotic in water (1 g/L), and water containing kombucha (80 ml/L).

D. Experimental procedure

The procedure included weighing of all the chickens at the age of 1, 10, 24, and 42 days, and keeping records of the feed intake for every growth period to determine the feed conversion ratio. Daily records were kept for chicken deaths and individual weights, and this data was utilized to make adjustments to body weight. The feed intake (FI), the feed conversion ratio (FCR) and the average body weight gain (BWG) were then measured from 1 to 10, 11 to 24 and 25 to 42 days of age. After six weeks of the experiment, 5 chickens per treatment were analyzed.

E. Plasma biochemical parameters, antibody titer

Using heparinized syringes, blood samples from the brachial veins of 5 chickens at the age of 42 days (2 chickens per replicate) were obtained per treatment. Samples were collected on ice before centrifugation. Until analysis, plasma was stored at −20 °C. The plasma metabolites concentrations including high-density lipoprotein (HDL), low density lipoprotein (LDL), total triglyceride, Alanine aminotransferase (ALT) and Aspartate transaminase test (AST), and uric acid have been measured. The measurements have been conducted by the use of spectrophotometer with standard kits. Standard Diagnostic Tests (OIE) manuals were used for the antibody titration methods.

F. Antioxidant enzymes in plasma and liver

On the 42th day of investigation, samples of liver tissue were collected from the slaughtered chicken and preserved at -80 °C. For further analysis, these samples were specifically gathered to assess the antioxidant enzymes activities and malondialdehyde (MDA) levels. Ice-cold isotonic physiological saline (0.1g/mL) was prepared; homogenization of the liver tissue was performed. Wing vein puncture was used to collect the blood samples which were transferred into EDTA-coated tubes immediately. Samples were then centrifuged at 3000 g for 15minutes at room temperature was conducted to separate the plasma which was then stored at -20 ℃ in aliquots until use. MDA levels, catalase (CAT) activities, superoxide dismutase (SOD) activities, and antioxidant enzyme activities were measured in the plasma samples by spectrophotometric methods. Xanthine oxidase method was used for the measurement of SOD activity by inhibiting the reduction of nitro blue tetrazolium and absorbance change at 560nm **(Sun *et al.* 1988)**. Determining the activities of the CAT was applied by observing the decline in absorbance at 240nm caused by the decomposition of hydrogen peroxide**. (Aebi, H. 1984)**. Determination of MDA level was applied at a 532nm wavelength for the absorbance assessment **(Placer *et al.* 1966).**

G. Intestinal morphology

All chickens were weighted separately on the 42th day of experiment and then transferred for the standard slaughter procedure to a local commercial chicken slaughterhouse. Chickens that had been electrically stunned were slaughtered. Intestinal fragments were taken from the individuals of three experimental groups (Antibiotic, Kombucha and Control Groups) after the commercial slaughtering procedure. Fragments were taken from duodenum, ileum and ceca for histological examination. These fragments were fixed in neutral buffered formalin (10%) for 72 h, then

dehydrated in progressively higher ethyl alcohol (70, 80, 90 and 100) and clarified in two benzene baths, and then placed in paraffin. For sectioning the paraffin blocks a manual rotary microtome was used **(Ştef *et al.*, 2015).**

Hematoxylin and Hematoxylin Eosin (HE) were used to stain the slides, and then the slide examination was done using the light microscope **(Bancroft and Layton, 2013).** For histometric measurements, a microscope micrometer was employed **(Luna, 1968).** The traits assessed were crypt depth, villus width, and villus height. The Histo-morphometric investigation was performed by ImageJ analysis software. The villi length, width, and inter-villi space of intestine were measured and expressed as µm whereas the goblet cells were expressed as no per mm^2.

Statistical analysis

All data of the determined parameters were calculated and stated as mean values with their standard. Analysis of variance (ANOVA) was estimated for investigating the differences between groups ($p<0.05$). All statistical analysis was performed using SPSS software (version 23; SPSS Institute Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Antibiotic residues incidence in chicken meat samples

From 41 samples that were collected from different regions in Cairo, 19 (46 %) gave +ve results for the existence of antibiotic residues using LC-MS/MS (Table 1). Only 4 (10 %) show +ve results for of antibiotic residues presence using well diffusion agar method. Microbiological methods are based on inhibition zone formation around the chicken tissue samples. Although microbiological methods are not precise, they are extremely appropriate for the detection of antibiotic residues, particularly as they are simpler and less expensive than chromatographic approaches.

Table 1. Antibiotic residue rates in samples of chicken meat by different techniques

Technique	Positive	Negative	x^2	p-value
LC-MS/MS	19 (46%)	22 (54%)	0.518	0.472
Well diffusion agar	4 (10%)	37 (90%)	51.840	<0.001**

x^2: Chi-square value for Number (%) or Fisher's exact value, whenever suitable

P-value >0.05 is insignificant difference; *p-value <0.05 is significant difference; **p-value <0.001 is highly significant differences between groups

Data in **Table 1** shows highly significant differences of negative results for well diffusion agar with 37 sample (90%) comparing to positive results for well diffusion agar was 4 samples (10%), with p-value ($p<0.001$).

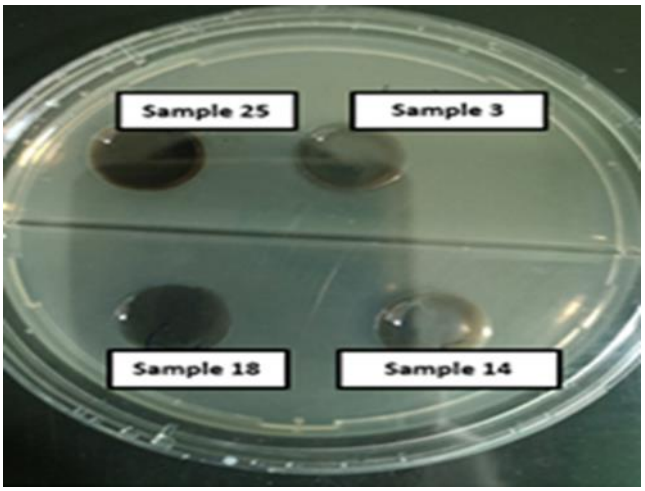

Figure 2. Chicken samples with *Bacillus Subtilis* to detect antibiotic residues samples according to Well diffusion agar test (microbiological method)

Detection of antibiotic residues by LC-MS/MS

To determine 22 antibiotics, a developed method was applied to different chemical antibiotic classes such as (sulfonamides, quinolones tetracyclines, and beta-lactams). Quality control was applied for the result validation on every batch of samples. The levels of various antibiotic residues in each raw chicken sample were measured in concentrations (µg/kg), revealing that 46% (n = 19) of samples were found to violate regulations which classified as those ones exceeding the substance MRL as outlined in the EU relevant legislation **(Table 2, Figure 3).** However, The Codex Alimentarius Commission **(CAC. 2017)** reported that there shouldn't be any residue left over after food from animals origin or chicken origin, that were treated with veterinary medicines that exceed the Maximum Residue Limit (MRL) that could pose a risk to consumer health. An evaluation of the sulfonamides group revealed that five antibiotic residues, including sulfacetamide, sulfamerazine, sulfathiazine, and sulfapyridine, were not detected in any of the samples, sulfathiazole. Our outcome aligns with a study carried out in Muscat, analyzing chicken liver and breast -that both are edible- for antibiotic residues existence showed different concentrations **(El Tahir *et al,* 2021).**A study conducted in Egypt and Bahrain aimed to evaluate the potential health hazards associated with antibiotics (specifically sulfadiazine and ciprofloxacin) that could be present in the meat of chicken and their offal.Quinolones were the less detected family than the other antibiotics families, the percentage of positive samples (5%; n = 1). The ciprofloxacin residual level was higher than the MRL levels (100 µg/kg) according to the **European Union EC (2010)**. Several researches commonly reported that the mean value of quinolones in chicken is about 30µg/kg. In comparison to the finding in our research, these researches have indicated that the improper use of quinolone in chicken farms has contributed to a resistant increase in strains of *Salmonella spp.* and *Campylobacter jejuni* that have been isolated from chicken meat. The improper use of quinolones in the Egyptian farms was confirmed by our study, noted that the recommended withdrawal times has an implementation lack. Thus, the intervention is necessary the evaluation of the tetracyclines family revealed a 16% positivity rate (n = 3), with the highest percentage of antibiotic residues found among all

samples, all three positive samples were exceeded the MRL for chlortetracyclines (100 µg/kg). Tetracycline family has an importance as indicated by a study conducted in the city of Porto using *Salmonella* spp. isolated from chicken products, the results in this study showed a high resistance percentage 36% **(Antunes *et al.* 2003)**. Tetracycline usage in Egypt should be adopted and become in control. Ampicillins (β-lactams) were shown positive in samples (11%; n=2). Ciprofloxacin (quinolones) represented the most reduced rate of +ve samples when compared to other antibiotic families. It was found that chlortetracycline (tetracyclines) showed the highest percentage (16%), followed by ampicillin (β-lactams), erythromycin, trimethoprim, tylosin, gentamycin and colistin that were the same percentage (11%)

Table 2. The residues of antibiotics in chicken meat samples compared with Maximum Residue Limits MRL

Antibiotic | families	Name of antibiotics	No. +ve samples	MRL (mg/kg)	Within MRL	Above MRL
β-lactams	**Ampicillin (AMP)**	2	50	35.32	75.15
Tetracyclines	**Chlortetracycline (CTC)**	3	100		260.325 225.317 104.465
Quinolones	**Ciprofloxacin (CIP)**	1	100		150.25
	Erythromycin (ERY)	2	200	46.34	236.61
	Sulfadiazine (SDA)	1	100		52.44
Sulfonamides	**Sulfmethoxazole (SMZ)**	2	100		99.633 121.509
	Trimethoprim (TMP)	2	100		190.32 210.311
	Tylosine (TYL)	2	100		210.61 276.14
Aminoglycosides	**Gentamycin**	2	50		246.132 225.325
Polymyxin	**Colistin (CT)**	2	150		245.07 251.32

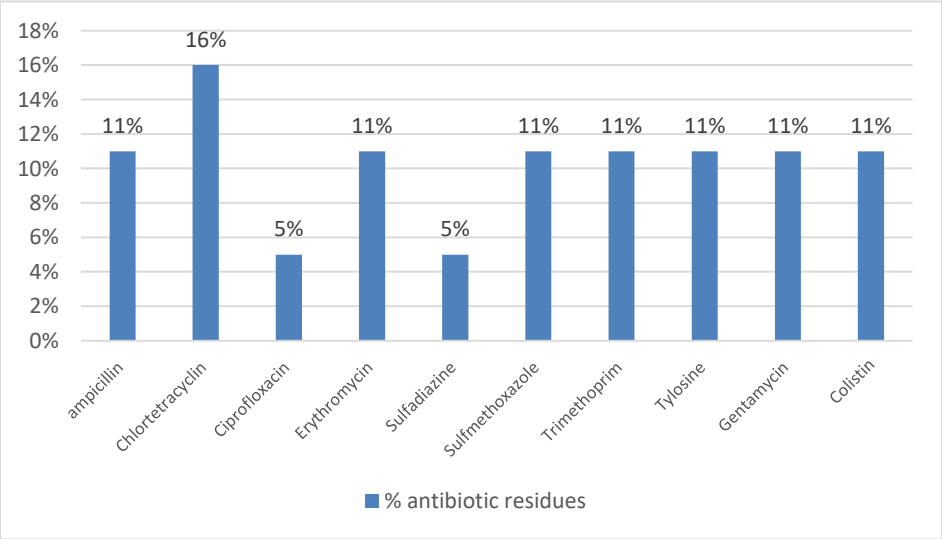

Figure 3. Method validation data for the antibiotics analyzed with LC–MS/MS technique from chicken meat samples.

Thermal stability of antibiotics stability under boiling and freezing treatments

The effect of freezing on the antibiotic residue detection with time has a data shortage in publication articles. Chicken meat samples has been frozen and stored (-18°C) for 4 weeks and the antibiotic residue has been stated **(Table 3)**. The results showed that the antibiotic residues after freezing (-18 °C) were not affected totally. On the other hand, other samples have been tested by boiling (100°C) for 20 minutes. These samples showed that the antibiotic residues in all samples were all affected. These results are in agreement with a study in 2015 **(Morshdy *et al.* 2015)** in which there was no positive frozen samples (breast muscles, thigh muscles and liver) detected after boiling for 30 minutes at 100°C. Taking into consideration that the violative levels of the drug residues is affected and diminished by heating the raw meat, therefore, it may be prevented by the heating process. Also, another study in 2015 **(Tian *et al*. 2015)** recorded the reduction of antibiotic residues concentration after thermal processing. Other taxological studies is suggested to be conducted to study the hazardous by-product degradation during boiling and to determine the food safety impact, as well as, the most residue was exerted from tissues into boiled cooking fluid. **Table 3** showed highly statistically significant high frequency of No. of affected samples after boiling was 19 sample (100%) comparing to No. of affected sample after freezing, with p-value ($p<0.001$).

Table 3. Effect of freezing (-18°C/ 4 weeks) & boiling (100°C/20 minutes) on antibiotic residues in samples of chicken meat.

Total samples	+ve samples	Freezing affected	Boiling affected	x^2	p-value
41	19	0 (0%)	19 (100%)	18.332	<0.001**

x^2: Chi-square value for Number (%) or Fisher's exact value, whenever suitable P-value >0.05 is insignificant difference; *p-value <0.05 is significant difference; **p-value <0.001 is highly significant differences between groups

Investigating the antimicrobial effect of the combined Probiotic and Kombucha on pathogenic bacteria

The results in figure 4 show that *Bifidobacterium* spp. had higher antimicrobial activity than *Lactobacillus* spp. The results also showed that kombucha tea had higher antimicrobial activity than all probiotics strains by using the well disk diffusion method. The highest efficacy of Kombucha was 25 mm for *E. coli*, then 18 mm for *Staphylococcus aureus*, 14 mm for *Proteus mirabilis*, 11 mm for *Klebsiella pneumoniae* not MDR and *Salmonella typhi* not MDR, 10 mm for *Listeria monocytogenes* and *MRSA*, 8 mm for *Enterobacter cloacae*, 7mm for *E.Coli* MDR, 6 mm for *Salmonella typhi* MDR and 5 mm for *Campylobacter jejuni.* Concerning the antimicrobial activity of probiotic on pathogenic bacteria we found that the higher efficacy of *Bifidobacterium* spp. was 7mm for E. coli, *Listeria monocytogenes* and *Proteus mirabilis*, then 6 mm for *Enterobacter cloacae*, 5 mm for *Staphylococcus aureus* and *Salmonella typhi* not MDR, 4 mm *Campylobacter jejuni* which is the same as *Lactobacillus* spp. The results showed the resistance of *Bifidobacterium* spp. against *Klebsiella pneumoniae* not MDR, *Klebsiella pneumoniae* MDR and *Salmonella typhi* MDR. Also, we found that *E. coli* MDR *Klebsiella pneumoniae* not MDR, *Klebsiella pneumoniae* MDR, *MRSA*, *Staphylococcus aureus* and *Salmonella typhi* MDR. Were resistant against *Lactobacillus* spp., While the *Campylobacter jejuni, Proteus mirabilis, Salmonella typhi, Listeria monocytogenes* and *Enterobacter cloacae.*

were sensitive against *Bifidobacterium* spp. The results also showed the resistance of *Klebsiella pneumoniae* MDR against Kombucha.

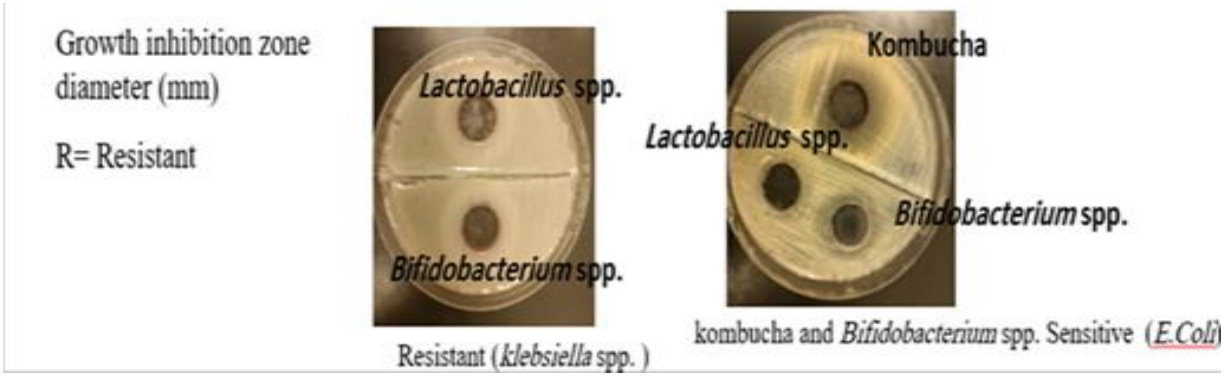

Figure 4. Evaluation of antibacterial activity of probiotic, *Lactobacillus* spp. and *Bifidobacterium* spp. compared kombucha in terms of zone of inhibition using the well diffusion method.

A. Growth Performance

Results of the study have been grouped into two phases starter (0-10 days) and grower (11-42 days). The experimental treatments have an impacts of on BWG, FI and FCR and were stated in **Figure 5**. The highest group in body weight was Group K (chicken received kombucha with drinking water) with a value of 3200.40±2.07, followed by Group A (chicken received Antibiotic with drinking water) which was 3008.60±5.94, then Group C (chicken received drinking water only) of 2905.80±58.96, with p-value ($p<0.001$). Also, Group C & A show higher mean value of feed intake with 4692.02±5.05 and 4638.34±73.76, respectively, followed by Group K of 4595.78±6.55, ($p<0.05$). Feed conversion ratio gain in Group C with 1.61±0.04, followed by Group A that was 1.54±0.02, then Group K was 1.44±0.01, with p-value ($p<0.001$). The experimental treatments didn't affect the mortality rate which was within the expected range.

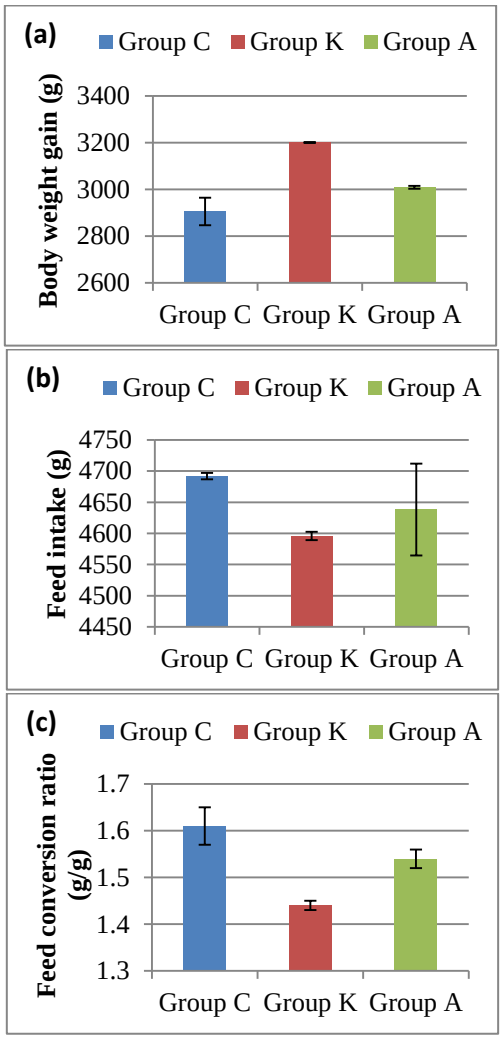

Figure 5. The effect of different treatments on (a) body weight gain (g), (b) feed intake (g), and (c) feed conversion ratio (g/g).

B. Antibody titer and biochemical parameters of plasma

From **Figure 6** it could be concluded that kombucha tea was shown to improve the blood parameters of chicken, and hence, it could be an alternative suitable for being a growth-promoting antibiotics. Results showed higher mean value of AST in Group A with 31.57±2.57, followed by Group K was 22.36±2.59, then Group C was 21.92±1.15, with p-value ($p<0.001$).Also, there was a higher mean value of triglyceride in Group A & C was 158.80±10.69 and 154.40±15.53, respectively, followed by Group K was 137.20±8.93, with p-value ($p<0.05$). Also, higher mean value of LDL in Group C was 75.55±7.81, followed by Group A was 70.92±8.93, then Group C was 58.43±5.48, with p-value ($p<0.05$). There was a higher mean value of uric acid in Group C was 6.51±0.71, followed by Group A was 6.05±0.63, then Group K was 5.18±0.37, with p-value ($p<0.05$). Also, a higher mean value of ALT in Group A was 25.91±0.96, followed by Group C was 17.26±1.91, then Group K was 13.46±2.90, with p-value ($p<0.001$).

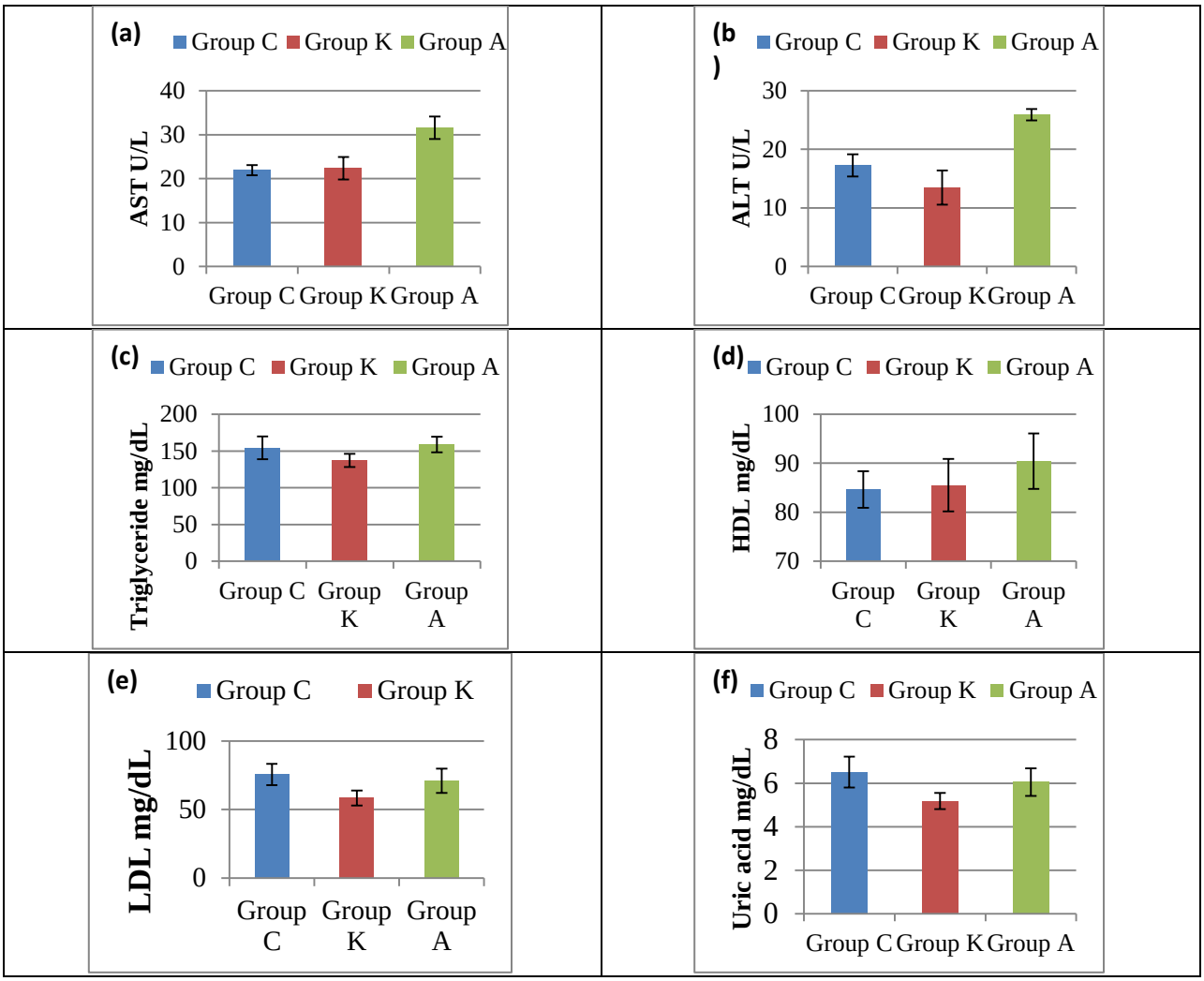

Figure 6. Effect of treatments on (a) AST U/L, (b) ALT U/L, (c) Triglyceride mg/dL , (d) HDL mg/dL, (e) LDL mg/dL. and (f) uric acid mg/dL at 42 days of age.

C. Liver and serum Antioxidant enzyme activities

From **Figure 8**, concentrations of SOD, CAT and the levels of MDA were reported, it is clear that the growth performance and the antioxidant status of chicken have been improved by kombucha tea. The figure shows high significant difference of SOD %of inhibition in K Group was 58.20±0.84, followed by A Group was 53.40±1.67, then C Group was 50.40±2.61, ($p<0.001$). Also, there was a higher mean value of CAT in K Group was 17.17±1.38, followed by A Group was 13.86±1.45, and then C Group was 11.73±0.53, ($p<0.001$). Higher mean value of MDA in C Group was 36.94±3.41, followed by A Group was 30.02±2.31, the K Group was 25.97±2.65, ($p<0.001$).

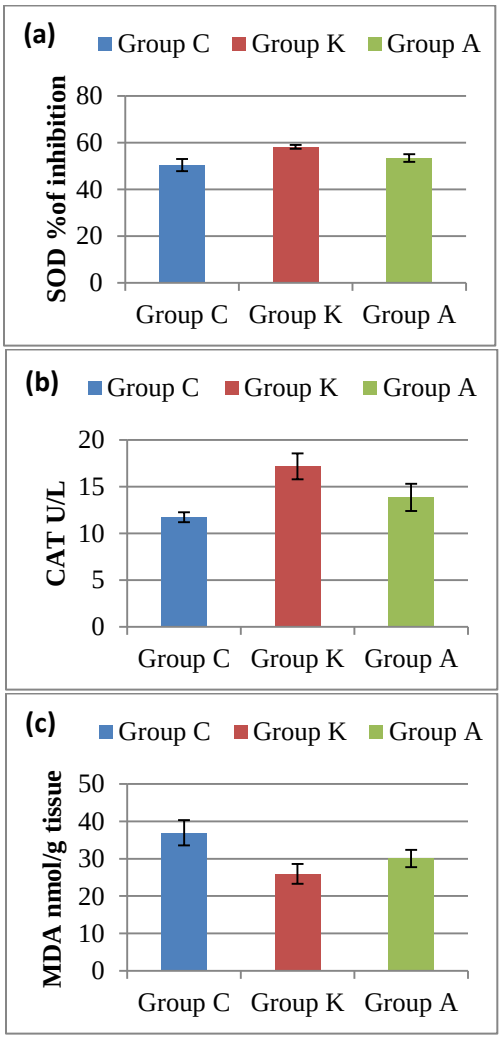

Figure 7. Effect of treatments on (a) SOD % of inhibition, (b) CAT U/L, and (c) MDA nmol/g tissue at 42 days of age.

D. Histomorphology of jejunum and ileum

The results in **figures (8, 9, and 10)** showed the intestinal morphology in chicken after 42 days of age, The height of jejunal villi expanded significantly in the antibiotic and kombucha treatment groups compared to the control group, with the most prominent villi height observed in the former to kombucha with drinking water expanded the width and surface zone of the jejunal villus.

Kombucha has rich contents of organic acids which are beneficial for intestinal morphology as it was reported in some studies **(Emami *et al.* 2017; Sabour *et al.* 2019; Saleem *et al.* 2020).** Organic acids have been hypothesized that it could decrease the pathogenic intestinal bacterial growth which diminishes the inflammatory responses and the infectious processes at the area of intestinal mucosa that in turn could increase the villus height **(Adil *et al.* 2010).**

Kombucha tea demonstrated to improve the morphometric parameters of the intestine, specially in the jejunum and ileum of chickens, these parameters are villus height, width, and surface area

And it could be regarded as a growth-promoting antibiotics alternative.

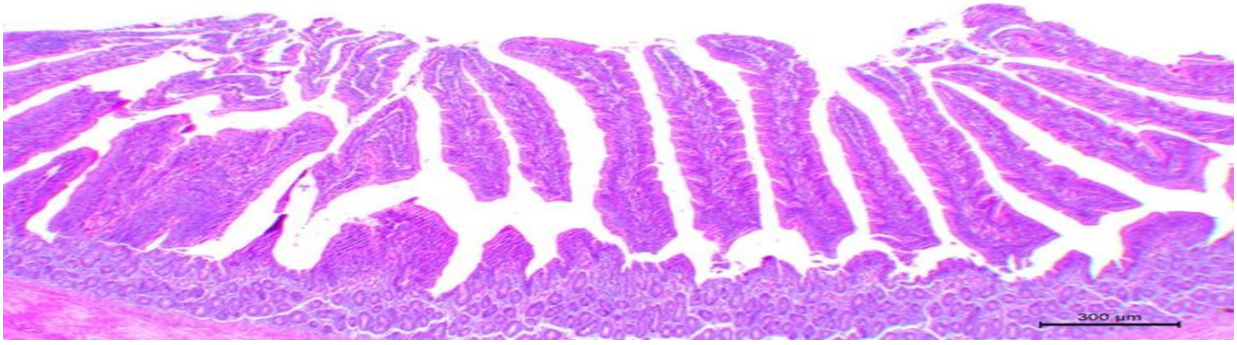

Figure 8. Intestine (duodenum) of chicken from group 1 (**control**). showing thin villi lined with pseudostratified epithelium with goblet cells, H&E, X50, bar= 300 µm.

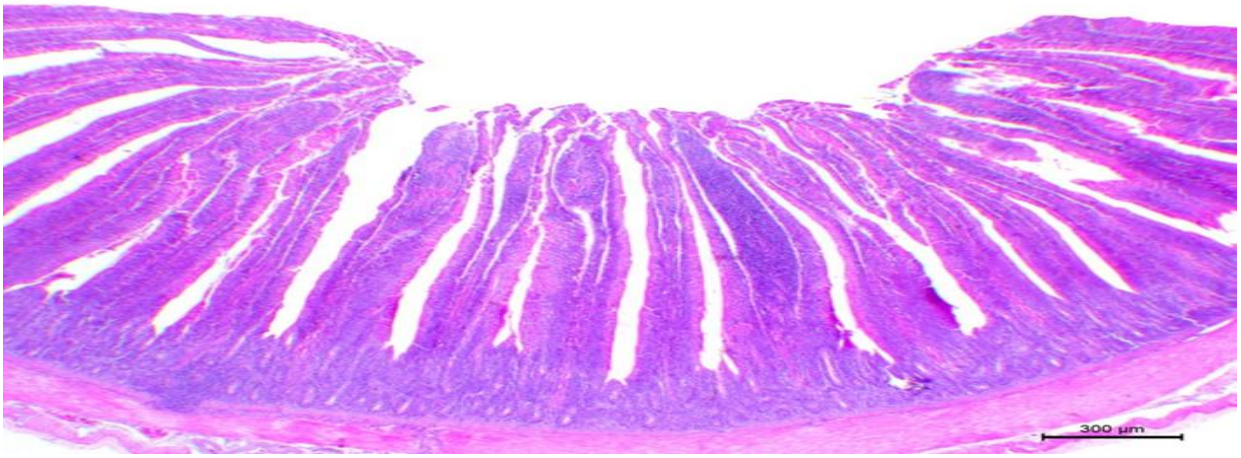

Figure 9. Intestine (duodenum) of chicken from group A (**Antibiotics**) showing normal intestinal villi with mild increase of their length, H&E, X50, bar= 300 µm.

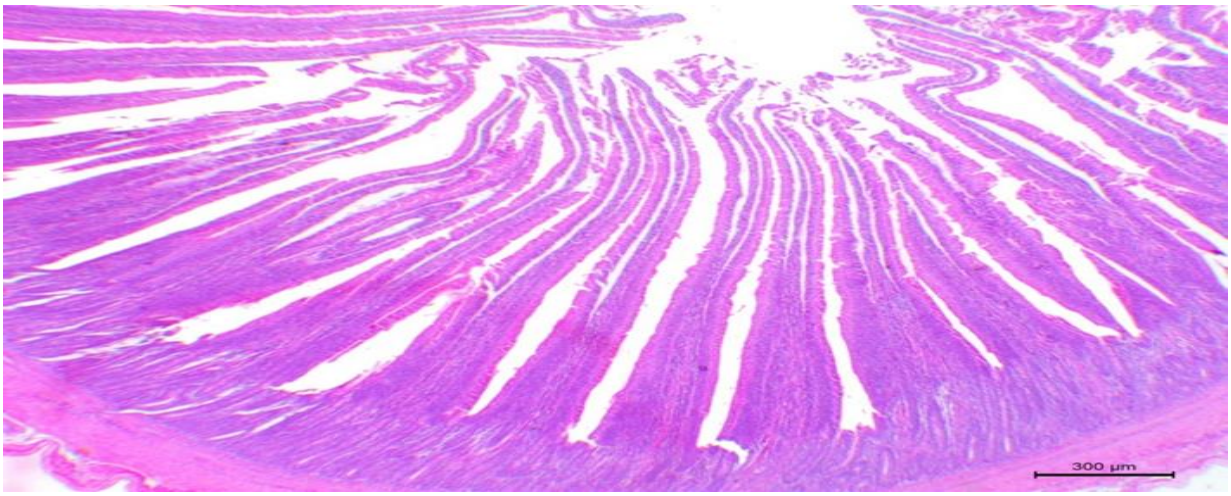

Figure 10. Intestine (duodenum) of chicken from group B ((**kombucha**) showing marked increase of intestinal villi length with decrease the inter-villus spaces, H&E, X50, bar= 300 µm.

CONCLUSION

Developing antibiotic-resistant bacterial strains is a global issue in infectious diseases treatments. Results showed that CTC, CT, AMP, CIP, ERY, SDA, SMZ, TMP, TYL, and gentamycin residues were existed and exceeded the MRLs in chicken samples. This may help in development of antibiotic resistance and also triggering allergies in human. This study is an attempt to answer 3 main questions concerning the problem of veterinary drug residues contaminating food of animal origin. The first is to select the best and most accurate method of choice to determine the residues of antibiotics. The 2nd is to present an actual image of antibiotic residues in fresh samples of chicken meat collected from local markets in Cairo. The 3rd is to shed more light on the effect of the most widespread thermal treatments of processing chicken meat on the initial level of contamination with the appropriate antibiotics. The Ministry of Agriculture and veterinary directorates should act to prevent the presence of veterinary antibiotic residues in chicken and chicken products, it is imperative to establish exacting monitoring programs and also should put in place some regulations to guarantee the appropriate withdrawal times before slaughter and marketing. Moreover, monitoring policy should be stated to ensure that chicken meat is going with the worldwide standards. It was clear that the processed kombucha could improve the growth performance. It also can ameliorate immune responses, antioxidant status, and blood parameters of chickens. All of these benefits make the processed kombucha as an appropriate elective for growth-promoting alternative to other used antibiotics in chickens. Our recommendation is 80ml of the fermented kombucha per 1000ml of water to get the best effect and to achieve the chickens' best performance.

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